CLINICAL STUDY

Is pituitary TSH an adequate measure of thyroid hormone-controlled homeostasis during thyroxine treatment?

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Abstract

Objective: In recognition of its primary role in pituitary–thyroid feedback, TSH determination has become a key parameter for clinical decision-making. This study examines the value of TSH as a measure of thyroid hormone homeostasis under thyroxine (T₄) therapy.

Design and methods: We have examined the interrelationships between free triiodothyronine (FT₃), free T₄ (FT₄) and pituitary TSH by means of i) a retrospective analysis of a large clinical sample comprising 1994 patients either untreated or on varying doses of L-T₄ and ii) independent mathematical simulation applying a model of thyroid homeostasis, together with a sensitivity analysis.

Results: Over a euthyroid to mildly hyperthyroid functional range, we found markedly different correlation slopes of log TSH vs FT₃ and FT₄ between untreated patients and L-T₄ groups. Total deiodinase activity (G_D) was positively correlated with TSH in untreated subjects. However, G_D was significantly altered and the correlation was lost under increasing L-T₄ doses. Ninety-five per cent confidence intervals for FT₃ and FT₄, when assessed in defined TSH concentration bands, differed significantly for L-T₄-treated compared with untreated patients. Higher doses were often needed to restore FT₃ levels within its reference range. Sensitivity analysis revealed the influence of various structural parameters on pituitary TSH secretion including an important role of pituitary deiodinase type 2.

Conclusion: The data reveal disjoints between FT₄–TSH feedback and T₃ production that persist even when sufficient T₄ apparently restores euthyroidism. T₄ treatment displays a compensatory adaptation but does not completely re-enact normal euthyroid physiology. This invites a study of the clinical consequences of this disparity.

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Introduction

Thyrotoxicosis and hypothyroidism have been traditionally defined as states where elevated or decreased concentrations of circulating free thyroid hormones exert their own appropriate influences on the numerous metabolic outputs and organ functions of the body (1). Cellular access to triiodothyronine (T₃) is tightly controlled by two mechanisms, an active transport into the cell and an enzymatic conversion of thyroxine (T₄) to T₃ by deiodinases, which exist in different subtypes (2, 3). Conversion is also the main source of T₃ in humans, making up about 80% of its production, the rest being directly supplied by the thyroid gland (4). Failure of the thyroid gland or any change in hormone production is sensitively reflected at the pituitary level via a negative feedback within the hypothalamus–pituitary–thyroid regulatory loop. As a result, statistically derived TSH reference ranges have shaped definitions of thyroid dysfunctions, introducing sub-clinical disease entities and thus have been given the

various roles of screening tool, therapeutic target and prognostic marker (5, 6, 7, 8, 9).

The TSH-centred definition of thyroid function has both merits and disadvantages. While TSH has become accurately and readily measurable, the exact reference values remain elusive (5, 10, 11, 12). Moreover, in an individual, normal distribution of thyroid hormone concentrations encompasses only a fraction of the span of the population reference range and the TSH set point is particularly tightly controlled (13).

On the other hand, appreciation of the interplay of TSH, free T₃ (FT₃) and free T₄ (FT₄) should facilitate a more individualised approach than relating analytic decisions to the broad inter-individual variation of each parameter. This concept requires a clear understanding of the interaction of these parameters under various physiological and pathophysiological conditions, in particular the implications of their homeostatically controlled equilibria.

We have therefore sought to assess the interrelation of FT₃, FT₄ and pituitary TSH, querying reliance on TSH

as a reliable measure for thyroid hormone homeostasis under T₄ therapy. To this end, we i) retrospectively analysed a broad clinical sample and ii) tested our conclusions by a mathematical simulation.

Materials and methods

Patients and laboratory samples

This study extends an earlier publication where full details of methods were described (14). Data were obtained from a large number of unselected patients referred for thyroid testing to the Departments of Endocrinology or Nuclear Medicine of the Klinikum Ludenscheid, Germany, between October 2006 and January 2007. Excluded from analysis were patients with pituitary or hypothalamic disorders, critically ill patients, patients with renal failure and pregnant women, as those conditions can interfere unpredictably with thyroid testing.

