Understanding the Healthy Thyroid State in 2015

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Abstract

Thyroid hormones (TH) are of crucial importance for the physiological function of almost all organs. In cases of abnormal TH signaling, pathophysiological consequences may arise. The routine assessment of a healthy or diseased thyroid function state is currently based on the determination of serum concentrations of thyroid-stimulating hormone (TSH), and the TH T₃ and T₄. However, the definition of a 'normal' TSH range and similarly 'normal' T₃ and T₄ concentrations remains the subject of debate in different countries worldwide and has important implications on patient treatment in clinics. Not surprisingly, a significant number of patients whose thyroid function tests are biochemically determined to be within the normal range complain of impaired well-being. The reasons for this are so far not fully under-

stood, but it has been recognized that thyroid function status needs to be 'individualized' and extended beyond simple TSH measurement. Thus, more precise and reliable parameters are required in order to optimally define the healthy thyroid status of an individual, and as a perspective to employ these in clinical routine. With the recent identification of new key players in TH action, a more accurate assessment of a patient's thyroid status may in the future become possible. Recently described distinct TH derivatives and metabolites, TH transporters, nongenomic TH effects (either through membrane-bound or cytosolic signaling), and classical nuclear TH action allow for insights into molecular and cellular preconditions of a healthy thyroid state. This will be a prerequisite to improve management of thyroid dysfunction, and additionally to prevent and target TH-related non-thyroid disease.

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Introduction

Thyroid hormones (TH) are critical in all phases of life and thus for development as well as for maintenance of homeostasis. The connection between thyroid dysfunction and disorders of the central nervous system (CNS), the cardiovascular system and the skeleton has been known for a long time. Furthermore, thyroid dysfunction is a frequent and reversible factor contributing to female and male infertility. Moreover, adipose tissue, which is now a major research focus due to the global increase in obesity, has long been established as an important TH effector organ.

TH Dysfunction – A Canonical Definition

Thyroid dysfunction, which results from disturbed TH synthesis, transport, metabolism and/or TH action, is among the most common endocrine disorders [1–3]. Of specific clinical importance is an adequate supply of TH during critical phases of life [4], e.g. in utero and in the neonatal period (for the child), during pregnancy (for the mother) or with older age. For example, congenital hypothyroidism, if left untreated, may lead to severe and irreversible mental retardation. In addition, hypothyroidism can result in impairment of growth and maturation of the skeletal system, while hyperthyroidism leads to accelerated linear growth in children which results in premature closure of the epiphyseal plate [5] if not treated properly [1–3].

Thyroid dysfunction in a pregnant woman may contribute to pregnancy loss or premature termination and maternal complications during delivery. For this reason, the recent European Thyroid Association (ETA) pregnancy guidelines committee is to be commended because they are the first, worldwide, to advocate screening for thyroid dysfunction in every pregnant woman [6]. In adulthood, hypothyroidism is associated with psychiatric disorders such as depression and dementia. The CNS is also commonly afflicted in hyperthyroidism, which typically presents as anxiety and agitation, while tremor and sweating are symptoms of increased TH action on the sympathetic autonomous nervous system. In this issue of *European Thyroid Journal*, Münte and colleagues [pp. 113–118] describe the effects of hyperthyroidism in the CNS using sophisticated functional imaging. In male probands they show that T_4 treatment resulting in hyperthyroidism affects grey matter structure of cerebellar regions that have been associated with sensorimotor functions and working memory.

Both hyper- and hypothyroidism are associated with increased cardiovascular morbidity (arrhythmia, cardiomyopathy, stroke) and mortality, particularly in the elderly. Likewise, hyperthyroidism is an established risk factor for osteoporosis in postmenopausal women. These epidemiological findings have recently led to a change in management recommendations, whereby persistent subclinical hyperthyroidism should now be treated in all patients above 65 years of age and treatment should also be considered in younger patients with known heart disease or risk for osteoporosis [7].

Importantly, classical clinical features of thyroid dysfunction are often obscured in elderly patients and hence diagnosis may be missed. The molecular mechanisms accounting for these variations upon aging are still under research and besides the pituitary-thyroid axis, various compartments, i.e. TH synthesis, TH transport in the blood and metabolism, transport through the plasma membrane and intracellular and nuclear TH action, may also be affected. In this issue, Engels and colleagues [pp. 81–86] report their findings on TH transporter expression pattern in liver tissues of young and old male mice with experimentally induced thyrotoxicosis. Besides an alteration in TH serum status, they found upregulation in gene expression of various TH transporters in liver tissue with aging, while similar patterns in TH-responsive genes and TH transporter expression were observed in young and old mice under chronic hyperthyroidism.

