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Homeostatic equilibria between free thyroid hormones and pituitary thyrotropin are modulated by various influences including age, body mass index and treatment

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Summary

Objective We examined the interrelationships of pituitary thyrotropin (TSH) with circulating thyroid hormones to determine whether they were expressed either invariably or conditionally and distinctively related to influences such as levothyroxine (L-T4) treatment.

Design and methods This prospective study employing 1912 consecutive patients analyses the interacting equilibria of TSH and free triiodothyronine (FT3) and free thyroxine (FT4) in the circulation.

Results The complex interrelations between FT3, FT4 and TSH were modulated by age, body mass, thyroid volume, antibody status and L-T4 treatment. By group comparison and confirmation by more individual TSH-related regression, FT3 levels were significantly lower in L-T4-treated *vs* untreated nonhypothyroid autoimmune thyroiditis (median 4·6 *vs* 4·9 pM, $P < 0{\cdot}001$), despite lower TSH (1·49 *vs* 2·93 mU/l, $P < 0{\cdot}001$) and higher FT4 levels (16·8 *vs* 13·8 pM, $P < 0{\cdot}001$) in the treated group. Compared with disease-free controls, the FT3-TSH relationship was significantly displaced in treated patients with carcinoma, with median TSH of 0·21 *vs* 1·63 ($P < 0{\cdot}001$) at a comparable FT3 of 5·0 pM in the groups. Disparities were reflected by calculated deiodinase activity and remained significant even after accounting for confounding influences in a multivariable model.

Conclusions TSH, FT4 and FT3 each have their individual, but also interlocking roles to play in defining the overall patterns of thyroidal expression, regulation and metabolic activity. Equilibria typical of the healthy state are not invariant, but profoundly altered, for example, by L-T4 treatment. Consequently, this suggests the revisitation of strategies for treatment optimization.

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Introduction

Multiple homeostatic mechanisms control and define the collective metabolic activity of the human body. As key contributors, thyroid hormones influence a plethora of metabolic effects. Their genomic actions depend mainly on their binding to specific nuclear ligands (thyroid hormone receptors, TR) that, in turn, regulate the expression of an array of genes.[1] Owing to its high affinity for TR, triiodothyronine (T3) is the biologically active thyroid hormone, whereas thyroxine (T4) is the predominant secretory product of the thyroid gland in humans.[2] To elicit its full genomic response, T4 requires prior conversion into T3 by enzymatic monodeiodination regulated by specific deiodinases.[3] The vast spectrum of actions and important metabolic role of thyroid hormones demand tight control and maintenance of narrow ranges in the circulation. This includes a hypothalamic pituitary thyroid feedback mechanism and secretion of a pituitary thyroid stimulator (thyrotropin, TSH) sensitively responding to changes in concentrations of circulating thyroid hormones.[4] While the basic principles have long been unravelled and exploited for diagnostic purposes, more recent studies have revealed the level of complexity of the system.[5–9] At the molecular level, variations have been found at all sites of action including different subtypes of T3 receptors involved in T3 binding and isoforms of deiodinases operating in central and peripheral organs.[10,11] For the feedback control, we have proposed a hierarchical model to replace the constant relationship hitherto assumed to exist between FT4 and TSH.[12]

The clinical consequences of these new concepts are still poorly understood. In this study, we hypothesized that systemic complexity may directly impact on the homeostatic equilibria existing between key contributors under various conditions. This proposes that the equilibria are not consistently maintained, but expressed

differently under various pathophysiological conditions. In our prospective study, we analysed the interrelations of FT3, FT4, FT4–FT3 conversion and TSH in various situations. We examined whether the expression of the interacting equilibria remained invariably constant or was conditionally related to influences such as age, obesity or levothyroxine (L-T4) treatment.

Materials and methods

Study design and setting

This trial was a prospective open observational study of patients referred to the Department of Nuclear Medicine at Klinikum Luedenscheid, Germany, for thyroid testing or treatment between July 2013 and February 2014. The Department of Nuclear Medicine is a specialized thyroid centre, and Klinikum Luedenscheid is a teaching hospital of the University of Bonn. Luedenscheid is located in a former area of mild iodine deficiency. The study was approved by the Ethics Committee of the University of Muenster, Germany, and the trial registered at www.ClinicalTrials.gov (Study ID IIFHT-161013). All adult persons were eligible to participate provided they were free of severe comorbidity and gave their written informed consent.

Routine diagnostic procedures included a detailed history supplemented by a standardized questionnaire, a thyroid-related physical examination, blood sampling for laboratory tests and thyroid imaging.

Outcome

The primary measure of interest was the interrelation of thyroid parameters under the various conditions observed.

Patients

A total of 1912 subjects were recruited. Most were seen on an ambulatory basis, and some were treated as inpatients in the radioiodine treatment unit. These patients were not severely ill, but their hospitalization stemmed from German legal requirements that preclude ambulatory treatment. However, as regular medication was withdrawn from some before hospitalization, treatment-related analyses were restricted to the ambulatory panel.

Excluded from analysis were the following situations: hypothalamic/pituitary diseases ($n = 5$), pregnancy ($n = 3$), use of antithyroid drugs ($n = 99$) or thyroid replacement regimes involving T3 ($n = 9$) rather than L-T4.

Characteristics of the remaining 1796 patients are given in Results. Documentation included gender, age, height, weight, smoking habits (disclosed by 75% of the participants), prior surgery or radioiodine treatment, current and past medication (brand and dosage), time between intake of thyroid medication and blood sampling, thyroid test results (FT3, FT4, TSH), autoantibody determination (only in case of diagnostic suspicion of or to exclude thyroid autoimmune disorders), thyroid volume, scintigraphical findings (in cases with larger nodules), diagnosis according to ICD-10 coding and free text.

