The Effect of Total Binding Capacity of Thyroxine Binding Globulin on the Free Thyroxine Index

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In search of a definite source of misleading free thyroxine index (FT_4I), the relationships between in vitro thyroid testing results and thyroxine-binding globulin (TBG) capacities were reexamined in sera from a population with a relatively high prevalence of serum TBG alterations. Sera from 21 subjects with different total thyroxine-binding globulin capacities (TTBG), were loaded with graded amounts of thyroxine (T_4) and assayed for T_4, T_3 uptake (T_3U), TTBG, and free T_4 concentration (FT_4). Serum T_4, T_3U, and the calculated FT_4 index (FT_4I) were able to separate efficiently the samples according to their FT_4, but their respective normal ranges varied with TTBG. Interpretation of the results of the in vitro tests, in the light of TTBG, greatly improved their operating characteristics in the study of 141 patients with a high prevalence of TBG alterations. The misleading FT_4I is not the outcome of reduced intrinsic sensitivities of the in vitro tests, but a consequence of a shift of their normal ranges caused by a change of TTBG. By estimating TTBG from the values of T_4 and T_3U, this problem is easily solved without adding cost.

The total thyroxine concentration (T_4) is a reliable indicator of thyroid function in most individuals, but its values are also affected by altered concentrations of thyroxine-binding globulin (TBG) in serum (*1*). The use of a free thyroxine index (FT_4I)—obtained as the product of a T_4 assay and the in vitro uptake of radioactive triidothyronine by a secondary binder (T_3U)—has been a valuable means of obtaining data regarding the actual free T_4 (FT_4) concentration in serum (*2*). This index has been shown to be superior to several FT_4 assays in accurately assessing the thyrometabolic status of clinically euthyroid subjects with relatively high or low T_3U (*3, 4*). Unfortunately, this index may render misleading results in some clinical circumstances characterized by serious alterations of TBG in serum (*5–8*). These limitations of the FT_4I have been ascribed to a reduction of the intrinsic sensitivities of T_3U (*3, 9*) and T_4 (*3*) assays when TBG is oversaturated by endogenous T_4. This situation may occur in sera from euthyroid individuals with abnormally reduced TBG concentration (*10*), or from thyrotoxic patients with normal or reduced total TBG binding capacity (TTBG) (*11*). Furthermore, the calculation of the FT_4I does not consider the association constant between T_4 and TBG demanded by the law of chemical mass action, and this may lead to an error (*10,11*).

In an effort to elucidate a definite source of the misleading FT_4I, the relationship between T_4, T_3U, FT_4I, FT_4, and TTBG were reexamined in sera from a population with a relatively high prevalence of serum TBG alterations.

MATERIALS AND METHODS

Experimental Study

This study was performed with sera from 21 subjects: seven euthyroid, six thyrotoxic, and eight hypothyroid. Diagnosis was made upon integrating the clinical findings, the clinical course, the serum FT_4 concentration as measured by equilibrium dialysis, and TTBG as measured by paper electrophoresis. Sera were selected in order to cover a wide range of values for TTBG (7.3–43.6 μg T_4/dl of serum) as follows: six sera with low TTBG ($<$ 15.0 μg T_4/dl), eight with normal TTBG (15–28 μg T_4/dl), and seven with high TTBG ($>$ 28 μg T_4/dl). All sera with reduced TTBG were collected from seriously ill cardiac patients with nephrosis and/or malnutrition, and all sera with high TTBG were collected from women under chronic treatment with various anovulatories.

Aliquots from all samples were prepared with the following increasing amounts of exogenous T_4: 0, 0.4, 0.8, 1.1, 2.2, 4.4, 5.8, 8.8, 11.6, and 17.6 μg/dl. To avoid the effects of dilution, the hormone solution was added to the test tube before the sample and kept at room temperature until totally dry. The serum aliquot was then added and maintained under constant shaking at room temperature for at least 1 hr to assure a proper distribution of the added T_4 in the sample.

