Integration of Peripheral and Glandular Regulation of Triiodothyronine Production by Thyrotropin in Untreated and Thyroxine-Treated Subjects

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Key words
- TSH
- triiodothyronine
- feedback control
- deiodinase
- homeostasis

Abstract

The objective of the study was to evaluate the roles of central and peripheral T3 regulation. In a prospective study involving 1796 patients, the equilibria between FT3 and TSH were compared in untreated and L-T4-treated patients with varying functional states, residual thyroid secretory capacities and magnitudes of TSH stimulation. T3 concentrations were stable over wide variations in TSH levels (from 0.2 to 7 mU/l) and endogenous T4 production in untreated patients, but unbalanced in L-T4-treated athyreotic patients where T3 correlated with exogenous T4 supply. T3 stability was related to TSH-stimulated deiodinase activity by clinical observation, as predicted by theoretical modelling. Deiodinase activity in treated patients was reduced due to both diminished responsiveness to TSH and lack of thyroidal capacity. Deiodinase activity was increased in high thyroid volume, compared to lower volumes in euthyroid patients (<5 ml, $p<0.001$). While deiodinase differed between euthyroid and subclinically hypothyroid patients in high volume, 26.7 nmol/s (23.6, 29.2), $n=214$ vs. 28.9 nmol/s (26.7, 31.5), $n=20$, $p=0.02$, it was equivalent between the 2 functional groups in low volume, 23.3 nmol/s (21.3, 26.1), $n=117$ vs. 24.6 nmol/s (22.2, 27.5), $n=38$, $p=0.22$. These findings suggest that the thyroid gland and peripheral tissues are integrated in the physiological process of T3 homeostasis in humans via a feed-forward TSH motif, which coordinates peripheral and central regulatory mechanisms. Regulatory and capacity deficiencies collectively impair T3 homeostasis in L-T4-treated patients.

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Introduction

Thyroid hormone homeostasis is tightly regulated by 2 major processes, hypothalamic pituitary thyroid feedback controlling thyroid hormone production and thyroxine (T4)-triiodothyronine (T3) conversion supplying the majority of the latter biologically active hormone. The conversion mechanism involves 3 types of deiodinases termed deiodinase 1 (D1), 2 (D2), and 3 (D3), which differ in both their functionalities and their expression depending on organ, developmental stage and thyroid function [1,2]. Deiodination of T4 is assumed to account for approximately 80% of the T3 production in humans [3,4]. However, challenging the essential physiological role that has been ascribed to this mechanism, knock-out mice deficient in either 1 or even all 3 deiodinases showed little phenotypic alterations, maintaining sufficient, if not optimum, thyroid hormone homeostasis for the continued functioning of vital developmental and metabolic processes [5–7]. The peripheral control of T3 homeostasis has been mostly viewed as auto-regulated and operating independently of the central hypothalamic pituitary regulation [4,8,9]. However, we have recently shown that the healthy equilibria between thyroid hormones and pituitary thyrotropin (TSH) appear not to be fixed, but adapt in different ways to various conditions including levothyroxine (L-T4) treatment [10].

We hypothesised that the peripheral and central pathways may be coordinated and, in particular, a functional TSH-deiodinase feed-forward motif may be operative in humans in vivo that could play a major role in both fine-tuning T3 homeostasis and maintaining optimum adaptive circulatory FT3 levels over a wide variation in thyroid production.

To examine the hypothesis, we used data obtained from a large prospective study to assess the contributions of thyroid secretory capacity and deiodinase activity to T3 homeostasis [10]. The findings confirm the hypothesis and suggest that circulatory T3 concentrations are jointly

regulated by central and peripheral pathways, in order to maintain the hormone in a tight concentration range over a wide variation in endogenous T4 supply. The situation sharply differs in L-T4-treated athyreotic patients where the normal homeostatic mechanisms are compromised.

Materials and Methods

Study design and objective

A prospective open observational study (ClinicalTrials.gov, Study ID IIFHT-161013, NCT01969552) was conducted at the Department of Nuclear Medicine at Klinikum Luedenscheid, Germany between July 2013 and February 2014, and approved by the Ethics Committee of the University of Muenster, Germany. All participants were adults, free of severe comorbidity and provided written informed consent.

