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Concentration of thyroxine-binding globulin: value of direct assay

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Summary

The concentration of thyroxine-binding globulin (TBG) in the serum can now be measured by direct assays that are simple and inexpensive. Comparison of a direct measurement of TBG concentration with a widely used indirect method (Thyopac-3) showed that the indirect method was inaccurate when TBG concentrations were high. This will result in an increase in the derived free thyroxine index (FTI), so that euthyroid patients with a raised TBG concentration may be at risk of being labelled thyrotoxic. Correction of serum total thyroxine (T4) concentration according to the actual TBG concentration (T4:TBG ratio) provided a better correlation with thyroid state than FTI.

Introduction

Thyroxine-binding globulin (TBG) binds about 70% of the total serum thyroxine (T4),[1] and serum TBG concentrations may vary widely under the influence of hormones, drugs, disease, and genetic factors.[2] Some estimate of TBG concentration is therefore necessary for the correct interpretation of serum T4 values. Measurement of TBG from the maximum T4-binding capacity[3] is unsuitable for routine use, and this has led to the development of indirect assays of TBG such as the triiodothyronine (T3) uptake tests. These tests assess the number of unoccupied T4-binding sites in a sample by partitioning radiolabelled T3 between the serum sample and red cells, ion-exchange resin, or Sephadex.[4] T3 uptake tests have proved clinically useful and have been combined with measurement of protein-bound iodine[5] or total T4[6] to yield a free thyroxine index (FTI; calculated as T4/T3 uptake), which has been found to correlate with both the clinical state of patients and the free T4 concentration as measured direct.[7]

T3 uptake tests are highly reproducible[8] but their sensitivity to changes in TBG concentration has not been critically evaluated. The possibility that T3 uptake may not be an accurate measure of high concentrations of TBG was suggested by the finding of a raised FTI in pregnancy[9] and during oral contraception.[10]

We recently described a rapid and simple immunoelectrophoretic assay of TBG.[11] Several radioimmunoassays of TBG have been described,[12-15] and a commercial kit assay is available in the USA.* In this study we examine the relation between the results of a T3 uptake test (Thyopac-3[16]) and TBG concentrations in healthy euthyroid people with widely differing concentrations of TBG, and compare the ability of the FTI and T4:TBG ratio to distinguish between thyrotoxic, myxoedematous, and euthyroid states.

Subjects and methods

The following groups were studied: 42 healthy euthyroid volunteers aged 18-50 years (29 men, 13 women); 10 pregnant women in the second and third trimesters; nine women taking oral contraceptives (the "pill"); 16 patients with inherited high levels of serum TBG (12 female, 4 male); and five male patients with inherited absence of TBG.

The patients with inherited abnormalities of TBG concentration were identified over 12 months by means of the TBG assay.[17] Serum was also obtained from 21 patients with clinically and biochemically proved thyrotoxicosis and 10 patients with myxoedema. These patients were selected retrospectively from a group of patients attending a thyroid clinic and seen by one of us (WAB).

Serum TBG was measured by rocket immunoelectrophoresis[18] with the use of monospecific antiserum to human TBG.[11] Serum T4 was measured by a radioimmunoassay method based on that of Ratcliffe *et al*[19] but modified to use a 20-µl sample with 900 µg 8-anilinonaphthalene-1-sulphonic acid per tube and a Dowex 1 × 8400 resin (Sigma Chemical Co, London) to separate antibody bound from unbound T4. T3 uptake was measured by a commercial kit method (Thyopac-3, Radiochemical Centre, Amersham), and FTI and T4:TBG ratio were calculated for each sample.

