The T₄-free thyroxine index as a test of thyroid function of first choice

P. J. N. HOWORTH¹ AND RICHARD L. WARD

From the Royal Infirmary, Blackburn

SYNOPSIS The T₄-free thyroxine index was used as the thyroid function test of first choice in a district general hospital for one year. Ninety-two patients were assessed both initially and at follow up by a single physician. The index agreed with clinical assessment in 69 out of 74 patients in whom diagnosis was unequivocal. There was initial doubt about thyroid status in 14 patients; after re-assessment the index agreed with status in seven cases, in three cases there is still some uncertainty, whilst the index was at variance with clinical status in four patients. Some possible causes of discrepancy between clinical thyroid status and the index are a low index in euthyroid patients due to a fall in serum thyroxine-binding prealbumin ('sick euthyroid'), a raised index in euthyroid patients due to latent thyroid heart disease, and a normal index in thyrotoxicosis due to preferential secretion of triiodothyronine.

Assessment of thyroid status is a clinical problem in which laboratory tests play an important but subsidiary role; of these the indirect assessment of free thyroxine as a free thyroxine index or factor (Table I) has been found to be a valuable approach. A good thyroid function test must be able to give an abnormal result in definite cases of under- or over-activity of the gland, be helpful in difficult or borderline cases, and be technically feasible for a normal work load. Preliminary results with the T₄-free thyroxine index (T₄-FTI, which is the product of

serum total thyroxine in $\mu\text{g}/100\text{ ml}$ multiplied by resin uptake expressed as a ratio of test uptake over control, using ¹²⁵I-thyroxine for both tests) suggested that it met these requirements (Howorth and MacLagan, 1969). This paper reports results for one year of this test performed on the patients under the care of a single physician in a district general hospital.

Patients and Methods

All (92) patients were assessed initially and followed up by the same physician at his thyroid clinic. Blood was taken at the first attendance and results of the

¹Present address: Department of Chemical Pathology, King's College Hospital Medical School, London, SE5.

Received for publication 21 September 1971.

	Serum Circulating Thyroid Hormone (1)	Serum Free Thyroxine-binding Protein Capacity (2)	Index or Factor
Osorio <i>et al</i> (1962)	Electrophoretic measurement of serum total free thyroxine-binding protein capacity or serum PBI	Figure derived from red cell uptake of ¹³¹ I-T ₄	Factor = $\frac{(1)}{(2)}$
Clark and Horn (1965)	Serum PBI	¹³¹ I-T ₄ -resin uptake ratio $\left(\text{Ratio} = \frac{\% \text{ test uptake}}{\% \text{ control uptake}} \right)$	Index = (1) $\times$ (2)
Goolden <i>et al</i> (1967)	Serum PBI	Figure derived from ¹³¹ I-T ₄ -resin uptake	Factor = $\frac{(1)}{(2)}$
Howorth and MacLagan (1969)	Serum total T ₄ (using ¹²⁵ I-T ₄)	¹²⁵ I-T ₄ -resin uptake ratio	Index = (1) $\times$ (2)

Table I The free thyroxine index or factor

T₄-FTI test were compared with clinical assessment at a follow-up clinic. Fourteen out of the 92 patients had a history of previous thyroidal illness or were already under treatment, and had been referred for reassessment of thyroid status. Ancillary tests were carried out on some patients in whom there was difficulty in classification.

The T₄-free thyroxine index was measured as published (MacLagan and Howorth, 1969). ¹³²I-neck uptake tests, including T₃-suppression and thyroid-stimulating hormone (TSH) tests, were carried out by the method of Hobbs, Bayliss, and MacLagan (1963). A TSH:T₄-FTI stimulation test was carried out by giving three consecutive daily injections of 5 iu TSH (Armour) and measuring the T₄-FTI on days 1, 2, and 5, ie, 48 hr after the third injection of thyroid-stimulating hormone. Serum protein-bound iodine (AutoAnalyzer, Technicon), cholesterol (Zlatkis, Zak, and Boyle, 1953), and T₃-resin uptake (T₃-RU) (Howorth, 1970) were sometimes measured also.