Furthermore, TH are fundamental to energy expenditure in adipose tissue. To get closer insights into how TH affect lipid storage and utilization, Krause and colleagues [pp. 59–66] studied the expression of acyl-CoA thioesterase in adipose tissues of hyper- and hypothyroid mice. They could show that acyl-CoA thioesterase expression is differentially regulated in white and brown adipose tissue in response to TH. This study highlights the necessity of taking a more precise look at the distinct compartments of white, brown and beige adipose tissues, which came into the spotlight of research more recently. Additionally, bile acids have been reported as having a role in human energy metabolism [8] and a link between TH and bile acid excretion has long been known. Zwanziger and colleagues [pp. 67–73] show an association between the tight junction protein claudin-1 in liver and the thyroid serum status in male and female mice. They were able to demonstrate that claudin-1 expression in the liver biliary system is influenced by TH and interestingly they observed a sex dependence of claudin-1 expression in euthyroid versus hypothyroid mice.

Intriguingly, thyroid disorders are approximately four times more frequent in females [9] than in males, but –

besides a most probable role of estrogen – no precise molecular explanation exists for this persistent finding.

In addition to common TH-associated diseases, thyroid storm and myxedema coma represent rare but life-threatening TH-associated dysfunction. In both conditions, an acutely altered TH action has been proposed to occur in the target organs and to contribute to multiorgan failure with very high mortality rates [10, 11].

Thyroid storm is a particularly good example of discrepant TH serum and organ status, whereby classical TH T_4 and T_3 in the blood do not differ from ‘uncomplicated’ thyrotoxicosis. Hence, diagnosis is often difficult and may be fatally delayed [10]. The so-called ‘low- T_3 syndrome’ observed in critically ill ICU patients demonstrates a further systemic alteration in TH action, and in case of persistence might prove suitable as a highly valid prognostic marker for poor outcome.

Laboratory TH Assessment

Classification of thyroid function and dysfunction is currently based on specific laboratory criteria. A constellation of normal TSH and free T_4 but reduced total T_4 points to a deficiency in thyroxine-binding globulin (TBG), which is the main TH-transporting molecule in the blood. In this supplement, Moeller and colleagues [pp. 108–112] report two new mutations in the TBG gene, namely a splice site variant and a large deletion mutation that were discovered in 2 children. These findings are interesting from the basic science perspective and point to the fact that abnormal TH values might be explained by genetic defects in TH-binding proteins and must not necessarily reflect diseased thyroid status. The evolutionary significance of TBG and its genetic variants has been highlighted in another recent study on a population of Australian indigenous people, where it was found that certain folds of TBG may contribute to adapting to life in hot climates [12, 13]. These recent observations on TBG mutations and altered TH-binding capacities are important to consider in cases where such mutations are prevalent in family history, in order to avoid unnecessary treatment which would otherwise be motivated by seemingly diseased serum TH concentrations.

Overt hyperthyroidism is biochemically defined as a constellation of reduced TSH serum concentrations and elevated FT_4 and/or FT_3 , whereas overt hypothyroidism is characterized by elevated TSH and decreased FT_4 serum concentrations [1, 2]. Combinations of reduced (hyperthyroidism) and elevated (hypothyroidism) TSH lev-

els with normal FT_4 serum concentrations are defined as subclinical thyroid dysfunction [3]. This definition is appropriate in humans for most cases of thyroid dysfunction, and the term ‘thyrotoxicosis’ is frequently applied for clinical situations associated with TH excess. However, for a more comprehensive and broader concept it must be added that hyper- or hypothyroidism rather mark augmented or reduced TH signaling, respectively, in a tissue- and/or cell-specific manner [14]. This may arise, besides from the pituitary-thyroid axis, from disturbed peripheral TH action through altered metabolism, transport, transmembrane and/or intracellular signaling.

