

## CLINICAL STUDY

# Pilot study on the assessment of the setpoint of the hypothalamus–pituitary–thyroid axis in healthy volunteers

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## Abstract

**Objective:** To determine the log-linear relationship between TSH and free thyroxine in healthy subjects, and the variation in baseline TSH/free thyroxine (FT<sub>4</sub>) combination in each individual.

**Subjects and methods:** Twenty-one healthy volunteers (nine males and 12 females; mean age 60 years, range 51–74) were randomized to receive at 2300 h with 2-week intervals a single dose of placebo, 125 µg T<sub>4</sub> and 250 µg T<sub>4</sub> (arm 1, *n* = 10), or placebo, 25 µg triiodothyronine (T<sub>3</sub>) and 50 µg T<sub>3</sub> (arm 2, *n* = 11). Blood samples were taken in the morning (0800–1100 h) before and following the administration of the drug for the assessment of TSH, FT<sub>4</sub> and T<sub>3</sub>.

**Results:** Intra- and inter-individual variation and the individuality index of the four baseline serum samples were respectively 21.6%, 41.9% and 0.52 for TSH; 9.9%, 16.5% and 0.60 for FT<sub>4</sub>; and 9.3%, 16.0% and 0.58 for T<sub>3</sub>. Substantial differences existed in the location of individual working points within the reference range. T<sub>4</sub> administration increased FT<sub>4</sub> (but not T<sub>3</sub>) and decreased TSH, resulting in a log-linear relationship ( $\log \text{TSH} = 1.50 - 0.059 \times \text{FT}_4$ , *P* < 0.05) for the whole group. T<sub>3</sub> administration increased T<sub>3</sub> and decreased TSH (but not FT<sub>4</sub>), resulting in a log-linear relationship ( $\log \text{TSH} = 0.790 - 0.245 \times \text{T}_3$ , *P* < 0.001) for the whole group. Log-linear relationships were not always significant when assessed for each subject separately.

**Conclusion:** Individuality indices of TSH, FT<sub>4</sub> and T<sub>3</sub> are all ≤ 0.6, thereby limiting the usefulness of the population-based reference values. Accurate assessment of individual setpoints of the HPT axis was not possible with the applied single doses of T<sub>4</sub> or T<sub>3</sub>, and will require either prolonged administration or higher single doses of thyroid hormone.

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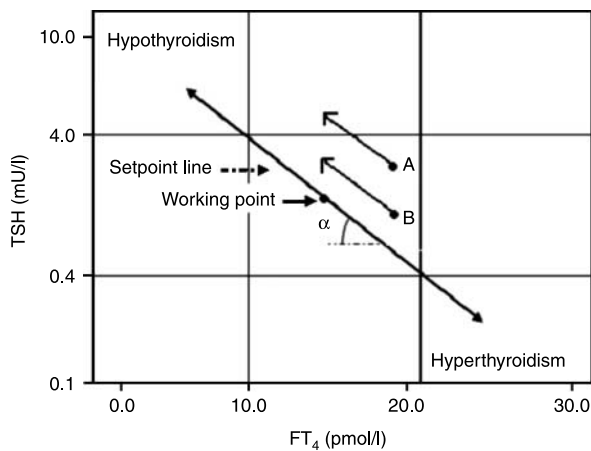
## Introduction

Many studies report a log-linear relationship between serum TSH and free thyroxine (FT<sub>4</sub>) over a wide concentration range (1, 2). This has been demonstrated in cross-sectional studies encompassing hyperthyroid, euthyroid and hypothyroid subjects (2), but also within individuals in longitudinal studies. When patients with primary hypothyroidism are treated with levoT<sub>4</sub> (L-T<sub>4</sub>), the interrelated changes in serum FT<sub>4</sub> and TSH move along a straight line (3). Conversely, when euthyroid subjects are treated with large doses of T<sub>4</sub> to render them hyperthyroid, serum FT<sub>4</sub> and TSH values also move along a straight line, but in the opposite direction (2). The straight line represents the setpoint of the hypothalamus–pituitary–thyroid (HPT) axis (Fig. 1). The slope of the line is an indicator of the sensitivity of the HPT axis for changes in ambient thyroid hormone concentrations. Indeed in patients with thyroid hormone resistance, the slope is flattened (4). Only small variations in the slope have been

observed between healthy individuals as evident from parallel straight lines representing individual setpoints (2), but studies on this subject are few. It is thus assumed that each individual has about the same slope for his/her log-linear TSH/FT<sub>4</sub> relation, but intercepts vary.

