Determination of Iodothyronine Absorption and Conversion of L-Thyroxine (T₄) to L-Triiodothyronine (T₃) using Turnover Rate Techniques

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ABSTRACT The absorption of L-thyroxine (T₄) and L-triiodothyronine (T₃) and the fractional rate of conversion of T₄ to T₃ were determined from the turnover rates of T₄ and T₃ in seven patients without endogenous thyroid function during separate treatment periods with these iodothyronines. Serum T₃ concentration was measured by a radioimmunoassay procedure in which the iodothyronines are separated from the plasma proteins before incubation with anti-T₃ antibody. Metabolic clearance rates were calculated by an integral (noncompartmental) approach since the use of single compartment kinetics led to a 40% overestimation of the metabolic clearance rate of T₃. Based on the amount of hormone ingested and the observed hormonal turnover rates, the absorption of T₄ and T₃ (iodothyronine turnover/iodothyronine ingested) in man could be estimated. Absorption of T₃ was complete in three subjects but decreased to 43% in a fourth who was suffering from mild congestive heart failure. Mean T₃ absorption was 48.0±2.6% (SEM) for seven subjects. The mean fractional rate of T₄ to T₃ conversion determined during T₄ replacement therapy (T₄ turnover/T₃ turnover) was 42.6% (range 30.7–50.8%). Thus, approximately one-half of the T₄, which was deiodinated was converted to T₃ suggesting that monodeiodination is an obligatory step in the peripheral metabolism of T₄. Calculations based on these results together with other available data suggest that under normal physiologic circumstances the major portion of the T₃ pool is derived from monodeiodination of T₄.

INTRODUCTION

The recent development of accurate methods for the determination of plasma L-triiodothyronine (T₃)¹ concentration (1–5) have for the first time allowed precise measurement of T₃ turnover in man. In the following report we describe studies in which both T₃ and L-thyroxine (T₄) turnover rates were measured in patients without endogenous thyroid function but maintained in the euthyroid state by the administration of synthetic T₃ or T₄. Since the amount of T₃ or T₄ administered was known and the turnover of these iodothyronines could be calculated it was possible to estimate both the absorption of T₄ and T₃ and the fractional conversion of T₄ to T₃ in man. Turnover was calculated from the product of the mean plasma iodothyronine concentration and the metabolic clearance rate. In the case of T₃, a newly developed radioimmunoassay technique was used for measurement of plasma concentration (6). Metabolic clearance rates were assessed by the application of noncompartmental assumptions to the analysis of the isotopic data (7).

The results of these studies, taken in conjunction with other available data suggest that (a) under normal conditions the human thyroid gland secretes largely T₄, (b) the source of circulating T₃ in normal man is largely the monodeiodination of T₄ in the peripheral tissues, and (c) monodeiodination in man, as in the rat (8), appears to be an obligatory intermediate step in the deiodination of T₄ by tissues. Moreover, these studies indicate that application of single compartment kinetics leads

¹Abbreviations used in this paper: CR, conversion ratio; MCR, metabolic clearance rate; T₄, triiodothyronine; T₃, thyroxine; TCA, trichloroacetic acid; TSH, thyrotropin.

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to a systematic overestimation of the metabolic clearance rate of T₃ and underscores the desirability of using multicompartamental or noncompartamental approaches to the analysis of metabolic data obtained with isotopic T₄ in man.

METHODS

The turnover rate of T₄ and T₃ was measured in seven hypothyroid patients during hormonal replacement treatment with synthetic T₃ (Synthroid, Flint Laboratories, Morton Grove, Ill.). Four were known to be athyreotic after surgical and radioiodine thyroidectomy for papillary-follicular thyroid carcinoma. They were without metastatic disease as assessed by total body scans and urinary excretion of radioiodine as well as routine roentgenography. The diagnosis in the remaining three patients was severe primary hypothyroidism. All subjects were clinically euthyroid at the time of study. Their serum concentrations of thyrotrophin (TSH) were less than 3 μU/ml based on Research Standard A (obtained through the courtesy of R. Bangham, National Institute for Medical Research, Mill Hill, London) (9). The human TSH and the rabbit anti-human TSH antiserum were gifts of the National Pituitary Agency.