Results

TBG CONCENTRATION

Fig 1 shows the serum TBG concentrations of the various groups studied. The mean concentration in normal men (±SE of mean) was

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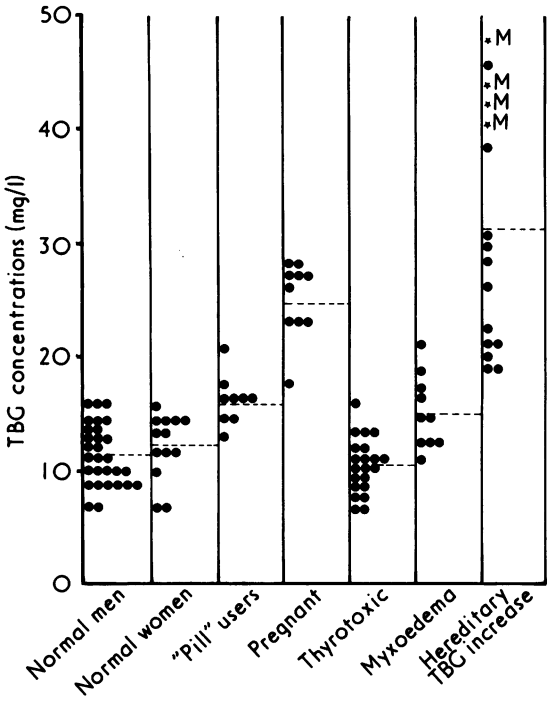

FIG 1—TBG concentrations of subjects studied. Male patients with familial increase in TBG are denoted by ★M. Dashed lines indicate mean values.

11·2±0·49 mg/l, and in normal women 12·5±0·75 mg/l, the difference not being significant. Women using oral contraceptives had a mean value of 16·1±0·73 mg/l, and pregnant women a mean value of 24·9±1·08 mg/l, both being significantly higher than normal ($P < 0.0025$ for pill users; $P < 0.0005$ for pregnant women). Thyrotoxic patients had a mean value of 10·4±0·5 mg/l, which was significantly less than normal ($P < 0.05$), while myxoedematous patients had a mean value of 14·9±1·01 mg/l, which was greater than normal ($P < 0.0025$).

Extremely high concentrations of serum TBG were found in patients with hereditary high levels, with mean values of 43·9±2·1 mg/l in male patients and 26·5±2·4 mg/l in female patients. These patients also had abnormally high concentrations of total serum T4, with mean values of 317±42 nmol (246±33 μg)/l and 204±14 nmol (159±11 μg)/l in male and female patients respectively. Patients with undetectable TBG had a mean serum T4 concentration of 20±1·6 nmol (15·5±1·2 μg)/l (see table).

Results of thyroid function studies in patients with undetectable TBG

Case No	T4 (nmol/l)	Thyopac-3 value	FTI
1	25	59	0·42
2	22	64	0·34
3	20	63	0·32
4	17	42	0·40
5	16	61	0·26
Mean±SE of mean	20±1·6	57·8±4·0	0·35±0·03
Reference values	60–130	92–120	0·58–1·37

Conversion: SI to traditional units—T4: 1 nmol/l ≈ 0·8 μg/l.

T3 UPTAKE AND TBG CONCENTRATION

Fig 2 shows the relation between the Thyopac-3 value and the TBG concentration in all euthyroid subjects studied. At high concentrations of TBG the relation became non-linear, the Thyopac-3 curve becoming a plateau at about 130, above which little or no increase occurred despite the TBG concentration doubling from 25 to 50 mg/l.

In the five patients with undetectable TBG the Thyopac-3 value ranged from 42 to 64 (table), presumably reflecting the contribution of thyroxine-binding prealbumin and other T4-binding proteins to the Thyopac-3 result.

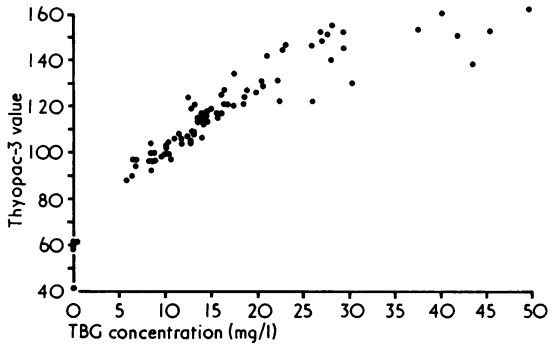

FIG 2—Relation between Thyopac-3 value and TBG concentration in euthyroid subjects.

