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Urinary triiodothyronine excretion

I. Orden*, J. Pié, M. G. Juste, A. Giner, M. E. Gómez and J. F. Escanero

Departamento de Fisiología
Facultad de Medicina y Hospital Clínico
Universitario
Zaragoza (Spain)

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The purpose of this work was to estimate the 24 h urinary excretion of free and conjugated triiodotironine (T3) using a direct radioimmunoassay and enzyme hydrolysis. Mean urinary values of free and total T3 (mean ± 1 SD) in euthyroid controls were 2074 ± 673 and 2819 ± 809 pmol/24 h respectively. In patients with hyperthyroidism, values of free hormone were about 4.2 times higher than the mean value of the euthyroid controls, and about one-third in patients with hypothyroidism. These results show this measurement to be useful as an indicator of thyroid function. Mean renal clearance of free T3 was 211.6 ± 62.8 ml/min (mean ± 1 SD) in euthyroid controls, 260.8 ± 87.5 ml/ml in hyperthyroid patients and 229 ± 98.7 ml/min in hypothyroid patients. The data show that T3 renal clearance is, in all cases, greater than glomerular filtration rate, suggesting tubular secretion of T3.

Key words: Urinary triiodothyronine, Renal clearance.

From the circulating triiodothyronine (T3), 99.7 % is normally bound to plasma proteins and 0.3 % circulates free (9). This unbound hormone together with the glucuronidated and sulphated T3, are the fractions that can be cleared by the kidney (10). Information on urinary T3 excretion is scanty, and the papers on urinary elimination of free and conjugated T3 reflect discordant results (2-8, 10-14, 16-19), which may be due to the different methods employed. The present methods to determine serum T3 are quite reliable, but not to measure urinary T3. The purpose of this work was to improve the methodology of urinary free and conjugated T3 determinations and to better if possible the knowledge of the renal handling of this hormone.

* To whom all correspondence should be addressed.

Materials and Methods

Subjects. — The study comprised 132 subjects divided into 3 groups. The first group consisted of 112 euthyroid controls (103 females and 9 males) with a mean age of 24.9 ± 7.6 years. The second one comprised 13 hyperthyroid patients (9 females and 4 males) with a mean age of 53.5 ± 17.7 years. And the third, consist-

ed of 7 hypothyroid patients (7 females) with a mean age of 55.7 ± 4.3 years. Endogenous creatinine renal clearance was estimated to ensure a normal renal function and to exclude gross errors in the collection of urine. The subjects with values above or below our reference values were excluted, thereby reducing the euthyroid group from 134 to 112 subjects. No one received drugs with possible effects on thyroid and renal functions or that could interfere with the employed techniques. None had proteinuria as demonstrated with commercial strips. Thyroid status was evidenced by the clinical picture and routine measurements of serum T3, T4 and TSH.

Experimental material. — Blood samples were obtained in fasting subjects between 08.00 and 10.00 h. Serum was immediately frozen at −20° C. Single 24 h urine collections were carried out beginning at 08.00 h. Total volume was measured and aliquots were kept at −20° C. Samples were thawed immediately before use. Assays were performed within 15 days of collection, since long conservation of frozen urine can unpredictably raise the concentration of T3. This increase has been attributed either to the hydrolysis of conjugates or to the degradation of T4 to T3 during storage (5). Others have affirmed that storage for 2 years does not affect the concentration of iodothyronines in serum or urine (10).

Urinary free T3 assay. — Reagents. We used [^{125}I]-triiodothyronine derivate solution (10^6 × 6.6 Bq/l) and T3 antibody suspension from Amersham International. The cross-reaction with L-thyroxine was < 0.35 %. The T3 standard stock solution was made with L-3,5,3' triiodothyronine, free acid (Sigma) (16). The working standards from 0 to 15.3 nmol/l were performed before each assay by dilution of the stock standard with 0.9 % NaCl. This range was selected from a previous trial. The zero standard was 0.9 % NaCl. Enzymatic hydrolysis was carried out as in a previous work in which T4 conjugates were hydrolized (15).

Procedure. — Urinary T3 values were determined by direct radioimmunoassay. Aliquots, 100 µl, of the standard and unknown urine samples were dispensed into the appropriate tubes. 500 µl of [^{125}I]-L-triiodothyronine derivate solution and 500 µl of T3 antibody suspension were sequentially added. All the tubes were mixed in a vortex and incubated in a water bath at 37° C for two hours. Separation of the antibody-bound fraction was made by centrifugation at 1,000 × *g* for 15 min. The supernatant was immediately decanted from all the tubes except the total count tubes. Thereafter, all tubes were counted in a LKB Wallac gamma counter. The assay sensitivity was 0.08 nmol/l. The coefficient of variation between assays was 6.56 %.

