

## Intra-Individual Variation of Thyroxin, Triiodothyronine, and Thyrotropin in Treated Hypothyroid Patients: Implications for Monitoring Replacement Therapy

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We measured total thyroxin (TT<sub>4</sub>), free thyroxin (FT<sub>4</sub>), total triiodothyronine (TT<sub>3</sub>), free triiodothyronine (FT<sub>3</sub>), and thyrotropin (TSH) in serum sampled before and 1, 2, 4, 6, and 8 h after administration of prescribed doses of thyroxin to 12 patients with proven primary hypothyroidism. At 2, 4, and 6 h post-dose, the mean values for TT<sub>4</sub> and FT<sub>4</sub> and also that at 8 h for FT<sub>4</sub> significantly ( $P < 0.05$ ) exceeded the corresponding pre-dose values. No significant changes were found for TT<sub>3</sub>, FT<sub>3</sub>, or TSH. The mean intra-individual CVs over the study period were TT<sub>4</sub> 4.9%, FT<sub>4</sub> 5.7%, TT<sub>3</sub> 8.7%, FT<sub>3</sub> 8.7%, and TSH 20.2%. Individual subjects showed small but predictable changes in TT<sub>4</sub> and FT<sub>4</sub>. Changes in TT<sub>3</sub> and FT<sub>3</sub> were greater but random. Fluctuations in TSH were greatest, but in all subjects with detectable concentrations the variations were of similar magnitude. We conclude that strict adherence to timing of specimen collection in relation to dosage is probably unnecessary.

Treated hypothyroid patients receive a single oral daily dose of thyroxin to replace the normal day-long controlled pulsatile release of thyroxin, and of lesser amounts of triiodothyronine, from the thyroid gland. The best means of assessing the correct dose of thyroxin replacement for individual hypothyroid patients is still unsettled. Fraser et al. (1) have suggested that clinical assessment replace biochemical investigation, because none of the analytes commonly used to assess thyrometabolic function—namely, total thyroxin (TT<sub>4</sub>), free thyroxin (FT<sub>4</sub>), total triiodothyronine (TT<sub>3</sub>), free triiodothyronine (FT<sub>3</sub>), and thyrotropin (TSH)—can distinguish "euthyroid" treated patients from those receiving inadequate or excessive doses of thyroxin.<sup>3</sup> In contrast, in other studies, various combinations of assays of thyroid hormones and of TSH and TRF stimulation tests have been advocated as appropriate investigations (2-9). Indeed, one author (7) has categorically stated that, where there is a discrepancy between clinical and biochemical findings, the latter should have precedence over the former!

The analytes measured in patients being maintained on replacement therapy are those also used to establish the diagnosis of thyroid dysfunction; however, interpretation of the results may require use of different criteria. For patients on replacement therapy the analyses are performed for therapeutic drug monitoring, and constraints regarding the relative timing of dosing and sampling may be required if analyte concentrations are influenced by the elapsed time

between these events. As has been shown (10-15), TT<sub>4</sub> and FT<sub>4</sub> increase somewhat in response to an oral dose of thyroxin, TT<sub>3</sub> and FT<sub>3</sub> show little consistent change, and TSH decreases.

Whether it is necessary to relate the timing of sample collection to time of dosing with thyroxin will depend upon the magnitude and predictability of the variation of the concentration, in response to the dose, of the analyte chosen to monitor therapy. Analytes that show large and predictable post-dose changes will necessitate close adherence to a fixed sampling schedule, whereas those showing predictable but small changes or random fluctuation will not.

We designed the following study to identify which, if any, of the analytes used to monitor patients receiving replacement thyroxin would require fixed time intervals between dosing and sample collection for accurate assessment of the patients' response to treatment.