Serial measurements in hypothyroid subjects before and after surgery

Serial measurements in patients with hypothyroidism were derived from a subgroup of a separate ongoing prospective longitudinal study of patients in open-loop situation, ethically approved and registered (NOMOTHETICOS Trial, UTN U1111-1122-3273, ClinicalTrials.gov ID NCT01145040). We investigated the calculated sum activity of peripheral deiodinases in a small cohort of 10 subjects [nine women, median age 45 (38, 50) years] before and after initiation of substitution therapy with L-T4 [median dose two (1·9, 2·2) μg/kg body weight] following thyroidectomy for benign or malignant disease.

Laboratory methods

Thyroid function tests for the main study were performed by the Institute of Laboratory Medicine, accredited by the German National Accreditation Body (DAkkS). Standard laboratory quality evaluation procedures were routinely employed, and regular participation at interlaboratory tests formed part of the quality management strategy. TSH was measured with an automated direct chemoluminescence method (TSH3-Ultra, Advia Centaur XP; Siemens Healthcare Diagnostics, Erlangen, Germany), based on the 3rd International Standard for TSH (WHO, IRP 81/565) with a linear range from 0·006 to 160·03 mU/l. CVs of interassay imprecision ranged from 0·9% to 2·4%. FT3 and FT4 were determined on the same system. The reference intervals used were 0·4–4·0 mU/l for TSH, 10–23 pM for FT4 and 3·1–6·8 pM for FT3, based on an extensive evaluation in the target population.[13] Equivalently to the FT3/FT4 ratio, a measure of conversion, the sum activity of peripheral deiodinases (deiodinase activity), was calculated as previously described.[14,15] Thyroid peroxidase antibodies (TPO-Ab) were determined by a competitive chemoluminescence method (ADVIA Centaur XP; Siemens Healthcare Diagnostics, reference range <60 U/ml) and TSH-receptor antibodies (TSH-R Ab) by a competitive ELISA (Euroimmun AG, Lübeck, Germany, reference range <2 U/l).

Biological variation assessed in 106 patients followed for a median of 7 months (IQR 6, 10) under unchanged conditions was 19·8% for TSH in untreated controls ($n = 72$) and 22·8% in L-T4-treated subjects and <5% in untreated or <8·5% in treated persons for FT3 and FT4. Variations of relationships are shown in Results.

For the longitudinal substudy, TSH, FT4 and FT3 were measured on fully automated immunoassay analysers (DxI800; Beckman Coulter, Krefeld, Germany). Locally defined reference ranges were 0·3–4·0 mU/l for TSH, 9–26 pM for FT4 and 3·7–6·9 pM for FT3.

Thyroid ultrasonography and scintigraphy

Thyroid ultrasonography (10 MHz transducer) assessed echogenicity, nodularity and thyroid volume (ellipsoid formula, reference values <18 ml for female and <25 ml for male

subjects). A volume <1 ml was considered athyreotic. Thyroid scintigraphy was performed in patients with larger nodules detected by ultrasonography.

Statistical methods

Descriptive data are reported as median plus interquartile range (IQR). Comparison of baseline characteristics was based on Wilcoxon's rank-sum or chi-square test in case of categorical variables. Paired Wilcoxon signed rank test was used to compare longitudinal data. A generalized linear model (GLM) was used for analysis of multiple variables and influences, as implemented by Deducer (version 0.7–6) in the R software package.[16] Nonlinear relationships between thyroid parameters that existed in the full thyroid functional spectrum were represented by third- or fourth-order polynomials. For restricted TSH intervals, a linear approximation sufficed. *P* values <0·05 were considered significant. Statistical analyses were performed using R statistical package (Mac version 3.02).[17]

Results

Thyroid function and adjusted multivariable relationships

Patient characteristics and descriptive statistics of the 1796 subjects included are given in Table 1.

As previous studies demonstrated,[7–9] a polynomial nonlinear fit resulted in a superior model for FT4-TSH or FT3-TSH relationships in untreated subjects over the full functional spectrum, compared to a log-linear model (GLM, ANOVA, $P < 0{\cdot}001$). As treatment significantly altered the relationships ($P < 0{\cdot}001$, Fig. 1), the treated and untreated group were analysed separately.

Various factors influenced the relations between TSH and FT4 or FT3 in untreated outpatients ($n = 824$), including age ($P < 0{\cdot}001$), BMI (FT4 $P = 0{\cdot}003$, FT3 $P < 0{\cdot}001$), aetiology of disease ($P < 0{\cdot}001$), TPO-Ab titres (FT4 $P = 0{\cdot}02$, FT3 $P = 0{\cdot}003$) and thyroid volume ($P < 0{\cdot}001$), but not gender (FT4 $P = 0{\cdot}40$, FT3 $P = 0{\cdot}21$) and smoking habits (where

Table 1. Demographics and thyroid status in the cohort

Parameter	Total	Untreated	Treated	*P* value
Patients (*n*)	1796	1126	670	
Outpatients (%)	1395 (78%)	824 (73%)	571 (85%)	<0·001
Inpatients (%)	401 (22%)	302 (26%	99 (15%)	
Females (%)	1365 (76%)	843 (75%)	522 (78%)	0·13
Males (%)	431 (24%)	283 (25%)	148 (22%)	
Nonsmokers (%)	1019 (76%)	582 (73%)	437 (80%)	0·002
Smokers (%)	321 (24%)	215 (27%)	106 (20%)	
Autoimmune thyroiditis	195 (11%)	85 (8%)	110 (16%)	<0·001
Thyroid carcinoma*	255 (14%)	3 (0·3%)	252 (38%)	
EuT goitre or nodule	512 (29%)	402 (36%)	110 (16%)	
HyperT, nodular disease	270 (15%)	270 (24%	0 (0%)	
HyperT, Graves' disease	69 (4%)	69 (6%)	0 (0%)	
Disease-free	146 (8%)	146 (13%)	0 (0%)	
Postintervention for benign disease	325 (18%)	132 (12%)	193 (30%)	
Others	24 (1%)	19 (2%)	5 (0·4%)	
Radioiodine treatment	358 (20%)	65 (6%)	293 (45%)	<0·001
Surgery	418 (23%)	43 (4%)	375 (57%)	<0·001
Age (years)	54 (43, 66)	53 (42, 67)	56 (45, 65)	0·02
BMI (kg/m^2)	26·6 (23·7, 30·5)	26·2 (23·5, 30·1)	27·4 (24·0, 31·1)	<0·001
FT3 (pM)	5·0 (4·5, 5·4)	5·1 (4·7, 5·5)	4·7 (4·1, 5·2)	<0·001
FT4 (pM)	15·1 (13·4, 17·1)	14·4 (13·2, 15·8)	17 (14·5, 19·6)	<0·001
TSH (mU/l)	1·07 (0·43, 2·06)	1·07 (0·46, 1·88)	1·08 (0·38, 3·0)	0·05
TPO-Ab (U/l), $n = 764$	44 (33, 134)	42 (33, 70)	59 (36, 1300)	<0·001
Positive (%)	31%	26%	50%	
TSH-R Ab (U/l), $n = 329$	0·2 (0·2, 1·0)	0·21 (0·2, 1·0)	0·2 (0·2, 0·7)	0·43
Positive (%)	9%	10%	6%	
Deiodinase activity (nmol/s)	30·6 (26·2, 35·2)	32·8 (30·0, 36·5)	25·6 (22·3, 29·4)	<0·001
Thyroid volume† (ml), $n = 1493$	13 (8, 21)	16 (11, 25)	7 (4, 12)	<0·001