Serum aliquots were then assayed in triplicate for total T_4 concentration* (normal: 4.5–11.5 μg/dl), $T_3U^{\dagger}$ (normal: 35%–45%), FT_4 concentration by equilibrium dialysis in 0.04 *M* veronal buffer (normal: 1.2–3.6 ng/dl), and TTBG by paper electrophoresis in 0.04 *M* veronal buffer (normal: 15–28 μg

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of T_4/dl). A FT_4I was calculated as the product of T_4 and T_3U divided by 100 (normal: 2.2–4.7). Results obtained in those aliquots with oversaturated TBG (more T_4 than TTBG) were excluded from the graphic analysis.

Clinical Study

This study was performed with sera from 141 subjects, including the 21 patients of the experimental investigation. Table 1 shows the distribution of these subjects according to thyroid function and TTBG. Diagnosis was achieved in each case upon integrating the clinical data with the information attained at the laboratory as follows: FT_4 as measured by equilibrium dialysis, and TTBG as measured by paper electrophoresis.

RESULTS

Experimental

Figure 1 shows the dependence of T_4, T_3U, and FT_4I on the total TBG capacity—the normal range for each parameter varies with TTBG. Although the limits of the normal ranges are visually drawn, accurate separation of those aliquots with normal FT_4 concentration from those with thyrotoxic and hypothyroid levels of the free hormone can be made independently of the magnitude of TTBG.

Clinical

Figure 2 shows the results obtained in sera from 141 patients (Table 1). A clear-cut distinction between euthyroid, thyrotoxic, and hypothyroid subjects are obtained with either T_4, T_3U, or FT_4I by using their variant normal limits according to the measured TTBG. The data from these figures are used to calculate the operating characteristics (sensitivity, specificity, and accuracy) for the in vitro thyroid tests by using both their usual fixed normal limits and those modulated by TTBG (Table 2). In the 141 clinical samples analyzed by the latter technique, the previously misleading FT_4I values of 25 (17.7%) sera are aligned with the true diagnostic condition of the patient, notably increasing its operating characteristics.

DISCUSSION

Our results seem to enhance the understanding of the importance of TBG as a regulator of the thyrometabolic status of the subject. Serum T_4 concentration, T_3U, and the FT_4I derived from them, are all dependent on the total binding capacity of TBG (Figs. 1 and 2). If it were not for the normal and abnormal variations on TTBG, either of these concentrations would be a good indicator of thyroid function. Serum T_4 and T_3U efficiently separate the serum samples according to the accepted thyrometabolic status of their donors at any of the TTBG levels included in this study (7.3–43.6 μg T_4/dl), but their normal ranges varied with TTBG. Significant shifts in their respective normal ranges were observed even within the normal spectrum for TTBG (15–28 μg T_4/dl). These effects were more significant in sera with abnormal TTBG. It is clear that, even at the extremes of the normal range for TTBG, these procedures would render erroneous results according to their conventionally established normal values.

The normal spectrum for total T_4 concentration in serum, rises and widens as TTBG is increased as shown in Figures 1A and 2A. The correlation equations governing its limits seem to indicate that euthyroidism is maintained whenever serum T_4 concentration is strong enough to saturate between 25% and 50% of the total binding sites in TBG. Whereas hypothyroidism is the outcome of the abnormally reduced FT_4 concentration when the total hormone concentration in serum is lower than 25% of the TTBG, thyrotoxicosis results from the abnormally increased FT_4 concentration in serum when the total hormone concentration is higher than half the total binding capacity of the protein.

