Total serum thyroxine and triiodothyronine; a comparison between Graves’ disease and hyperthyroxinaemia due to thyroxine replacement

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Abstract. Serum concentrations of total thyroxine (T₄) and total triiodothyronine (T₃) were measured in a group of patients (n = 113) presenting with untreated hyperthyroidism due to Graves’ disease and in subjects receiving oral T₄ replacement (n = 93) in whom the total T₄ concentration was supraphysiological (> 150 nmol/l). The mean total T₄ concentration in the hyperthyroid group was 226 nmol/l, sd 59, range 151–420, and the mean total T₃ concentration was 6.8 nmol/l, sd 2.73, range 3.1–17.5. For the group receiving T₄ the mean total T₄ concentration was 175 nmol/l, sd 25, range 150–258, and the mean total T₃ concentration was 2.66 nmol/l, sd 0.45, range 1.7–4.2. In the hyperthyroid group a highly significant linear correlation was found between total T₄ and total T₃. T₃ = 0.0354 T₄ + 1.21, r = 0.761, P ≤ 0.001, while in the patients taking T₄ this correlation was less close. T₃ = 0.0073 T₄ + 1.39, r = 0.398, P ≤ 0.001. The two groups are readily distinguished by expressing total T₄ as a molar ratio of total T₃. In the hyperthyroid group the mean T₄:T₃ ratio was 35.6, sd 7.8, range 19.9–56.1, compared to the patients on T₄ where the mean T₄:T₃ ratio was 67.0, sd 11.7, range 44.3–114 (t = 22.5, P ≤ 0.0001). An arbitrarily chosen value of 50 for the T₄:T₃ ratio affords a simple and convenient means of distinguishing the two categories: in only 3 patients with Graves’ disease (2.6%) was this ratio above this, and it was below in only 5 patients (5.4%) taking T₄. Where there is doubt as to the aetiology of hyperthyroxinaemia this simple operation will differentiate between hyperthyroidism caused by Graves’ disease and surreptitious ingestion of T₄. In other clinical situations where symptoms of hyperthyroidism are associated with a T₄:T₃ ratio greater than 50 the combination may suggest subacute thyroiditis or iodide-induced thyroid dysfunction.

Thyroxine (T₄) replacement therapy for hypothyroidism may be accompanied by total T₄ concentrations in serum at or above the normal range but total serum triiodothyronine (T₃) levels are rarely elevated and the patients are clinically euthyroid. If large doses of T₄ are taken however, both total T₄ and total T₃ may be supraphysiologically and the patient may report symptoms of hyperthyroidism. In the clinical context it can be difficult to distinguish between patients with genuine hyperthyroidism due to thyroid disease and a small number of individuals who consume excessive doses of T₄ surreptitiously. Because the underlying pathophysiology in the two conditions is quite different with respect to T₃ production, distinction between the two groups is possible by relating total T₄ to total T₃ as their molar ratio in serum (Pearce & Himsworth 1982). Graves’ disease is characterised by an excess secretion of T₃ from the thyroid gland itself, while ingestion of T₄ will suppress pituitary TSH and therefore thyroidal secretion, and all the T₃ present is derived from the peripheral metabolism of T₄. The net effect of this fundamental difference is that relatively more T₃ in serum is found in the genuinely hyperthyroid patient than in cases of factitious thyrotoxicosis due to T₄ ingestion.

It was not possible to assemble data from a large cohort of patients with thyrotoxicosis factitia as this is a relatively unusual condition. However, a large number of patients treated for hypothyroidism with T₄ has been identified in whom the total T₄ in
serum was supraphysiological, often markedly so, and these subjects have been used to examine the relationships of total serum \(T_4\) and \(T_3\) under conditions of hyperthyroxinaemia due to \(T_4\) therapy. A comparison is made with a large group of patients with hyperthyroidism due to untreated Graves' disease. The results confirm and extend the original suggestion (Pearce & Himsworth 1982) that distinction between the two groups is accurately made on the basis of the ratio of total \(T_4\) to total \(T_3\) in serum. Clinical situations where this ratio may give misleading results are discussed.

