Diagnostic Value of Free Triiodothyronine in Serum

Ed Avalos, Ph. De Nayer, and C. Beckers

Centre de Médecine Nucléaire, University of Louvain Medical School, Belgium

Serum free T_3 concentration has been assessed in various thyroid conditions by a T_3 analog method and the results compared with those obtained by equilibrium dialysis in the same individuals. The methodology is easy to perform and reproducible. FT_3 determination appears to be especially valuable in detecting borderline thyrotoxicosis as in cases previously cured from thyrotoxicosis but suspected of relapse, or in nontoxic goitrous patients overtreated with T_4.

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The determination of circulating free thyroid hormones is of great value for evaluating the physiopathology of thyroid function, especially in those patients presenting alterations of the thyroxine-binding proteins, or demonstrating subclinical hypothyroidism or thyrotoxicosis. It is well recognized that even if some of the methods for measuring free T_4 (FT_4) levels remain open for criticism they have largely contributed to the diagnosis and the management of thyroid diseases (*1–5*). In the case of free-T_3 (FT_3) determination, many methodological problems have been encountered delaying the assessment of such measurement in thyroid disturbances.

The aim of this study has been to evaluate a recently developed kit for FT_3 measurement using a T_3 analog (Amerlex FT_3, Radiochemical Centre, Amersham, UK). The assay of FT_3 by equilibrium dialysis was used as reference method. The results support the concept that FT_3 determination appears most valuable in the diagnosis of borderline thyrotoxicosis.

MATERIALS AND METHODS

Patients

Serum samples were obtained from 271 subjects. According to clinical and chemical findings they were classified as follows. Euthyroid controls: 99 euthyroid healthy subjects (41 males, 58 females); patients with thyroid disease: 21 hyperthyroid patients (2 males and 19 females) and 11 hypothyroid patients (all females); 22 subjects on T_4 supplementation therapy (6 males, 16 females); 103 euthyroid subjects with alterations in thyroid-hormone binding proteins comprising ten women using contraceptive pills, 11 subjects (7 males, 4 females) with low TBG concentrations; 19 subjects (2 males, 17 females) with high TBG concentration; 15 women at the first-trimester of pregnancy (gestational age: range 6 to 13 wk); 15 women at the second-trimester pregnancy (gestational age range: 14 to 26 wk), and 18 women at the third-trimester pregnancy, (gestational age: range 27 to 38 wk) and ten subjects with familial dysalbuminemic T_4-excess. Nineteen other subjects with clinical and chemical data suggesting borderline thyrotoxic hyperthyroidism were also analyzed.

Hormone Measurements

The serum concentration of total T_4 and total T_3 was measured by the Clinical Assays Gammacoat method (MA), Serum TSH was measured by the IRMA Immophase Corning method (Medfield), and the T_3 uptake ratio by the MAA Amerlex method (Radiochemical Centre, Amersham, UK) Serum TBG was measured using the RIA-Gnost-Kit (Hoechst, FRG). The free T_4 and T_3 indices were calculated from the absolute amounts (nmol/l) of either T_4 or T_3 and the T_3 uptake ratio from the percent calculated uptake in a given sample to the percent uptake in the reference serum. The serum free T_3 was determined by the Amerlex method (Amersham International, Ltd., Amersham, UK) and by the equilibrium dialysis method as described by Oppenheimer et al. (*1*), as adapted by ourselves (*5*).

RESULTS

The data obtained in euthyroid subjects and in patients suffering from either hypo- or hyperthyroidism

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For reprints contact: Prof. C. Beckers, MD, Centre de Médecine Nucléaire, UCL 54.30, University of Louvain Medical School, Avenue Hippocrate, 54, B-1200 Brussels, Belgium.

TABLE 1

Mean Values (± s.d.) of Circulating Thyroid Hormones with Special Reference to Free T_3 Concentrations as Measured by Free-T_3 Amerlex and Free-T_3 Dialysis Methods

	n	Total T_4 (nmol/l)	Free T_4 index*	Total T_3 (nmol/l)	Free T_3 index†	Free T_3 Amerlex (pmol/l)	Free T_3 dialysis (pmol/l)
Euthyroid	99	115 ± 23	109 ± 22	2.1 ± 0.2	2.0 ± 0.3	5.8 ± 1.2	4.3 ± 0.8
Hyperthyroid	21	251 ± 70	269 ± 75	6.7 ± 2.2	7.2 ± 3.0	25.5 ± 1.0§	18.1 ± 8.3§
Hypothyroid	11	17 ± 13	14 ± 11	1.0 ± 0.2	0.9 ± 0.3	2.2 ± 0.5§	1.6 ± 0.5§
Borderline thyrotoxic	19	166 ± 32	156 ± 3.0	3.3 ± 0.8	3.1 ± 1.0	9.8 ± 2.8§	7.2 ± 2.3§
T_4 therapy	22	155 ± 27	146 ± 25	2.2 ± 0.4	2.1 ± 0.5	8.9 ± 3.8‡	—

* T_3 uptake ratio × T_4.
† T_3 uptake ratio × T_3.
‡ $p < 0.05$.
§ $p < 0.001$.

are presented in Table 1. In the same table are also listed the results obtained in the patients on T_4 therapy for nontoxic goiter and in those treated for hyperthyroidism but presenting chemical evidence of residual borderline thyrotoxicosis according to the serum total T_4 and T_3 values but a normal TSH response to TRH.