The primary study outcome, the interrelation of thyroid parameters under various conditioning influences such as higher age, obesity, and L-T4 treatment has been reported [10]. The present analysis focuses on investigating additional homeostatic mechanisms, particularly an integrating role of TSH stimulation, thyroid capacity and deiodinase activity on T3 homeostasis.

Patients

The study involved 1796 consecutive patients, after excluding from analysis patients with hypothalamic/pituitary diseases (n=5), pregnancy (n=3), on antithyroid drugs (n=99) or T3/T4 combination therapy (n=9). Hospitalisation of some patients (22%) stemmed from German legal requirements that preclude ambulatory radioiodine application. L-T4 medication was withdrawn from some before hospitalisation.

Diagnostic procedures included a detailed history supplemented by a standardised questionnaire documenting gender, age, height, weight, smoking habits (75% answered), prior surgery or radioiodine treatment, medication (duration, brand, dosage, time of intake), physical examination, laboratory tests (FT3, FT4, TSH, and, if indicated, TPO-Ab or TSH-R Ab) and thyroid imaging.

Laboratory methods

TSH, FT3 and FT4 were measured with an automated direct chemoluminescence method (Advia Centaur XP, Siemens Healthcare Diagnostics, Erlangen, Germany). For TSH, the 3rd International Standard for TSH (WHO, IRP 81/565) was used, the range from 0.006 to 160.03 mU/l was linear, and CVs of inter-assay imprecision ranged from 0.9% to 2.4%. Laboratory-established and extensively evaluated reference intervals were used, 0.4–4.0 mU/l for TSH, 10–23 pmol/l for FT4, and 3.1–6.8 pmol/l for FT3.

Thyroid peroxidase antibodies (TPO-Ab) were determined by a competitive chemoluminescence method (ADVIA Centaur XP, Siemens Healthcare Diagnostics, Erlangen, Germany, 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).

Deiodinase activity and thyroid secretory capacity

We calculated the sum activity of peripheral deiodinases (termed deiodinase activity thereafter, nmol/s), from equilibrium levels of FT4, FT3 and estimated constant parameters for plasma protein binding, distribution and elimination with

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

as previously described [11].

Deiodinase activity serves as a measure of conversion, similarly to the FT3-FT4 ratio, which is also given for comparison.

The maximum functional secretory capacity of the thyroid gland, amount of T4 released into the circulation per unit of time (pmol/s or nmol/d) (termed T4 output or supply), was estimated according to a previously published mathematical model of thyroid hormone homeostasis [11–13].

In the absence of a thyroid gland in L-T4-treated patients, we estimated the T4 supply from the daily hormone intake (T4 dose expressed in molar units), assuming a standard absorption rate of 70%. The theoretical relationship between FT3 values and thyroid hormone output (GT) was simulated with the use of the application SimThyr 3.3 (developed by one of the authors and downloadable from sourceforge.net). The entire mathematical model including the parameters and equations used here have been made publicly available, validated in previous publications and were conceptualised in a recent review [11–13].

Thyroid ultrasound and scintigraphy

Thyroid volume was sonographically (10 MHz transducer) determined according to the ellipsoid formula. Reference values were < 18 ml for females and < 25 ml for males. A volume < 1 ml was considered athyreotic. Larger nodules were further examined by scintigraphy.

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. Multiple variables and influences were analysed by a generalised linear model (GLM). Non-linear relationships that existed over a wide range were approximated by locally weighted smooth regression, simpler relationships over restricted intervals fitted by linear regression. Statistical analyses were performed using Deducer (version 0.7-7) and the R statistical package (Mac version 3.1.0) [14, 15]. p-Values < 0.05 were considered significant.

Results

Untreated subjects

Characteristics of patients are given in **Table 1**. In untreated subjects (n=1126), FT3 concentrations were maintained in a tight range over a wide span of TSH concentrations (from approximately 0.2 to 7 mU/l TSH) as reflected by a flat FT3-lnTSH gradient (**Fig. 1**). In contrast, FT4 and particularly deiodinase activity varied to a greater extent over the TSH range, made apparent when comparing the mean standardised parameters (**Fig. 1**). T3 homeostasis was better preserved than FT4 levels, while deiodinase activity increased at both functional extremes, thereby contributing to both T3 excess in hyperthyroidism and T3 restoration in hypothyroidism in untreated patients (**Fig. 1**).