Serum samples. — Serum total and free T3 were estimated by RIA using commercial kits (Abbott Laboratories and Amersham International respectively).

Serum and urine creatinine were assayed by the alkaline picrate method (1).

Statistic. — Statistical evaluation of the data was performed by dispersion analysis.

Results

The results of 24 h urinary T_3 excretion (mean ± 1 SD), T_3 renal clearance and endogenous creatine renal clearance in euthyroid controls, hyperthyroid and hypothyroid patients are shown in table I.

Mean urinary free T_3 excretion in hyperthyroid subject was 4.2 times higher than in euthyroid. In the hypothyroid group, the values was 3 fold lower.

Table I. *Serum total and free T_3, urinary T_3, free T_3 renal clearance and endogenous creatinine clearance values in controls and patients with thyroid disease.*
Values are given as mean ± 1 SD. Reference values (mean ± 2 SD) are given only for the control group; N = number of subjects.

Age in years	Serum total T_3 (nmol/l)	Serum free T_3 (pmol/l)	Urinary free T_3 (pmol/24 h)	Free T_3 clearance (ml/min)	Creatinine clearance (ml/min)	Urinary total T_3 (pmol/24 h)
Euthyroid						
24.9±7.6	2.04±0.36	6.85±1.32	2074±673	211.6±62.8	113.4±21.3	2819±809
N = 112	(1.32–2.76)	(4.21–9.49)	(728–3420)	(86–337.2)	(70.8–156)	(1201–4437)
Hyperthyroid						
53.5±17.7	6.14±5.36	22.21±11.42	8794±7227	260.8±87.5	117.1±33.8	11018±9657
N = 13						
Hypothyroid						
55.7±14.3	0.89±0.36	2.12±1.04	674±439	229±98.7	81±27.2	1309±511
N = 7						

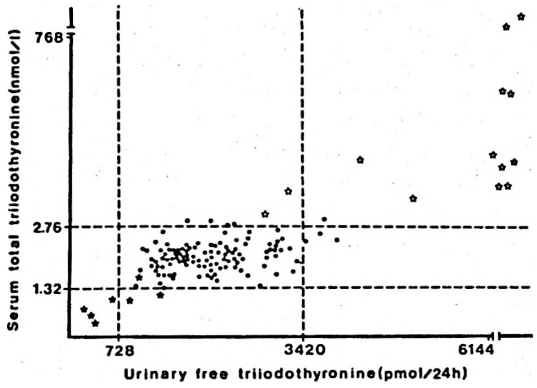

Fig. 1. *Correlation of serum total T_3 and urinary free T_3 values.*
Correlation coefficient, r = 0.95. The areas between dotted lines represent our reference values. • Euthyroid. ☆ Hyperthyroid. ★ Hypothyroid.

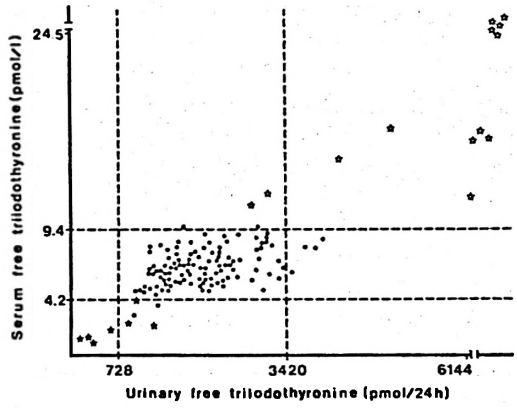

Fig. 2. *Correlation of serum total T_3 and urinary free T_3 values.*
Correlation coefficient, r = 0.90. The areas between dotted lines represent our reference values. (mean ± 2 SD). • Euthyroid. ☆ Hyperthyroid. ★ Hypothyroid.

Positive correlations were found between serum total T_3 and urinary free T_3 r = 0.95 (fig. 1) and between serum free T_3 and urinary free T_3 r = 0.90 (fig. 2).

Discussion

The present results on 24 h urinary excretion of free T3, evaluated by direct radioimmunoassay in healthy euthyroid subjects (table I), are in agreement with several previous studies (2, 8, 11-13), a similar method for T3 estimation being employed by most of them.