The focus of this study was on treatment-related aspects of the thyroid–pituitary relationship. To this end, 1994 patients and as many samples (including only the first sample per patient) were included for analysis. Of them, 1159 patients were untreated, receiving neither thyroid medication nor any other drugs at the time of sampling. Others were on levo T₄ (l-T₄) or iodine in varying doses of 50–200 µg/day ($n=785$) or 100 or 200 µg/day ($n=50$) respectively. The small group of iodine-treated patients was deliberately separated from untreated subjects to determine any effects of supplementation, as iodine status is of known influence on the FT₃:FT₄ ratio. Median patient age was 61 years (range 18–90 years). The majority of patients were women (78%) and seen in an ambulatory setting (80%). Thyroid peroxidase antibodies (TPO Abs) or TSH receptor antibodies (TSH R Ab) were measured only when clinically relevant to confirm or exclude suspected thyroid autoimmune disease. In the majority of patients with hypothyroidism in its overt or subclinical form ($n=190$), the condition was due to thyroid ablative therapy, surgery and/or radioiodine treatment; 53/190 patients (28%) suffered from thyroid autoimmunity, as evidenced by elevated serum levels of TPO Abs above the reference level. For classification purpose, subclinical thyroid disorders were defined by a TSH value outside reference limits, plus corresponding FT₃ and FT₄ values within the reference interval. The study was approved by the local ethics committee.

Laboratory methods

TSH was measured using an enzyme immunoassay (Abbott), with a working range of 0.01–100 mU/l and laboratory-evaluated reference range of 0.4–4.1 mU/l. FT₃ and FT₄ were determined by non-analogue, two-step immunoassays from the same manufacturer,

reference ranges being 2.23–5.36 and 9.5–25 pmol/l respectively. TPO Abs were determined by RIA (reference range <60 IU/ml) and TSH-R Abs by the second-generation TRAK assay (reference range <2 U/l; BRAHMS, Berlin, Germany). Quality management included standard laboratory evaluation procedures and regular participation in inter-laboratory tests.

Calculations, models and statistical methods

A physiologically based mathematical model (MiMe-NoCoDI model) of thyroid homeostasis has been previously developed and validated in healthy volunteers and cohorts of patients with defined thyroid disorders, including hypothyroid and hyperthyroid conditions, by one of the authors (15, 16). This model has also been made available as an open-source software (SimThyr 3.2 for Mac OS X, Windows and Linux) at sourceforge.net. It allows mapping of the physiological and biochemical interplay to the various parameters involved. With this simulative approach, sensitivity analysis was performed to study the relationship between certain structural parameters such as type 2 deiodinase (D₂) and thyroid parameters (17). The mathematical equation used for empirical curve fitting was as follows: $TSH = s / (1 + L_S \times FT_4)$, with s referring to maximum secretory capacity and L_S to the brake constant of non-competitive inhibition at the pituitary site. Theoretical sum activity of peripheral deiodinases (G_D) was calculated from the equation:

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

from equilibrium levels for FT₄ and FT₃ and from constant parameters for kinetics, plasma proteins and dissociation constants as previously defined and described (17).

Basic descriptive statistics, correlations and linear models were performed using the statistical software packages R 2.15.0 for Mac and Deducer 0.7 with JGR 1.7–10 (18, 19). Kruskal–Wallis rank sum test was used to compare values among groups and analysis of covariance (ANCOVA) was used for comparison of regression lines. For comparison of models, F-statistics and Akaike criteria were applied. In the setting of multiple testing, P values were corrected with the Benjamini–Hochberg method. P values <0.05 were considered significant.

Results

The 1994 patients studied were grouped together according to their treatment. Group 1 comprised untreated subjects, with groups 2–4 subjects on 50–75, 100–125 and 150–200 µg/day l-T₄ respectively. Group 5 comprised patients on iodine

supplementation. Table 1 provides an overview of treatment-related functional states in the five groups. Thyroid parameters differed significantly among groups in the euthyroid state, not in hypothyroidism, and partly in the other conditions shown (Table 1).