Values shown represent median and IQRs or relative frequencies. Comparisons were made using Wilcoxon's test or chi-square test. 59 patients taking 100–200 μg/day iodine were classified as untreated. 618 of the treated patients were on L-T4 and 52 on L-T4 and iodine, none on T3/T4 (nine had been excluded, see Methods).

BMI, body mass index; deiodinase activity, calculated sum activity of peripheral deiodinases, an equivalent of the T3/T4 ratio; EuT, euthyroid; HyperT, hyperthyroid.

*47 (18%) of the patients with carcinoma were in stadium 1 according to TNM classification.

†Excluding volumes of athyreotic patients (<1 ml).

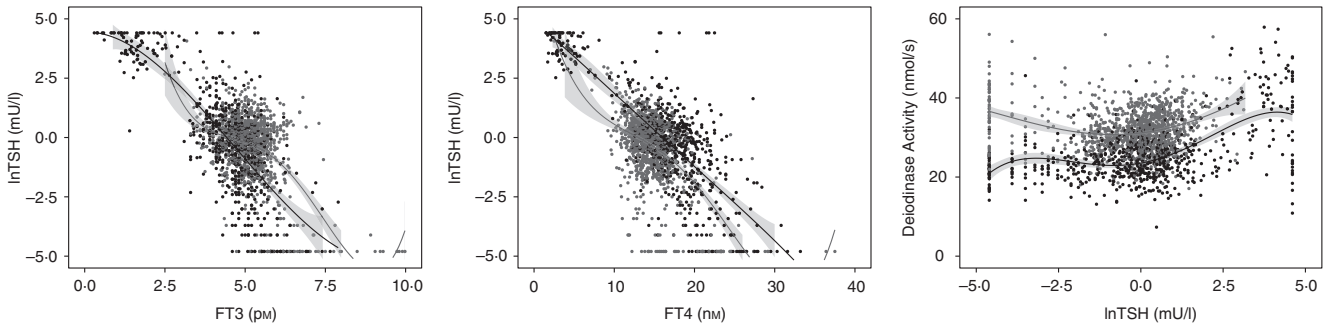

Fig. 1 Relationships between TSH and FT4, FT3 or deiodinase activity stratified by treatment. The fitting lines were described as a quartic polynomial and differed from each other (see Results). Grey dots or lines represent untreated subjects, and black symbols treated patients in all figures. Treatment refers to L-T4 administration, deiodinase activity to the sum activity of peripheral deiodinases (see Methods).

available, FT4 $P = 0{\cdot}67$, FT3 $P = 0{\cdot}92$). The contributing entities remained independently significant after adjustment by multivariable modelling. Age significantly reduced the FT4- or FT3-adjusted pituitary TSH response, while TSH at any given FT4 or FT3 concentrations was higher in obesity. Table 2 displays the sizes of some effects for increasing age and BMI in the entire functional spectrum and the euthyroid TSH range. Increasing thyroid volume was associated with a −0·15 mU/l FT3-adjusted TSH change per 10 ml ($P < 0{\cdot}001$). Whilst other influences were shaping the TSH response, TSH was uncorrelated with FT3 in the euthyroid range ($P = 0{\cdot}19$). In treated patients ($n = 571$), gender ($P = 0{\cdot}02$), disease aetiology ($P = 0{\cdot}03$) and volume ($P = 0{\cdot}01$), but not age ($P = 0{\cdot}55$) or BMI ($P = 0{\cdot}37$), influenced the relationship between TSH and FT4, even after correcting for the significant influence of L-T4 dose (except for aetiology which was no longer significant). When relating TSH to FT3, gender, age, BMI, thyroid volume and L-T4 dose all remained independently influential ($P < 0{\cdot}003$).

Influence of L-T4 treatment

Given heterogeneity in the treatment group (Table 1), interrelationships were further assessed by stratifying for disease aetiology and restricting the panel to a compensated stable thyroid state (FT4 > 10 pM with no history of change in medication over the last 4 weeks). In patients with autoimmune thyroiditis selected by these criteria ($n = 188$), FT3 was significantly lower in treated, compared with untreated subjects with the disease and similar TPO-Ab titres, although TSH levels were higher in the latter group (Table 3). When relating FT3 levels to lnTSH, the regression line in treated patients was placed below that for untreated subjects over the euthyroid range; the two lines converged in proximity to the lower TSH reference limit (Fig. 2a). The gradients differed significantly in height and slope (ANCOVA, $P < 0{\cdot}001$), before ($P < 0{\cdot}001$) and after adjusting ($P = 0{\cdot}04$) for covariates (age, BMI, volume, TPO-Ab, smoking). TSH-adjusted FT4 levels in treated patients were placed above the respective untreated subjects, whereas deiodinase activity was significantly below parity (Fig. 2a).