SERUM T4, T3 UPTAKE, AND TBG CONCENTRATION

Fig 3 shows the relation between serum T4 and T3 uptake in euthyroid, thyrotoxic, and myxoedematous subjects. In the euthyroid people a good correlation ($r = 0.81$) was obtained between the two values, although with high serum T4 concentrations, in pregnant patients, and in those with high congenital concentrations of TBG there was a tendency to deviate from the linear relationship, so that increments in T4 concentration were associated with progressively smaller increases in Thyopac-3 values. The ability of serum T4 and T3 uptake to distinguish between euthyroid, thyrotoxic, and myxoedematous states, when the results are plotted graphically, is also shown in fig 3.

Serum T4 concentration relative to serum TBG is shown in fig 4

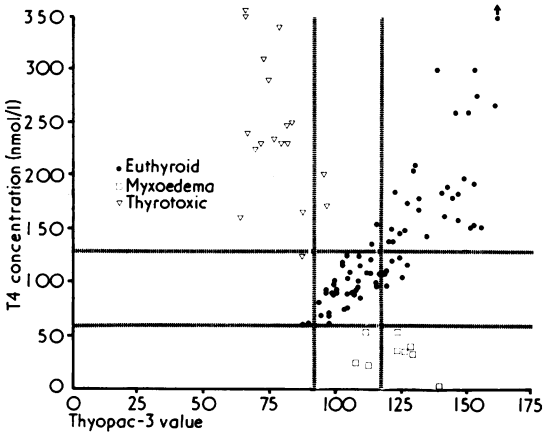

FIG 3—Relation between serum T4 concentration and Thyopac-3 value.
Conversion: SI to traditional units—T4: 1 nmol/l ≈ 0·8 μg/l.

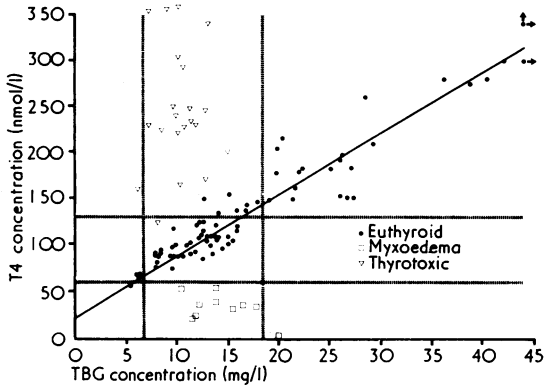

FIG 4—Relation between serum T4 and TBG concentrations.
Conversion: SI to traditional units—T4: 1 nmol/l ≈ 0·8 μg/l.

With these two values there was a linear relationship throughout the range of TBG concentrations in the euthyroid subjects, and a good correlation was found ($r = 0{\cdot}94$). Serum T4 and TBG concentrations also distinguish between euthyroid, thyrotoxic, and myxoedematous states when plotted graphically. From the graph of T4 against TBG it may be calculated that the T4 concentration corresponding to a TBG concentration of zero is 11·3 nmol (8·8 μg)/l. This is somewhat lower than the mean of 20 nmol (15·5 μg)/l observed in the five patients with undetectable TBG.

FTI AND T4:TBG RATIO

FTI (fig 5) differentiated well between thyrotoxic, myxoedematous, and euthyroid subjects with normal TBG concentrations. In the pill users and pregnant women the FTI was within 2 SD of the normal mean, although the mean value was higher than normal. Of the 16 patients with congenital high concentrations of TBG, however, 13 had an FTI within the thyrotoxic range.

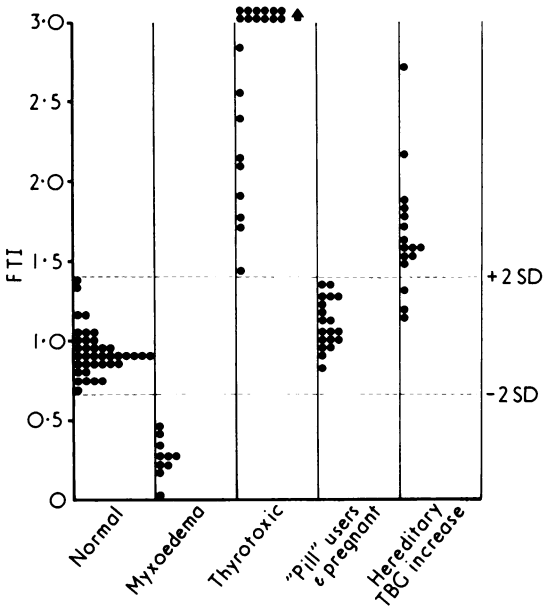

FIG 5—FTI in abnormalities of thyroid function and TBG concentration.