The relationships of log TSH serum levels and peripheral hormone concentrations were analysed over a comparable functional range from the euthyroid to mildly hyperthyroid spectrum defined by $FT_4 \geq 9.5$ pmol/l and $TSH > 0.01$ and < 100 mU/l (Fig. 1). The correlations revealed marked differences in the relationships among the groups, particularly between patient groups untreated and on $L-T_4$.

Gradients of the log TSH– FT_3 regressions were relatively flat in untreated patients and those taking iodine, but much steeper in $L-T_4$ treatment (Fig. 1). The log TSH– FT_4 relationship also displayed significantly higher gradients in treatment groups 3 and 4, but compared with log TSH vs FT_3 , changes were less pronounced (Fig. 1). G_D was positively correlated with log TSH only in untreated subjects (Fig. 1).

Group differences in TSH, FT_3 , FT_4 and G_D were further assessed in defined bands of TSH concentrations (Fig. 2). Over a wide functional range, medians and 95% confidence interval (CIs) for FT_4 and partly FT_3 differed significantly from untreated or iodine-treated subjects,

Table 1 Treatment groups and thyroid function states.

Thyroid status	Parameter	Group 1 no Tx <i>n</i> = 1159	Group 2 $L-T_4$ 50–75 µg/day <i>n</i> = 254	Group 3 $L-T_4$ 100–125 µg/day <i>n</i> = 343	Group 4 $L-T_4$ 150–200 µg/day <i>n</i> = 188	Group 5 iodine <i>n</i> = 50
Hypothyroid	<i>n</i>	41	15	7	1	0
	FT_3 (pmol/l)	2.25 1.49–3.29	2.41 1.65–2.91	2.09 1.85–2.32	3.67 –	–
	FT_4 (pmol/l)	5.91 4.45–8.25	7.91 7.01–8.84	7.64 6.55–7.93	9.04 –	–
	G_D (nmol/s)	35.9 27.6–45.0	28.2 24.0–35.5	24.4 20.9–34.7	37.5 –	–
	TSH (mU/l)	18.6 7.24–43.0	28.3 9.35–55.48	15.3 7.28–38.55	22.8 –	–
Subclinically hypothyroid	<i>n</i>	54	28	29	6	0
	FT_3 (pmol/l)	3.34 2.65–3.83	2.78 2.30–3.36	3.19 2.12–3.82	2.89 2.79–3.60	–
	FT_4^* (pmol/l)	10.9 10.4–12.3	13.1 11.9–14.1	13.9 12.1–15.5	14.9 13.8–16.0	–
	G_D^* (nmol/s)	27.5 19.8–32.4	19.2 17.5–21.8	18.9 13.8–25.5	16.6 16.4–18.7	–
	TSH (mU/l)	5.62 4.88–7.53	7.24 5.11–15.3	5.87 5.32–9.54	5.32 4.49–18.10	–
Euthyroid	<i>n</i>	731	154	153	31	42
	FT_3^* (pmol/l)	3.64 3.13–4.23	3.47 2.97–3.94	3.29 2.82–3.77	3.65 3.13–4.01	3.81 3.21–4.18
	FT_4^* (pmol/l)	13.0 11.8–14.6	14.1 12.9–15.8	16.14 14.5–18.7	17.0 15.4–19.1	13.3 13.0–14.4
	G_D^* (nmol/s)	25.8 21.8–30.1	21.4 18.6–26.3	18.0 14.6–22.0	19.7 17.1–22.0	26.0 22.8–29.0
	TSH* (mU/l)	1.07 0.64–1.64	1.21 0.88–2.00	1.09 0.75–1.91	0.92 0.59–1.87	1.01 0.86–1.59
Subclinically hyperthyroid	<i>n</i>	314	55	135	114	8
	FT_3^* (pmol/l)	3.77 3.03–4.41	3.56 2.99–4.21	3.93 3.25–4.43	4.07 3.52–4.53	4.13 3.63–4.66
	FT_4^* (pmol/l)	14.7 12.9–16.5	15.4 13.4–17.5	19.4 17.0–21.6	21.5 19.7–23.5	14.5 12.6–16.7
	G_D^* (nmol/s)	23.5 19.1–27.7	21.7 17.7–24.2	18.3 16.2–21.3	17.4 15.5–19.5	24.2 21.7–31.9
	TSH (mU/l)	0.21 0.07–0.31	0.21 0.08–0.30	0.05 0.01–0.19	0.01 0.01–0.06	0.27 0.20–0.37
Hyperthyroid	<i>n</i>	19	2	19	36	0
	FT_3^* (pmol/l)	7.68 4.90–12.0	4.49 4.28–4.71	4.71 3.93–5.20	4.77 4.31–5.54	–
	FT_4 (pmol/l)	31.8 26.1–38.4	34.5 30.8–38.2	26.5 25.9–28.3	28.4 26.5–29.8	–
	G_D^* (nmol/s)	22.5 17.6–30.3	12.9 10.9–14.8	16.3 13.1–17.8	15.5 13.5–17.6	–
	TSH (mU/l)	0.01 0.01–0.18	0.01 0.01–0.01	0.01 0.01–0.01	0.01 0.01–0.04	–

