

# Replacement therapy with levothyroxine plus triiodothyronine (bioavailable molar ratio 14 : 1) is not superior to thyroxine alone to improve well-being and cognitive performance in hypothyroidism

W. Siegmund\*, K. Spiekert†, A. I. Weike‡, T. Giessmann\*, C. Modess\*, T. Dabers\*, G. Kirsch§, E. Sanger¶, G. Engel¶, A. O. Hamm‡, M. Nauck\*\* and W. Meng†

\*Department of Clinical Pharmacology of the Peter Holtz Research Centre of Pharmacology and Experimental Therapeutics, †Department of Internal Medicine, ‡Department of Psychology, §Department of Nuclear Medicine, ¶Hospital Pharmacy and \*\*Department of Clinical Chemistry, University of Greifswald, Greifswald, Germany

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## Summary

**OBJECTIVES** There is evidence from recent controlled clinical studies that replacement therapy of hypothyroidism with T4 in combination with a small amount of T3 may improve the well-being of the patients. As the issue is still the subject of controversial discussion, our study was assigned to confirm the superiority of a physiological combination of thyroid hormones (absorbed molar ratio 14 : 1) over T4 alone with regard to mood states and cognitive functioning.

**DESIGN AND PATIENTS** After a run-in period with the T4 study medication for 4 weeks, a controlled, randomized, double-blind, two-period (each 12 weeks), cross-over study without washout between the treatment periods was performed in 23 hypothyroid patients (three males, 20 females, age 23–69 years, 21 subjects after surgery/radioiodine, two with autoimmune thyroiditis) to compare the effects of the previous individual T4 dose (100–175 µg) with a treatment in which 5% of the respective T4 dose was substituted by T3.

**MEASUREMENTS** Standard hormonal characteristics and standardized psychological tests to quantify mood

and cognitive performance were measured after the run-in period and at the end of each treatment period. In 12 subjects, the concentration–time profiles of fT3 and fT4 were compared after the last administration of the respective study medication. TSH, fT3 and fT4 were measured with immunological assays.

**CLINICAL RESULTS** Replacement therapy with T4 and T4/T3 was not different in all steady-state hormonal, metabolic and cardiovascular characteristics except for TSH, which was more suppressed after T4/T3. The efficacy of replacement therapy with the T4/T3 combination was not different from the T4 monotherapy with regard to all psychological test scores describing mood and cognitive functioning of the patients. Mood was even significantly impaired by the T4/T3 combination in eight subjects, with TSH < 0.02 mU/l, compared to patients with normal TSH (Beck Depression Inventory: 8.25 ± 5.01 vs. 4.07 ± 5.60,  $P = 0.026$ ).

**PHARMACOKINETIC RESULTS** The area under the concentration–time curve ( $AUC_{0-8h}$ ) of fT3 was significantly higher after T4/T3 compared to the T4 monotherapy (42.8 ± 9.03 pmol × h/l vs. 36.3 ± 8.50 pmol × h/l,  $P < 0.05$ ) and was significantly correlated to serum TSH ( $r_s = -0.609$ ,  $P < 0.05$ ). After T4/T3, patients with a history of Graves' disease or autoimmune thyroiditis had significantly higher serum trough levels of fT3 whereas the fT4 concentrations were significantly lower in patients with a nonautoimmune background. **CONCLUSION** Replacement therapy of hypothyroidism with T4 plus T3 does not improve mood and cognitive performance compared to the standard T4 monotherapy. There is even a higher risk of signs of subclinical hyperthyroidism associated with impaired well-being of the patients, which is clearly caused by significant fluctuations in the steady-state fT3 serum concentrations.

Correspondence: Werner Siegmund, Department of Clinical Pharmacology, Ernst Moritz Arndt University, Friedrich Loefflerstr. 23d, D-17487 Greifswald, Germany. Tel.: + 49 3834865632; Fax: + 49 3834865631; E-mail: siegmuw@mail.uni-greifswald.de

Levothyroxine (T4) is widely accepted in replacement therapy of patients with hypothyroidism (Wiersinga, 2001). However, administration of oral doses equivalent to the physiological daily production rate of T4 clearly does not fully mimic the endogenous production of thyroid hormones to supply all organs and

tissues with adequate amounts of T4 and of the more potent 3,5,3'-triiodothyronine (T3), 80% of which is formed by deiodination of T4. Not all tissues that need thyroid hormones are equally able to convert T4 to T3 (Visser *et al.*, 1982). Recent data in thyroidectomized rats have shown that replacement therapy with T4 alone normalizes tissue T3 levels only in supraphysiological doses except in the brain cortex, cerebellum and brown fatty tissue whereas replacement therapy with a physiological amount of T4 plus T3 maintains physiological T3 levels in all tissues (Escobar-Morreale *et al.*, 1995, 1996). There are also differences in susceptibility of the pituitary gland and peripheral tissues, for example suppression of TSH to normal values does not indicate adequate substitution of the periphery (Meier *et al.*, 1998; Tigas *et al.*, 2000).