Similarly, we compared thyroidectomized (100%) and radioiodine-treated (97%) carcinoma outpatients on a stable L-T4 regime ($n = 158$) to a control group without evidence of thyroid disease ($n = 146$) (Table 3). FT3 was nearly identical between the groups, even after adjusting for age, BMI and smoking habits, but TSH was considerably lower and FT4 higher in the treatment group (Table 3). In a subgroup of patients with carcinoma with a TSH level within the reference interval ($n = 50$), median FT3 [4·6 pM (4·2, 5·1), $P < 0{\cdot}001$] was lower than in controls ($n = 146$, see Table 3), as was TSH [1·25 mU/l (0·66, 2·11), $P = 0{\cdot}01$], while FT4 was higher [18·9 pM (16·5, 20·3) $P < 0{\cdot}001$]. Unadjusted and adjusted FT3 in patients with carcinoma was significantly below parity in controls at the same TSH over the entire euthyroid TSH range (Fig. 2b). FT4 and, importantly, deiodinase activity also differed between the two groups (Fig. 2b).

Role of peripheral deiodinases

Deiodinase activity was significantly reduced with lower thyroid volume in treated patients with autoimmune thyroiditis or thyroid carcinoma (Fig. 3), but the two parameters were uncorrelated in controls ($n = 146$, $r = -0{\cdot}08$, $P = 0{\cdot}30$). Deiodinase activity was inversely correlated with L-T4 dose per kg body weight administered to patients with autoimmune thyroiditis ($r = -0{\cdot}40$, $P < 0{\cdot}001$), not thyroid carcinoma ($r = -0{\cdot}08$, $P = 0{\cdot}29$). Weight adjusted L-T4 dose was positively correlated with FT4 ($r = 0{\cdot}45$, $P < 0{\cdot}001$), inversely with TSH ($r = -0{\cdot}21$, $P = 0{\cdot}03$), but uncorrelated with FT3 ($r = -0{\cdot}01$, $P = 0{\cdot}89$) in autoimmune thyroiditis and correlated with all three parameters in carcinomas (FT4 $r = 0{\cdot}39$, $P < 0{\cdot}001$, TSH $r = -0{\cdot}22$, $P = 0{\cdot}005$, and FT3 $r = 0{\cdot}34$, $P < 0{\cdot}001$).

Influence of sampling time to L-T4 intake

FT4 was associated with the time interval between L-T4 ingestion and sampling. In patients with thyroid carcinoma, FT4 reached at median 20·6 pM (18·1, 23·3, $P = 0{\cdot}01$) in an early sampling interval (<5 h, $n = 122$), compared with 18·9 pM (15·6, 20·9) in late sampling (≥5 h, $n = 24$). TSH was

Table 2. *Effect sizes of age and body mass index on pituitary TSH response in untreated subjects*

Parameter	All Mean FT4-adjusted ΔTSH (mU/l) $n = 824$	Euthyroid range Mean FT4-adjusted ΔTSH (mU/l) $n = 716$	All Mean FT3-adjusted ΔTSH (mU/l) $n = 824$	Euthyroid range Mean FT3-adjusted ΔTSH (mU/l) $n = 716$
Age increase 20–80 years	−0·84 (95% CI −1·19, −0·51) $P < 0·001$	−0·58 (95% CI −0·54, −0·80) $P < 0·001$	−1·14 (95% CI −1·51, −0·79) $P < 0·001$	−0·63 (95% CI −0·88, −0·40) $P < 0·001$
BMI increase 25–35 kg/m^2	0·28 (95% CI 0·12, 0·53) $P < 0·001$	0·18 (95% CI 0·04, 0·35) $P < 0·001$	0·32 (95% CI 0·07, 0·56) $P < 0·001$	0·19 (95% CI 0·03, 0·36) $P < 0·001$

ΔTSH refers to the mean adjusted difference in TSH that occurs with a change in age or BMI over the specified interval, in the presence of the other parameter and FT4 or FT3 in a generalized linear model.
A polynomial representation of thyroid hormones was used in the complete sample, and a more parsimonious linear model in the euthyroid TSH range. TSH was used after logarithmic transformation.

similarly suppressed in both groups [0·20 mU/l (0·03, 1·18) *vs* 0·26 mU/l (0·04, 2·0, $P = 0·34$), FT3 5·1 pM (4·6, 5·7) *vs* 4·6 pM (4·1, 4·9, $P < 0·01$)]. Differences between sampling periods were not significant in patients with autoimmune thyroiditis for any parameter [FT4 17·2 pM (15·5, 18·6, $n = 48$) *vs* 16·0 pM (13·8, 19·0, $n = 59$), $P = 0·13$]. FT4 levels rose with time (peaking at 3–4 h) and were dose dependent (nonsignificant below 100 μg/day L-T4) and, owing to the complex responses, difficult to model, while there was no appreciable FT3 peak.

Serial measurements

Follow-up in a control group with unchanged conditions showed closely concurring regression lines of the relationships between FT3, FT4 or deiodinase activity and lnTSH (Fig. S1). Measurements preceding surgery and on L-T4 replacement after thyroidectomy were available from a separate longitudinal study in 10 patients. On L-T4 replacement, deiodinase activity was markedly reduced [from 37·7 (36·9, 41·3) nmol/s to 28·3 (26·0, 30·1) nmol/s, $P = 0·002$] in the same patient, FT3 comparable at a nonsignificantly lower TSH and a significantly higher FT4 ($P = 0·01$) (Fig. S2).

Discussion

The present study has shown that the adaptive equilibria defining the relationships between FT3, FT4 and TSH are not invariant, but depend on the underlying factors controlling the expression of body function in particular circumstances. This provides a conceptual counterpoint to the current paradigm of TSH-centred thyroid function testing, which is based on the implicit assumption that pituitary TSH accurately and universally reflects the thyroidal status of the body in various physiological and pathophysiological conditions.[4–6] Our study challenges the rationale of deriving appropriate treatment targets from universally applicable diagnostic criteria.