Results

Results of the T₄-FTI for 74 patients, in whom the diagnosis was unequivocal, are given in Figure 1. Results and details of 14 patients in whom there was initial doubt or unresolved findings are given in Table II. The remaining four patients were all taking L-thyroxine orally and are considered separately.

Discussion

Patients were usually referred by family doctors for assessment owing to a suspicion of thyroid disease, or for confirmation of diagnosis and advice about treatment. Although most patients were initially thought to be euthyroid (64 out of 92), all had one or more features making assessment of their thyroid status necessary, eg, anxiety state or thyrotoxicosis during pregnancy (one case each), proximal myopathy (two cases), ophthalmic form of Graves's disease (four cases). Unexplained weight loss and

Patient No.	Age	Sex	T ₄ -FTI ¹	Final Status	Notes and Results for Other Tests
A Patients thought possibly hypothyroid on initial examination					
8	23	F	7.7	Eu	Initially thought possibly hypothyroid; PBI 2.0, ² cholesterol 525, ³ ¹³² I-neck uptake TSH test: ⁴ (1) 12%; (2) after TSH 63%. Euthyroid at follow up: cholesterol 175, PBI 5.1.
13	36	M	3.1	Hypo	Initially thought only possibly hypothyroid; good response to oral T ₄ . PBI 2.3; cholesterol 376; ¹³² I-neck uptake TSH test: (1) 19%; (2) after TSH (i) 15%; (3) (ii) 14%. T ₄ -FTI:TSH test: (1) 3.1; after TSH (2) 3.2; (5) 5.0.
43	68	F	4.4	Hypo	Hypothyroidism confirmed after careful follow up, when PBI was 2.2, T ₃ -resin uptake ⁵ 0.73.
B Patients thought possibly thyrotoxic on initial examination					
7	74	F	14.6	Probably Eu	Depressed and anorexic at follow-up; to be admitted to psychiatric unit. PBI 8.7; cholesterol 210, ¹³² I-neck uptake 21%, initially; PBI 10.0, T ₃ -resin uptake 0.88 at follow up.
52	22	F	8.2	Eu	Possible thyrotoxicosis during pregnancy; euthyroid at follow up after delivery.
114	65	M	9.5	Probably Eu	Carcinoma bronchus removed before follow up, when PBI 9.2; T ₃ -resin uptake 0.96. Still possibility of mild Graves's disease.
124	69	M	14.0	Toxic	Thought only possibly toxic initially; good response to carbimazole. T ₃ -resin uptake 1.58.
C Patients thought probably euthyroid on initial examination					
50	72	F	16.5	Toxic	Good response to carbimazole.
55	41	F	13.6	Eu	Possible relapse of thyrotoxicosis but euthyroid at follow up. ¹³² I-neck uptake 23%, initially; PBI 9.7, T ₃ -resin uptake 0.9 at follow up.
84	58	M	14.1	Eu	Ophthalmic Graves's disease; on steroids. PBI 11.7, ¹³² I-neck uptake 16% initially; PBI 6.2, T ₃ -resin uptake 1.0 at follow up.
91	64	F	17.1	Possibly toxic	Still unclear after follow up; weight loss, tremor, and atrial fibrillation present. PBI 10.0; T ₃ -resin uptake 1.0 at follow up.
90	48	F	17.0	Toxic	Relapse of Graves's disease; initially euthyroid (T ₄ -FTI 7.2) and carbimazole withheld.
109	64	F	9.0	Toxic	On carbimazole. Before treatment: PBI 6.0; T ₃ -resin uptake 1.42; after: PBI 4.6; T ₃ -resin uptake 1.12.
117	60	F	13.4	Possibly toxic	Anxious and only possibly toxic at follow up; referred for psychiatric assessment. T ₃ -resin uptake 1.32 initially, 1.14 at follow up. PBI 13.7 at follow up.