When relating the estimated secretory capacity of the thyroid gland to circulating FT3 levels a similar pattern emerged, showing that FT3 was maintained at stable level over a wide variation in endogenous T4 supply (**Fig. 2a**). The observed relationship

Table 1 Patient characteristics.

Parameter	Untreated patients n = 1126	L-T4-Treated patients n = 670	L-T4-Treated athyreotic patients n = 137
Gender (female)	75 %	78 %	69 %
Age (years)	53 (42, 67)	56 (45, 65)*	53 (47, 62)
BMI (kg/m^2)	26.2 (23.5, 30.1)	27.4 (24.0, 31.1)**	28.4 (24.7, 33.6)**
No thyroid disease[a]	132 (12 %)	0 (0 %)	0 (0 %)
Euthyroid goitre or nodule	402 (36 %)	110 (16 %)	0 (0 %)
Hyperthyroid nodular disease	270 (24 %)	0 (0 %)	0 (0 %)
Hyperthyroid Graves' disease	69 (6 %)	0 (0 %)	0 (0 %)
Autoimmune thyroiditis	146 (13 %)	110 (16 %)	0 (0 %)
Post intervention for benign disease	85 (8 %)	193 (30 %)	0 (0 %)
for thyroid carcinoma	19 (2 %)	252 (38 %)	137 (100 %)
Others	3 (0.3 %)	5 (0.4 %)	0 (0 %)
Intervention: surgery, radioiodine treatment	43 (4 %), 65 (6 %)	375 (57 %)**, 293 (45 %)**	137 (100 %)**, 134 (98 %)**
L-T4 dose (µg/d)	0	100 (75, 125)**	150 (125, 175)**
Weight-adjusted L-T4 dose (µg/kg BW)	0	1.37 (0.97, 1.70)	1.71 (1.52, 2.03)
Thyroid volume (ml)	16 (11, 25)	3 (0, 9)**	<1**
FT4 (pmol/l)	14.4 (13.2, 15.8)	17.0 (14.5, 19.6)**	20.9 (18.9, 23.2)**
FT3 (pmol/l)	5.1 (4.7, 5.5)	4.7 (4.1, 5.2)**	5.1 (4.7, 5.7)
TSH (mU/l)	1.07 (0.46, 1.88)	1.08 (0.38, 3.04)*	0.12 (0.02, 0.69)**
TPO-Ab (U/ml), n = 764	42 (33, 70)	59 (36, 1300)**	–
TPO-Ab positive (%)	26 %	50 %	
TSH-R Ab (U/l), n = 329	0.21 (0.2,1.0)	0.2 (0.2, 0.7)	–
TSH-R Ab positive (%)	10 %	6 %	
FT3- FT4 ratio (%)	36 (32, 39)	28 (24, 32)**	25 (22, 28)**
Deiodinase activity (nmol/s)	32.8 (30.0, 36.5)	25.6 (22.3, 29.4)**	23.2 (20.8, 25.5)**
T4 output (pmol/s)	3.90 (2.60, 7.92)	–	–

* Significant difference <0.05; ** Significant difference <0.001, Wilcoxon test, compared to untreated patients

[a] For referencing purposes, thyroid parameters in disease-free individuals with a median age of 38 (26, 49) years were as follows: FT3 5.0 (4.8, 5.2) pmol/l, FT4 14.0 (13.0,15.1) pmol/l, TSH 1.62 (1.12, 2.25) mU/l, thyroid volume 10 (8,13) ml, deiodinase activity 32.8 (30.0, 36.2) nmol/s, and T4 output 2.88 (2.30, 3.72) pmol/s

Athyreotic patients shown here were in a compensated non-hypothyroid state on a stable L-T4 dose. For estimation of deiodinase activity and T4 output, refer to Methods

was strikingly different from what might be expected on theoretical grounds, and predicted by a theoretical model assuming peripheral autoregulation, that is, absence of a postulated T3-stabilising TSH-deiodinase interaction (**Fig. 2b**).