There is a considerable variation in serum TSH and thyroid hormone concentrations in healthy subjects, in whom the inter-individual variability is much greater than the intra-individual variability (5–12). It looks like every individual has his/her own peculiar combination of TSH and FT<sub>4</sub>, which is determined by genetic and environmental factors (13). We like to refer to this specific TSH/FT<sub>4</sub> combination as the working point on the straight line depicting the log-linear TSH/FT<sub>4</sub> relationship which represents the setpoint of the HPT axis.

Determination of the working point and the setpoint of the HPT axis is of much theoretical interest, but it may also have clinical relevance (14). For instance, the distance along the setpoint line to reach supranormal TSH values (that is the decrease in serum FT<sub>4</sub>) is much greater when



**Figure 1** Log-linear relationship between serum TSH and  $FT_4$  depicted as a straight line, representing the setpoint of the hypothalamus–pituitary–thyroid axis. The slope  $\alpha$  indicates the sensitivity of the HPT axis for changes in  $FT_4$ . The working point at the line represents the actual thyroid state. The position of the working point of healthy euthyroid subjects varies greatly within the normal reference range (TSH 0.4–4.0 mU/l and  $FT_4$  10–21 pmol/l). This may have clinical relevance, e.g. subclinical hypothyroidism (TSH > 4.0 mU/l but normal  $FT_4$ ) will be diagnosed at an earlier time in subject A than in subject B despite an equal decrease in serum  $FT_4$ .

the working point is located in the lower part of the reference area as compared to the upper part (Fig. 1). Up to now, very few studies have assessed individual setpoints of the HPT axis. The aim of the present study was to develop a feasible and practical method for assessing not only the individual working point but also the setpoint of the HPT axis in healthy subjects.

## Subjects and methods

### Subjects

We included healthy volunteers aged 50 years or older who were recruited by advertisements in local newspapers. Exclusion criteria were past or present thyroid

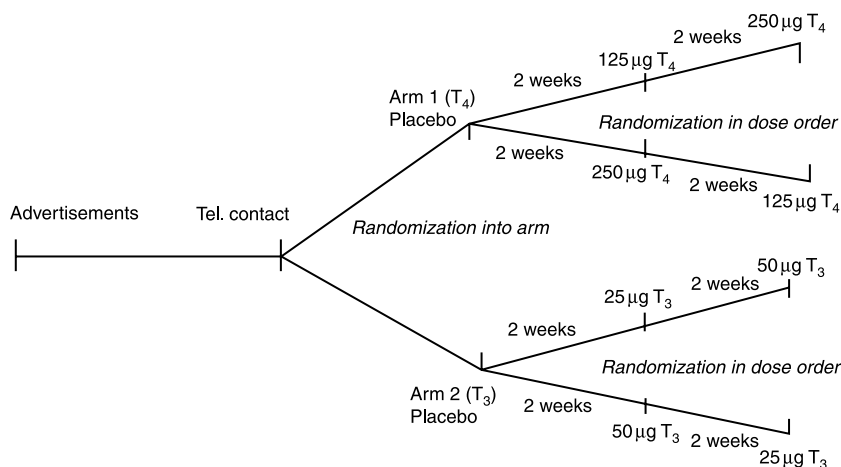
disease, thyroid function tests outside the reference range, active cardiovascular disease, severe illness and thyroid-influencing medication or oestrogens. All subjects lived in the iodine-replete region of Amsterdam, The Netherlands. An electrocardiogram (ECG) was performed before inclusion to exclude patients with cardiac arrhythmias. This study was carried out with the approval of the local ethics committee of the Academic Medical Centre of the University of Amsterdam. All subjects gave written consent before entering the study.

### Study design

Subjects were randomized in a 1:1 ratio to either the  $T_4$  or triiodothyronine ( $T_3$ ) intervention arm (Fig. 2). In each arm, oral medication was given three times: either placebo, 125  $\mu g$   $T_4$  and 250  $\mu g$   $T_4$  (arm 1), or placebo, 25  $\mu g$   $T_3$  and 50  $\mu g$   $T_3$  (arm 2). The subjects always started with placebo, followed as determined by a second randomization by the low dose or the high dose of  $T_4$  or  $T_3$ . In both arms, there was a minimum 2-week interval between the medication intakes (washout period). Subjects were instructed to take the medication at 2300 h. A blood sample was collected in the morning (0800–1100 h) of the day before and in the morning of the day after the medication was taken. Pulse rate, blood pressure and body weight were measured during each visit. Subjects were blinded with regard to the prescribed drugs and doses.