Turnover rates were calculated as the product of the metabolic clearance rates and mean plasma concentrations of each hormone. In the four athyreotic patients (R. W., A. F., V. DiG., and V. P.) the metabolic clearance rate of T₃ was determined during treatment with synthetic T₃ (Cytomel; Smith, Kline & French Laboratories, Philadelphia, Pa.) 4 wk before the metabolic clearance rate of T₄ was measured. The metabolic clearance rates of T₄ and T₃ were measured simultaneously in the other patients. The plasma concentration of T₄ was assessed both by competitive protein binding (10) and T₄-I by column (11) methods (Bioscience Laboratories, Van Nuys, Calif.). Plasma T₃ was measured by radioimmunoassay as previously described (6). The average value of plasma hormone concentrations from three to eight different plasma samples was used in the calculation of turnover rates. Plasma samples for determination of hormone concentrations were obtained between 8 and 9 a.m. just prior to the administration of the daily dose of T₄. They were stored at −20°C until the assays were performed.

L-thyroxine labeled with ¹³¹I (Tetramet,²⁸¹I), specific activity = 40-60 μCi/μg, and L-triiodothyronine, labeled with either ¹²⁵I (Triomet,²⁵¹I), specific activity = 70-90 μCi/μg, or ¹³¹I (Triomet,²³¹I), specific activity = 30-50 μCi/μg were obtained from Abbott Laboratories, North Chicago, Ill. For the determination of metabolic clearance rates 20 μCi of either ¹²⁵I]T₄ or [¹³¹I]T₃ or a combined dose of 20 μCi [¹²⁵I]T₄ and 40 μCi [¹³¹I]T₃ were injected intravenously. Plasma samples were generally obtained every 2 h for the first 12 h after injection and at 24-48 h intervals thereafter. Plasma was obtained for 5 days for the T₄ studies and for 12 days for the T₃ studies. Five drops of Lugol’s solution were administered twice a day throughout the study. All plasma samples were treated with trichloroacetic acid (TCA) to remove inorganic iodide (7). In the T₃ metabolic clearance rate determinations, the plasma nonextractable iodine was measured by extraction with ethanol as previously described (7). Plasma radioiodothyronine concentration was calculated as the difference between the TCA-precipitable radioactivity and the nonextractable radioactivity. Radioactivity in all samples was measured in a two-channel Packard Autogamma Spectrometer to a statistical precision of ±2%. Metabolic clearance rates were calculated by both single compartment kinetics and the integral approach first described by Tait (12) for the steroid hormones and more recently applied to the iodothyronines (7).

RESULTS

Plasma T₃ concentration was within the normal range for all patients treated with 150-200 μg T₄/day (Table I). The range of values for individual samples collected during a 12 day period was ±10% of the mean value for each patient. Moreover, determinations of T₃ concentration by competitive protein binding and by iodine analysis were in close agreement. Plasma T₃ concentrations on different days during the study were within ±15% of the mean T₃ concentration for each patient (Table I). The mean plasma T₃ concentration for all of the subjects, 172±9.3 (SE) ng/100 ml, was somewhat higher than the mean of untreated euthyroid individuals in our laboratory, 146±24 ng/100 ml.

Data for the metabolic clearance rates of T₄ and T₃ are shown in Table I and Fig. 1. Calculation by single compartment kinetics resulted in a larger estimate of metabolic clearance rate of T₄ and T₃ than by the integral (noncompartamental) approach. The difference was small (4.5%) in measurements of T₄ metabolic clearance rate, but still significant statistically (P < 0.01, paired t test). For T₃, however, the mean metabolic clearance rate calculated by single compartment kinetics was 40.6% greater than by integral calculations (range 16.6-61.9%). The metabolic clearance rate derived from the integral (noncompartamental) calculation was therefore used in all calculations (Table I).