This prospective observational study employing a large sample of consecutive patients has investigated the many influences on the interrelations of thyroid hormone parameters under various defined conditions. This supports previous reports indicating a nonlinear complex relationship of TSH with free circulating thyroid hormones.[7–9,12] After adopting an improved nonlinear model of the primary relationships and adjusting for confounding entities as well as thyroid status, the influence of age and body mass index (BMI) was assessed. Pituitary responsiveness appeared to decline with increasing age, demonstrated by a decreasing TSH response in untreated patients to a given FT4 concentration. In obesity, meanwhile, the pituitary setpoint was shifted towards higher TSH levels. Age- and BMI-related effects remained significantly independent of each other. This agrees with another study showing a diminished TSH response in elderly subjects and numerous reports of univariate statistical associations between thyroid parameters and age or body mass.[18–22] Possible mechanisms that may account for the modulation of the setpoint for feedback regulation involve among

Table 3. Comparison of untreated *vs* L-T4-treated patients with nonhypothyroid autoimmune thyroiditis and L-T4-treated patients with thyroid carcinoma *vs* disease-free controls

Parameter	Autoimmune thyroiditis No Tx $n = 81$	Autoimmune thyroiditis L-T4 Tx $n = 107$	*P* value	Thyroid carcinoma L-T4 Tx $n = 158$	Controls No Tx $n = 146$	*P* value
Gender	Female 89%	Female 92%	0·53	Female 68%	Female 72%	0·40
Smoking	Smokers 23%	Smokers 19%	0·63	Smokers 15%	Smokers 31%	0·003
L-T4 (μg/day)	0	100 (75, 112)	–	125 (112, 175)	0	–
L-T4 (μg/kg BW)		1·25 (0·94, 1·63)		1·67 (1·48, 2·00)		
Age (years)	47 (30, 59)	51 (41, 62)	0·014	53 (45, 62)	38 (26, 49)	<0·001
BMI (kg/m^2)	24·6 (22·0, 29·5)	27·2 (23·5, 29·7)	0·18	28·6 (24·8, 33·0)	25·2 (22·8, 29·5)	<0·001
FT3 (pM)	4·9 (4·7, 5·2)	4·6 (4·2, 5·0)	<0·001	5·0 (4·4, 5·6)	5·0 (4·7, 5·3)	0·88
FT4 (pM)	13·8 (12·7, 15·1)	16·8 (14·9, 18·8)	<0·001	20·2 (17·6, 22·9)	14·1 (13·1, 15·2)	<0·001
TSH (mU/l)	2·93 (1·88, 4·47)	1·49 (0·63, 2·5)	<0·001	0·21 (0·03, 1·49)	1·63 (1·13, 2·25	<0·001
TPO-Ab (U/l)	704 (106, 1300)	850 (81, 1300)	0·76	–	–	–
Deiodinase activity (nmol/s)	33·6 (30·2, 36·7)	25·3 (22·2, 28·3)	<0·001	23·2 (21·0, 25·6)	32·9 (30·0, 36·3)	<0·001
Thyroid volume (ml)	11 (8, 15)	7 (4, 11)	<0·001	0 (0, 0)	10 (8, 13)	<0·001

Values shown represent median and IQRs or relative frequencies. Patients with thyroid carcinoma included were all thyroidectomized, underwent radioiodine therapy in 97%, were in a compensated nonhypothyroid thyroid state on stable L-T4 dosage (no change in medication for the past 4 weeks) and were seen as outpatients. Controls were untreated euthyroid disease-free subjects.
Tx, treatment; BW, body weight.