L-T4-treated patients

L-T4-treated patients showed lower median FT3 levels, compared to untreated subjects (**Table 1**), and their T3 relationship was unbalanced. This followed virtually the same descending gradient as FT4 from lower to higher TSH levels, with a steep decline towards the hypothyroid end (**Fig. 3a**). FT3 levels were closely associated with both the daily weight-adjusted T4 dose (**Fig. 3b**) and the estimated total exogenous T4 supply (**Fig. 3c**) in a subgroup of athyreotic thyroid carcinoma patients that were non-hypothyroid on stable medication (n = 137, **Table 1**). For comparison, FT3 was stable and unrelated to similar changes in endogenous T4 supply in untreated controls (**Fig. 3c**).

In euthyroid L-T4-treated patients with low thyroid volume (< 5 ml, n = 117), deiodinase activity was lower, compared to the higher volume group (≥ 5 ml, n = 214), 23.3 nmol/s (21.3, 26.1) vs. 26.7 nmol/s (23.6, 29.2), p < 0.001. Deiodinase activity did not differ between euthyroid and subclinically hypothyroid subjects in the low volume group, 23.3 nmol/s (21.3, 26.1), n = 117 vs. 24.6 nmol/s (22.2, 27.5), n = 38, p = 0.22, but did so at higher volumes, 26.7 nmol/s (23.6, 29.2), n = 214 vs. 28.9 nmol/s (26.7, 31.5), n = 20, p = 0.02 (**Fig. 4**). In the low volume group, 82 % of the patients had surgery, 68 % radioiodine treatment, and 8.5 % suffered from autoimmune thyroiditis. In a multivariable model, thyroid volume and thyroid state defined as either euthyroidism, subclinical hypothyroidism or hypothyroidism remained independently influential on deiodinase activity (p < 0.001 for each parameter).

Discussion

In the present study we have shown that maintenance of T3 homeostasis is a preeminent goal in thyroid hormone metabolism that apparently requires a coordinated interaction of central and peripheral regulatory mechanisms. We present clinical and modelling evidence that the 2 processes are more closely interlocked than previously thought. Their intricate interrelation contrasts with the prevailing view where T4 production is assumed to be subject to TSH control and T3 generation predominantly under peripheral "autoregulation" [4,8,9]. Conversely, we find TSH acts as a main integrator, technically feed-forward motif, that not only regulates T4 production by the thyroid gland, but also controls T3 conversion from T4 thereby fine-tuning and stabilising FT3 levels.

The present in vivo results expand on previous experimental findings showing that deiodinases respond sensitively to intracellular cAMP concentrations, which, in turn, are modified by TSH [16–23]. Our observation that deiodinase activity is equally enhanced at the hypothyroid and hyperthyroid extreme may be explained in part by the same mechanisms, because, both TSH receptor stimulating antibodies in Graves' disease and TSH receptor activating mutations in toxic adenomas are known, like TSH, to enhance intracellular cAMP levels [24,25].