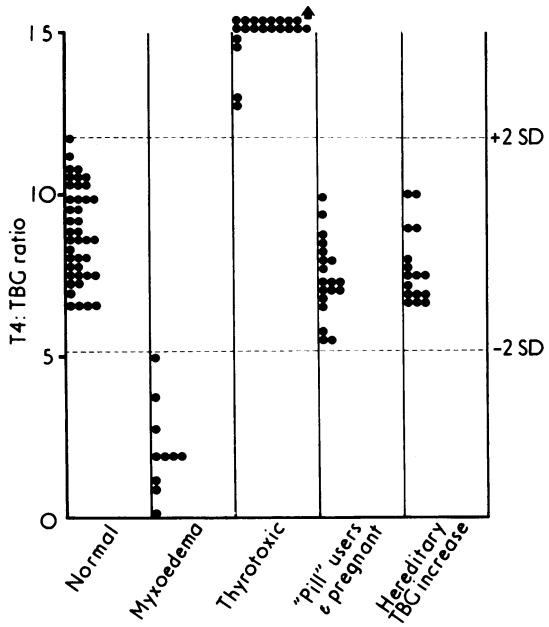

FIG 6—T4:TBG ratio in abnormalities of thyroid function and TBG concentration.

The T4:TBG ratio (fig 6) appeared to be able to differentiate between euthyroid subjects with normal TBG concentrations and those with thyrotoxicosis and myxoedema, at least as well as FTI. In euthyroid patients with raised TBG concentrations the T4:TBG ratio gave values in the euthyroid range, even in the group with extreme increases in TBG and T4 due to hereditary factors, in which the FTI gave "thyrotoxic" results.

In those patients with absent TBG, who were clinically euthyroid the FTI was in the myxoedematous range in all cases (table). T4:TBG could not be applied to these patients, but the knowledge that they had undetectable TBG helped in interpreting the low T4 concentration.

Discussion

Direct measurement of serum TBG will probably soon be available as a routine diagnostic aid. The assay we used is both simple and inexpensive and may be applied to large numbers of samples. The normal values for serum TBG we found are comparable with those reported by some investigators,[14 15] although higher values have been suggested by others.[12 13] Direct comparisons are difficult owing to the lack of an international standard for TBG. We did not find a significant difference in TBG concentrations between men and women, although the numbers in each group were rather small, and larger numbers might have confirmed a tendency for higher values in women. The lower TBG values in thyrotoxicosis and raised values in myxoedema confirm reports made on the basis of binding-capacity measurements.[20] Our myxoedematous patients tended to be older than the thyrotoxic and euthryoid subjects, and this might have contributed to the higher TBG values found, as suggested by Hesch *et al.*[21]

The relation between T3 uptake and TBG concentration was non-linear at high concentrations of TBG, with the result that T3 uptake tended to underestimate TBG concentration. This was most pronounced in the patients with hereditary high concentrations of TBG but in some cases occurred with T3 uptakes as low as 130, which may be found during oral contraception and pregnancy. An underestimate of TBG concentration would explain the high values of FTI found by other workers in patients with raised TBG concentrations due to the "pill" and pregnancy.[9 10] Similarly, the high FTI found in our patients with a hereditary increase in TBG could have been due to the inability of T3 uptake to measure high TBG concentrations. Another possible explanation would be that free thyroxine concentration is actually increased in such patients, but in most reports of a hereditary increase in TBG the free thyroxine concentration has been normal.[22-24] We measured the free thyroxine concentration in our patients and found it to be normal.[17]