Recent analyses of two large cohort studies performed in Germany [15–19] have demonstrated that serum concentrations of TSH above the respective assay-specific ranges occurred in as many as 4.3% (SHIP, Study of Health in Pomerania) and 14.1% (KORA, Cooperative Health Research in the Augsburg Region) of adults above 65 years of age. In addition, TSH concentrations below reference ranges were found in 3.5% (SHIP) to 1.7% (KORA) of the study populations [16]. Overall, the prevalence of TSH concentrations outside the assay reference ranges was higher in women than men, and this has been observed in most epidemiological cohorts. However, detection of thyroid function values outside the assay reference ranges must not necessarily indicate a thyroid disorder. For this reason, there have been heated debates on the so-called ‘normal TSH values’ for some years on both national and international stages [4, 20, 21]. Determination of TSH serum concentrations in clinical chemistry largely depends on the assay(s) used and usually manufacturer-derived reference ranges. In Germany alone, at least 38 different TSH assays were evaluated in Interlaboratory Reference Institute for Bioanalytics (RfB), but most laboratories will rely on 1 of only 4 assays with which approximately 80% of TSH measurements are currently conducted (Interlaboratory RfB 4/2013). On the other hand, TSH reference ranges vary greatly depending on the population analyzed.

An important factor which influences TSH concentrations is age [22–24]. Several epidemiological studies have suggested that the TSH levels increase with age physiologically [25–27], indicating that novel diagnostic tools are required in order to implement adequate age-related reference values. Additional factors which impact on a more comprehensive definition of healthy TSH ranges within a given population comprise gender; BMI; exclusion of incident thyroid disease, particularly autoimmune thyroiditis and thyroid autonomy; ethnicity, and iodine and selenium intake [20, 22]. In addition, there is currently a

debate on whether the influence of anthropogenic substances, so-called ‘endocrine disruptors’ such as estrogen derivatives and polychlorinated biphenyls, potentially contribute to an altered prevalence of thyroid dysfunction in various populations [28].

In clinical practice, it is well known that TH serum status often poorly correlates with the expected and observed effects of TH in target tissues. This applies to both overt and subclinical thyroid dysfunction. Furthermore, the biologically relevant intracellular hormone levels may be altered in, for example, intensive care patients and patients with specific comorbidities or medication (based on clinical observation). Therefore, it is likely that intracellular variations of TH levels do not necessarily reflect changes of classical TH serum status. Additionally, genome-wide studies have demonstrated genetic variability in enzymes and other proteins responsible for TH synthesis and metabolism [29], thereby implicating that an individually characteristic value determines TH action in a person, patient or particular group of patients.

The consequences of these variable and most likely genetically based dispositions are rather impressively observed in patients under TH treatment who, despite ‘normal’ laboratory parameters, often complain of impaired physical and neurocognitive function and suffer from impaired quality of life [30]. In fact, very recently a study demonstrated that patients with thyroid disorders, despite adequate and prompt treatment, are at higher risk for long-term absence from work or, more drastically, early disability pensioning or unemployment compared to the normal population [31]. Here, individual genotype-phenotype analyses must be performed in the future in order to provide a more thorough understanding of the risk assessment of individually characteristic values that determine TH signaling in a person, patient or particular group of patients.

The findings and considerations described above underline that the assessment of normal or abnormal thyroid function is rather complex in the individual case, and clearly surpasses the presently practiced and available serum TH status.

In search of new thyroid-derived molecular players that might help to obtain a more comprehensive picture of healthy versus diseased thyroid status, additional TH and their derivatives are increasingly being studied. Very recently, the determination of the TH derivative 3,5-diiodothyronine (3,5-T₂) by a monoclonal antibody-based immunoassay was reported [32]. To further unravel the physiological role of 3,5-T₂ in this issue, Pietzner and colleagues [pp. 92–100] investigated the association between

3,5-T₂ serum concentrations and urine metabolomics in a cohort of healthy individuals. In their study based on the SHIP cohort, they propose an association of 3,5-T₂ serum concentrations with glucose and lipid metabolism. Most interestingly, the authors describe that 3,5-T₂ levels correlate to a urine metabolic profile that is very different to that of elevated concentrations of classical TH.

Moreover, in this issue, Ittermann and colleagues [pp. 101–107] performed an association study in three different cohorts (SHIP-2, SHIP-TREND and CARLA) to analyze a potential relationship between thyroid function (determined by TSH and 3,5-T₂) and peripheral arterial disease, which was evaluated by determination of the ankle-brachial index. However, they could not delineate any correlation of low, normal or enhanced TSH and 3,5-T₂ serum concentrations with the ankle-brachial index, and did not confirm such an association reported in previous studies, albeit with smaller proband numbers.

Future research is therefore required to establish novel and precise high-throughput assays that are suited to comprehensively analyze the spectrum of TH metabolites including derivatives in body fluids. Thus, assessing the ‘thyronome’ is at stake. Mass spectrometry analysis will have a pivotal role here, and in this issue, Köhrle and colleagues [pp. 51–58] report on quantitative analysis of TH metabolites in cell culture samples using LC-MS/MS. Their approach is a prerequisite for comprehensive analysis of TH and TH derivatives in more complex biological samples such as blood or tissues of humans and/or animal models.