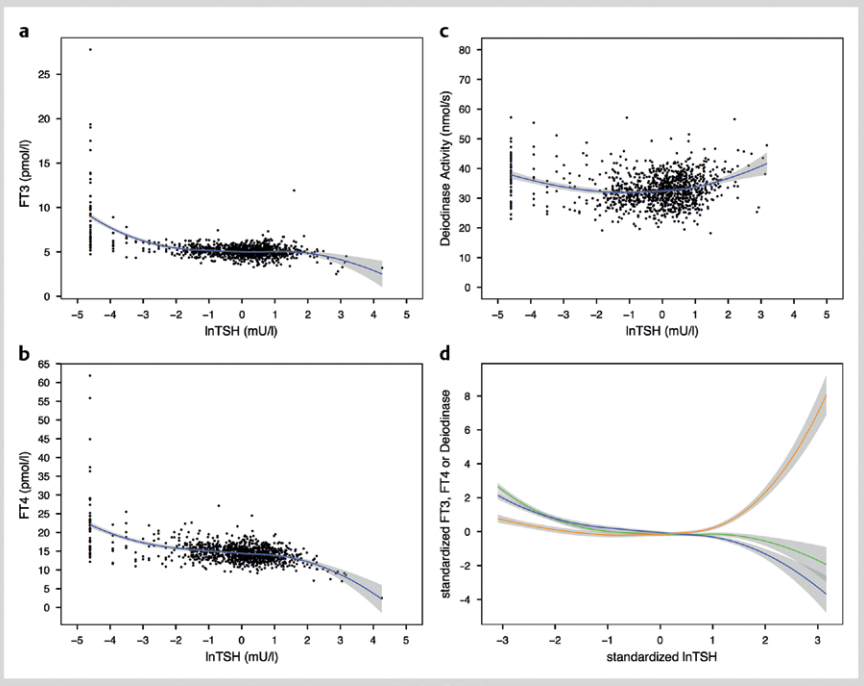

Fig. 1 Relationships between FT3 **a**, FT4 **b**, and deiodinase activity **c** and TSH in untreated subjects (n = 1 126) and comparison of the mean standardised relationships among all thyroid parameters **d**. A blue line represents the fitted curve by locally weighted smooth regression and the shaded area the 95 % confidence interval surrounding the curve **a**–**c**. In panel **d**, the green middle line indicates lnTSH vs. FT3, blue lower line FT4, and orange upper line deiodinase activity. (Color figure available online only).

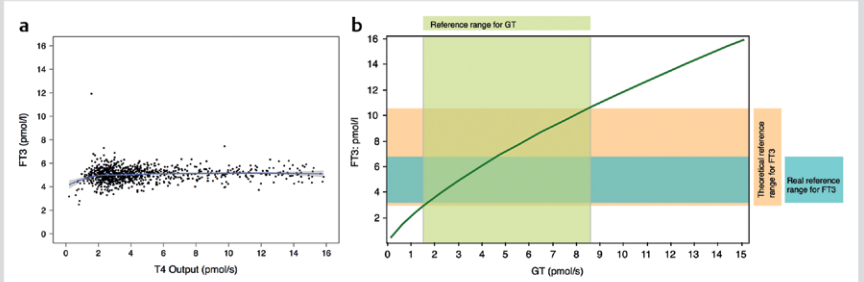

Fig. 2 Relationship between serum FT3 and glandular T4 output, as observed in 982 untreated patients **a**, and theoretically expected with no TSH-deiodinase interaction **b**. See Methods for the calculation of T4 output (GT) and theoretical modelling. The difference between the right panel and the left panel illustrates the T3-stabilising influence of the proposed additional TSH control over deiodinase activity. (Color figure available online only).

While both deiodinases, D1 and D2, are upregulated by TSH/c-AMP, they respond differently to T3 [4, 18, 19]. In hyperthyroidism, T3 upregulates D1 and downregulates D2, whereas in hypothyroidism T4 deficiency mainly increases the efficiency of D2 [4]. A marked increase in the T3 to T4 ratio is clinically evident in both T3-dominant hyperthyroidism and many cases of overt hypothyroidism where FT4 is low, but FT3 found within the reference range. In addition to the regulation by cAMP/TSH and influence of thyroid state, we found that deiodinase activity was associated with thyroid volume, as detailed below, introducing a higher level of complexity and enabling differentiated control.

We conclude that the TSH effect takes place predominantly in the thyroid gland. Although certain peripheral tissues such as skeletal muscle or brown adipose tissue express both a functional TSH receptor and deiodinases our results suggest that they may play only a minor role for systemic T3 generation, compared to thyroid [26, 27].