Clinical studies have shown that patients on T4 replacement, even with normal TSH levels, complain of persistent lethargy and related symptoms. Psychological well-being occurs often just at T4 doses higher than necessary to normalize the plasma levels of TSH (Carr *et al.*, 1988; Saravanan *et al.*, 2002). There is evidence from recent controlled clinical studies suggesting that substitution of T3 for a small portion of the T4 replacement dose, which does not change TSH, leads to a significantly better quality of life for most patients with hypothyroidism (Bunevicius *et al.*, 1999; Bunevicius & Prange, 2000). However, this issue is still under controversial discussion because of several theoretical and methodological objections. Therefore, the present randomized, double-blind, cross-over study in hypothyroid patients on replacement with 100–175 µg T4 was assigned to confirm the superiority of replacement therapy with a combination of T4/T3 over the monotherapy with T4 alone. In the combination, 5% of the T4 replacement dose was substituted by T3 to achieve the bioavailable molar T4/T3 ratio of 14 : 1, which is equivalent to the mean endogenous production rate of 101 µg T4 and 6 µg T3 in healthy subjects (Pilo *et al.*, 1990).

## Materials and methods

### Subjects

Twenty-six patients with hypothyroidism (21 women, five men, age 23–69 years, body weight 58–120 kg) who attended our outpatient Department of Endocrinology were enrolled into the study after they had given their written informed consent. A sample size of 24 subjects has been calculated to be sufficient to confirm a decrease in the Global Mood Score by 27% as in the study of Bunevicius *et al.* (1999), assuming a significance level of  $P < 0.05$  and a power of 80% of a nonparametric Wilcoxon test for paired samples (n-Query Advisor 3.0, Statistical Solutions Ltd, Cork, Ireland). The subjects were on stable long-term replacement therapy with 100 µg (five subjects), 125 µg (12 subjects), 150 µg (eight subjects) or 175 µg (one subject) of T4

because of autoimmune thyroiditis ( $n = 2$ ), surgery or radioiodine therapy ( $n = 24$ ), among them six subjects with Graves' disease and one with hypertrophic autoimmune thyroiditis. The subjects were ascertained to be of good health by means of history, physical examination, routine clinical chemical and haematological screening and assessment of a 12-lead electrocardiogram. The patients were monitored by measurement of serum levels of free T4 (fT4), free T3 (fT3), TSH and SHBG. All subjects were negative for hepatitis B virus antibody, human immunodeficiency virus (HIV) and drugs. Nine patients were smokers. Those who consumed more than 40 g alcohol per day were not included. Eleven subjects were hypertensive and took  $\beta$ -adrenoreceptor blocking drugs, ACE-inhibitors and diuretics. Six patients suffered from allergies, one had type 2 diabetes mellitus and three had migraine attacks. Concomitant medication that may have interfered with mood ratings was not allowed, e.g. neuroleptics, antidepressants, benzodiazepines or sedatives. Therapeutic required co-medication was not changed in kind and dose during the entire study. The study protocol was approved by the local ethics committee.

### Study protocol

The patients were initially subjected to a run-in phase of 4 weeks to replace the former commercial T4 medication with identical doses of T4 in capsules that were manufactured for the study by our Hospital Pharmacy. After adaptation to the potential minor galenic influence of the study medication, the baseline hormonal, metabolic and psychological characteristics were obtained. The patients were then randomly allocated to the respective treatment sequence of the cross-over study. After 6 weeks, the treatment was visited to control the hormonal and metabolic parameters and to assess drug safety. After 12 weeks, the patients were admitted to our department to obtain basic hormonal and metabolic parameters, to perform a second psychological examination and, in 12 subjects, to measure serum concentration–time profiles of fT4 and fT3 after final administration of the study medication.

The second 3-month treatment period with the alternative study medication was performed in an identical manner. Thereafter, the medication was changed to the former replacement therapy with marketed T4, the efficacy and safety of which was controlled during the poststudy examination within the next 4 weeks and after a further 6 weeks and thereafter in standard intervals by our outpatient Department of Endocrinology.