### Patients and Methods

We studied 12 patients (11 women, one man) with clinically and biochemically proven primary hypothyroidism who were receiving replacement therapy with thyroxin. All had been taking a constant dose of thyroxin for at least 10 weeks. No patient was clinically overtly hypo- or hyperthyroid, but no attempt was made to optimize the dose of thyroxin before the study. Table 1 gives details of the duration of hypothyroidism, its etiology, and the current dose of thyroxin. All patients gave informed consent for the study, which was approved by the hospital ethical committee. Patients reported to the hospital at 0830 hours, having refrained from taking the prescribed dose of thyroxin for that day. At 0900 hours, 10 mL of venous blood was collected from each seated patient—without anticoagulant, with minimal stasis, and by the same phlebotomist—for measurement of TT<sub>4</sub>, FT<sub>4</sub>, TT<sub>3</sub>, FT<sub>3</sub>, and TSH. This pre-dose specimen was collected approximately 24 h after the dose taken on the previous day. Each patient was then given the appropriate dose of thyroxin and five further blood specimens were collected 1, 2, 4, 6, and 8 h later, under the same

Table 1. Characteristics of the 12 Patients Studied

Age, y	Etiology of hypothyroidism	Thyroxin treatment	
		Duration, months	Current dose, µg/day
75	Primary autoimmune	5	50
65	Post <sup>131</sup> I therapy	11	150
32	Idiopathic	26	100
39	Primary autoimmune	6	100
50	Post <sup>131</sup> I therapy	3	100
43	Primary autoimmune	12	50
38	Primary autoimmune	7	100
41	Post <sup>131</sup> I therapy	4	100
56	Primary autoimmune	4	100
76	Primary autoimmune	25	150
32	Primary autoimmune	16	150
44	Primary autoimmune	16	150

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<sup>3</sup> Nonstandard abbreviations: TT<sub>4</sub>, total thyroxin; TT<sub>3</sub>, total triiodothyronine; FT<sub>4</sub>, free thyroxin; FT<sub>3</sub>, free triiodothyronine; TSH, thyrotropin; TRF, thyroliberin (thyrotropin-releasing factor); SD<sub>A</sub><sup>2</sup>, analytical variance; SD<sub>I</sub><sup>2</sup>, intra-individual variance; CV<sub>I</sub>, intra-individual coefficient of variation; SD<sub>(A+I)</sub><sup>2</sup>, total (analytical + intra-individual) variance; CV<sub>(A+I)</sub>, total (analytical + intra-individual) coefficient of variation.

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conditions as described above. All specimens were centrifuged at  $3000 \times g$ , and the serum was removed and stored in aliquots at  $-20^\circ\text{C}$  until assay.

For each analyte, all specimens from a single patient were assayed, in duplicate, in the same batch.  $\text{TT}_4$  and  $\text{TT}_3$  were measured with in-house radioimmunoassays, with use of

antisera from the Scottish Antibody Production Unit (Carluke, ML8 5ES, Scotland).  $\text{FT}_4$  was determined with the Gamma-Coat two-stage method (American Hospital Supplies, Compton, Berkshire, RG16 0QW, U.K.),  $\text{FT}_3$  with the Coat-A-Count  $\text{FT}_3$  analog assay (Diagnostic Products [UK] Ltd, Wallingford, Oxfordshire, OX10 9DA, U.K.). Because there is controversy regarding the accuracy of currently available commercial assay kits for free thyroid hormones (16), the results obtained should be taken to be relative to, rather than the absolute amounts of, free hormone. TSH was measured with the "RIAgnost" TSH assay (Hoechst (UK) Ltd, Hounslow, Middlesex, TW4 6JH, U.K.).

#### Statistical Analysis

For each analyte we calculated the mean values ( $\pm$  SE) for all 12 patients at each sampling time. Log transformation of individual TSH values was performed before calculation to convert the intra-individual data to a gaussian distribution. To identify significant changes in analyte concentration after administration of thyroxin we used Student's paired *t*-test to compare each post-dose value for an individual with the pre-dose value.

The analytical ( $\text{SD}_A^2$ ) and intra-individual ( $\text{SD}_I^2$ ) variances were calculated by analyses of variance (17-19) and the intra-individual coefficient of variation ( $\text{CV}_I$ ) was derived.