The absorption of the iodothyronines was estimated from the turnover rates of T₄ or T₃ during separate treatment periods with these preparations. Since, in these patients, the only source of iodothyronine is that absorbed from the enteral tract it follows that:

\[
\text{iodothyronine turnover, } \mu g/\text{day} = \text{iodothyronine absorbed, } \mu g/\text{day},
\]

\[
\text{absorption } (\%) = 100 \times \frac{\text{iodothyronine turnover, } \mu g/\text{day}}{\text{iodothyronine ingested, } \mu g/\text{day}}.
\]

During treatment with T₃ from 39.8 to 54.6% of the ingested T₃ was absorbed in these patients (mean 48.0± 2.6%) (Table I). Similar calculations of T₄ absorption were made in four patients during a separate treatment period with 50-75 μg T₄/day (Table II). In these subjects, plasma T₄ concentration increased 300-500% after T₄ ingestion, falling thereafter to pre-dose values. The

¹ These data have been reported previously (6).
mean plasma T3 concentration was calculated by integrating the area under a curve describing T3 concentration vs. time. The average absorption of T3 was 102.8% in three of the four subjects studied (range 92.9-113.3%) (Table II). In V. P., who was in mild congestive heart failure at the time of study, T3 absorption was 43.2%.

Since, during treatment with T4, the only source of T3 is from the metabolism of T4, a minimal estimate of T4 to T3 conversion can be calculated from the T3 and T4 turnover rates. Thus, the T4 to T3 conversion ratio (CR), representing the percentage of the T4 turnover converted to T3 is

\[
\text{CR} = \frac{100 \times \frac{T3 \text{ turnover, } \mu mol/day}{T4 \text{ turnover, } \mu mol/day}}{\text{Conversion rate}}
\]

The mean CR for the seven patients studied was 42.6±3.1% (range 30.7-50.8%) (Table I).
**TABLE II**

**Turnover and Absorption of L-Triiodothyronine (T₃) in Hypothyroid Patients during Treatment with T₄**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose of T₄</th>
<th>Mean plasma T₃</th>
<th>Metabolic clearance rate</th>
<th>Turnover ‡</th>
<th>Absorption ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. W.</td>
<td>75</td>
<td>322 (8)§</td>
<td>23.9</td>
<td>76.6</td>
<td>102.1</td>
</tr>
<tr>
<td>A. F.</td>
<td>75</td>
<td>303 (8)</td>
<td>23.0</td>
<td>69.7</td>
<td>92.9</td>
</tr>
<tr>
<td>V. DiG.</td>
<td>50</td>
<td>237 (8)</td>
<td>23.9</td>
<td>56.7</td>
<td>113.3</td>
</tr>
<tr>
<td>V. P.</td>
<td>75</td>
<td>282 (7)</td>
<td>11.5</td>
<td>32.4</td>
<td>43.2</td>
</tr>
<tr>
<td>Mean</td>
<td>286</td>
<td>20.6</td>
<td>10.9</td>
<td>87.9</td>
<td>15.5</td>
</tr>
<tr>
<td>SEM</td>
<td>18.3</td>
<td>3.0</td>
<td></td>
<td>10.9</td>
<td>15.5</td>
</tr>
</tbody>
</table>

* Calculated by integration of the curve described by plotting plasma T₃ concentration against time.
‡ See footnote to Table I for calculations.
§ Numbers in parentheses indicate the number of plasma samples analyzed.