### Assays

Non-fasting venous blood samples were taken for thyroid hormone measurements and stored at  $-20^\circ C$  until assay. Serum  $T_3$  was measured with in-house RIA. Serum  $FT_4$  was assayed by time-resolved fluoroimmunoassay (Delfia, Turku, Finland). Serum TSH was determined with a fluoroimmunometric assay



**Figure 2** Study design.

(Delfia hTSH, Perkin–Elmer, Turku, Finland). All samples of one individual were measured in the same assay run. Reference values are 0.4–4.0 mU/l for TSH, 10–21 pmol/l for FT<sub>4</sub> and 1.30–2.70 nmol/l for T<sub>3</sub> (15).

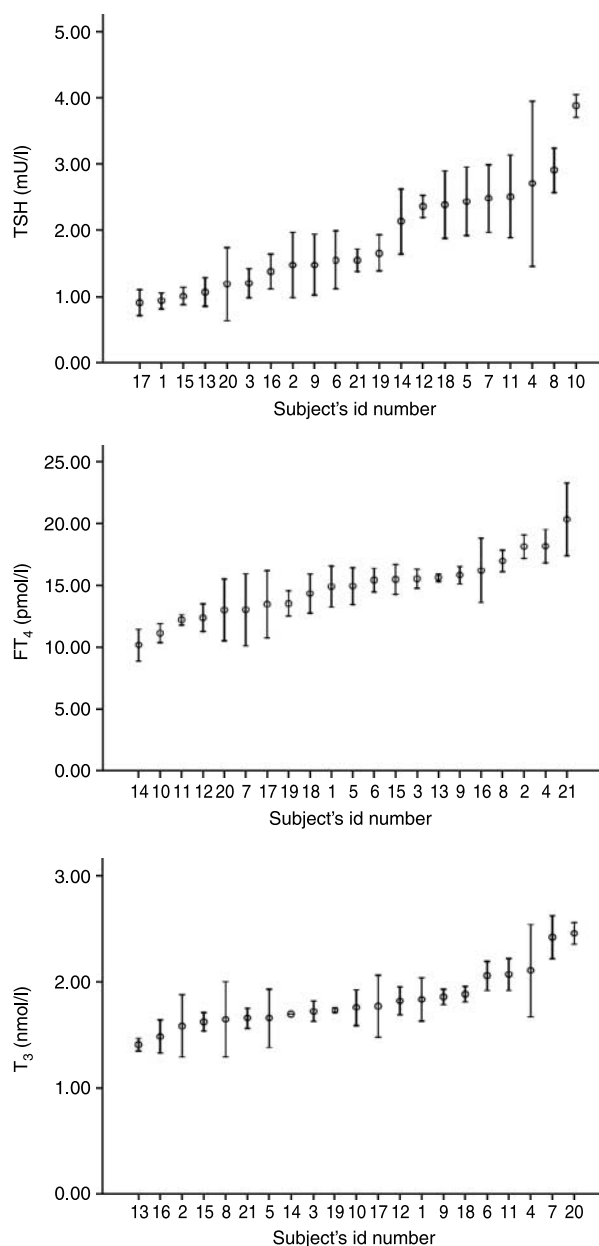
### Statistical analysis

Serum FT<sub>4</sub> values were normally distributed. TSH distribution was skewed, but it became normal after transformation (natural logarithm) evaluated by the Shapiro–Wilk test and normality plots. The intra-individual variance of baseline thyroid function tests (four measurements for each individual) was calculated by the variation for each individual, and the inter-individual variance was calculated by the variation of individual means. The individuality index was the ratio of intra- to inter-individual variation (16). Calculations using ANOVA gave similar variances. Differences in thyroid function level tests within each arm were evaluated with the paired *t*-test. The log-linear relationship between lnTSH and FT<sub>4</sub> or T<sub>3</sub> was tested by mixed model analysis, exponential model analysis and linear regression analysis for the whole group as a total and for each intervention arm separately. A *P* value of <0.05 was considered statistically significant. All statistical analyses were performed using the statistical package for social sciences version 15.0 (SPSS Inc., Chicago, IL, USA).