Because significant heterogeneity among intra-individual variances may be masked by the use of the average intra-individual variance, we calculated an index of heterogeneity (20). This ratio of the observed CV of a set of total intra-individual variances ( $\text{SD}_{A+I}^2$ ) to the theoretical CV,  $[2/(n-1)]^{1/2}$  where *n* is the number of observations per individual, allows assessment of the variability of the intra-individual variances of a group of individuals. If there was no heterogeneity of intra-individual variances, this ratio would be 1.00; the larger the ratio, the greater the heterogeneity. Heterogeneity is considered significant when the index value exceeds  $1.00 + 2 \text{SD}$  where  $\text{SD} = (2n)^{-1/2}$ . In this study, in which we measured a wide range of analyte concentrations, we substituted  $(\text{CV}_{A+I})^2$  for  $(\text{SD}_{A+I})^2$ .

#### Results and Discussion

Figure 1 shows the mean ( $\pm$  SE) for each analyte at each sampling time. Considered as a group, the subjects show a post-dose increase in  $\text{TT}_4$ ,  $\text{FT}_4$ , and  $\text{FT}_3$  with maximum values at 4 h; a decrease in TSH with the nadir at 2 h; and a slight downward trend in  $\text{TT}_3$  during the study period. By paired *t*-test the values at 2, 4, and 6 h after thyroxin administration are significantly higher ( $P < 0.05$ ) than the pre-dose value for either  $\text{TT}_4$  and  $\text{FT}_4$ , the 8-h value being significantly higher ( $P < 0.05$ ) only for  $\text{FT}_4$ . We saw no significant differences between pre- and post-dose values for  $\text{TT}_3$ ,  $\text{FT}_3$ , or TSH. These observations are in keeping with most previous studies (10-12, 14, 15), although one group (10) failed to observe a significant increase in  $\text{TT}_4$ , and another (15) found a significant increase in  $\text{TT}_3$  after oral administration of thyroxin.

Failure to demonstrate a significant change between any post-dose value and the corresponding pre-dose value may be either because: (a) little change has in fact occurred or (b) the pattern of the response varies greatly among patients. Conversely, a significant change may be demonstrated when either: (c) a small but consistent pattern of change occurs or (d) a few subjects show major changes and others show little or no change. To identify to which category each of the analytes— $\text{TT}_4$ ,  $\text{FT}_4$ ,  $\text{TT}_3$ ,  $\text{FT}_3$ , and TSH—belonged,