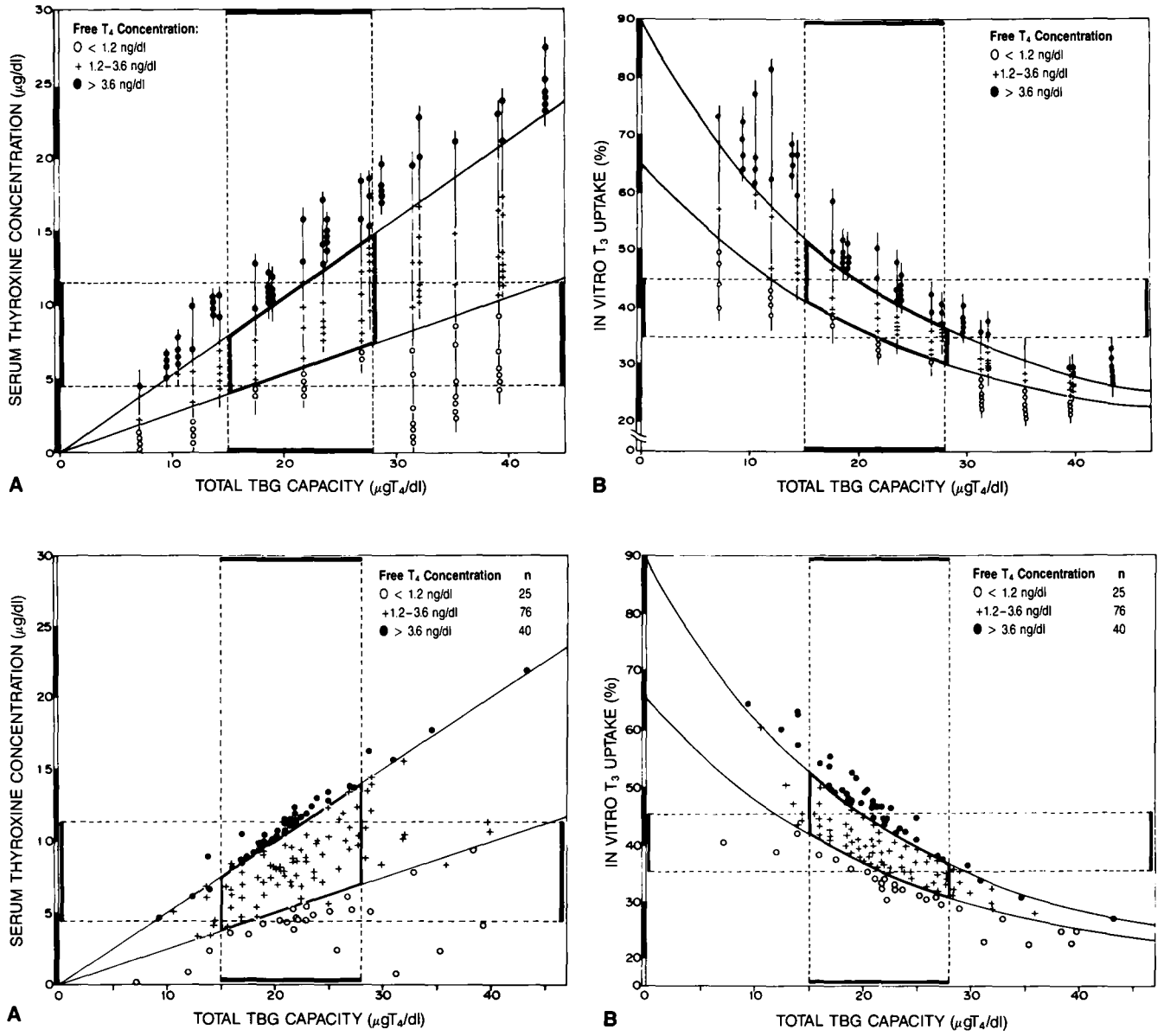
The peculiar features of the normal band for T_4 along the full scale of TTBG means that the normal range for T_4 is lower and narrower (3.75–7.5 μg/dl) when TTBG is at its lower normal value (15 μg T_4/dl) than when it is at its higher normal level (28 μg T_4/dl). In this case, T_4 should be between 7.0–14.0 μg/dl in order to sustain a euthyroid state. This behavior explains not only the low sensitivity of T_4 for thyrotoxicosis and its low specificity for hypothyroidism at relatively reduced levels of TTBG, but also its low sensitivity for the latter and its low specificity for the former at relatively high TTBG values when the diagnostic decision is based on its fixed conventional normal values (4.5–11.5 μg/dl) (Table 2).

The conduct of T_3U in relation to TTBG is the inverse of that of T_4: its normal spectrum narrows and reduces as TTBG is increased (Figs. 1B and 2B). This is a clear consequence of the increase of free binding sites in the protein and the consecutive reduction of the fraction of radioactive T_3 available to the secondary binder (*5, 10, 11*). At relatively low TTBG levels (15 μg T_4/dl), the normal range for T_3U is 43%–52%, but it shifts to 30%–36% at relatively high levels of TTBG (28 μg T_4/dl). This behavior explains its low sensitivity for