### Results

We recruited 21 healthy subjects (9 males and 12 females) with an average age of 60 years (range 51–74); all except one were Caucasians. The average body mass index was 25.4 (range 18.4–31.1). All volunteers completed the study without complaints or side effects.

Figure 3 depicts the mean values  $\pm$  S.E.M. of individual baseline thyroid function tests derived from four separate blood samples, three taken the morning before the administration of the study drug and one taken the morning after the administration of placebo the previous evening. The intra-individual variation coefficients (mean and range) are 21.6% (4.4–46.9) for TSH, 9.9% (1.9–22.4) for FT<sub>4</sub>, and 9.3% (0.0–22.1) for T<sub>3</sub>. Taking the mean value of each individual, the mean value  $\pm$  S.D. of the whole group of 21 healthy subjects are  $1.86 \pm 0.78$  mU/l for TSH,  $14.83 \pm 2.45$  pmol/l for FT<sub>4</sub> and  $1.81 \pm 0.29$  nmol/l for T<sub>3</sub>. The mean group intra- and inter-individual variation is listed in Table 1. The within-individual differences of all three thyroid function tests are lower than the between-individual differences, resulting in an intra-individual to inter-individual variation coefficient ratio  $\leq 0.60$ . The particular combination of each individual mean TSH and FT<sub>4</sub> or mean TSH and T<sub>3</sub> values reflects the actual working point of the HPT axis in that individual, which



**Figure 3** Mean values  $\pm$  S.E.M. of baseline serum TSH, FT<sub>4</sub> and T<sub>3</sub> concentrations in 21 healthy subjects, as calculated from four separate morning blood samples withdrawn over a 4-week period and ranked from lowest to highest mean value.

is graphically depicted in Fig. 4. Although all working points lie within the reference area, their location is widely scattered.

The results of thyroid function tests before and after the administration of T<sub>4</sub> (arm 1, *n* = 10) or T<sub>3</sub> (arm 2, *n* = 11) are shown in Fig. 5. In both intervention arms, the serum TSH, FT<sub>4</sub> and T<sub>3</sub> levels were similar in the three blood samples withdrawn in the morning before the study drugs were taken. This indicates that the 2-week washout interval was sufficient. In treatment

**Table 1** Individuality indices of serum TSH, FT<sub>4</sub> and T<sub>3</sub> in healthy subjects as reported in the literature.

|                                  | 2008 Present study | 2002 Ref. (5)     | 1997 Ref. (6) | 1993 Ref. (7) | 1990 Ref. (9) | 1986 Ref. (10) |
|----------------------------------|--------------------|-------------------|---------------|---------------|---------------|----------------|
| Study characteristics            |                    |                   |               |               |               |                |
| Number of subjects               | 21                 | 15                | 26            | 47            | 15            | 12             |
| Number of samples per subject    | 4                  | 12                | 12            | 5             | 7             | 7              |
| Sampling period, weeks           | 4                  | 52                | 52            | 2             | 1             | 5              |
| TSH variation                    |                    |                   |               |               |               |                |
| Intra-individual variation, %    | 21.6               | 22.4              | 29.3          | 29.0          | 19.3          | 16.2           |
| Inter-individual variation, %    | 41.9               | 43.9              | 48.4          | 80.5          | 19.7          | 31.7           |
| Individuality index <sup>a</sup> | 0.52               | 0.51              | 0.60          | 0.36          | 0.98          | 0.50           |
| FT <sub>4</sub> variation        |                    |                   |               |               |               |                |
| Intra-individual variation, %    | 9.9                | 10.7 <sup>b</sup> | 7.1           | 12.2          | —             | 9.5            |
| Inter-individual variation, %    | 16.5               | 20.3 <sup>b</sup> | 9.1           | 20.3          | —             | 12.3           |
| Individuality index <sup>a</sup> | 0.60               | 0.53              | 0.78          | 0.60          | —             | 0.78           |
| T <sub>3</sub> variation         |                    |                   |               |               |               |                |
| Intra-individual variation, %    | 9.3                | 12.0              | 9.4           | 11.4          | 8.7           | 10.4           |
| Inter-individual variation, %    | 16.0               | 20.4              | 18.5          | 21.5          | 18.0          | 20.4           |
| Individuality index <sup>a</sup> | 0.58               | 0.59              | 0.51          | 0.53          | 0.48          | 0.51           |