G_D , sum activity of peripheral deiodinases. Values indicate median and interquartile range (IQR). An asterisk indicates significant differences ($P < 0.05$) of the parameter among the groups using Kruskal–Wallis rank sum test and P value correction for multiple testing according to Benjamini–Hochberg method.

as did G_D , because FT_3 and FT_4 trends were moving in opposite directions (Fig. 2). In the euthyroid TSH range, represented by TSH bands 4 and 5, medians of FT_3 for patients receiving moderate $L-T_4$ doses (Groups 2 and 3) declined and the lower 95% CI limits were placed outside the reference range (Fig. 2). Only high $L-T_4$ doses (Group 4), frequently accompanied by suppressed TSH,

placed FT_3 values within its reference limits. Patients regarded euthyroid by TSH criteria (TSH bands 4 and 5) would sometimes be classified differently by FT_3 and considered hypothyroid in 13/164 (8%) of the cases in Group 2 on 50–75 $\mu\text{g/day}$ $L-T_4$, 18/154 (12%) in Group 3 on 100–125 $\mu\text{g/day}$ $L-T_4$ and 0/33 (0%) in Group 4 on 150–200 $\mu\text{g/day}$ $L-T_4$.

Theoretical mathematical modelling was performed independently from the analysis of the clinical sample. We have initially assessed the MiMe-NoCoDI model by fitting it to our data sample. This may best be done in an open-loop situation, i.e. in overtly hypothyroid untreated patients, as shown in Fig. 3. For comparison, fitting with the conventional log TSH FT_4 model is also shown in Fig. 3. As the MiMe-NoCoDI model is based on TSH values rather than their log transformed derivatives, which are commonly applied and used in the latter model, the two models were depicted in terms of TSH vs FT_4 (Fig. 3). When statistically comparing the compatibility of the two models with the data (F-statistics and Akaike criteria), their goodness of fit did not significantly alter (Fig. 3).

When applying the MiMe-NoCoDI model, sensitivity analysis revealed that pituitary TSH secretion, in addition to responding to circulating FT_3 and FT_4 concentrations, is subject to the influence of a variety of structural parameters (Fig. 4, left panel). This importantly includes pituitary D_2 (Fig. 4, left panel). In this model, the divergence of central T_3 and peripheral T_3 production becomes evident. Increased central T_3 by D_2 activation suppresses TSH and indirectly (via TSH) reduces FT_4 and FT_3 production (Fig. 4, right panel).

Discussion

Originating from a basic understanding of pituitary thyroid feedback, the perspective of the diagnostic role of TSH has shifted in importance and the parameter has been promoted into a separate statistical measure in its own right (5, 6, 7, 8, 9, 10, 11, 12, 20). Limitations in this approach include analytical issues, genetic variability, uncertainty about the reference range, high ratio of the basic intra-individual to inter-individual variability and considerable natural intra-individual fluctuations (10, 11, 12, 13, 21, 22, 23, 24, 25, 26, 27, 28, 29). A lack of correlation with clinical symptom scores occurs particularly in patients with subclinical hypothyroidism (30). However, the implicit assumption

of a fixed relationship between peripheral thyroid hormone homeostasis and the pituitary set point in health and disease has not been seriously questioned. In this study, we show that as a result of the attainment of a hypothyroid state, there is an imbalance and disjoint involving peripheral thyroid hormone homeostasis and the pituitary set point that persists and is particularly evident under T_4 monotherapy. The treatment-related disjoint between peripheral thyroid hormone status and pituitary response became apparent by retrospective correlative analysis of a large clinical sample and was further corroborated by fundamental modelling and independent simulation.