### Study medication

The study medication in hard capsules was manufactured by the Hospital Pharmacy using the capsule-filling machine MS6N (Multigel, Florence, Italy). The T4 study medication (Reference) was prepared in steps of 25 µg, i.e. 100, 125, 150 and 175 µg. The T4/T3 medication (Test) contained 95% of the respective T4

mass and 5% of T3. This composition is equivalent to a bioavailable molar mixture of 14 : 1 under the precondition that T3 is completely absorbed and the bioavailability of T4 is about 90%. The T4 and T3 granulates were obtained by grinding L-thyroxine Henning® and Thybon® tablets (Henning-Berlin, Berlin, Germany) and mixing with adequate amounts of filling mass consisting of mannitol and silicium dioxide.

### Psychological assessment

Mood states were assessed using the Beck Depression Inventory (BDI; Beck *et al.* 1995), which measures the severity of depressive symptoms, and the state portion of the Spielberger State-Trait-Anxiety Inventory (STAI; Laux *et al.*, 1981), which measures the severity of anxiety symptoms. The Symptom-Check-List-90 (SCL-90, Franke, 1995) was used to screen for general psychopathology. A special questionnaire was used to assess physical well-being (FAW; Frank 1991). Furthermore, the German version of the Profile of Mood Scales (EWL; Janke & Debus, 1978), in which participants rate the extent of which they have experienced a certain mood state on a five-point scale, and the standardized version of a semantic differential using a list of bipolar adjectives (von Zerssen, 1975) were selected to measure pure mood states.

Cognitive functioning was assessed using the Digit Span Test and the Digit Symbol Test from the Wechsler Adult Intelligence Scale (Wechsler, 1997) and the Visual Scanning Test d2 test of attention (Brickenkamp & Zillmer, 1998). In the first part of the Digit Span Test, the subject is instructed to repeat spoken numbers with increasing numbers of digits. In the second part of this test, the subject is required to repeat the spoken numbers in reversed order. In the Digit Symbol Test, a key is provided that pairs numbers (1–9) with nine symbols. Then, without the key, the subject is asked to recall which symbol matched each number. The number of correct pairs is counted as a measure of memory. In the d2 test, the subject has to mark each letter d with two lines (either above, below or one above and one below the letter d) in a stream of the letters d and p with either none, one or two lines above or below the letters or both. This test measures processing speed, rule compliance and quality of performance, which are suitable neuropsychological characteristics of visual attention.

### Evaluation of bioavailability

Concentration–time profiles of serum fT4 and fT3 were measured in a subgroup of 12 patients after the last administration of the study medication in the morning of the respective treatment period. After overnight fasting, the hard capsule was swallowed with 200 ml noncarbonated mineral water. Venous blood was collected via an indwelling forearm cannula before and then hourly up to 8 h after administration. Drinking of mineral water was allowed after 3 h, and a standard lunch was eaten after 4 h.

### Analytical methods

Serum TSH and fT4 were quantified with radioimmunoassays (Brahms, Berlin, Germany). The intra-assay coefficient of variation of the TSH assay was 2.7% at 1.06 mU/l and of the fT4 assay 2.4% at 23.59 pM. fT3 and SHBG were measured by immunometric assays on an Immulite 2000 analyser (DPC Biermann, Bad Nauheim, Germany). The intra-assay coefficient of variation of the fT3 assay at 4.7 pM was below 9% and of the SHBG assay at 3.9 mg/l was below 6%.

### Pharmacokinetic and statistical evaluation

All serum fT4, fT3 and TSH values were obtained at steady state immediately before administration of the last dose of the respective study medication (trough levels). Maximum ( $C_{max}$ ) and minimum ( $C_{min}$ ) serum concentration of fT4 and fT3 was taken from the concentration–time profiles of fT4 and fT3. The area under the serum concentration–time curve was calculated with the trapezoidal rule from 0 to 8 h ( $AUC_{0-8h}$ ). Arithmetic means and standard deviations (SD) are given. Data sets were evaluated with the nonparametric Mann–Whitney *U*-test, Wilcoxon's signed rank test or with Spearman's rank correlation, as appropriate.