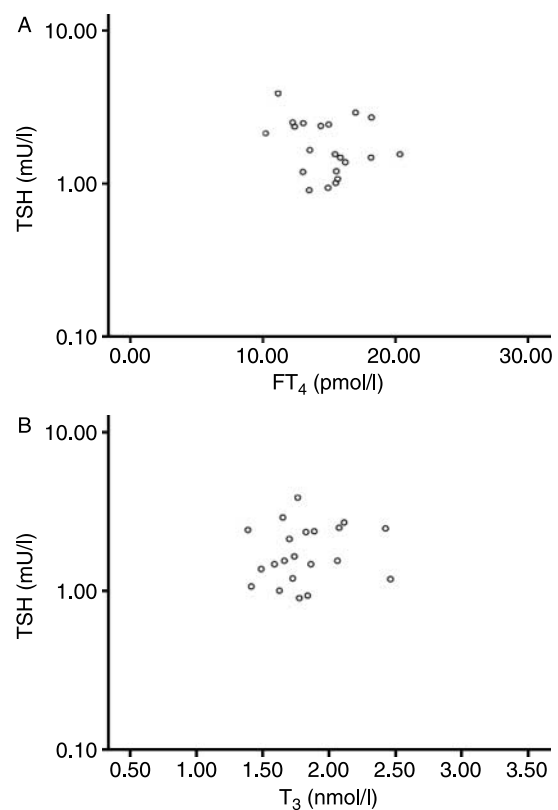
<sup>a</sup>Individuality index = ratio of intra- to inter-individual variation.<sup>b</sup>FT<sub>4</sub> index.

arm 1, serum FT<sub>4</sub> increased (significantly) and TSH decreased (not significantly) after T<sub>4</sub> administration, but T<sub>3</sub> remained unchanged (Fig. 5). There existed a significant log-linear relationship between TSH and FT<sub>4</sub> in this group as a whole ( $\log \text{TSH} = 1.50 - 0.059 \times \text{FT}_4$ ,  $P$  for slope  $< 0.05$ ; Fig. 6A), but the relationship was not always significant if evaluated in each individual separately. In treatment arm 2, serum T<sub>3</sub> increased and TSH decreased after T<sub>3</sub> administration, but FT<sub>4</sub> remained unchanged. A significant log-linear relationship between TSH and T<sub>3</sub> was observed in this group as a whole ( $\log \text{TSH} = 0.790 - 0.245 \times \text{T}_3$ ,  $P$  for slope  $< 0.001$ ; Fig. 6B), but not in every individual subject.

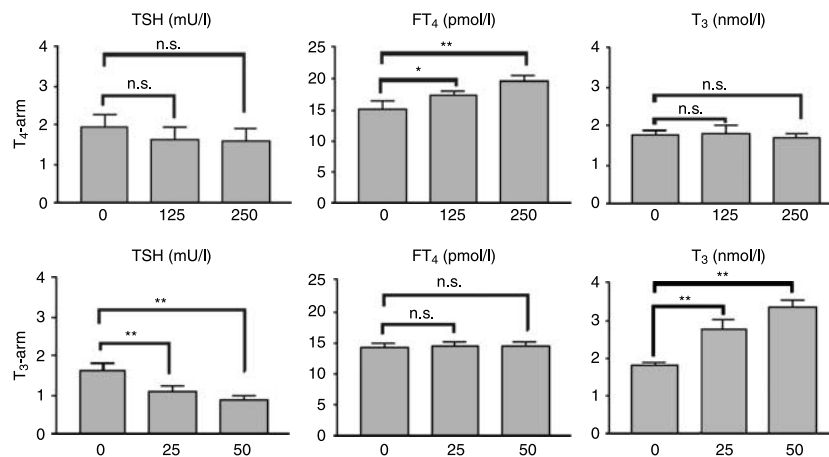
## Discussion

The first finding of our study is that the working point of the HPT axis in a particular individual can be determined reasonably well from four separate morning blood samples withdrawn over a 4-week period. The intra- and inter-individual variation coefficients and the individuality index of TSH, FT<sub>4</sub> and T<sub>3</sub> in our study are in close agreement with those observed in other studies in which 12 samples were collected over a 52-week period (Table 1). Only one study reports much higher individuality indices (9), which is likely explained by a very short sampling period of 1 week. The data further suggest a very limited effect of seasonal variation in thyroid function tests on the working point of the HPT axis (14). Estimation of an individual's working point becomes more precise by full standardization of the conditions under which blood samples are drawn. In this respect, the intra-individual variation observed in the present study could have been narrower by sampling at more precisely the same hour in the

morning, thereby avoiding the circadian variation (especially in TSH) occurring in the presently allowed sampling period of 3 h between 0800 and 1100 h.