In untreated euthyroid subjects, the gradient of the correlation line relating FT_3 and log TSH is flat, but in subjects treated by T_4 monotherapy, the relationship is different in all cases, the gradient increasing with hormone dosage. This suggests that the balanced relationship between the thyroid hormones themselves and TSH, evident in untreated euthyroids, no longer applies and shows that in monotherapy added increments of T_4 are progressively more effective in suppressing TSH production, but simultaneously less effective in restoring FT_3 . Obviously, this is a consequence of the decreasing ability of thyroid homeostasis to maintain the normal interrelationships between T_3 , T_4 and TSH.

Hence, the results indicate that the observed disjoint between the thyroid/pituitary FT_4 -TSH feedback mechanism and T_3 production noted in hypothyroidism (whether inadequately or untreated) is not fully restored even when sufficient T_4 is given to regain an apparently euthyroid state. In several cases, this results in a classification mismatch, for example placing ~10% of patients on moderate T_4 doses that are judged euthyroid according to their TSH measurements below the FT_3 reference range. Higher L - T_4 doses maintained FT_3 within its reference limits, though frequently only in conjunction with suppressed TSH. Gullo *et al.* (31) have recently reported the same phenomenon in a large homogeneous group of athyreotic patients, whereas Jonklaas *et al.* (32) reported that total T_3 was not

Figure 1 Correlations of log TSH vs FT_4 , log TSH vs FT_3 and G_D vs log TSH in various treatment groups. Group 1: untreated subjects, Groups 2–4: L - T_4 -treated patients with doses of 50–75 μ g/day (Group 2), 100–125 μ g/day (Group 3) or 150–200 μ g/day (Group 4), Group 5: patients taking iodine supplementation. *P* values given were corrected for multiple testing by the Benjamini–Hochberg method. Equations of regression lines ($\pm$ s.e.m.) depicted are as follows: left panels, Group 1: log TSH = $-0.069 (\pm 0.005) * FT_4 + 0.80 (\pm 0.07)$, $r = -0.39$, $P < 0.0001$, $n = 1061$. Group 2: log TSH = $-0.058 (\pm 0.015) * FT_4 + 0.86 (\pm 0.21)$, $r = -0.25$, $P < 0.0001$, $n = 234$. Group 3: log TSH = $-0.11 (\pm 0.011) * FT_4 + 1.64 (\pm 0.19)$, $r = -0.52$, $P < 0.0001$, $n = 284$. Group 4: log TSH = $-0.095 (\pm 0.016) * FT_4 + 1.23 (\pm 0.32)$, $r = -0.51$, $P < 0.0001$, $n = 109$. Group 5: log TSH = $-0.06 (\pm 0.025) * FT_4 + 0.75 (\pm 0.35)$, $r = -0.32$, $P = 0.02$, $n = 50$. Middle panels, Group 1: log TSH = $-0.019 (\pm 0.016) * FT_3 - 0.066 (\pm 0.06)$, $r = -0.04$, $P = 0.27$, $n = 1061$. Group 2: log TSH = -0.18 ± 0.040 , $FT_3 + 0.65 \pm 0.14$, $r = -0.29$, $P < 0.0003$, $n = 234$. Group 3: log TSH = $-0.32 (\pm 0.044) * FT_3 + 0.87 (\pm 0.16)$, $r = -0.40$, $P < 0.0003$, $n = 284$. Group 4: log TSH = $-0.17 (\pm 0.086) * FT_3 - 0.05 (\pm 0.33)$, $r = -0.19$, $P = 0.08$, $n = 109$. Group 5: log TSH = -0.05 ± 0.052 , $FT_3 + 0.12 \pm 0.20$, $r = -0.13$, $P = 0.37$, $n = 50$. Right panels, Group 1: $G_D = 3.2 (\pm 0.44) * \log TSH + 25.60 (\pm 0.22)$, $r = 0.22$, $P = 0.0005$, $n = 1061$. Group 2: $G_D = -1.5 (\pm 0.70) * \log TSH + 21.86 (\pm 0.40)$, $r = -0.14$, $P = 0.06$, $n = 234$. Group 3: $G_D = -0.10 (\pm 0.46) * \log TSH + 18.90 (\pm 0.36)$, $r = 0.01$, $P = 0.83$, $n = 284$. Group 4: $G_D = 1.74 (\pm 0.54) * \log TSH + 18.98 (\pm 0.56)$, $r = 0.30$, $P = 0.004$, $n = 109$. Group 5: $G_D = 0.69 (\pm 2.68) * \log TSH + 26.21 (\pm 0.91)$, $r = 0.04$, $P = 0.83$, $n = 50$. Slopes were significantly different compared to the untreated Group 1 (ANCOVA), for FT_4 in Group 3 ($P < 0.001$) and Group 4 ($P < 0.03$), for FT_3 in Group 2 ($P < 0.001$), Group 3 ($P < 0.001$) and Group 4 ($P < 0.01$), for G_D in Group 2 ($P < 0.001$), Group 3 ($P < 0.03$) and Group 4 ($P < 0.001$).