### Results

Twenty-three patients passed the clinical study according to the protocol. One subject was withdrawn for personal reasons, another because of surgical treatment of a disc prolapse, and a third subject because of atrial fibrillation with absolute arrhythmia in association with TSH suppression below zero after treatment with the T4/T3 combination. The per protocol samples were homogeneous and comparable with respect to the basic hormonal, metabolic and cardiovascular characteristics. Treatment with the T4/T3 combination caused significantly lower TSH values independent of whether the combination was given before or after the monotherapy with T4. Six weeks after the beginning of the treatments, the TSH effect was not fully expressed. The serum concentrations of fT4 and fT3 remained stable during the study. Three months after administration of the respective study medication, there were no significant differences in all hormonal, metabolic and cardiovascular parameters except for TSH (Table 1). TSH was suppressed more strongly by the T4/T3 combination than by the T4 monotherapy. In eight subjects after T4/T3 compared to two patients after T4, TSH was not detectable at all ( $< 0.02$  mU/l).

The pharmacokinetic evaluation of the study medication on the respective last treatment days showed markedly higher serum levels of fT3 for several hours after administration of the T4/T3 combination (Fig. 1). After treatment with T4/T3,  $AUC_{0-8h}$  and  $C_{max}$  of fT4 were significantly lower and of fT3 significantly

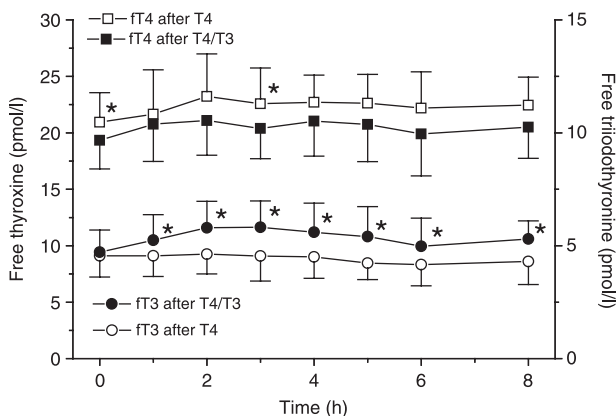
**Table 1** Hormonal, metabolic and cardiovascular characteristics of 23 patients with hypothyroidism after the run-in period with T4 (baseline) and after cross-over replacement therapy with T4 and T4/T3 for 3 months

	Baseline	T4	T4/T3	Normal range
TSH (mU/l)	1.72 ± 1.23	1.5 ± 1.3	0.5 ± 0.6*	0.3–4.0
TSH = 0†	3	2	8	
Free T3 (pmol/l)	5.10 ± 1.48	4.53 ± 1.31	4.98 ± 1.60	3.4–7.1
Free T4 (pmol/l)	22.1 ± 3.37	20.9 ± 2.31	20.14 ± 2.62	10–25
Cholesterol (mmol/l)	5.93 ± 1.36	5.92 ± 1.20	5.61 ± 1.93	< 6.0
HDL-cholesterol (mmol/l)	1.36 ± 0.35	1.35 ± 0.61	1.32 ± 0.62	> 1.40
LDL-cholesterol (mmol/l)	3.88 ± 1.24	3.57 ± 1.44	3.55 ± 1.47	< 4.0
Triglycerides (mmol/l)	1.50 ± 0.68	1.47 ± 1.10	1.57 ± 1.17	< 1.9
SHBG (mg/l)	4.71 ± 3.07	3.80 ± 2.16	4.27 ± 2.88	F 1.68–10.7; M 1.21–6.64
Heart rate (min <sup>-1</sup> )	69.3 ± 12.5	66 ± 5.4	67 ± 6.1	
Systolic BP (mmHg)	144 ± 19.4	118 ± 9.9	125 ± 16.2	
Diastolic BP (mmHg)	87.2 ± 9.5	77 ± 4.4	80 ± 9.1	

Values are means ± SD. The statistical comparisons were performed with the Mann–Whitney test. \* $P < 0.05$  compared to T4.

†Number of patients with undetectable TSH (< 0.02 mU/l).

HDL, high density lipoprotein; LDL, low density lipoprotein; BP, blood pressure; F, females, M, males.



**Fig. 1** Serum concentration–time profiles of fT4 and fT3 at steady state after cross-over replacement therapy of 12 patients with hypothyroidism with T4 and the combination T4/T3. Values are means ± SD. \* $P < 0.05$ , Mann–Whitney  $U$ -test.

higher than after T4 alone (Table 2). Interestingly, the serum levels of TSH were significantly correlated with the  $AUC_{0-8h}$  of fT3 after T4/T3 ( $r_s = -0.609$ ,  $P = 0.036$ ) but not after T4 (Fig. 2).