The values found by others (10, 14, 16, 18, 19), using RIA on Sephadex columns, were slightly lower, although relatively

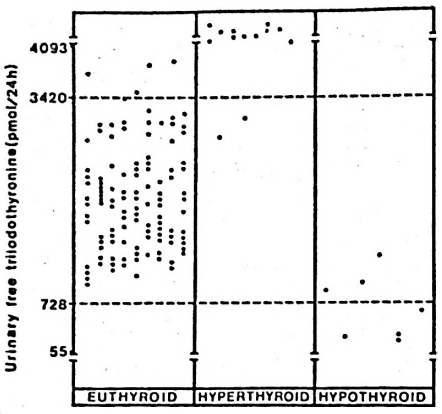

Fig. 3. *Individual values of urinary free triiodothyronine in euthyroid, hyperthyroid and hypothyroid subjects.*
The area between dotted lines represents our reference values (mean ± 2 SD).

close to the ones presented here. The slight discrepancies might be explained either by methodological differences or by the frozen urine storage time; this time may influence the amount of measured hormone (5).

The amount of urinary free T3 excretion reported by Chan et al (7), was higher than the present one. RIA was used by them ethyl-acetate extraction, which included additional material that competes in the assay system. Their greater values can be explained by hydrolysis of conjugates and deiodination of T4 during the extraction step, or to the non-specificity of the T3 antibody or to radiochemical impurities in the labelled hormone employed.

The present results confirm the fact that the daily urinary T3 measurement is useful as a diagnostic indicator of the thyroid function (fig. 3).

The correlations between serum and urinary T3 in our study indicate a better correlation of urinary free T3 with serum total T3 than with the serum unbound fraction. This contrasts with the relationships found with T4 in a previous study where the correlation was better between both free fractions (15).

Our results indicate that 73.6 % of urinary T3 in euthyroid subjects is excreted free, whereas 26.4 % is excreted as glucuronidated and sulphated T3 conjugates. The hyperthyroid patients excreted 79.8 % free T3 and 20.2 % conjugated T3. In the hypothyroid group, 51.5 % of T3 was excreted free and 48.5 % was excreted conjugated. Statistical differences between the percentage of free and conjugated hormone in the euthyroid and hyperthyroid subjects were not significant ($p > 0.05$). However, the statistical difference between euthyroid and hypothyroid patients was highly significant ($P < 0.01$). A similar statistical significance between the percentages of urinary free and conjugated T4 was found, as described in a previous study (15). These percentages of free and conjugated hormone excreted in the urine do not agree with those of BURKE *et al.* (2), GAITAN *et al.* (11) and SHAKESPEAR and BURKE (19). These investigators using acid hydrolysis, obtained percentages of free and conjugated hormone about fifty fifty. The present results are different but quite close to those of FABER *et al.* (10) who also employed enzyme hydrolysis and differentiated between glucuronide and sulphate conjugates. Their findings suggest that T3 and T4 are excreted in the urine, mainly in the free and glucuronidated form, while the excretion of the sulphate form was very low. For that reason and because the enzyme solution used here contained both sulphatase and glucuronidase enzymes differences between glucuronide and sulphate conjugates were not attempted. The discrepancy or similarity between the present results and those of the previously mentioned authors, could be attibuted to the type of hydrolysis employed.

Free T3 renal clearance was on average 211.6 ± 62.8 ml/min in euthyroid sub-

jects, 260.8 ± 87.5 ml/min in hyperthyroid and 229 ± 98.7 ml/min in hypothyroid patients. There are significant differences ($P < 0.05$) between euthyroid and hyperthyroid values, but the statistical difference between euthyroid and hypothyroid subjects was not significant ($P > 0.05$). The endogenous creatinine renal clearance was determined simultaneously (table I), as an indicator of the glomerular filtration rate (GFR). Free T3 renal clearance was in all cases, significantly higher than GFR. This indicates that at any time the mean amount of T3 eliminated is higher than by simple filtration. After evaluation of both clearance values the results indicate that in euthyroid patients about 53.7 % of free T3 eliminated in the urine coincides with that filtered by the glomeruli, while 46.3 % will be added to the tubular flow after filtration. The authors reaching similar conclusions (3-5, 19) suggested an apparent tubular secretion of T3. Nevertheless, this interpretation may be in error. In a previous study (15) by us a percentage of renal reabsorption of T4 was found very close to the apparent T3 secretion of now. BURKE and SHAKESPEAR (6), who obtaind similar results, suggested that the additional urinary T3 could proceed from deiodination of reabsorbed T4, an unlikely theory, because the correlation found by us between the supposable reabsorbed T4 and the secreted T3 was low ($r = 0.52$). The authors previously mentioned arrived at a similar conclusion although by different reasoning. According to them much urinary T3 cannot be ascribed to the re-excretion of deiodinated T4 due to a lack of effect of very low blood T4 levels on apparent T3 clearance. An overestimation of urinary T3 in the present results is considered to be unlikely because of the high specificity of the T3 antibody employed.