Figure 2 Comparison of medians and 95% confidence intervals (95% CI) of thyroid parameters in various treatment groups and functional bands defined by TSH values. The TSH bands on the x-axis were defined as follows: Band 1: TSH >50 mU/l, Band 2: TSH 10–50 mU/l, Band 3: TSH 4–10 mU/l, Band 4: TSH 2–4 mU/l, Band 5: TSH 0.4–2 mU/l, Band 6: TSH 0.1–0.4 mU/l and Band 7: TSH <0.1 mU/l. The error bars (95% CI) depicted in each TSH band refer to Groups 1–5 respectively. Group 1: untreated subjects, Group 2: L-T₄-treated patients with doses of 50–75 µg/day, Group 3: L-T₄-treated patients with doses of 100–125 µg/day, Group 4: L-T₄-treated patients with doses of 150–200 µg/day and Group 5: patients taking iodine supplementation. An asterisk indicates the presence of a significant difference ($P < 0.05$) among the groups, as established by Kruskal–Wallis test and Benjamini–Hochberg correction for multiple testing. Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-12-0819>.

reduced in such patients, but did not study FT₃. In contrast, Ito *et al.* (33) showed that in patients who have undergone thyroidectomy, a moderately TSH-suppressive dose of L-T₄ is required to postoperatively restore serum FT₃ to the preoperative level. Interestingly, earlier studies have also indicated that L-T₄ replacement aiming at a normal serum TSH may not invariably result in an appropriate normalisation of serum FT₃ (34).

Additionally, we observed a positive correlation between TSH and G_D in untreated patients. This could possibly stem from the stimulation by TSH of both type 1 (D₁) and type 2 (D₂) deiodinases mediated by a signalling process using cAMP (35, 36, 37, 38, 39). Interestingly, the correlation was strongest in untreated subjects and impaired in T₄-treated patients, suggesting a role of TSH-modulated deiodination within the thyroid gland itself.

The pituitary responds primarily to changes in circulating FT₄ by the action of type 2 deiodinase (D₂) on the local production of T₃ (central T₃) from T₄ (2). Circulating FT₃ derived mainly from peripheral deiodinase activities has less influence (2). Remarkably, the pituitary retains its ability to respond to increasing FT₄ levels in T₄-treated subjects, permitting appropriate TSH feedback responses. This may be achieved by a mechanism unique to thyrotrophs, which in contrast to other cells expressing D₂, are capable of increasing D₂ expression to maintain high T₃ production at high T₄ concentrations (2). By sensitivity analysis, we have shown that central T₃ levels at the pituitary level, as opposed to T₃ in the periphery, are up-regulated by the activation of D₂. This, in turn, results in suppression of TSH concentrations and, as a further consequence, in a decrease in circulating FT₄ and FT₃ concentrations. The divergence in the central response from the peripheral equilibrium may explain the observed phenomenon that FT₃ serum levels remain disproportionately low even when a supposedly normal TSH has been achieved by means of exogenous L-T₄ administration. This means that the thyroid status and dose adequacy of T₄ substitution cannot readily be defined by the putative gold standard TSH in these conditions because TSH concentrations within the accepted reference range may not invariably signal optimum concentrations of the peripherally active hormone FT₃.