The pharmacokinetic differences of the T4 and T4/T3 study medication, respectively, were not associated with any significant differences in mood and cognitive functioning of our patients (Table 3). A small increase in cognitive performance as a result of the practice indicated that no ceiling effect occurred. This increase, however, was not related to the study medication (data are not shown). Interestingly, in the subgroup of patients with TSH < 0.02 mU/l, significantly more depressive symptoms after treatment with T4/T3 were reported than in patients with normal TSH (Beck Depression Inventory: 8.25 ± 5.01 vs. 4.07 ± 5.60,

**Table 2** Area under the curve (AUC), maximum ( $C_{max}$ ) and minimum ( $C_{min}$ ) serum concentrations of fT4 and fT3 at steady-state after cross-over replacement therapy of 12 patients with T4 and T4/T3, respectively

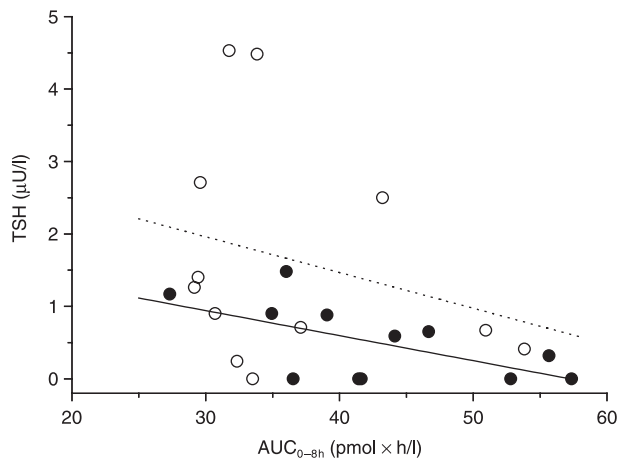
	T4	T4/T3
<b>fT4</b>		
$AUC_{0-8h}$ (pmol × h/l)	180 ± 21.1	163 ± 28.1*
$C_{max}$ (pmol/l)	24.3 ± 2.92	22.3 ± 3.23*
$C_{min}$ (pmol/l)	20.1 ± 2.57	18.5 ± 2.80*
<b>fT3</b>		
$AUC_{0-8h}$ (pmol × h/l)	36.3 ± 8.50	42.8 ± 9.03*
$C_{max}$ (pmol/l)	5.36 ± 1.18	6.47 ± 1.13*
$C_{min}$ (pmol/l)	3.77 ± 0.99	4.20 ± 1.52*

Values are geometric means ± SD.

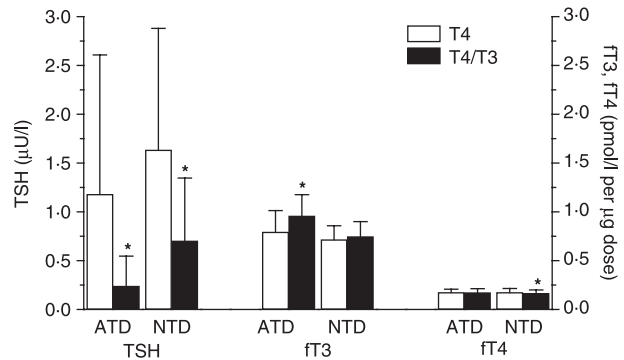
\* $P < 0.05$ , Mann–Whitney  $U$ -test.

$P = 0.023$ ). There was also a tendency towards increased general psychopathology indicated by the SCL-90 questionnaire (total positive symptoms 30.4 ± 13.2 vs. 19.1 ± 18.2;  $P = 0.081$ ).

A subgroup analysis according to the pathogenic background of the hypothyroidism is given in Fig. 3 (the fT3 and fT4 values are dose corrected). Patients with an autoimmune background (six subjects with Graves' diseases, three patients with autoimmune thyroiditis) had significantly higher fT3 serum levels after T4/T3 (compared to T4 monotherapy) whereas the fT4 concentrations were significantly lower in patients with a nonautoimmune background. TSH in these groups was not significantly different. In general, patients with Graves' disease or autoimmune thyroiditis reported more psychopathological symptoms and negative mood states. Furthermore, they performed worse in the



**Fig. 2** Correlation between the  $AUC_{0-8h}$  of fT3 and serum TSH after replacement therapy with T4 (open circles) and the combination T4/T3 (filled circles) for 12 weeks. The full line describes the correlation after administration of T4/T3 ( $r_s = -0.609$ ,  $P = 0.036$ ) and the dotted line after T4 ( $r_s = -0.336$ , not significant).