As a limitation of the study, some comparisons were made using thyroid volumes of patients spread over various etiological diagnoses, such as thyroid carcinoma, autoimmune thyroiditis or uni- and multi-nodular goitre. However, we have previously shown that a TSH-FT3 disjoint exists in L-T4-treated patients even when stratifying by defined TSH categories and disease entities, comparing treated with untreated patients with autoimmune thyroiditis or athyreotic carcinomas with normal controls [10]. We calculated the sum activity of peripheral deiodinases, but were unable to discriminate activities by type of deiodinase, as the source of T3 could not be traced back. It has, however, been shown that the activities of D1 and D2, though they may respond differently to T3 and T4, are both cAMP-dependent and generally correlated [4, 18, 19, 28]. Only indirectly therefore can we infer, from a drop in FT3 levels in athyreotic patients, and lack of TSH response, that the thyroid gland itself is a major player in T3 homeostasis. The loss of the thyroid-derived T3 pool remained relatively uncompensated in L-T4-treated patients. Any bias from the low T3 syndrome appears negligible, because the thyroid patients were otherwise healthy and none of the included subjects suffered from severe comorbidity. For the same reason, we would not envisage any issues with FT3 measurements [29]. FT4, but not FT3, was affected by a minor bias due to variable sampling intervals from T4 ingestion [10]. TSH levels after L-T4 withdrawal of some carcinoma patients were not in full equilibrium, but this, while affecting central equilibria, should not prevent a response of peripheral deiodinases. Our prospective observational study permitted analysis of L-T4-treatment in a large sample, as this is the generally recommended mode of therapy, but patients on T3/T4 combinations were too few for a meaningful comparison and excluded from analysis. The study's non-randomised design, while introducing some uncontrolled variables, permitted the analysis of a broad functional spectrum.

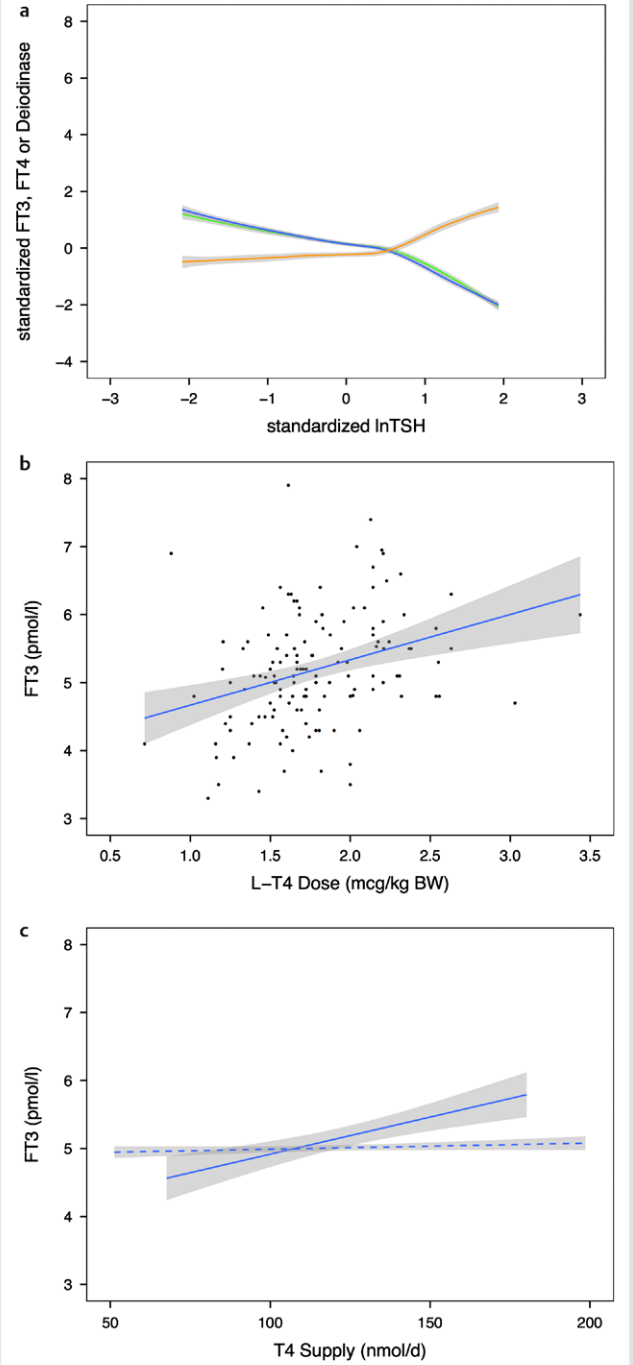

Fig. 3 Relationships between mean standardised FT3, FT4 or deiodinase activity and TSH in L-T4-treated patients (**a**, n = 670), serum FT3 and weight-adjusted daily L-T4 dose (**b**, n = 137, r = 0.33, p < 0.0001), and serum FT3 and estimated daily exogenous T4 supply (**c**, solid line, n = 137, r = 0.33, p < 0.001) in L-T4-treated athyreotic patients with thyroid carcinoma, compared with untreated subjects over a similar range of endogenous T4 supply (**c**, dashed line, n = 701, r = 0.03, p = 0.45). All thyroid carcinoma patients were non-hypothyroid on stable L-T4 medication. T4 supply was estimated as described in Methods. BW, body weight. In panel **a**, the green line indicates lnTSH vs. FT3, blue line FT4, and orange line (starting below the others and crossing over) deiodinase activity. (Color figure available online only).