**Patients and Methods**

The records of all patients who have attended Northwick Park Hospital with untreated hyperthyroidism due to Graves' disease were scrutinised and patients selected for this study on the grounds that serum concentrations of both total \(T_4\) and total \(T_3\) were available. The diagnosis was based on conventional clinical criteria; patients with a clinically nodular goitre were excluded. Patients attending the outpatient clinic who had been stabilised on oral \(T_4\) replacement therapy with \(T_4\) for hypothyroidism were seen and selected for study if the total serum \(T_4\) concentration was above the upper limit of normal for our laboratory (>150 nmol/l). \(T_4\) was taken on rising and blood sampling performed in the afternoon. All these subjects were in good health and none were taking any medication known to interfere with the protein binding or peripheral metabolism of \(T_4\). There were 113 patients with Graves' disease and 93 subjects on \(T_4\) with supraphysiological \(T_4\) concentrations. Total \(T_4\) and total \(T_3\) were measured by immunoassay using sheep or rabbit antisera and polyethylene glycol 6000 to separate bound from free fractions. The inter- and intra-assay coefficients of variation for the \(T_4\) assay were 5.4 and 6.8\%, and for the \(T_3\) assay 3.7 and 4.9\%, respectively. Student's \(t\)-test was used to determine statistically significant differences between the various groups, and linear regression analysis was performed by the method of least squares.

**Results**

The serum concentrations of total \(T_4\) and total \(T_3\) in all the samples analysed are shown graphically in Fig. 1. In the hyperthyroid group the mean total \(T_4\) concentration was 226 nmol/l, median 207, SD 59, range 151—420, and the mean total \(T_3\) concentration was 6.8 nmol/l, median 6.2, SD 2.73, range 3.1—17.5. In the patients receiving \(T_4\) replacement the mean total serum \(T_4\) concentration was 175 nmol/l, median 164, SD 25, range 151—258, and the mean total serum \(T_3\) concentration was 2.66 nmol/l, median 2.60, SD 0.45, range 1.7—4.2. In the hyperthyroid patients total \(T_3\) correlated closely with total \(T_4\), the regression equation being \(T_3 = 0.0354 \times T_4 - 1.21\), \(r = 0.761, P < 0.001\); in the patients on \(T_4\) the correlation between total \(T_3\) and total \(T_4\) was less good although still significant, the regression equation being \(T_3 = 0.0073 \times T_4 + 1.39\), \(r = 0.398, P < 0.001\). When the data were analysed using Spearman's rank method the correlation coefficients (rho) between \(T_3\) and \(T_4\) were 0.766 and 0.300 for the Graves' patients and those on \(T_4\), respectively. The difference between these two coefficients is highly significant (\(u = -4.99, P < 10^{-6}\)). The difference in the respective slopes of the regression lines is obvious from inspection of Fig. 1, and this is confirmed by formal analysis \((t = 12, P < 0.001)\).
The diagonal line in Fig. 1 is drawn arbitrarily to divide the two sets of data; only four (2%) points from the patients on T₄ lay above this line and none of those from the hyperthyroid group were below. The formal description of this divisor is given by the expression \( T₃ = 0.0164 \times T₄ + 0.624; \) for practical purposes however, it is more convenient to relate total T₄ to total T₃ by calculating their molar ratio. Because the line of division in Fig. 1 does not pass through the origin, the \( T₄:T₃ \) ratio is an approximation for distinguishing between the two groups. The frequency distribution of this ratio in both sets is shown in Fig. 2. In the patients with Graves’ disease the mean \( T₄:T₃ \) ratio was 35.6, SD 7.8, range 19.9–56.1, compared to the group receiving T₄ in whom the mean ratio was 67.0, SD 11.7, range 44.3–114, the difference being highly significant \( (t = 22.5, P < 0.001) \). The arbitrary and easy to remember figure of 50 affords a practical means of distinguishing between the two groups: in only three (2.6%) of the hyperthyroid patients was the ratio greater than this (50, 52.3 and 56.1), and only five (5.4%) of those receiving T₄ were less (44.3, 46.7, 47.5, 48.4 and 49.7).

**Discussion**

The concept of T₄ as a prohormone requiring conversion to the active thyromimetic compound 3,5,3’-triiodothyronine before a biological effect occurs is widely accepted (Larsen et al. 1981). In health the contribution of the thyroid gland to daily T₃ production is estimated as 20% (Surks et al. 1973), but in hyperthyroidism due to Graves’ disease abnormal and prolonged stimulation of the thyroid leads to intrathyroidal iodine depletion and preferential T₃ production. In contrast adequate replacement therapy for hypothyroidism is associated with suppression of pituitary TSH and residual thyroid function, and in consequence T₃ production in these circumstances is entirely dependent on the peripheral deiodination of T₄. When serum T₄ levels are supraphysiological due to T₄ replacement the handling of T₄ and its fractional conversion to T₃ are unknown. A degree of control over the peripheral conversion has been proposed such that preferential production of the inert metabolite reverse T₃ occurs has been proposed (Kurtz et al. 1980), but direct kinetic evidence for this is
lacking. In any event the data in this paper show that quite marked increases in serum $T_4$ produce only modest increases in $T_3$ in patients receiving thyroxine replacement therapy.