**Fig. 3** Serum levels of TSH and of dose-corrected fT3 and fT4 in nine hypothyroid patients with previous autoimmune forms (ATD) and 14 patients with nonimmune forms (NTD) of thyroid disease after replacement with T4 and the combination T4/T3. Values are means  $\pm$  SD. \* $P < 0.05$  compared to replacement with T4, Mann-Whitney  $U$ -test.

neuropsychological tests independent of the kind of replacement therapy. However, these differences did not reach the level of statistical significance because of the small sample sizes and low power of the statistical assessment, respectively (Table 4).

	Baseline	T4	T4/T3
<b>Mood scores</b>			
Beck Depression Inventory (BDI)	7.8 $\pm$ 5.9	6.9 $\pm$ 6.7	5.5 $\pm$ 5.7
State-Trait-Anxiety Inventory (STAI-GX)	36.4 $\pm$ 9.3	34.0 $\pm$ 6.9	33.8 $\pm$ 7.8
Symptom Check List (SCL-90)*	31.7 $\pm$ 17.0	23.9 $\pm$ 18.1	23.0 $\pm$ 17.2
Profile of Mood Scale (EWL 60 S)			
• arousal	10.0 $\pm$ 3.0	9.9 $\pm$ 2.9	10.3 $\pm$ 3.0
• fatigue	6.0 $\pm$ 2.3	6.2 $\pm$ 2.5	6.0 $\pm$ 2.9
• anger	5.2 $\pm$ 1.3	4.7 $\pm$ 1.2	4.9 $\pm$ 2.4
• anxiety	5.4 $\pm$ 1.5	5.0 $\pm$ 1.5	4.9 $\pm$ 1.4
• depression	5.8 $\pm$ 1.9	5.6 $\pm$ 2.2	5.4 $\pm$ 2.7
Global mood score (Bf-S)	15.13 $\pm$ 11.9	10.4 $\pm$ 9.9	12.2 $\pm$ 13.7
Physical well-being (FAW)			
• contentment	19.0 $\pm$ 5.6	19.4 $\pm$ 4.8	19.3 $\pm$ 6.4
• vitality	15.4 $\pm$ 6.4	15.2 $\pm$ 7.2	16.5 $\pm$ 6.6
• pleasure	16.4 $\pm$ 7.8	15.8 $\pm$ 8.6	16.6 $\pm$ 8.6
• freshness	26.6 $\pm$ 5.6	27.5 $\pm$ 5.7	27.2 $\pm$ 5.3
<b>Cognitive performance</b>			
Memory (Digit Symbol Test)	50.1 $\pm$ 11.2	53.2 $\pm$ 12.1	53.0 $\pm$ 11.9
Working memory (Digit Span Test)			
• recall of digits	11.3 $\pm$ 2.2	12.1 $\pm$ 2.4	12.4 $\pm$ 2.8
• forward recall of digits	6.1 $\pm$ 1.5	6.7 $\pm$ 1.3	6.9 $\pm$ 1.6
• backward recall of digits	5.2 $\pm$ 1.1	5.4 $\pm$ 1.7	5.6 $\pm$ 1.8
Visual attention (Visual Scanning Test d2)†			
• total	54.0 $\pm$ 28.5	60.8 $\pm$ 28.0	65.5 $\pm$ 28.7
• total correct	59.3 $\pm$ 31.3	75.3 $\pm$ 27.8	72.1 $\pm$ 26.7

\*Number of symptoms (positive symptoms total, PST). †Scores are converted to normative percentiles.

**Table 3** Psychological characteristics of 23 patients with hypothyroidism after the run-in period with T4 (baseline) and after cross-over replacement therapy with T4 and T4/T3 for 3 months

**Table 4** Selected psychological characteristics obtained in nine hypothyroid patients with autoimmune thyroid diseases (ATD) and in 14 patients with nonautoimmune thyroid diseases (NTD) in history after replacement therapy with T4 and T4/T3, respectively