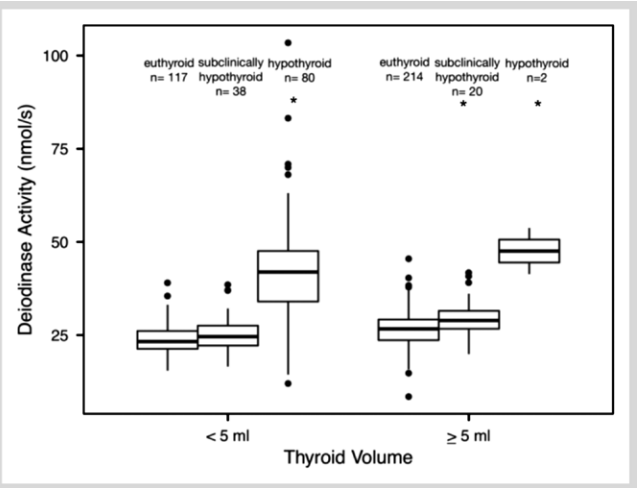

Fig. 4 Deiodinase activity in L-T4-treated patients from 2 thyroid volume groups (cut-off 5 ml), stratified by thyroid function. The difference between the 2 euthyroid panels was significant. Asterices indicate significant differences ($p < 0.05$), compared to euthyroid patients in each group (see Results for details). Euthyroid was defined by FT4 and TSH values within their reference limits, subclinically hypothyroid by elevated TSH together with an FT4 value within the reference range, and hypothyroid according to elevated TSH and FT4 values below the lower reference limit.

We found important homeostatic physiological mechanisms to be compromised in L-T4-treated patients. Patients, with a mostly post-interventional low residual thyroid volume (< 5 ml, defined as a cut-off associated with a decline in deiodinase activity in a previous study [10]), 1) have significantly reduced deiodinase activity and lower FT3 levels, compared to either patients with a higher residual capacity or untreated subjects, 2) show relatively reduced deiodinase activities and FT3 concentrations based on their serum TSH levels (TSH-FT3 disjoint), 3) lack the ability to raise their deiodinase activity appropriately upon TSH stimulation, and 4) in contrast to L-T4-treated patients with larger volumes, who responded to TSH, are unable to fine-tune their FT3 levels in the subclinically hypothyroid state. They still respond to T4 deficiency by raising their deiodinase activities in the overtly hypothyroid state in order to partly restore FT3 levels. As might be expected, overt hypothyroidism was too infrequent in the high volume group for a conclusive comparison.

We conclude that residual thyroid capacity, deiodinase response to TSH and deiodinase reaction to a decline in T4 are all contributing to fine-tuning the T3 equilibria.

These findings augment our growing understanding of the complexity of the thyroid pituitary regulatory processes, as expressed in recent publications [9,13,30]. It is remarkable to note that the interacting equilibria diverged on L-T4 treatment, whilst optimum healthy physiological FT3, FT4, and TSH levels in the circulation were not maintained [10]. Our results confirm earlier studies by Escobar-Morreale et al. in the rat where T4 alone supplied by infusion, as opposed to a combination of T4 and T3, could not simultaneously achieve normal plasma levels of T3, T4, and TSH, and was unable to ensure tissue euthyroidism [31,32]. The studies in the rat have been questioned as to their relevance to human physiology by pointing out differences between species in deiodinase types and activities, in particular that the assessment of the proportion of T3 contributed by the human thyroid is much lower than in the rat thyroid (approx. 20% vs. 50%) thereby facilitating a superior compensation in the absence of a thyroidal T3 pool [3,4]. However, the maintenance of optimum T3 levels in various tissues on L-T4 replacement in athyreotic patients has never been proven in humans and the