Table II T₄-FTI in cases with initial doubt or unresolved findings

¹Normal range 4.5-11.5.

²Normal range 3.5-7.5 μg/100 ml.

³Normal range 100-300 mg/100 ml.

⁴Normal range 11-33% initial uptake, > 30% after TSH.

⁵Normal range 0.85-1.30.

Fig. 1. T_4 -free thyroxine index in 74 patients classified unequivocally. Dotted lines indicate normal limits.

atrial fibrillation were common. Many patients were taking drugs known to interfere with thyroid function tests, such as carbimazole and chlorthalidoxepoxide which interfere with the radioiodine neck uptake test and lower T_4 and T_4 -FTI, or oral contraceptive tablets which raise the T_4 and PBI but not the T_4 -free thyroxine index.

Assessment of thyroid status was relatively simple in 74 patients, whose T_4 -FTI results are given in Figure 1. All patients diagnosed as having over- or underactivity of the thyroid had correspondingly abnormal T_4 -FTI levels and were considered to require treatment. Of those diagnosed as euthyroid 48 had normal T_4 -FTI levels, but five had low levels. Ten out of the 74 patients had a misleading serum T_4 result (four toxic and two hypothyroid patients had a normal serum T_4 : four euthyroid patients had a low T_4). Nine had a misleading result for the resin uptake test (eight results from euthyroid patients were outside the normal range: five low and three high; one hypothyroid patient had a normal result). However, one of the virtues of the free thyroxine index is that a marginal result from either the serum T_4 or resin uptake test in euthyroid patients still usually gives a normal free thyroxine index, except in some 'sick euthyroid' patients in whom a diminution in serum thyroxine-binding pre-albumin con-

centration may result in a low T_4 and hence low T_4 -free thyroxine index. The reason why there is not a corresponding rise in the resin uptake test in such 'sick euthyroid' patients is probably that the test depends mainly on the degree of saturation of the thyroxine-binding globulin and the percentage free thyroxine, and not at all on thyroxine-binding pre-albumin since the test is carried out under conditions which inhibit thyroxine binding to prealbumin (Myant and Osorio, 1960; Tata, Widnell, and Gratzner, 1961). Thus resin uptake tests are usually carried out at either acid pH or at pH 8.6 in barbitone buffer. This may be the explanation of the five low T_4 -FTI results in euthyroid patients.

Initial assessment was difficult for the T_4 -FTI level discrepancy in 18 patients, who fall into two main groups.

One group consists of four patients taking oral L-thyroxine who were clinically euthyroid but had abnormal T_4 -FTI levels of 3.6, 12.7, 16.8, and 17.8. The test is therefore unreliable in such patients. The high values might be explained on the grounds that normally at least half the total plasma thyroid hormone activity is due to T_3 and that, if thyroid hormones are replaced by T_4 only, plasma T_4 needs to be higher than normal to maintain a euthyroid state. It may be, therefore, that treatment with a combination of T_3 and T_4 in physiological proportions would make T_4 -FTI estimations more reliable for controlling therapy (Taylor, Kapur, and Adie, 1970). However recent work indicates that exogenous T_4 is converted to T_3 in the body, and that plasma T_3 levels in patients on T_4 replacement therapy may be normal or even above normal (Braverman, Ingbar, and Sterling, 1970).

The other group of 14 cases, in all of which the initial clinical assessment was doubtful, can be subdivided into three subgroups (Table II). In subgroup A all three patients were on balance judged initially to be hypothyroid; two were finally classified as hypothyroid and did in fact have low T_4 -FTI levels; the other was finally diagnosed as euthyroid and had a normal T_4 -FTI level. In subgroup B, all four patients were initially judged on balance to be hyperthyroid; only one was finally placed in this category and did in fact have a high T_4 -FTI level, whereas the other three were finally classified as euthyroid and of these two had normal T_4 -FTI levels, the other having a raised level. In subgroup C all seven patients were initially considered to be euthyroid; of these two were finally classified as euthyroid though in each case the T_4 -FTI was raised whereas five were finally classified as hyperthyroid and all but one had high T_4 -FTI levels, the other having a normal level.