**DISCUSSION**

The accuracy of the rates of iodothyronine absorption and T₄ to T₃ conversion determined by turnover rate techniques as described herein depends on the precision of measurement of the mean plasma concentration and metabolic clearance rates of T₃ and T₄. Whereas the methods employed for T₄ determination are well established, only a limited experience is available with published methods for the determination of plasma T₃. There is general agreement, however, that radioimmunoassay procedures with specific anti-T₃ antibodies provide the most reliable measurements. Moreover, available evidence suggests that the presence of the plasma binding proteins in the assay mixture results in high T₃ values (14). In the radioimmunoassay used for measurement of T₃ in this study, T₃ is separated from the plasma binding proteins before incubation with antibody (6). The mean plasma T₃ concentration of euthyroid individuals by this method, 146±24 ng/100 ml, is in good agreement with that of some published methods in which the binding of T₃ to plasma proteins in the assay mixture is blocked by addition of other agents (2-5) but somewhat greater than that of other reports in which the mean plasma T₃ of euthyroid individuals is in the range of 100-110 ng/100 ml (4, 5). The effect of a possible overestimation of plasma T₃ by our method on the conclusions of the present study is discussed below.

Since the mean plasma concentration is required for the calculation of turnover rates, the relationship of the observed plasma T₃ and T₄ concentrations to the mean iodothyronine concentration must be considered. We have previously shown that the plasma T₃ concentration may increase transiently after T₄ ingestion in some patients (6). Since the increase is relatively small in magnitude (20–40%) and short in duration (2-4 h), it does not influence significantly the mean plasma T₄ concentration. In contrast to T₄, plasma T₃ concentration remains relatively constant after a dose of T₃ is ingested (6). Thus, during treatment with T₄ the plasma concentration of T₃ and T₄ in samples obtained prior to administration of the daily T₄ dose adequately represents the mean plasma iodothyronine concentration. It is notable that the mean plasma T₄ concentration during T₄ replacement therapy, 172±9.3 ng/100 ml, is higher than that of euthyroid subjects in our laboratory (6). Similar data have been reported by Lieblich and Utiger (3). The possibility that the dose of T₃ administered to these patients may be somewhat greater than necessary to produce euthyroidism is currently under investigation.

During T₄ treatment, the large and sustained (8-12 h) increase in T₃ concentration which occurs after the hormone is ingested necessitates repeated sampling of plasma throughout the day to assess the mean plasma concentration (6). An important component in the determination of the turnover rate is the measurement of the clearance rate by isotopic techniques. Conventionally, estimates of the clearance rates of iodothyronines have been made from the product of the apparent distribution volume as determined from the reciprocal of the zero-time extrapolation of the terminal plasma disappearance curve and the terminal fractional plasma removal rate (15). This procedure makes the tacit assumption of the existence of a single rapidly mixing compartment. The results of our studies clearly indicate that whereas no major error is introduced in estimating the T₄ clearance rate in this fashion, a systematic 40% overestimation of the clearance rate is introduced when such analytic techniques are applied to T₃. The reason why T₄ clearance rate can be measured by single compartmental methods may be related to the fact that the fractional rate of distribution of T₄ is relatively rapid in comparison to the fractional rate of metabolism. In the case of T₃, the relatively more rapid rate of fractional hormone metabolism in relationship to its rate of distribution appears to invalidate the assumptions of single compartment kinetics. Our observations are at variance with those of Nicoloff, Low, Dussault, and Fisher (16) and Cavalieri, Steinberg, and Searle (17) who reported no difference in the clearance rates of T₃ determined by single compartmental kinetics and by a noncompartmental approach in constant infusion experiments employing euthyroid subjects. However, an overestimation of the T₃ clearance rate by single compartmental methods was observed by Cavalieri et al. (17) in thyrotoxic subjects. The basis of these discrepancies may perhaps be related to an inadequate duration of the T₃ infusion in the euthyroid subjects. Under any circumstances, the integral ap
approach first used by Tait for steroid clearance measurements (12) and later applied by us to the iodothyronines (7) appears to be both useful and a convenient technique for measuring the clearance rate of T₃ by isotopic techniques. Although the T₃ clearance measurements in some of the subjects was determined prior to measurement of T₄ clearance and during treatment with T₂, mean T₃ clearance and conversion ratios in this group were not significantly different from the values of the remaining three subjects in whom T₂ and T₄ clearance rates were measured simultaneously during T₃ treatment.