assumption of this proportion relies on a few reports only, prominently a study by Pilo et al. [3,4]. Although this study was well designed, participants were given high doses of iodine (Lugol solution), which is known to act as a blocker of thyroid hormone secretion and deiodination [3]. This might have led to an underestimation of the thyroid-derived hormone, casting doubt on the study's physiological relevance. Indeed, our data indicate a significantly greater direct contribution of T3 from the thyroidal pool and, more importantly, suggest that its loss in athyreotic patients may not be readily compensated owing to an impaired T3-homeostatic regulation. Circulatory FT3 concentrations clearly varied with exogenous T4 supply, showing no sign of compensation or successful "autoregulation".

The proposed overarching T3-homeostatic control may be regarded as an instrument of central-peripheral balance. On the one hand, peripheral T3 generation is influenced by TSH, as shown here, but on the other hand, the pituitary responds to both circulating FT3 and FT4 converted to T3 by D2 [1]. This does, however, not preclude short-term adjustments, additional corrections or responses to circumstantial events such as inflammation at the respective tissue level, which may serve as another protective layer [33]. In euthyroidism, the factors involved in regulation are balanced, each exerting its own stabilising influence, so that the quantitative contribution by the TSH-T3 tuning is at its smallest in this state. However, in unbalanced situations the TSH-T3 cross-talk becomes increasingly important. In athyreotic patients on L-T4, the physiological T3-homeostatic regulatory process is distorted and maintenance of adequate FT3 concentrations becomes a function dependent on exogenous T4 supply.

The functional consequences of the suspected TSH-deiodinase feed-forward motif were further elucidated by comparing clinical outcomes in L-T4-treated patients with predictions of our theoretical model, which would suggest a stronger dependence of T3 generation on thyroid secretory capacity, if TSH did not control deiodinase activity. The model serves as an adjunct to the clinical data on anatomical thyroid capacity, providing estimates on functional thyroid capacity. It has been validated to a considerable extent in previous studies, which we refer to for further details [11–13] (see review [11] for additional references).

Overall, the homeostatic system seems ill-prepared for L-T4 monotherapy, which produces an unnatural event of T4 excess without appropriate provision of T3, subsequently enforcing a situational integrating compromise (high FT4, low TSH, low FT3), though failing to restore T3 stability and "normality".

Although our study has shown evidence of the break-down of important T3-homeostatic mechanisms we cannot answer the question whether the hormonal dysequilibria extend to tissues in L-T4-treated athyreotic patients. However, it is commonly assumed that a long-term equilibrium exists between plasma and intracellular T3 concentrations [9]. As T3 is largely created intracellularly and contributes to the circulating T3 pool following its active transport across the plasma membrane, reduced T3 levels in the circulation are likely to reflect T3 deficiency within the bulk of the T3-producing tissues. Experimental studies in the rat, discussed above, and the high dissatisfaction rate with the current L-T4 standard treatment patients expressed in many trials point in the same direction [34,35]. Our clinical data on homeostatic regulation, further supported independently by theoretical modelling, at least cast doubt on an "autoregulated" and guaranteed optimum tissue supply of T3 by L-T4 treatment, and encourage further study of this important issue.

In summary, our results suggest that 1) the thyroid gland physiologically plays a direct essential and significant part in maintaining T3 homeostasis and fine-tuning circulating T3 concentrations, 2) TSH is involved in coordinating central and peripheral mechanisms that cooperate to facilitate T3 homeostasis, and 3) regulatory and capacity deficiencies together may contribute to an apparent inadequacy of T3-homeostatic mechanisms in patients on L-T4 treatment. The novel findings point to an intricate interactive relationship between T4–T3 conversion and thyroid hormone production, rather than confirming a divided two-step regulation and peripheral "autoregulation".

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Conflict of Interest

JWD is co-owner of the intellectual property rights for the patent "System and Method for Deriving Parameters for Homeostatic Feedback Control of an Individual" (Singapore Institute for Clinical Sciences, Biomedical Sciences Institutes, Application Number 201208940e20120895). All other 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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