Another possible source of urinary T3 might be the conversion of thyroxine to triiodothyronine. This is likely to happen in the urinary tract or in the bladder, where the frequent low pH values may be an important factor. It is probable that T4 deiodination occurs more easily in an acid pH than in a neutral or basic medium (11). Nevertheless it is likely that urinary T3 elimination involves both glomerular filtration and renal tubular secretion.

Resumen

Se cuantifica la triiodotironina (T3) urinaria, libre y conjugada excretada en 24 h, utilizando RIA directo e hidrólisis enzimática. Los valores promedio de T3 libre y total en el grupo control eutiroideo son 2074 ± 673 y 2819 ± 809 pmol/24 h respectivamente. En los pacientes con hipertiroidismo, los valores de hormona libre son 4,2 veces superiores a los valores del grupo control y en el caso de los hipotiroideos 3 veces inferiores. Estos resultados muestran que dicha cuantificación es útil como indicador de la función tiroidea. El valor promedio del aclaramiento renal de T3 libre es de 211,6 ± 62,8 ml/min en el grupo eutiroideo control, 260,8 ± 87,5 ml/min en los pacientes hipertiroideos y 229 ± 98,7 ml/min en los hipotiroideos. Los resultados muestran que el aclaramiento renal de T3 es, en todos los casos, mayor que la velocidad de filtración glomerular, sugiriendo una secreción tubular de T3.

Palabras clave: Triiodotironina urinaria, Aclaramiento renal.

References

1. Bonsnes, R. W. and Taussky, H. H.: *J. Biol. Chem.*, 158, 581-591, 1945.
2. Burke, C. W., Shakespear, R. A. and Fraser, R.: *Lancet*, 2, 1177-1179, 1972.
3. Burke, C. W., Shakespear, R. A. and Fraser, R.: *Clin. Sci.*, 44, 5-6, 1973.
4. Burke, C. W.: *J. R. Coll. Physicians* (London): 8, 335-354, 1974.
5. Burke, C. W. and Eastman, C. J.: *Br. Med. Bull.*, 30, 93-99, 1974.
6. Burke, C. W. and Shakespear, R. A.: *J. Clin. Endocrinol. Metab.*, 42, 504-513, 1976.
7. Chan, V., Landon, J., Besser, G. M. and Ekins, R. P.: *Lancet*, 2, 253-265, 1972.

8. Chan, V.: *Ann. Clin. Biochem.*, 11, 120-129, 1974.
9. Chopra, I. J., Hershman, J. M., Pardridge, W. M. and Nicoloff, J. T.: *Ann. Inter. Med.*, 98, 946-957, 1983.
10. Faber, J., Busch-Sørensen, M., Rogowski, P., Kirkegaard, C., Sierbaek-Nielsen, K. and Friis, T.: *J. Clin. Endocrinol. Metab.*, 53, 587-593, 1981.
11. Gaitan, J. E., Wahner, H. W., Gorman, C. A. and Jiang, N. S.: *J. Lab. Clin. Med.*, 86, 538-546, 1975.
12. Hüfner, M. and Hesch, R. D.: *Lancet*, 1, 101-102, 1973.
13. Iversen, E. and Pedersen, K. O.: *Scand. J. Clin. Lab. Invest.*, 39, 7-13, 1979.
14. Kølendorf, K., Møller, B. and Rogowski, P.: *Acta Endocrinol.* (Copenh), 89, 80-88, 1978.
15. Orden, I., Pié, J., Juste, M. G., Marsellá, A. and Blasco, C.: *Acta Endocrinol.* (Copenh), 114, 503-508, 1987.
16. Rogowski, P. and Siersbaek-Nielsen, K. O.: *Scand. J. Clin. Lab. Invest.*, 37, 729-734, 1977.
17. Rogowski, P., Siersbaek-Nielsen, K. O. and Mólholm Hansen, J.: *Acta Endocrinol.* (Copenh), 87, 525-534, 1978.
18. Rogowski, P. Faber, J. and Siersbaek-Nielsen, K. O.: *Acta Endocrinol.* (Copenh), 94, 337-340, 1980.
19. Shakespear, R. A. and Burke, C. W.: *J. Clin. Endocrinol. Metab.*, 42, 494-503, 1976.