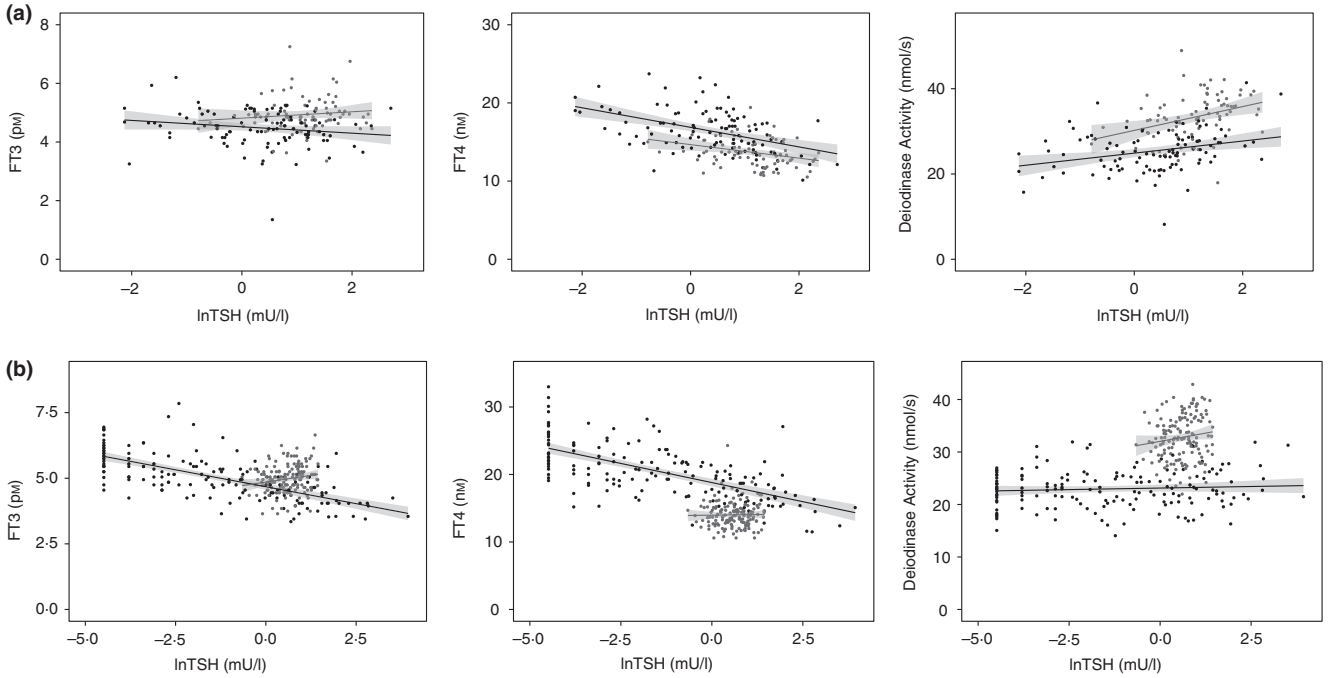

Fig. 2 (a, b) TSH-adjusted FT3, FT4 and deiodinase activity in (a) nonhypothyroid treated patients with autoimmune thyroiditis (black symbols, $n = 107$), compared with untreated autoimmune thyroiditis (grey symbols, $n = 81$), and (b) L-T4-treated thyroidectomized plus radioiodine-treated patients with thyroid carcinoma (black symbols, $n = 158$), compared with untreated disease-free controls (grey symbols, $n = 146$). Regression lines for each group were fitted using a linear model. The shaded area indicates the 95% confidence level of the regression. The two corresponding lines proved statistically different for all parameters shown (see text).

others TRH, central deiodinases and leptin, thereby integrating thyroid function and energy metabolism.[23]

Within the euthyroid TSH range, the lnTSH–FT3 relationship displayed a flat gradient. Influences other than TSH, such as age, BMI, thyroid volume and smoking habits fine-tuned the equilibria. This suggests that while pituitary TSH reacts sensitively to a deteriorating thyroidal response, the appropriate equilibria still acting within the euthyroid state are shaped by a variety of influences and adjusted to the prevailing conditions. The adaptation of homeostatic equilibria among the

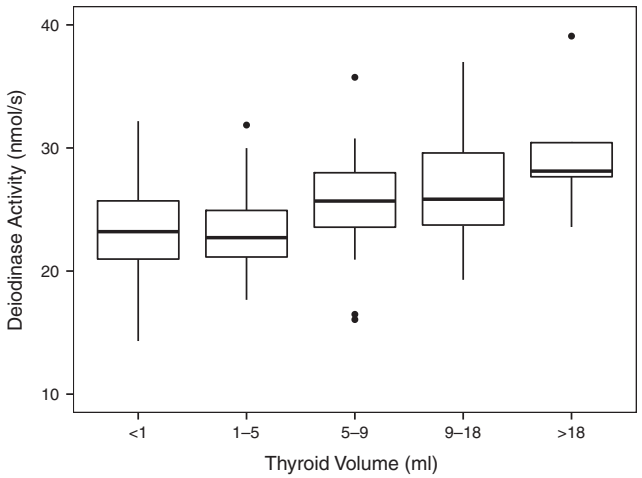

Fig. 3 Association of deiodinase activity with thyroid volume in treated patients with autoimmune thyroiditis or thyroid carcinoma ($n = 265$). Numbers per group were as follows, <1 ml $n = 159$, 1–5 ml $n = 32$, 5–9 ml $n = 33$, 9–18 ml $n = 36$, >18 ml $n = 5$. The overall difference in deiodinase activity among the volume groups was highly significant (Kruskal–Wallis test, $P < 0{\cdot}001$), and the <1 ml and 1–5 ml group differed from each of the higher volume groups by paired comparison (Wilcoxon's test, $P < 0{\cdot}02$).

contributors satisfies the demand for tight control of metabolically important regulators typified by the thyroid hormones themselves.

In L-T4-treated patients, the equilibria involving FT3, FT4 and TSH differed markedly from those in untreated subjects, confirming an earlier retrospective analysis.[24] When comparing L-T4-treated and untreated nonhypothyroid patients with autoimmune thyroiditis, FT3 was lower in treated patients, although accompanied by a lower TSH. Between treated athyreotic thyroid carcinoma patients and disease-free controls, median FT3 levels were comparable, but TSH was suppressed in the first group. When relating FT3 to TSH levels in a more individualized set-up, regression lines in both treated groups were displaced below control levels over the entire euthyroid TSH spectrum. The disparity was related to conversion, as assessed by the calculated deiodinase activity, remaining significant after adjusting for various covariates such as age and BMI. The cross-sectional findings were supported by a small prospective series comparing the various levels including deiodinase activity before and after thyroidectomy in the same patient. This confirms a recent report that to maintain presurgical thyroid hormone levels, a lower TSH is required postsurgery in the same patient.[25]

Aspects of variation and bivariate referencing have been widely discussed.[15,26] TSH is further affected by circadian variability, but this is probably a random influence in a large sample of consecutive patients. Overall biological variation was directly investigated in a control group. It could not explain the magnitude of the effect and observed systematic alteration of relationships, further supported by significant change in conversion (calculated deiodinase activity). Biases from variation in sampling intervals after L-T4 ingestion have to be taken into account in this and other studies, affecting mainly FT4 levels. The observed effect was, however, minor and could not account for the larger differences reported. It also implies overestimating, rather than underestimating, true FT3 levels in treated patients over controls.

In patients on L-T4, changes in central (D2) and peripheral deiodinase activities appear to be definitive elements in adjusting setpoints and equilibria. Fonseca and co-workers described a key role of the D2 pathway in regulating TSH expression involving thyrotrophs and tancytes.[27] Genetic variation may also play a role.[28] Although the complexity of the feedback loop is incompletely understood, central (D2) and peripheral deiodinases appear to differ in their sensitivities towards T4.[3,24] As for peripheral deiodinases, the gradient of their relationship with lnTSH was greater in L-T4-treated patients.[24] Higher L-T4 doses were associated with suppression of deiodinase activity in both our cross-sectional study and longitudinal series comparing postablative values on L-T4 replacement with presurgical levels in the same patient. An association with thyroid volume was present in L-T4-treated patients, suggesting a direct role for intrathyroidal deiodinases, presumably activated via TSH stimulation.[24,29,30]

Together, the above findings suggest that the system is rebalanced during L-T4 monotherapy, compared with the physiological state. The newly formed equilibria seem to maintain a centrally overcompensated and peripherally undercompensated state, with TSH levels partly suppressed while FT3 levels are still relatively low. Thyroidectomized/radioiodine-treated patients are most affected because a diminishing residual capacity, which reduces the T3 pool derived from intrathyroidal conversion, progressively augments the uncompensated failure to maintain homeostatically adequate FT3 levels. Indeed, FT3 levels achieved by L-T4 monotherapy have been reported to be below the lower reference limit in 15% of athyreotic patients.[31]

Consequently, L-T4 treatment represents a situation where physiological equilibria are not maintained, but conditionally modified. Thyroid functional states appear to be distinctly and differentially regulated.[12] As a major clinical implication, the optimal treatment target for TSH cannot be inferred from the physiological optimum observed in disease-free subjects.