Previous estimates of iodothyronine absorption in man have been based on measurements in the plasma or whole body of T₂ or T₃ radioactivity from isotopically labeled iodothyronine preparations which were administered orally (18-20). As pointed out by Hays (19), differences in the composition of the solution in which T₂ is ingested may result in different values for absorption. The measurement of absorption by turnover rate techniques described in this report allows for the first time measurement of the absorption of hormone in the actual pharmaceutical preparations which patients ingest for replacement therapy. In agreement with previous reports, T₂ absorption was essentially complete in patients without gastrointestinal disease (20, 21). The observation that T₂ absorption was reduced to 50% in one patient suffering from mild congestive heart failure suggests that a relatively minimal degree of intestinal dysfunction may reduce T₂ absorption, whereas published reports suggest that T₂ absorption is reduced only in severe malabsorption (19). The average T₂ absorption (50%) was somewhat less than that observed by Oddie, Fisher, and Epperson (18) (63.4%) who administered the dose in a capsule and by Hays (19) (74.4%) who gave the dose in a liquid form, but greater than observed by Hays for doses in capsules (41.7%) (19). Some of these differences may be due to the relatively small groups of subjects studied or to geographical factors.

The extrathyroidal conversion of T₂ to T₃ in man was first clearly demonstrated by Braverman, Ingbar, and Sterling (22). Subsequently, Sterling, Brenner, and Newman (23) and Pittman, Chambers, and Read (24) confirmed their observations and estimated the extent of conversion by measuring the concentration of radioactive T₂ in plasma after injection of radioactive T₂. Both groups reported that as much as 33% of the T₂ production was converted to T₃. Technical problems, however, may offer serious obstacles to this approach. Since only a small portion of the T₂ pool is in the plasma (7) isotopically labeled T₂ constitutes only 1-3% of the plasma radioactivity after injection of isotopically labeled T₂. The accurate measurement of such small amounts of labeled T₂ in the presence of a large excess of labeled T₂ is a formidable problem with inherent difficulties in the estimation of chromatographic paper background radioactivity and in the assessment of overlap of a small fraction of T₂ or tetraiodothyroacetic acid radioactivity into the T₃ region of chromatograms or conversion of isotopic T₂ to T₃ during sample processing. These factors thus necessitated numerous adjustments (24). The conversion rate of 42% determined by turnover techniques which obviate these technical difficulties would therefore appear to be a more reliable estimate of this metabolic pathway. It is theoretically possible that the conversion rates in the three subjects with primary hypothyroidism were somewhat overstated due to residual thyroidal secretion of T₂. This is unlikely since serum TSH concentration was undetectable on hormonal replacement therapy and since the mean conversion rate in this group did not differ significantly from that of the four athyreotic subjects.

Based on measurements of the conversion rate and the known biological activity of T₂ in the rat, we have previously indicated that essentially all of the biological activity of T₂ can be attributed to the T₃ which is generated and suggested that T₂ should be considered a prohormone (25). The more recent observation that propylthiouracil treatment causes a decrease in T₄ to T₂ conversion which fully accounts for the anti-T₂ effect of this agent strengthens this conclusion (8). The conversion rate of 42% observed in the current experiments in conjunction with a two to three fold greater biological activity of T₂ compared with T₄ (13) suggests that T₂ effects all thyroidal activity in man as well as in the rat. The conclusion that T₂ is the biologically active thyroid hormone is supported further by the recent demonstration of stereospecific low capacity, high affinity binding sites for T₂ only in the rat anterior pituitary (26) and in the nuclei of liver and kidney (27).