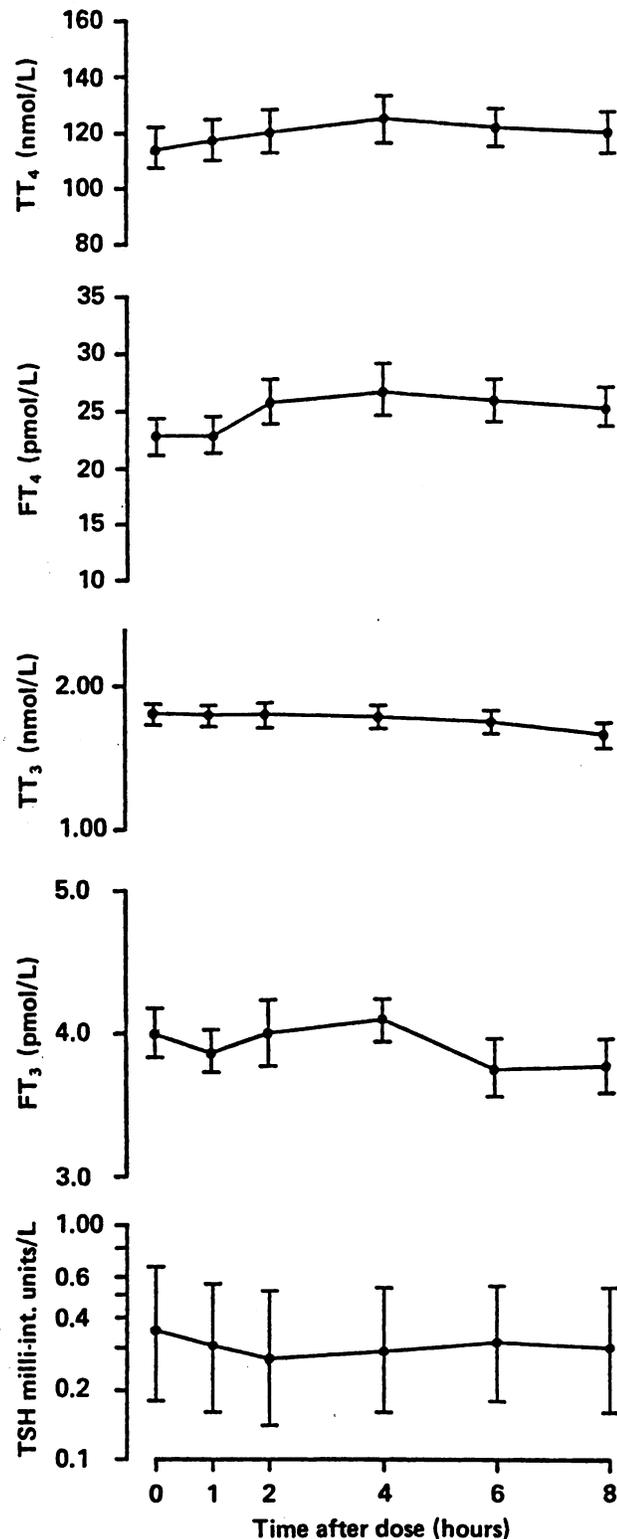


Fig. 1. Mean ( $\pm$  SE) values for  $\text{TT}_4$ ,  $\text{FT}_4$ ,  $\text{TT}_3$ ,  $\text{FT}_3$ , and TSH before and after the oral administration of thyroxin

**Table 2. Estimated Average Intra-Individual Variance (CV<sub>I</sub>) and Index of Heterogeneity of Thyroid Hormones in Hypothyroid Patients Maintained on Oral Thyroxin<sup>a</sup>**

	CV <sub>I</sub> , %		Index of heterogeneity <sup>b</sup>	
TT <sub>4</sub>	4.9	(5.1)	1.07	(1.31)
FT <sub>4</sub>	5.7	(9.5)	1.04	(1.21)
TT <sub>3</sub>	8.7	(10.4)	1.61	(2.03)
FT <sub>3</sub>	8.7	(7.9)	0.82	(1.06)
TSH	20.2	(16.2)	1.13	(1.09)

<sup>a</sup> Values in parentheses are the corresponding values for between-day intra-individual variation (19) and indices of heterogeneity in healthy subjects.

<sup>b</sup> Significant when the value exceeds 1 plus 2 SD, i.e., >1.58 for treated hypothyroid patients and >1.44 for healthy subjects.

we assessed the variations in their concentrations in serum during the study period. Analytical imprecision varies both for different assay procedures and across the concentration range for any one assay. To eliminate this variable component of the overall intra-subject variation, and to allow comparison of the biological variation of each analyte, we derived the intra-individual coefficient of variation (CV<sub>I</sub>) for each analyte for each subject. We calculated the overall CV<sub>I</sub> for each analyte and found for TT<sub>4</sub>, 4.9%; FT<sub>4</sub>, 5.7%; TT<sub>3</sub>, 8.7%; FT<sub>3</sub>, 8.7%; and TSH, 31%. Four subjects had TSH values at or below the detection limit of the assay (0.05 milli-int unit/L). Their results were omitted from the calculation of the overall CV<sub>I</sub> for TSH (Table 2). The intra-individual variation of both TT<sub>4</sub> and TT<sub>3</sub> are greater than has been reported (18) for healthy euthyroid subjects studied over 6 h, but for all analytes the intra-individual variations were similar to those previously reported (19) for healthy subjects studied over a period of weeks.