Accordingly, further assessment of the interrelationships and homeostatic equilibria could aid in defining disease status and estimating dose adequacy of replacement therapy. Carefully planned randomized clinical trials could assess the potential clinical benefit from optimizing serum FT3 in conjunction with well-defined TSH targets.[32] Biological long-term effects of alterations in deiodinase activity and an unbalanced FT3/FT4 ratio also require further study. Higher FT4 was associated with low bone mineral density in perimenopausal women, lower physical function and higher mortality in elderly men independently of TSH, but L-T4 users had been excluded.[33] Low patient satisfaction has been expressed in many L-T4 trials, but does not preclude establishing more objective measures.[32,34] End-point determinations indicating true euthyroidism in a given person are required, but are in need of better definition themselves.[35]

However, we have neither directly assessed T3/T4 therapy nor deliberately entered the present controversial debate, apart from

showing some unphysiological consequences and adaptive shifts in equilibria in L-T4-treated patients.[32,34–36] TSH suppression may have unwanted consequences.[35] Studies relating TSH suppression to adverse cardiovascular or skeletal outcomes have not completely distinguished between exogenous and endogenous causes and are frequently lacking corresponding FT3 and FT4 values.[35] In some studies, only patients with completely suppressed TSH were adversely affected, but not those below the reference limit. Together, this indicates a narrow therapeutic range of L-T4 at the lower reference limit of TSH. This suggests a revisitation through multivariate analysis rather than relying on univariate correlations, while taking into account appropriate equilibria for the condition and considering additional markers of tissue euthyroidism.

Our study design was uncontrolled thereby imposing some limitations, though allowing analysis of homeostatic equilibria over a wide range of pathophysiological conditions. A need for better assay harmonization including immunometric methods and liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based techniques has been recognized.[37] However, particularly in outpatients, where critical states such as nonthyroid illness, pregnancy or pituitary disease were excluded, we would not expect methodological issues with a well-standardized conventional immunometric method.[38] LCMS/MS-based techniques are currently not practicable with such a large patient panel.[39]

In conclusion, our study has shown that all thyroid hormones (TSH, FT4 and FT3) and deiodinases have their individual though interlocking parts to play in realizing the condition-related patterns of thyroidal expression, regulation and metabolic activity. Equilibria among thyroid parameters cannot be simplistically interpreted using the limits of normality established in complete thyroid health, questioning the role of universally applicable diagnostic criteria for TSH. It also suggests revisiting the regimes for treatment optimization.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contributions

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References

1 Cheng, S.Y., Leonard, J.L. & Davis, P.J. (2010) Molecular aspects of thyroid hormone actions. *Endocrine Reviews*, **31**, 139–170.
2 Pilo, A., Iervasi, G., Vitek, F. *et al.* (1990) Thyroidal and peripheral production of 3,5,3'-triiodothyronine in humans by multicompartmental analysis. *American Journal of Physiology Endocrinology and Metabolism*, **258**, E715–E726.
3 Gereben, B., Zavacki, A.M., Ribich, S. *et al.* (2008) Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. *Endocrine Reviews*, **29**, 898–938.
4 Larsen, P.R. (1982) Thyroid-pituitary interaction: feedback regulation of thyrotropin secretion by thyroid hormones. *The New England Journal of Medicine*, **306**, 23–32.
5 Baloch, Z., Carayon, P., Conte-Devolx, B. *et al.* (2003) Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. *Thyroid*, **13**, 3–126.
6 Garber, J.R., Cobin, R.H., Gharib, H. *et al.* (2012) Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. *Thyroid*, **22**, 1200–1235.
7 Hoermann, R., Eckl, W., Hoermann, C. *et al.* (2010) Complex relationship between free thyroxine and TSH in the regulation of thyroid function. *European Journal of Endocrinology*, **162**, 1123–1129.
8 Clark, P.M., Holder, R.L., Haque, S.M. *et al.* (2012) The relationship between serum TSH and free T4 in older people. *Journal of Clinical Pathology*, **65**, 463–465.
9 Hadlow, N.C., Rothacker, K.M. & Wardrop, R. (2013) The relationship between TSH and free T4 in a large population is complex and nonlinear and differs by age and sex. *The Journal of Clinical Endocrinology & Metabolism*, **98**, 2936–2943.
10 Lazar, M.A. (1993) Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocrine Reviews*, **14**, 184–193.
11 Arrojo, E., Drigo, R. & Bianco, A.C. (2011) Type 2 deiodinase at the crossroads of thyroid hormone action. *The International Journal of Biochemistry & Cell Biology*, **43**, 1432–1441.
12 Midgley, J.E.M., Hoermann, R., Larisch, R. *et al.* (2013) Physiological states and functional relation between thyrotropin and free thyroxine in thyroid health and disease: in vivo and in silico data suggest a hierarchical model. *Journal of Clinical Pathology*, **66**, 335–342.
13 Larisch, R., Tawo, P.L. & Hoermann, R. (2010) TSH, fT3 und fT4 bei Personen ohne Schilddrüsenfunktionsstörung. *Jahrestagung der Deutschen Gesellschaft für Nuklearmedizin*, P31.
14 Dietrich, J.W., Stachon, A., Antic, B. *et al.* (2008) The AQUA-FONTIS study: protocol of a multidisciplinary, cross-sectional and prospective longitudinal study for developing standardized diagnostics and classification of non-thyroidal illness syndrome. *BMC Endocrine Disorders*, **8**, 13.
15 Dietrich, J.W., Landgrafe, G. & Fotiadou, E.H. (2012) TSH and thyrotropic agonists: key actors in thyroid homeostasis. *Journal of Thyroid Research*, **2012**, 1–29.