An excellent correlation was found in euthyroid subjects between total T4 and TBG concentrations. This suggests that serum TBG is the major determinant of total T4 concentrations in euthyroid people and that the contribution of other binding proteins may be ignored for practical purposes. The linearity of the euthyroid T4:TBG relationship compared with the non-linear relationship between T4 and T3 uptake also explains the ability of the T4:TBG ratio to correct for high concentrations of TBG, as opposed to the failure of the FTI. In fact, the T4:TBG ratio, using binding-capacity measurement of TBG, was found by Harvey[25] to be the best diagnostic index of thyroid function. The mean T4 concentration of 20 nmol (15·5 μg)/l found in patients with undetectable TBG presumably represents the T4 associated with thyroxine-binding prealbumin, albumin, and other T4-binding proteins. The observed value was closely similar to the theoretical value of 11·3 nmol (8·8 μg)/l derived from the graph of T4 and TBG concentration, and the small difference may be attributed to inaccuracy of the T4 radioimmunoassay at this level and the presence of undetectable amounts of TBG (< 100 μg/l).

In 12 months we detected 21 patients with inherited abnormalities of TBG concentration, suggesting that the condition is not rare. The conflicting results obtained with conventional

thyroid function tests in such patients are readily resolved by the direct measurement of TBG.

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Effect of eating liquorice on the renin-angiotensin aldosterone axis in normal subjects

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Summary

The effect of confectionery liquorice on electrolyte status and the renin-angiotensin-aldosterone (RAA) axis was studied in 14 healthy volunteers. They ate liquorice in daily doses of 100 g or 200 g (equivalent to 0·7-1·4 g glycyrrhizinic acid) for one to four weeks.

Plasma potassium concentrations fell by over 0·3 mmol/l in 11 people, including four who had to be withdrawn from the study because of hypokalaemia. One or more values of the RAA axis, especially plasma renin activity and urinary aldosterone concentrations, were considerably depressed in all subjects. These results show that potentially serious metabolic effects may occur in some people who eat modest amounts of liquorice daily for less than a week.

Introduction

The syndrome of pseudo-primary aldosteronism is a well-known complication of chronic liquorice ingestion.[1-4] Glycyrrhizinic acid, the active component of liquorice, has mineralocorticoid effects on electrolyte balance in normal people when taken in high doses (4-6 g),[5] but the extent to which liquorice as sweets affects the electrolyte balance and the renin-angiotensin-aldosterone (RAA) axis in normal people is not known. Such information is important in view of the amount that liquorice is eaten in the community and the serious nature of chronic liquorice intoxication. We have attempted to answer some of these questions.

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Subjects and methods

Fourteen volunteers were studied. There were nine women aged 19 to 40 years and five men aged 21 to 46 years. None weighed more than 15% above ideal body weight. All subjects were normotensive and none had taken any medication, oral contraceptives, or liquorice in the preceding month. All subjects were studied when eating their usual home diets. Five men and four women ate 100 g of liquorice (two confectionery twists) daily and five women ate 200 g (four confectionery twists) daily for one to four weeks. Subjects were examined before the study, each week during it, and one (13 subjects) and two (11 subjects) weeks after liquorice was withdrawn. At each assessment lying and standing blood pressure and body weight were measured. Blood was drawn at 10 am for measurement of plasma aldosterone, plasma renin activity (PRA), plasma angiotensin II, and plasma electrolytes. On the same day a 24-hour urine collection for determining aldosterone, sodium, and potassium concentrations was taken.

PRA, angiotensin II, and aldosterone were measured by radioimmunoassay.[6-9] All samples from a single subject were measured in the same assay, and when possible several subjects' samples were included in the same assay.

Results

Eight subjects (five men and three women) completed four weeks of liquorice ingestion. Liquorice was prematurely withdrawn from six women because of either hypokalaemia (in four) or uncomfortable oedema of the face, hands, and ankles (in two). Another four people (one man and three women developed mild transient generalised oedema. Other side effects were headache in three subjects and lethargy in four. Blood pressure did not rise significantly in any subject. Ten people suffered a weight gain greater than 1 kg, and the two subjects with the most pronounced oedema gained 2·3 kg and 4 kg in one and three weeks. The mean weight gain for the whole group was 1·5±(SD) 0·7 kg. In all cases weight returned to control levels within one week of liquorice withdrawal.

Electrolyte imbalance was shown in most subjects on both dose