For patients maintained on oral thyroxin the concentrations of TT<sub>4</sub> or FT<sub>4</sub> in serum reflect the balance between absorption and clearance of the administered drug; both TT<sub>4</sub> and FT<sub>4</sub> increase significantly after administration of thyroxin yet have the lowest intra-individual variation. Calculation of the indices of heterogeneity (Table 2) for TT<sub>4</sub> and FT<sub>4</sub> shows no significant heterogeneity in their intra-individual variances. We therefore conclude that TT<sub>4</sub> and FT<sub>4</sub> display only small changes of a consistent and predictable pattern in patients maintained on oral thyroxin.

We detected no significant differences between pre- and post-thyroxin specimens for serum TT<sub>3</sub> or FT<sub>3</sub>. The intra-individual variations over the 8-h period were greater than for TT<sub>4</sub> and FT<sub>4</sub>. Calculation of the indices of heterogeneity shows significant heterogeneity in the intra-individual variances for TT<sub>3</sub> but not for FT<sub>3</sub>. A similar pattern of significant heterogeneity in TT<sub>3</sub> has been observed in healthy subjects (Table 2). We therefore conclude that TT<sub>3</sub> and FT<sub>3</sub> display a variable response pattern following oral thyroxin and that there is interindividual variation in the magnitude of the response of TT<sub>3</sub> but not FT<sub>3</sub>.

In athyroidal patients, peripheral metabolism of thyroxin is the only source of triiodothyronine. Nicoloff et al. (21) suggested that autoregulation of this step maintains triiodothyronine within the normal reference interval despite wide fluctuations in the thyroxin concentration in the blood (22). The intra-individual variation in TT<sub>3</sub> or FT<sub>3</sub> concentration in serum therefore contains components attributable to variation in thyroxin and to variation in the triiodothyronine formed by metabolism of thyroxin. Consequently, intra-individual variation in TT<sub>3</sub> and FT<sub>3</sub> must be greater than that for TT<sub>4</sub> or FT<sub>4</sub>.

We saw no significant differences in TSH between pre- and post-thyroxin specimens, and the intra-individual variation during the 8 h was greatest for TSH. Subjects with detectable TSH showed no evidence of heterogeneity in the individual responses. We therefore conclude that TSH displays a variable response pattern, although the magnitude of the response is similar in all subjects. Production of TSH by the anterior pituitary gland is stimulated by TRF produced by the hypothalamus. The response to TRF is modulated by concentrations of circulating triiodothyronine and thyroxin, and by the intra-pituitary metabolism of thyroxin to triiodothyronine. The intra-individual variation of TSH contains components of variation due to absorption and metabolism of the administered thyroxin, to peripheral and intra-pituitary conversion of thyroxin to triiodothyronine, and to the production of TSH. Predictably, the variation in TSH is greater than that of TT<sub>3</sub>, FT<sub>3</sub>, TT<sub>4</sub>, or FT<sub>4</sub>.

From this study we conclude that the benefits of standardizing the time interval between dosing and sample collection when TT<sub>4</sub> or FT<sub>4</sub> is used for serial monitoring are greatly outweighed by the convenience of using randomly timed specimens. TT<sub>3</sub> and FT<sub>3</sub> are insensitive to even major changes in thyroxin dosage (22) and this, plus greater random fluctuations in their concentration in serum and, for TT<sub>3</sub>, significant variation between subjects, makes them unsuitable for use as a first-line test to assess therapy with thyroxin. The variable pattern in the responses of TSH makes it difficult to use results for this analyte either to identify the effect of modifying therapy or to assess any variation in the response of patients maintained on the same dose.

Measurement of TT<sub>4</sub> and FT<sub>4</sub> as a first-line test, backed up *where indicated* by measurement of either TT<sub>3</sub>, FT<sub>3</sub>, or TSH, is recommended for the biochemical monitoring of therapy with thyroxin.

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