	ATD		NTD	
	T4	T4/T3	T4	T4/T3
<b>Mood scores</b>				
Beck Depression Inventory (BDI)	8.2 ± 6.6	8.3 ± 7.1	6.1 ± 6.9	3.7 ± 3.8
State-Trait-Anxiety Inventory (STAI-GX)	35.9 ± 7.7	36.7 ± 9.1	32.7 ± 6.3	32.0 ± 6.6
Symptom Check List (SCL-90)*	29.2 ± 20.9	28.9 ± 21.7	20.4 ± 15.9	19.3 ± 13.1
Global mood score (Bf-S)	12.9 ± 8.6	14.4 ± 17.4	8.8 ± 10.6	10.8 ± 11.3
<b>Cognitive performance</b>				
Memory (Digit Symbol Test)	49.7 ± 12.6	49.2 ± 13.9	55.4 ± 11.6	55.5 ± 10.3
Working memory (Digit Span Test)				
• recall of digits	12.4 ± 3.0	12.7 ± 3.9	11.9 ± 2.1	12.3 ± 1.9
Visual attention (Visual Scanning Test d2)†				
• total	53.9 ± 25.6	55.2 ± 31.8	65.3 ± 29.5	72.2 ± 25.6
• total correct	87.2 ± 12.0	76.6 ± 24.5	67.6 ± 32.4	69.3 ± 28.5

\*Number of symptoms (positive symptoms total, PST). †Scores are converted to normative percentiles.

## Discussion

Monotherapy of hypothyroidism with levothyroxine has been undisputed since the launch of the synthetic drug on the market and the discovery of the T4 conversion, and after TSH monitoring was introduced to adapt the T4 dose to individual needs (Wiersinga, 2001). However, there is ample evidence from experimental and clinical studies that oral thyroxine alone does not fully mimic the physiological disposition and regulation of the endogenous thyroid hormones. First, the human thyroid gland secretes on average 101 µg T4 and 6 µg T3, which accounts for about 25% of the total daily production (Pilo *et al.*, 1990). Second, the fT3 serum levels in patients on T4 replacement with normal serum fT4 and normal TSH levels account for only 80% of those in healthy subjects (Larsen & Ingbar, 1992). Third, a subset of hypothyroid patients complain of fatigue, lack of energy, discrete cognitive disturbances and depressive mood despite apparently adequate T4 replacement (Saravanan *et al.*, 2002). Well-being often occurs only with doses 50 µg above those necessary to normalize TSH (Carr *et al.*, 1988; Meier *et al.*, 1998), a situation that may result in adverse effects such as bone demineralization and atrial fibrillation (Wiersinga, 2001).

A Lithuanian group recently presented data from a controlled clinical study suggesting that the combination of T4 with a small proportion of T3 is superior to T4 alone in improving the well-being of the patients (Bunevicius *et al.*, 1999). However, the study is subject to controversial discussion because of too short treatment periods, variable and high T3 proportions (absorbed molar ratio, T4 : T3 = 3–15 : 1) and the inclusion of patients with thyroid carcinoma who require higher (suppressive) doses than

other hypothyroid patient. Furthermore, the psychological findings after high T3 proportions (3–6 : 1) were not significantly different from the results with smaller T3 proportions (9–15 : 1), and depressed patients benefited from the T4/T3 combination no more than other patients. Re-evaluation of the data without depressive patients revealed the same results (Bunevicius & Prange, 2003). In patients with previous Graves' disease, however, the same investigators could not verify better well-being and cognition after substitution of 50 µg T4 by 10 µg T3 in a study with the same questionable design (Bunevicius *et al.*, 2002).

Our patients were treated with capsules containing a T4/T3 mixture that delivers a bioavailable molar T4 : T3 ratio of 14 : 1. The duration of the cross-over treatment periods was 12 weeks instead of 5 weeks in the studies of Bunevicius *et al.* (1999, 2002). The length of our treatment periods was sufficient to establish a new hormonal steady state after dose adaptations (Helfland & Capro, 1990).

Substitution of 5% of the T4 dose by T3 was without significant influence on the serum trough concentrations of fT4 and fT3 compared to treatment with T4 alone. Nevertheless, TSH was significantly suppressed by the combination; in eight subjects even below the detection limit. In our opinion, this phenomenon results from the different concentration–time profiles of T3 and T4 during the administration intervals at steady state. Administration of the T4/T3 combination leads to distinct serum concentration maxima of fT3 about 2–3 h after the morning administration, as shown in Fig. 1. By contrast, fT3 converts from the T4 study medication very slowly and therefore maintains nearly constant serum levels during the entire administration interval. Because of the much longer half-life of T4, scarcely any

fluctuations of fT4 occur. Therefore, we believe that the fT3 concentration peak (but not the trough level) is the decisive stimulus to suppress TSH. The values the AUC of fT3 after treatment with the T4/T3 combination were negatively related to TSH activity, as shown in Fig. 2.