16 Fellows, I. (2013) Deducer: Deducer R package version 0.7-6. Retrieved from http://CRAN.R-project.org/package=Deducer (accessed 15 December 2013).
17 R Core Team (2013) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, ISBN 3-900051-07-0. Retrieved from http://www.R-project.org/ (accessed 15 December 2013).
18 Carlé, A., Laurberg, P., Pedersen, I.B. *et al.* (2007) Age modifies the pituitary TSH response to thyroid failure. *Thyroid*, **17**, 139–144.
19 Knudsen, N., Laurberg, P., Rasmussen, L.B. *et al.* (2005) Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. *The Journal of Clinical Endocrinology & Metabolism*, **90**, 4019–4024.
20 Roef, G.L., Rietzschel, E.R., Van Daele, C.M. *et al.* (2014) Triiodothyronine and free thyroxine levels are differentially associated with metabolic profile and adiposity-related cardiovascular risk markers in euthyroid middle-aged subjects. *Thyroid*, **24**, 223–231.
21 Vadiveloo, T., Donnan, P.T., Murphy, M.J. *et al.* (2013) Age- and gender-specific TSH reference intervals in people with no obvious thyroid disease in Tayside, Scotland: the Thyroid Epidemiology, Audit, and Research Study (TEARS). *The Journal of Clinical Endocrinology & Metabolism*, **98**, 1147–1153.
22 Agnihothri, R.V., Courville, A.B., Linderman, J.D. *et al.* (2014) Moderate weight loss is sufficient to affect thyroid hormone homeostasis and inhibit its peripheral conversion. *Thyroid*, **24**, 19–26.
23 Lechan, R.M. & Fekete, C. (2006) The TRH neuron: a hypothalamic integrator of energy metabolism. *Progress in Brain Research*, **153**, 209–235.
24 Hoermann, R., Midgley, J.E.M., Larisch, R. *et al.* (2013) Is pituitary thyrotropin an adequate measure of thyroid hormone-controlled homeostasis during thyroxine treatment? *European Journal of Endocrinology*, **168**, 271–280.
25 Ito, M., Miyauchi, A., Morita, S. *et al.* (2012) TSH-suppressive doses of levothyroxine are required to achieve preoperative native serum triiodothyronine levels in patients who have undergone total thyroidectomy. *European Journal of Endocrinology*, **167**, 373–378.
26 Laurberg, P., Andersen, S., Carlé, A. *et al.* (2011) The TSH upper reference limit: where are we at? *Nature Reviews Endocrinology*, **7**, 232–239.
27 Fonseca, T.L., Correa-Medina, M., Campos, M.P.O. *et al.* (2013) Coordination of hypothalamic and pituitary T3 production regulates TSH expression. *The Journal of Clinical Investigation*, **123**, 1492–1500.
28 Hoftijzer, H.C., Heemstra, K.A., Visser, T.J. *et al.* (2011) The type 2 deiodinase ORFa-Gly3Asp polymorphism (rs12885300) influences the set point of the hypothalamus-pituitary-thyroid axis in patients treated for differentiated thyroid carcinoma. *The Journal of Clinical Endocrinology & Metabolism*, **96**, E1527–E1533.
29 Maia, A.L., Goemann, I.M., Meyer, E.L.S. *et al.* (2011) Type 1 iodothyronine deiodinase in human physiology and disease: deiodinases: the balance of thyroid hormone. *The Journal of Endocrinology*, **209**, 283–297.
30 Murakami, M., Kamiya, Y., Morimura, T. *et al.* (1990) Thyrotropin receptors in brown adipose tissue: thyrotropin stimulates type II iodothyronine deiodinase and uncoupling protein-1 in brown adipocytes. *The American Journal of Physiology*, **258**, 1195–1201.
31 Gullo, D., Latina, A., Frasca, F. *et al.* (2011) Levothyroxine monotherapy cannot guarantee euthyroidism in all athyreotic patients. *PLoS One*, **6**, e22552.
32 Biondi, B. & Wartofsky, L. (2012) Combination treatment with T4 and T3: toward personalized replacement therapy in hypothyroidism? *The Journal of Clinical Endocrinology & Metabolism*, **97**, 2256–2271.
33 Yeap, B.B., Alfonso, H., Hankey, G.J. *et al.* (2013) Higher free thyroxine levels are associated with all-cause mortality in euthyroid older men: The Health in Men Study. *European Journal of Endocrinology*, **169**, 401–408.
34 Grozinsky-Glasberg, S., Fraser, A., Nahshoni, E. *et al.* (2006) Thyroxine-triiodothyronine combination therapy versus thyroxine monotherapy for clinical hypothyroidism: meta-analysis of randomized controlled trials. *The Journal of Clinical Endocrinology & Metabolism*, **91**, 2592–2599.
35 Biondi, B. & Wartofsky, L. (2014) Treatment with thyroid hormone. *Endocrine Reviews*, **35**, 433–512.
36 Wiersinga, W.M. (2014) Paradigm shifts in thyroid hormone replacement therapies for hypothyroidism. *Nature Reviews Endocrinology*, **10**, 164–174.
37 Thienpont, L.M., Van Uytfanghe, K., Beastall, G. *et al.* (2010) Report of the IFCC Working Group for Standardization of Thyroid Function Tests; part 2: free thyroxine and free triiodothyronine. *Clinical Chemistry*, **56**, 912–920.
38 Midgley, J.E.M. (2001) Direct and indirect free thyroxine assay methods: theory and practice. *Clinical Chemistry*, **47**, 1353–1363.
39 Jonklaas, J. & Soldin, S.J. (2008) Tandem mass spectrometry as a novel tool for elucidating pituitary-thyroid relationships. *Thyroid*, **18**, 1303–1311.

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