In addition, ‘OMICS’ tools may help to uncover further systemic TH action. Using a proteomics approach, Engelmann and co-workers [pp. 119–124] show in this issue that experimental thyrotoxicosis results in selective thyroxine-induced changes in coagulation markers in humans. This technology may represent a future avenue to identify and define more specific biomarkers for thyroid hormone effects in health and disease.

Nonclassical Aspects of TH Actions

In changing a century-old paradigm, it was demonstrated that TH molecules enter cells via specific transporter proteins [33]. With the identification of mutations in the monocarboxylate transporter 8 (*MCT8*) gene in 2004 [33, 34], it was demonstrated that the MCT8 TH transporter protein is responsible for T₃ uptake into neurons; hence, impairment of MCT8 function was found to account for a severe neurodevelopmental disorder first

described by Allan, Herndon and Dudley in 1944. This hereditary form of impaired sensitivity to TH is now classified as a TH cell membrane transport defect [35].

In patients with the Allan-Herndon-Dudley syndrome (AHDS), laboratory assessment of serum levels is characterized by elevated T_3 concentrations and normal or decreased TSH and T_4 values [36]. The affected patients demonstrate a complex phenotype of severe psychomotor retardation and muscular hypotonia. Interestingly, due to the distinct relevance of MCT8 in different organs, AHDS patients exhibit signs of both hypothyroidism (CNS phenotype) and hyperthyroidism (liver phenotype). In contrast, no heart-related pathological manifestations have been described despite the strikingly elevated T_3 levels [33]. It is thus concluded that TH transporters may have a different impact on TH target tissues depending on their expression pattern. Not surprisingly, the underlying molecular causes that explain the specifically altered T_4 -to- T_3 ratios in the serum of AHDS patients are the subject of intense research. In this issue, Wirth and colleagues [pp. 87–91] provide evidence that the characteristic serum TH profile in Mct8-deficiency is not caused by increased hepatic conversion through the type I-deiodinase selenoprotein enzyme.

Astonishingly, Mct8-deficient mice show a much weaker neurological phenotype despite a TH serum status comparable with the situation in AHDS patients [37]. These findings illustrate that the regulation of TH transport into the target cells may differ not only from cell type to cell type, but also between different species.

Very recently a new mouse model that resembles the human phenotype of MCT8 deficiency with regard to the neurological symptoms, the Mct8/Oatp1c1 double-knockout mouse, was reported by Heuer and colleagues [38]. These findings explicitly demonstrate that the quest for physiologically relevant TH transporters in mice and man needs our utmost attention in the years to come. Careful considerations of species-specific variations in TH transport and TH metabolism are mandatory, including investigations on pathogenic TH transporter mutations and their effects on cellular trafficking to the correct location, which is the cell surface in the case of MCT8 [39].

Besides primary TH transporters like MCT8, also secondary transporters such as the L-type amino acid transporter 2 (LAT2) or the organic anion-transporting polypeptide 1c1 (Oatp1c1) exist. In this issue, Kinne and colleagues [pp. 42–50] describe a powerful oocyte cell system that the authors established to facilitate studies on the precise transport characteristics of LAT2. Of note, they

could show that LAT2 is capable mediating transport of $3,3'$ - T_2 across the plasma membrane, while also enabling transport of T_3 , although to a much lower extent. These findings underline that LAT2 cannot be regarded as a canonical T_4/T_3 transporter, but might be important in eliminating TH degradation products from cells by enabling its export from TH target cells. Thus, TH transport involves both import and export by transporter-mediated translocation across membranes, which is instrumental for TH uptake or TH release from both TH-generating and TH target cells [40]. To date, the precise nature of TH transport in many relevant TH target tissues is still largely unknown and this applies also to intestinal TH transporter systems, where the quest for the apical TH transporter is still ongoing, although this organ is the main entry point in TH replacement therapy.

We conclude that the discovery of the TH transporters has revolutionized the concept and interpretation of TH-evoked effects. However, the mechanisms of TH uptake into the target cells and their elimination after having exerted cellular TH action are still to be fundamentally refined for each and ultimately all TH target organs.

In addition to the classical genomic effects of T_3 , a number of studies have demonstrated initiation of a fast transmembrane signal transduction by T_4 which triggers downstream events via binding to integrins; in addition, intracellular T_3 signaling via the PI3-K cascade was described as well (for a review, see Brix et al. [41]). The importance of such nongenomic and nonclassical TH effects requires further characterization. Based on the available experimental data, nonclassical intracellular TH effects may be particularly relevant in the context of tumor-associated angiogenesis (for a review, see Moeller et al. [42]).