The clinical findings prompted us to seek independent confirmation on theoretical grounds. To this end, we applied a physiologically based mathematical model of thyroid homeostasis, which had previously been developed by one of the authors (15, 16). Apart from the fact that this model permits simulation of the influence of various structural parameters and sensitivity analysis, which the standard log linear model does not, another advantage is that it does not make a preconceived assumption that the TSH–FT₄ relationship is log linear but relies on Michaelis–Menten kinetics and non-competitive divisive inhibition, thus reflecting the physiological and biochemical interplay of the various

Figure 3 Fit of models to observed data in the subgroup of untreated overtly hypothyroid patients: i) MiMe-NoCoDI model (solid line), ii) log linear standard model (dashed line). The fitting curves to the data points ($n=50$) were described by the following equations and parameters ($\pm$ S.E.M.): a) $\text{TSH} = 88 (\pm 22) / (1 + 0.35 (\pm 0.16) * \text{FT}_4)$ and b) $\log \text{TSH} = -0.12 (\pm 0.02) * \text{FT}_4 + 2 (\pm 0.12)$. For reasons of comparability to a, b is also shown in a non-logarithmic representation of TSH vs FT_4 . Comparisons by F-statistics (sum of residuals 31312 vs 30934, $P=\text{NS}$) and Akaike criteria (AIC 469 vs 466, $P=\text{NS}$) revealed no significant differences in the goodness of the fits of the two models.

parameters involved (15). The model has previously been validated in a clinical setting (16). It was briefly re-evaluated in the present data sample and provided a reasonably good fit to the data, as did the log linear model. Proof of superiority of one or the other model is a more complex task and beyond the scope of this article, but the question is currently explored in an ongoing prospective trial (NOMOTHETICOS study), the protocol of which has been published (ClinicalTrials.gov NCT01145040, German Clinical Trials Register DRKS00003153, UTN U1111-1122-3273). Sensitivity analysis revealed a dependency of TSH levels on multiple structural parameters of the feedback control system, including the sum activity of pituitary type 2 deiodinase ($\text{G}_{\text{D}2}$), which in turn is modulated by a whole variety of factors (2). TSH-stimulated deiodination, on the other hand, seems to be largely restricted to patients with a functional thyroid gland. Finally, our observation could explain the existence of a subgroup of patients with normal TSH but low FT_3 levels.

Although our study may be affected by heterogeneity and its retrospective design, we have been careful to exclude those patients with known problems of assay interpretation. No patient was considered to have a systemic illness of a magnitude likely to initiate a significant 'low T_3 syndrome' typical of this condition, though such possibilities must always be envisaged when using FT_3 as a diagnostic tool. Although the study group was heterogeneous, relevant comparisons were made in defined TSH segments. While TSH disequilibrium may have been present in some subjects, it does not account for a low FT_3 /'normal' TSH pair because peripheral hormone levels are normalised before TSH in the treatment of hypothyroidism. Apart from

acknowledged limitations of a retrospective study design and assay techniques, importantly, there is a second line of independent supportive evidence. Mathematical modelling was found to be in good accord with our empirical findings, supporting a significant disparity between the central FT_3 /TSH and the peripheral T_3 under L- T_4 .