Hence in the 74 patients where the clinical status was clear the T_4 -FTI level was consistent in all but

five. In the 14 patients where the clinical status was initially doubtful, the T_4 -FTI level was consistent with the final diagnosis in all but four. The discrepant results in these four cases may not be due to analytical error. Thus, case 109 (subgroup C), thyrotoxicosis with normal serum PBI and T_4 -FTI, could be due to preferential secretion of excess T_3 (' T_3 thyrotoxicosis'), whilst the euthyroid patients, nos. 7, 55, and 84 (subgroups B and C), all had a raised PBI as well as a raised index. Thyroid heart disease, in which euthyroid patients may have a raised PBI, was considered in all these latter three patients but excluded on the basis of normal heart size and sinus rhythm.

The T_4 -FTI is within the scope of a busy laboratory in a district general hospital. It meets most of the needs for a routine thyroid function test since it was accurate (Fig. 1) and helpful in most of the difficult borderline cases (Table II). T_4 is preferable to PBI as a basis for the free thyroxine index test as it is less affected by drugs or by contamination. However, 'demand inflation' on laboratory services has led to the perpetuation of less accurate tests such as the serum PBI for screening purposes because they are more suitable for mechanization.

We thank the scientific and technical staff of the Pathology Department at the Royal Infirmary, Blackburn, for carrying out many of the ancillary tests, and Dr L. Parker, Crumpsall Hospital,

Manchester, for results for serum protein-bound iodine.

References

- Braverman, L. E., Ingbar, S. H., and Sterling, K. (1970). Conversion of thyroxine (T_4) to triiodothyronine (T_3) in athyreotic human subjects. *J. clin. Invest.*, **49**, 855-864.
- Clark, F., and Horn, D. B. (1965). Assessment of thyroid function by the combined use of the serum protein-bound iodine and resin uptake of ^{131}I -triiodothyronine. *J. clin. Endocr.*, **25**, 39-45.
- Goolden, A. W. G., Gartside, J. M., and Sanderson, C. (1967). Thyroid status in pregnancy and in women taking oral contraceptives. *Lancet*, **1**, 12-15.
- Hobbs, J. R., Bayliss, R. I. S., and MacLagan, N. F. (1963). The routine use of ^{131}I in the diagnosis of thyroid disease. *Lancet*, **1**, 8-13.
- Howorth, P. J. N. (1970). *In vitro* thyroid function tests using ^{125}I -labelled triiodothyronine and thyroxine. *Ann. clin. Biochem.*, **7**, 55-59.
- Howorth, P. J. N., and MacLagan, N. F. (1969). Clinical application of serum-total-thyroxine estimation, resin uptake, and free-thyroxine index. *Lancet*, **1**, 224-228.
- MacLagan, N. F., and Howorth, P. J. N. (1969). Thyroid function studies using resin uptake of radioactive thyronines from serum and total thyroxine assay: the free thyroxine index. *Clin. Sci.*, **37**, 45-60.
- Myant, N. B., and Osorio, C. (1960). Paper electrophoresis of thyroxine in tris-maleate buffer. *J. Physiol. (Lond.)*, **152**, 601-612.
- Osorio, C., Jackson, D. J., Gartside, J. M., and Goolden, A. W. G. (1962). The assessment of free thyroxine in plasma. *Clin. Sci.*, **23**, 525-530.
- Tata, J. R., Widnell, C. C., and Gratzer, W. B. (1961). A systematic study of factors affecting the binding of thyroxine and related substances to serum proteins. *Clin. chim. Acta*, **6**, 597-612.
- Taylor, S., Kapur, M., and Adie, R. (1970). Combined thyroxine and triiodothyronine for thyroid replacement therapy. *Brit. med. J.*, **2**, 270-271.
- Zlatkis, A., Zak, B., and Boyle, A. J. (1953). A new method for the direct determination of serum cholesterol. *J. Lab. clin. Med.*, **41**, 486-492.