**Figure 4** Individual TSH/FT<sub>4</sub> (panel A) and TSH/T<sub>3</sub> (panel B) combinations representing the actual working point of the HPT axis in 21 healthy subjects. Horizontal and vertical lines indicate clinical reference intervals of TSH and FT<sub>4</sub>/T<sub>3</sub> respectively.



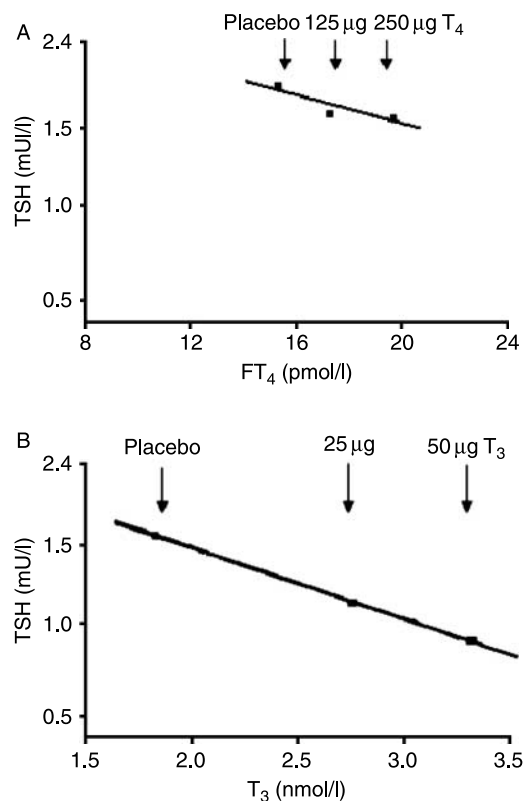
**Figure 5** The mean ( $\pm$  S.D.) TSH, FT<sub>4</sub> and T<sub>3</sub> concentrations in each intervention arm before and after oral supplementation of placebo, T<sub>4</sub> (125 and 250 μg) or T<sub>3</sub> (25 and 50 μg). \* $P < 0.05$ ; \*\* $P < 0.001$ ; <sup>S</sup>T<sub>4</sub>-arm represents the treatment arm 1 and T<sub>3</sub>-arm represents the treatment arm 2.

Our second finding is that substantial differences exist between individuals in the location of their working point of the HPT axis within the reference area (Fig. 4), limiting the usefulness of population-based reference ranges in the identification of disease in an individual as noted before (5). When the individuality index is  $< 0.6$ , the population-based reference range is an insensitive measure in the large majority of individuals; when the index is  $> 1.4$ , the reference range works as intended (16). The individuality indices of TSH, FT<sub>4</sub> and T<sub>3</sub> are mostly below 0.6 and below 1.0 in all the studies published so far (Table 1). Consequently, values within the population-based reference ranges do not necessarily indicate a normal thyroid function in that individual.

As a third finding, the present study suggests that the setpoint of the HPT axis can be determined from the TSH/FT<sub>4</sub> response to the separate administration of three single doses of oral T<sub>4</sub> (0, 125 and 250 μg respectively), at least at a group level. Assessment of the setpoint per individual was less reliable in the present setting because the individual log-linear relationship was not significant in all subjects.

Possible explanations for the failure to establish individual setpoints of the HPT axis in our study are i) differences in intestinal absorption of the administered L-T<sub>4</sub> between individuals, ii) too low doses of the administered L-T<sub>4</sub>, and iii) differences in particular genotypes affecting the ambient TSH and FT<sub>4</sub> levels between individuals. The latter possibility would indicate real differences in the setpoint of the HPT axis between our subjects. Indeed ethnic differences in ambient TSH values but not in FT<sub>4</sub> values suggest an effect of race (17, 18), and slightly lower TSH values have been observed in carriers of the Asp727Glu polymorphism in the TSH receptor gene occurring in about 20% of a Caucasian population (19). The low prevalence of this TSH receptor polymorphism does not

favour possibility iii) in our (mostly Caucasian) subjects. Possibility i) also is an unlikely explanation because the TSH/FT<sub>4</sub> relationship is determined by their inter-dependent serum concentrations, irrespective of the extent of intestinal T<sub>4</sub> absorption. Therefore, we consider a too low dose of administered T<sub>4</sub> as the most