In parallel with the discovery of the TH transporters in 2004, it was also described for the first time that thyronamines are biologically active, decarboxylated and deiodinated TH derivatives [43]. In particular, a fundamental opposing effect on T_4 and T_3 is attributed to the thyronamines 3- T_1 AM and T_0 AM. The terms of so-called 'cold' versus classical 'hot' TH were coined in consequence [43]. The most important effect of thyronamine administration first appeared to reside in lowering the body temperature; however, this notion is currently rather controversial because it was not reproduced in mice that lack the putative thyronamine receptor, the G protein-coupled trace amine-associated receptor Taar1 [44]. Furthermore, there is increasing evidence for an inhibitory effect of thyronamines on metabolic regulation [45, 46] in addition to previously described effects on glucose homeostasis [47]. However, the data known so far indicate an

urgent need for further studies to unravel overlapping, cooperative or competitive effects of thyronamines and their metabolic derivatives.

Current knowledge indicates that thyronamines can trigger their effect, in principle, via Taar1 which had been demonstrated in the first seminal paper on thyronamine-Taar interactions [48]. In this supplement, Cöster and colleagues [pp. 9–20] approached the evolutionary relatedness of 3-T₁AM function at TAAR1 in vertebrates with the aim to further unravel the biological significance of TAAR1. They identified an open-reading frame for over 100 different species and investigated evolutionary conservation of 3-T₁AM and β -phenylethylamine action at TAAR1 in a broad range of different species. Still, GPCRs other than Taar1 must be considered in explaining the observed thyronamine effect [44]. In this context, the work of Dinter and colleagues [pp. 21–29] in this issue shows that 3-T₁AM affects the function of the β -adrenergic receptor 2 (ADRB2) and the transient receptor potential melastatin channel 8 (TRPM8) and indicates that indeed more targets exist for circulating 3-T₁AM.

While the physiological roles of thyronamines have been approached in several studies, cell biological investigations towards thyronamine actions via Taar1 or related receptors have proven difficult. In this issue, Szumska and colleagues [pp. 30–41] employed immunofluorescence on the polarized rat thyroid epithelial cell line FRT to describe Taar1 transport along the compartments of the secretory route from the ER via the Golgi and trans-Golgi network to the apical plasma membrane, where it was present at cilia. These results suggest considering alternative pathways by which thyronamines or biogenic amines can activate Taar1, at least in thyroid follicle cells, namely by acting from within the thyroid follicle lumen.

Finally, new guidelines have been published for investigations of TH action in rodent models [49], thereby providing standardized protocols to ensure comparability between studies, thus enabling more general conclusions. Schmohl and colleagues [pp. 74–80] provide us with a revised protocol for radioiodine ablation of the thyroid gland, in which the combination of low-iodine-containing diets for stimulation of the thyroid gland and low-grade radioiodine ablation were sufficient to achieve hypothyroidism in mice.

Last but not least, new recommendations were published in 2014 for classification of inheritable forms of impaired sensitivity to TH enlarging our perception of the complexity of nonclassical TH action. Thus, besides the defects in TH cell membrane transporters discussed above, mutations in the selenocysteine insertion se-

quence-binding protein 2 have been identified, resulting in inactivation of sequence-binding protein 2 function and hence classified as TH metabolism defect. Affected patients show elevated T₄ and rT₃ but low T₃ serum concentrations with normal-to-elevated TSH. Clinically, patients appear hypothyroid with delayed development, impaired bone maturation and growth, impaired hearing and azoospermia, amongst other features. Furthermore, besides the ‘Refetoff syndrome’ of classical resistance to TH (RTH) by a THRB mutation, novel TH action defects have been identified in the form of RTH- α that involves dominant negative mutations of the THRA gene. RTH- α patients show a unique phenotype of skeletal dysplasia, delayed bone and dental development, cognitive impairment, constipation, and anemia, and a TH serum status that is characterized by low serum T₄/T₃ ratio and low rT₃. These few examples illustrate that impaired sensitivity, but most likely also a condition of hypersensitivity to TH, can arise from very different players relevant to TH signaling, and many of these defects have yet to be discovered. This, however, requires a meticulous and vigilant assessment of patients’ clinical features, suggesting abnormal tissue TH signaling in combination with sophisticated interpretation of serum TH status [35], and accompanied and integrated with basic science research.

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The authors have nothing to disclose.

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