TABLE 1. Case Distribution According to Thyroid Function and Total TBG Capacity

Total TBG Capacity	Clinical Diagnosis			Total	Prevalence
	Thyrotoxicosis	Euthyroidism	Hypothyroidism		
Low	4	6–5	3	12	0.850
Normal	32	60	16	108	0.766
High	4	11	6	21	0.149
Total	40	76	25	141	—

*The prevalence of thyrotoxicosis, euthyroidism, and hypothyroidism are 0.284, 0.539 and 0.177, respectively.



hypothyroidism and its low specificity for thyrotoxicosis when TTBG is relatively reduced. Thus, its low sensitivity for the latter and its low specificity for the former, when TTBG is relatively high, and the fixed conventional normal values are (35%-45%) in interpretation.

The normal spectrum for the FT_4I is also affected by TTBG in that it rises and widens as TTBG is increased, reaching asymptotic values when TTBG is well beyond its physiologic levels (Figs. 1C and 2C). The normal limits for the FT_4I are 1.45-3.85 at relatively low values of TTBG (15 μg T_4/dl), but these levels shift to 2.15-5.20 when TTBG is relatively high (28 μg T_4/dl). These variances explain why, when interpreted according to its conventional fixed limits (2.2-4.7), the FT_4I shows a low sensitivity for thyrotoxicosis and a low specificity for hypothyroidism at relatively low values of TTBG (15 μg T_4/dl). The operating characteristics of FT_4I are nominally affected by relatively high levels of TTBG (Table 2).

These results stress the need for data regarding TTBG for proper interpretation of these tests. These results also demonstrate that the limitations of the FT_4I, when TBG is seriously altered by nonthyroidal illnesses, cannot be ascribed to a reduction of the intrinsic sensitivities of T_3U and T_4 as proposed by others (*3,9*), but to an erroneous choice of their normal values according to the corresponding TTBG. Besides, the FT_4I, originally derived by Clark and Horn (*2*), correctly assumes that the free TBG capacity (FTBG) would be inversely related to T_3U (FTBG = $1/T_3U$). Clark and Horn, however, never considered the actual proportionality between FTBG and $1/T_3U$ or the association constant between T_4 and TBG, which should divide the product of T_4 and T_3U to achieve more significant proportionality between the actual concentration of FT_4 in serum and the calculated FT_4I.

Specific measurment of TTBG by electrophoresis is time-consuming and not practical for a daily routine, but this problem would be overcome by using the empirical equation derived by Nusynowitz and Benedetto (*12*) to estimate TTBG

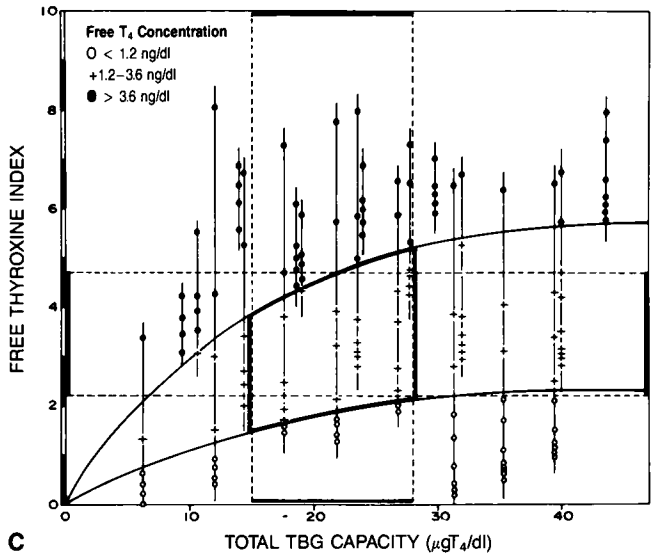


FIG. 1. Effects of added amounts of thyroxine on in vitro thyroid function tests results obtained in 21 sera with different total TBG binding capacity (TTBG). (A) Serum thyroxine concentration (T_4). Upper normal limits are T_4 = 0.5 TTBG; lower normal limits are T_4 = 0.25 TTBG. (B) In vitro uptake of T_3 by silicate (T_3U). Upper normal limits are $T_3U = 69\ e^{-TTBG/18} + 21$; lower normal limits are $T_3U = 43\ e^{-TTBG/18} + 21$. (C) Free thyroxine index (FT_4I). Upper normal limits are $FT_4I = 5.9\ (1-e^{-TTBG/12})$; lower normal limits are $FT_4I = 2.4\ (1-e^{-TTBG/13})$.