Despite higher concentrations peaks of fT3 and the more stringent suppression of TSH, replacement therapy with the T4/T3 combination was not superior in efficacy on mood and cognitive performance of our patients. Adequate tissue concentrations of T3 in the brain are obviously maintained over a wide range of fT4 and fT3 in the blood, as was observed in an experimental study in thyroidectomized rats (Escobar-Morreale *et al.*, 1995). Reviewing the clinical data from the literature results in similar conclusions. The patients in the first study of Bunevicius *et al.* (1999) with beneficial results were treated with  $175 \pm 53 \mu\text{g}$  T4 vs.  $125 \mu\text{g}$  T4 plus  $12.5 \mu\text{g}$  T3 (mean molar absorbed T4/T3 ratio 8 : 1), the second study without influence on mood (Bunevicius *et al.*, 2002) was performed with replacement doses of  $115 \pm 24 \mu\text{g}$  T4 vs.  $65 \pm 24 \mu\text{g}$  T4 plus  $10 \mu\text{g}$  T3 (mean T4/T3 proportion 5 : 1). Our patients received  $129 \pm 21 \mu\text{g}$  T4 vs.  $123 \pm 20 \mu\text{g}$  T4 plus  $6.5 \pm 1.0 \mu\text{g}$  T3 (T4/T3 ratio 14 : 1); that is patients who responded with a significantly improved quality of life (Bunevicius *et al.*, 1999) received an even lower proportion of T3 than the patients of the second study who did not benefit from the T3 substitution (Bunevicius *et al.*, 2002). The matter is puzzling as TSH in both studies was not influenced by the additional T3.

In our clinical study with a far lower proportion of T3 (14 : 1), however, TSH dropped significantly, in eight subjects even below the detection limit, despite normal fT4 and fT3 serum trough levels. This state of subclinical hyperthyroidism resulted in significantly impaired well-being of these patients and increased the risk of severe side-effects as observed in our study in one subject who was withdrawn because of atrial fibrillation and absolute arrhythmia.

Thyroid hormones regulate growth, development and metabolic functions through complex biological pathways including biosynthesis, cell-specific uptake and/or export, cellular conversion, as well as nuclear receptor interactions (Shi *et al.*, 2002). The fate of the thyroid hormones is under the control of physiological factors that might be disturbed by the pathogenetic process that has caused the hypothyroidism. Recent data have shown that the transmembranal transport of thyroid hormones during absorption and distribution is mediated by active transporter proteins of the OATP-family, by amino acid transporters, the MDR1 gene product P-glycoprotein, and/or other stereoselective, verapamil-sensitive carriers (Abe *et al.*, 2002; Pizzagalli *et al.*, 2002; Hagenbuch & Meier, 2003). There is evidence, at least for P-glycoprotein, that transporter proteins are upregulated in diseases with an inflammatory/immunological background such as systemic lupus erythematoses, rheumatoid arthritis or inflammatory bowel diseases (Diaz-Borjon *et al.*, 2000; Farrell *et al.*, 2000; Llorente *et al.*, 2000).

We have shown clearly that the dose-corrected serum fT3 after replacement with the T4/T3 combination was significantly higher in our subset of patients with immunogenic thyroid disease (Graves' disease, autoimmune thyroiditis). Significantly lower fT4 after the combination was measured in nonimmunogenic forms. In euthyroid patients affected by an autoimmune background, the tissue uptake of exogenous T3 might be restricted, in contrast to T4, which is more widely distributed. Further evaluation is needed to understand whether these particularities in thyroid hormone disposition are related to higher (although not significantly) mood scores in our patients with an autoimmune thyroid disease. However, there is evidence from euthyroid postnatal women with positive thyroid antibodies and from patients with Hashimoto encephalopathy that thyroid autoimmunity may be an independent risk factor for impairment of mood and cognitive function (Harris *et al.*, 2002; Kuijpers *et al.*, 2001; Chong *et al.*, 2003). Therefore, we believe that both the disposition and psychological effects of thyroid hormones are also influenced by the pathogenetic background that has caused hypothyroidism.

In conclusion, replacement therapy with a physiological combination of levothyroxine and triiodothyronine does not improve mood or cognitive functioning of patients with hypothyroidism compared to the traditional replacement therapy with levothyroxine alone. There is even a higher risk of signs of subclinical hyperthyroidism associated with impaired well-being of the patients, which is clearly caused by significant fluctuations in the steady-state serum concentrations of free triiodothyronine.

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