In the hypo- and hyperthyroid patients, both the FT_3 Amerlex and the T_3 dialysis methods yielded values significantly different from those observed in the euthyroid group ($p < 0.001$).

In the cases of borderline clinical thyrotoxicosis, the mean value with the FT_3 Amerlex and the FT_3 dialysis value were significantly higher than in the normal group ($p < 0.001$). Out of these 19 patients, nine patients had values of T_3 within the normal range (90–200 ng/dl) (Fig. 1).

In the patients on T_4 therapy, the FT_3 Amerlex value of 8.9 ± 3.8 pmol/l was significantly higher than the mean free T_3 concentration in the normal group, whereas the total T_3 values were within the normal range ($0.01 < p < 0.05$).

The data collected in the patients with alterations in TBG are listed in Table 2. In the low TBG euthyroid patients, both the FT_3 index and the FT_3 dialysis values yielded results higher than in the control ($p < 0.001$), but such was not the case with the FT_3 Amerlex method. In the euthyroid high-TBG subjects (not on contraceptives or pregnant), the FT_3 Amerlex values and the FT_3 indices gave results similar to those of the normal group whereas the mean FT_3 dialysis values were higher ($p < 0.01$), even if four out of the ten patients had values within the normal range.

In the women on contraceptive drugs, the enhanced TBG concentration did not result in any significant increase of the FT_3 levels by either method. In pregnancy, a slight but not significant free T_3 decrease was observed with the dialysis method while with the Amerlex method, the mean FT_3 was increased in the 1st trimester ($p < 0.01$) and significantly decreased in the third trimester ($p < 0.001$), but not during the second trimester.

In the group of patients with familial dysalbuminemic-T_4 excess, the FT_3 values (6.5 ± 1.4 mol/l) were slightly but not significantly increased as compared to the normal group.

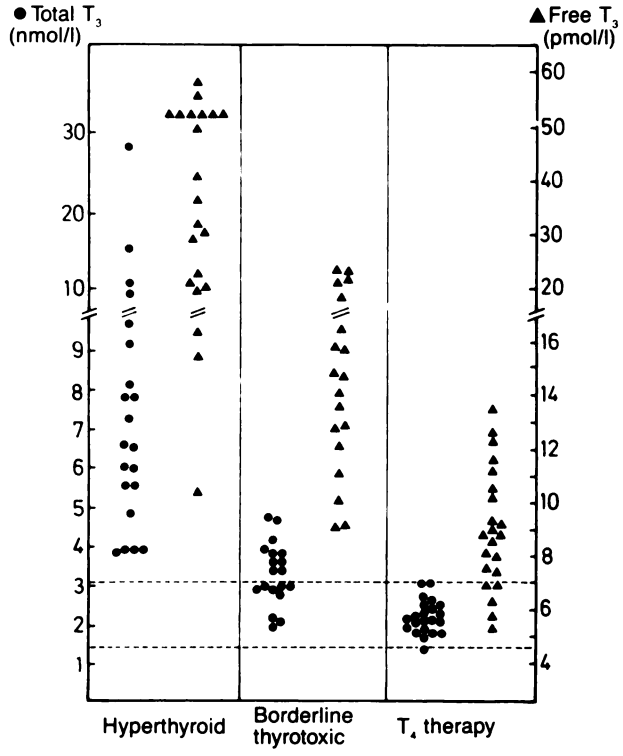

FIGURE 1

Total T_3 (●) and free T_3 (▲), as measured by Amerlex FT_3 method in hyperthyroidism and borderline thyrotoxic patients and in patients on T_4 therapy

TABLE 2

Mean Values (± s.d.) of Circulating Thyroid Hormones with Special Reference to Free T_3 Concentrations as Measured by Free-T_3 Amerlex and Free-T_3 Dialysis Methods