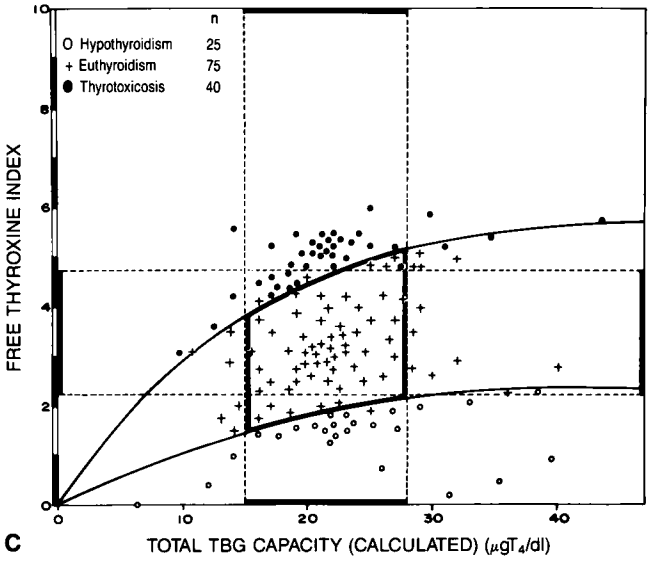


FIG. 2. Correlation of the in vitro testing results in 141 sera with their corresponding value for total TBG binding capacity (TTBG). (A) Serum thyroxine concentration (T_4). (B) In vitro uptake of T_3 by silicate (T_3U). (C) Free thyroxine index (FT_4I). Their variant normal limits, according to TTBG, are the same as those in Figure 1. Fixed conventional normal limits are shown at their respective coordinate.

from T_3U and T_4 values. We have found a highly significant linear correlation between this estimate of TTBG and the actual values measured by electrophoresis (*13*). We are confirmed in our previous belief that the combined use of T_4 and T_3U, with the estimated values for FT_4I and TTBG, would solve most of the diagnostic problems found in clinical practice without adding any further cost.

It is noted that the normal variant ranges for T_4, T_3U, and FT_4I described in this paper for the various levels of TTBG apply only to the particular in vitro system employed herein. It should also be emphasized that the equation for estimating TTBG from T_3U and T_4 can only be used exactly when these same kits are used. Clinical laboratories employing other test systems should develop exact values for this equation from the experimental values arising from their kits.

We believe that this approach to the interpretation of the in vitro thyroid tests represents a clearly defined and executed resolution of a clinical diagnostic problem that has made endocrinologists uneasy for many years.

TABLE 2. Operating Characteristics and Predictive Values for In Vitro Thyroid Tests According to the Interpretation Modality

		Thyroid Illness			
		Thyrotoxicosis		Hypothyroidism	
Test	Item*	Fixed Limits	Variant Limits	Fixed Limits	Variant Limits
T_3U	Sensitivity	0.600	0.900	0.760	1.000
	Specificity	0.950	1.000	0.767	1.000
	Accuracy	0.850	0.972	0.766	1.000
	PV+	0.828	1.000	0.413	1.000
	PV-	0.857	0.962	0.937	1.000
T_4	Sensitivity	0.425	0.950	0.480	1.000
	Specificity	0.901	0.970	0.966	0.974
	Accuracy	0.766	0.965	0.879	0.979
	PV+	0.630	0.927	0.750	0.804
	PV-	0.798	0.980	0.896	1.000
FT_4I	Sensitivity	0.750	0.925	0.960	1.000
	Specificity	0.931	0.970	0.922	0.983
	Accuracy	0.879	0.957	0.929	0.986
	PV+	0.811	0.925	0.727	0.926
	PV-	0.904	0.970	0.991	1.000

*Sensitivity = TP/TP+FN; Specificity = TN/TN+FP; Accuracy = TP+TN/TP+FP+TN+FN; PV+ = TP/TP+FP; PV- = TN/TN+FN.

FOOTNOTES

*Tetra-Tab RIA, Nuclear Medical Laboratories, Dallas, TX.
†Tri-Tab, Nuclear Medical Laboratories, Dallas, TX.

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