Since peripheral T₂ to T₃ conversion results from monodeiodination of T₂, the relationship of the amount of T₂ converted to the amount of T₃ deiodinated may help define the pathways in T₂ metabolism which result in T₃ formation. Approximately 85% of the T₂ turnover is metabolized by deiodination (28-31). In the current studies the ratio: T₂ converted/T₂ deiodinated was 0.5 (range 0.35-0.6). Using a different technique to measure the T₂ to T₃ conversion rate we recently reported a similar relationship between T₂ converted and T₃ deiodinated both in normal rats and in animals in which the conversion rate was reduced by treatment with propylthiouracil (8). Thus, in man as well as in the rat, approximately one-half of the T₂ deiodinated is converted to T₃. In earlier studies we have shown that during T₂ metabolism there is no significant difference between the appearance in urine of iodide from the phenolic ring and tyrosyl ring and have suggested that T₂ deiodination might be a random process (32). Since removal of either
phenolic ring iodine atom from T₄ would result in T₃ formation, provided that the side chain remains unaltered, it is apparent that if T₄ is metabolized by random monodeiodination a maximum of one-half of the T₃ molecules deiodinated will form T₃. The excellent agreement between the observed rate of T₃ formation and the theoretical maximum for random monodeiodination suggests that random monodeiodination is an obligatory metabolic pathway in T₄ metabolism. This formulation predicts that another iodothyronine, 3,3',5'-triiodothyronine, would also be formed during T₄ metabolism. Since this compound appears to be metabolized at a greater rate than T₃ (33) its detection by current radiochemical techniques would be exceedingly difficult.

The observed conversion ratio also allows estimation of the fraction of the T₃ pool which is derived from T₄ from the following expressions:

\[(\text{turnover T₃})_{\text{obs}} = \frac{\text{CR}}{100} \times (\text{turnover T₄})\]

where \((\text{turnover T₃})_{\text{obs}}\) represents the T₃ turnover due to conversion.

Since the turnover is equal to the product of the mean plasma concentration, \([\text{T₃}]\), and the metabolic clearance rate, MCR, it follows that:

\[\frac{\text{CR} \times [\text{T₃}] \times \text{MCR}_T}{100 \times \text{MCR}_T} = \text{MCR}_T\]

If we now substitute normal values for \([\text{T₃}]\) (80 μg/liter), \(\text{MCR}_T\) (23.0 liters/day), \(\text{MCR}_T\) (1.1 liter/day) and the conversion rate observed in these studies (42.6%) the mean concentration of T₃ in plasma due to conversion = 136 ng/100 ml (range 100-163 ng/100 ml). The close accord of this value with the normal mean T₃ concentration in our laboratory, 146±24 (SD) ng/100 ml (6) suggests that under normal conditions the major portion of T₃ pool is derived from peripheral T₄ metabolism and that the contribution from thyroidal secretion under normal conditions is minor. This is not necessarily the case in iodine deficiency (34) and in pathological conditions such as Graves' disease. These calculations differ from those of Sterling et al. (23) and Pittman et al. (24) who estimated that from 30 to 40% of the T₃ pool is derived from extrathyroidal T₄. However, our conclusion is strengthened by recent reports that T₃ constitutes less than 9% of the iodothyronine content of human thyroglobulin (35, 36). Based on this observation and assuming indiscriminate hydrolysis of thyroglobulin, the thyroid secretion in man appears to be primarily T₃.

As indicated above, the precise concentration of plasma T₃ in euthyroid individuals is still a matter of controversy. If the mean euthyroid plasma T₃ is in the range of 100-110 ng/100 ml as suggested by some reports (4, 5), the T₄ to T₃ conversion rate in the present studies would be reduced to approximately 30-35%. The lower conversion rate is still of sufficient magnitude to ascribe thyroidal activity in man predominantly to T₃ but is too low to be consistent with the random monodeiodination pathway of T₄ metabolism unless some of the T₃ generated from T₄ is irreversibly metabolized within the cell before entering the plasma. For either conversion rate, however, the T₃ pool would be derived principally from peripheral T₄ and not from thyroidal secretion.

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