	n	Total T_4 (nmol/l)	Free T_4 index*	Total T_3 (nmol/l)	Free T_3 index†	Free T_3 Amerlex (pmol/l)	Free T_3 dialysis (pmol/l)
Euthyroid	99	115 ± 23	109 ± 22	2.1 ± 0.2	2.0 ± 0.3	5.8 ± 1.2	4.3 ± 0.8
Contraceptive	10	167 ± 35	125 ± 9	2.6 ± 0.4	1.6 ± 0.1	5.5 ± 1.1‡	4.0 ± 0.6‡
Euthyroid low TBG	11	91 ± 41	108 ± 5	2.0 ± 0.6	2.6 ± 0.1	6.5 ± 2.2‡	6.4 ± 1.7"
Euthyroid high TBG	19	165 ± 41	122 ± 2	2.8 ± 0.6	2.1 ± 0.02	5.8 ± 1.5‡	5.7 ± 2.2¶
Pregnancy							
1st trimester	15	128 ± 25	110 ± 3	2.9 ± 0.9	2.5 ± 0.1	6.8 ± 1.7¶	4.3 ± 1.4‡
2nd trimester	18	149 ± 31	107 ± 2	3.1 ± 0.6	2.2 ± 0.03	5.2 ± 0.8‡	4.0 ± 0.8‡
3rd trimester	16	149 ± 31	110 ± 2	2.9 ± 0.7	2.1 ± 0.04	4.6 ± 0.9"	3.5 ± 0.9‡

*T_3 uptake ratio × T_4.
†T_3 uptake ratio × T_3.
‡N.S.
§$p < 0.05$.
¶$p < 0.01$.
"$p < 0.001$.

DISCUSSION

Free hormones levels are a better reflection of the peripheral activity of the thyroid hormones than total circulating levels. As for FT_4, this holds true for FT_3 if abnormalities of the carrier proteins—particularly of TBG—occur. Moreover, when alterations of the T_3/T_4 ratio are suspected in conditions as iodide deficiency, subtotal thyroidectomy or suspicion of relapse of thyrotoxicosis, determination of the free-T_3 level should provide a better diagnostic tool (*6–8*).

The measurement of FT_3 has long remained restricted because of the methodological problems related to equilibrium dialysis. The calculation of a FT_3 index appears to be helpful but not entirely satisfactory in some clinical situations (*9–11*). Therefore, it was of great interest to evaluate the recently developed procedures with FT_3 analog tracers.

FT_3 determination appears to be of special interest in cases of borderline clinical thyrotoxicosis. Indeed out of 19 patients, nine had a normal T_3 level, whereas 15 had elevated FT_3 values by the analog method and 16 by equilibrium dialysis. The assay of FT_3 in patients supplemented with T_4 emphasizes the observation already discussed by Braverman et al. (*12*): in 22 patients only six had elevated T_4 values whereas in 18 patients the FT_3 was in the hyperthyroid range. In patients with altered TBG levels, the determination of FT_3 also contributes in the evaluation of the thyroid function. From our data, it appears that in patients with congenitally high or low TBG, FT_3 as measured by the analog method remains within the normal range. In low TBG patients our results differ from the data of Franklyn et al. (*13*) and Wilke et al. (*14*), who using the same analog method, reported a significant decrease of FT_3. It should be noted that in the same patients, FT_3 measured by equilibrium dialysis are higher than in controls, in agreement with Smals et al. (*15*).

In oral contraceptive users, FT_3 remains normal as assessed either by dialysis or the analog method. However, Wilke et al. (*14*) found an increased mean FT_3 value in these subjects but they point out that out of 20 values, all but one fell within the normal reference range. An alternative explanation to be proposed to account for this discrepancy is a possible change in the binding properties of TBG (e.g., microheterogeneity) which is not reflected in the standards of the assay.

A slight but not significant decrease of FT_3 was observed during pregnancy using the dialysis method; the same trend was recorded using the analog method. During the third trimester of pregnancy, Amerlex FT_3 values were significantly decreased in agreement with previous reports (*8,14*). In our study, the mean FT_3 value was significantly increased during the first trimester of gestation using Amerlex FT_3 but not with dialysis whereas in the study of Wilke et al. (*14*) the FT_3 were similar to controls. A possible explanation for the decrease in FT_3 during pregnancy not associated with clinical hypothyroidism has been proposed by Franklyn et al. (*13*): the low FT_3 levels would reflect a homeostatic mechanism due to increased occupancy of nuclear receptors as the consequence of the hyperestrogenic status. Direct evidence for increased nuclear binding capacity for thyroid hormones during pregnancy has recently been provided (*16*).

The differences between FT_3 levels recorded with both methods do not appear to be due to abnormal binding of the T_3-analog tracer to albumin. Indeed, in familial dysalbuminemic hyperthyroxinemia subjects, no discrepancy in FT_3 levels was seen, indicating that

the abnormal T_4 binding sites in albumin present in these patients do not interfere with T_3 binding.

In conclusion, the assay of serum FT_3 using analog methods appears to yield satisfactory results in the clinical conditions tested, and may thus represent an interesting addition to total T_3 assay. However, in view of the observed discrepancy between the serum FT_3 Amerlex and the dialysis values in congenital alterations of TBG and during pregnancy, the possibility is to be considered that either the analog method yields binding-proteins dependent errors or alternatively, that the dialysis method—using diluted samples—does not represent the suitable reference method.

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