**Figure 6** Log-linear relationship between TSH and FT<sub>4</sub>/T<sub>3</sub> in 10 healthy subjects treated with oral T<sub>4</sub> (panel A) and in 11 healthy subjects treated with oral T<sub>3</sub> (panel B).

likely explanation for our failure to determine the setpoint of the HPT axis in each individual separately. Supporting this view is the absence of a difference in TSH and  $T_4$  at individual values before and after receiving 125  $\mu\text{g}$   $T_4$ . Only within a group ( $T_4$ -arm), there was a significant increase in the measured  $FT_4$  not in the TSH and  $T_3$  (Fig. 5). The relatively small differences in TSH and  $FT_4$  values after ingestion of 125 or 250  $\mu\text{g}$   $T_4$  might be related to the increase of  $L-T_4$  metabolic clearance rate when the  $L-T_4$  dose exceeds 2.0  $\mu\text{g}/\text{kg}$  per day (3). We conclude from our pilot study that prolonged administration of  $L-T_4$  or higher single doses of  $T_4$  (e.g. 250 and 500  $\mu\text{g}$ ) are required to determine accurately one's individual setpoint of the HPT axis.

Nevertheless, the log-linear TSH/ $FT_4$  relationship was significant when the ten subjects in the  $T_4$  intervention arm were considered together:  $\log \text{TSH} = 1.50 - 0.059 \times FT_4$ ,  $P < 0.05$ . Extrapolating this relationship, TSH will be 32 mU/l at a  $FT_4$  of 0 pmol/l, and  $FT_4$  will be 59.3 pmol/l at a given TSH value of 0.01 mU/l. The data can be compared with those of Spencer *et al.* (2) who reported the following log-linear TSH/ $FT_4$  relationship in 505 stable ambulatory patients who were either hypothyroid, euthyroid or hyperthyroid:  $\log \text{TSH} = 2.56 - 0.022 \times FT_4$  index,  $r = -0.84$ ,  $P < 0.001$ . In this formula, TSH will be 360 mU/l at a  $FT_4$  index of 0, and  $FT_4$  index is 207 at a given TSH value of 0.01 mU/l. From clinical practice, it is evident that undetectable  $FT_4$  values are associated with much higher TSH values than 32 mU/l. Consequently, our study underestimates the slope of the setpoint of the HPT axis, which is most likely caused by too low doses of administered  $T_4$ .

We encountered similar problems in the  $T_3$  intervention arm. Although the log-linear relationship between TSH and  $T_3$  was significant for all 11 subjects together, this was not true for each individual. We selected the doses of 25 and 50  $\mu\text{g}$   $T_3$  in view of the biological potency ratio of 5:1 for  $T_4$  and  $T_3$  on a weight basis (20). It would be of much interest to see whether the sensitivity of the pituitary to suppress TSH is similar for equivalent doses of exogenous  $T_4$  and  $T_3$ . In the study of Fish *et al.* (3), suppression of pituitary TSH secretion was more clearly related to serum  $T_3$  than to serum  $T_4$ , whereas Silva *et al.* (21) and Larsen (22) proposed that  $T_4$  rather than  $T_3$  has a dominant role in regulating pituitary TSH secretion in view of active conversion of  $T_4$  into  $T_3$  within the pituitary accounting for about 50% of nuclear  $T_3$  in the pituitary. Burmeister *et al.* reported that the relationship between free  $T_3$  index and TSH in stable hypothyroid patients on  $L-T_4$  treatment is similar to that between TSH and  $FT_4$  index (23). The most appropriate study design in this respect would be the administration of various doses of  $T_4$  and  $T_3$  to the same subjects.

In summary, four separate blood samples taken over a 4-week period allow for a reasonable estimate of an

individual's working point at the HPT axis. Substantial differences between individuals in the location of their working point within the reference area limit the usefulness of the population-based reference ranges. Accurate assessment of the log-linear relationship between TSH and  $FT_4$  (the setpoint of the HPT axis) was not possible by the administration of single doses of  $T_4$  (125–250  $\mu\text{g}$ ) or  $T_3$  (25–50  $\mu\text{g}$ ), but it requires either prolonged administration or higher single doses of thyroid hormones.

## 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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