The fundamental difference with respect to $T_3$ production between the patients with Graves' disease and those taking $T_4$ results in relatively higher $T_3$ concentrations in serum in the former group. This may be simply shown by relating total serum $T_4$ to total serum $T_3$ as their molar ratio, and was suggested as a useful means of differentiating genuine thyrotoxicosis from that caused by surreptitious ingestion of $T_4$ (Pearce & Himsworth 1982) in the absence of more sophisticated investigative facilities. The results in the present expanded series of patients confirm and extend this suggestion. If an arbitrary value of 50 is chosen for the $T_4$:$T_3$ ratio then only 3 of the patients with untreated Graves' disease (2.6%) were greater than this (50, 52.3 and 56.1) and of the patients receiving $T_4$ only 5 lower (5.4%), viz. 44.3, 47.5 48.4, 49.7 and 46.7.

Previous workers have calculated the $T_3$:$T_4$ ratio in Graves' disease in terms of nanograms per cent to micrograms per cent (Amino et al. 1978). After conversion to molar units the $T_4$:$T_3$ ratio agrees well with the present findings (mean 32.1, range 21.5–39.9). Another group reported a mean ratio of 40 for the molar $T_4$:$T_3$ ratio in hyperthyroidism (Nikolai et al. 1980), but in approximately 20% of these patients the ratio was greater than 50. The reason for this discrepancy is unclear but may relate to differences in iodine intake in the two populations.

The value of radioisotope uptake studies and the more recently described serum thyroglobulin assay are not in doubt in the differential diagnosis of thyrotoxicosis factitia (van Herle et al. 1982; Mariotti et al. 1982a), but these facilities, particularly the thyroglobulin assay, are not universally available. The simple expedient of expressing $T_4$ as a ratio of $T_3$ reliably discriminates between the two groups in 96% of the sera examined in this study, and offers a convenient clue to the differential diagnosis.

It has been pointed out that certain conditions exist when the use of this ratio may prove misleading (Mariotti et al. 1982b). The example given was hyperthyroidism induced by the anti-arrhythmic agent amiodarone. In this complex situation hyperthyroidism is probably induced by the release of large mounts of iodide during metabolism of the drug. Because amiodarone itself inhibits the peripheral conversion of $T_4$ to $T_3$ the net effect is a relatively lower $T_3$ concentration, and consequently the $T_4$:$T_3$ ratio is often in excess of 50. Similar considerations apply to other cases of iodine induced hyperthyroidism (Jod-Basedow): depletion of thyroidal iodine does not occur and preferential $T_3$ secretion is reduced. A careful drug history and the presence of a goitre in the majority of these patients should avoid any confusion.

A major difficulty in determining the aetiology of raised $T_4$ and $T_3$ levels can occur however, in patients presenting with the painless variant of acute thyroiditis. In this condition local pain is absent and often no goitre is palpable (Woolf & Daly 1976). Elevated levels of both $T_4$ and $T_3$ are found with symptoms of hyperthyroidism. It had been proposed earlier that this so-called destruction induced thyrotoxicosis could be differentiated from that of Graves' disease by calculating the $T_3$:$T_4$ ratio (Amino et al. 1978), but this was not confirmed in two subsequent reports (Walfish 1978; Nikolai et al. 1980). Thyroidal radioisotope studies are unhelpful in differentiating this condition from factitious thyrotoxicosis as in both little or no uptake occurs. It is in this situation that estimation of serum thyroglobulin may be of real value, as markedly elevated concentrations may be present in thyroiditis, but not in thyrotoxicosis factitia.

If medication other than $T_4$ is taken to produce signs and symptoms of hyperthyroidism, for example $T_3$ or thyroid extract, then the $T_4$:$T_3$ ratio will be misleading. In the case of $T_3$ ingestion $T_4$ production will be suppressed while in the case of thyroid extract the resulting serum concentrations will depend on the relative amounts of $T_4$ and $T_3$ in this notoriously variable preparation.

In conclusion, providing the limitations described above are understood, the data in this study show that expressing total serum $T_4$ to total serum $T_3$ will helpfully distinguish between patients with hyperthyroidism due to Graves' disease and those in whom elevated thyroid hormone concentrations are the result of ingestion of thyroxine.

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References


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