The described disjoint between FT_4 -TSH feedback and T_3 production implies that T_4 treatment constitutes a novel compensatory adaptive state of its own and therefore cannot be considered a complete re-enactment and restoration of the previous euthyroid state. This poses the question as to the possible long-term consequences of this disparity and whether thyroid hormone replacement, as it is currently performed, is truly optimal in all cases. Combined T_3 / T_4 therapy has generally not been found superior in many short-term trials including a meta-analysis (40, 41). However, it is possible that certain subjects, e.g. those with limited endogenous thyroid reserve and consecutive hypodeiodination, may benefit, and most studies have used more heterogeneous populations (40, 41). Genetic variation, in particular D_2 polymorphism, could also play a role (25, 42). On the other hand, a TSH value within the reference limits does not guarantee the absence of an adverse clinical outcome (43). This question remains unanswered, but our study encourages the need for further examination of adjunctive T_3 therapy in selected patients, as suggested by Biondi & Wartofsky (40) in a recent review. Further, preferably prospective, studies are required to corroborate and extend these findings: i) to relate the described 'disjoint' to a host of clinically important parameters (e.g. metabolic rate, thermogenesis, lipid profiles and psychological well-being); ii) to elaborate on the possible influence of genomic factors and other influences (such as age, sex, BMI, thyroid volume, disease etiology, iodine intake, selenium status); and iii) to address the remaining methodological concerns by applying equilibrium dialysis or mass spectrometry for hormone measurement (29). Theoretical modelling, as demonstrated here, may prove helpful in guiding the design of adequate clinical trials. There are a few randomised controlled trials or cross-sectional studies that have addressed some of these aspects. Appelhof *et al.* (44) in a double-blind randomised trial on combined T_3 / T_4 therapy made the point that satisfaction with study medication was not correlated with either absolute TSH values or with the change in TSH. Saravanan *et al.* (45) showed that strong correlations between FT_4 levels and well-being persisted even when TSH values were within the reference range. To complicate things further, a recent study by Wang *et al.* (46) suggests that not only may thyroid hormones act independently of TSH but TSH within its reference limits may also exert more direct thyroid hormone-independent effects, for instance on lipid profiles.

In conclusion, our data suggest that, in order to fully understand the implications of T_4 monotherapy in the

Figure 4 Mathematical model of sensitivity of TSH, central T₃, FT₃ and FT₄ to changes in structural parameters. According to sensitivity analysis (see Materials and methods section), shown in the left panel, a 20% variation in various structural parameters' changes, in the absence of any change in circulating FT₃ or FT₄ concentrations, pituitary TSH secretion by about 5 to 20%. Type 2 deiodinase (D₂) represented by G_{D2} and K_{M2} is among the more influential parameters in this respect. In the right panel, the relationships between G_{D2} activity and central T₃ and circulating FT₃ and FT₄ concentrations are shown. Central T₃ rises with increasing G_{D2} activity, thereby suppressing TSH and, as a consequence, circulating FT₃ and FT₄ levels decline. G_{D1}, maximum activity of type I deiodinase; K_{M1}, dissociation constant of 5'-deiodinase I; G_{D2}, maximum activity of type II deiodinase; K_{M2}, dissociation constant of 5'-deiodinase II; G_T, secretory capacity of thyroid gland; D_T, damping constant (EC₅₀) of TSH at the thyroid gland; G_H, secretory capacity of the pituitary; D_H, damping constant (EC₅₀) of TRH at the pituitary; S_S, brake constant of TSH ultrashort feedback; D_S, EC₅₀ for TSH at the pituitary; GR, maximum gain of TRβ receptors; D_R, EC₅₀ for central T₃; beta_S, clearance exponent for TSH; beta_{S2}, clearance exponent for central TSH; beta_T, clearance exponent for T₄; beta₃₁, clearance exponent for T_{3P}; beta₃₂, clearance exponent for central T₃; L_S, brake constant (adopted from Dietrich *et al.* (15, 17)). Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-12-0819>.

hypothyroid patient, the normalisation of T₃ production for maintenance of the metabolic processes should be considered to be equally as relevant as the control of the mechanisms of T₄-TSH feedback that currently dominate functional diagnostic procedures. The two cannot be regarded as equivalent owing to their treatment-related disparity of response. This challenges the role of TSH as an exclusive standard in assessing dose adequacy in thyroid hormone replacement and invites further study.

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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