CALCULATION OF AN ESTIMATE OF  
THYROXINE-BINDING GLOBULIN CAPACITY  

Martin L. Nusynowitz and Anthony R. Benedetto
William Beaumont Army Medical Center, El Paso, Texas  

An estimate of the serum thyroxine-binding globulin (TBG) may be computed from determinations of serum thyroxine and triiodothyronine uptake. A general equation for this computation is presented and a computer program for the calculation of the estimating parameters is discussed. With these methods, the regression equation for the calculated TBG and the observed TBG is the line of identity, and the correlation coefficients from determinations on data from two laboratories were +0.88 and +0.96. The calculated TBG may be used as a screening test for abnormalities of thyroxine-binding protein and as an aid in the proper interpretation of thyroid function studies.

Alterations in the concentration of thyroxine-binding protein, especially thyroxine-binding globulin (TBG), result in abnormal values of serum thyroxine concentration. Knowledge of the TBG concentration would be of value in the clinical interpretation of abnormal serum tests of thyroid function, in the evaluation of the effects of drugs and disease states on such tests, and in the detection and elucidation of inherited TBG abnormalities (1). Direct measurements of TBG are not readily available and the test is expensive. Determinations of the triiodothyronine resin (or red cell or surface adsorbent) uptake (T3U) and the total serum thyroxine (T4), which are used to compute the free thyroxine index, are routinely available for the evaluation of thyroid function. These same two tests may also be used to estimate the capacity of thyroxine-binding protein since there is a linear inverse relationship between unbound TBG and T3U and a direct relationship between bound TBG and T4. Total TBG should therefore be related to a summation of some function of 1/T3U and T4, allowing computation of an estimate of TBG from them. The particular form that the function assumes is highly dependent on the specific method employed in the measurement of T3U. This is true because the magnitude of change differs whereas the direction of change caused by a particular disordered state is the same among the various T3U methods. Thus, the general equation describing TBG as a function of T3U and T4, where T3U and T4 are determined by specific methods, is:

\[ \text{TBG} = a \left[ \frac{1}{T3U} \right]^m + b[T3U]^n + c. \]  

The problem evolves into the determination of values for the coefficients and exponents, a, m, b, n, and c, which yield the best estimate of TBG for the specific analytic methods employed. The purpose of this paper is to describe how this can be done.

METHODS

T3U was performed using a surface-adsorbent technique (Tri-Tab®, Nuclear Medical Laboratories) and T4 was measured using a competitive binding assay employing a surface adsorbent (Tetra-Tab®, Nuclear Medical Laboratories). Total TBG (as maximum binding capacity of T4) was determined by the method of Elzinga, et al (2) by Bio-Science Laboratories. Data from two laboratories were analyzed, with our own laboratory providing one set. Our normal range of T3U is 25–35% as we use a modification of the Tri-Tab kit in which samples are counted against a pooled normal control serum arbitrarily assigned a value of 30%. Nuclear Medical Laboratories provided the second set of data in which the normal range of T3U using Tri-Tab is 35–45%. If the T3U and T4 terms are expressed as

Received March 24, 1975; revision accepted May 5, 1975. For reprints contact: Col. M. L. Nusynowitz, P.O. Box 70014, William Beaumont Army Medical Center, El Paso, Texas 79920.
IN VITRO NUCLEAR MEDICINE

010 DIMENSION W0(100),X(100),Y(100),AM(100),AN(100),A(100),
020 BC(100),C(100),VC(100)
025 CONTINUE
030 PRINT 10
040 10 FORMAT(IX,"'THIS PROGRAM EVALUATES THE EQUATION'")
050 IX,'VC=AX**M+BY**N+C, WHERE X AND Y ARE EXPERIMENTALLY'
060 IX,'OBSERVED VALUES AND A, M, B, N AND C ARE VARIABLE'
070 IX,'COEFFICIENTS. THE COMPUTED VALUE WC IS COMPARED TO A'
080 IX,'KNOWN VALUE W0 AND THROUGH AN ITERATIVE PROCESS THE'
090 IX,'VARIABLES A, M, B, N AND C ARE CHANGED IN ORDER TO'
100 IX,'Determine the COEFFICIENT VALUES WHICH YIELD THE MIN'
110 IX,'NOM DIFFERENCE BETWEEN THE COMPUTED AND OBSERVED'
120 IX,'VALUES'/'
130 PRINT 11
140 11 FORMAT(IX,"READ IN A TWO DIGIT FIXED POINT NUMBER")
150 IX,'CALLED NUM WHICH SPECIFIES THE NUMBER OF SETS OF'
160 IX,'OBSERVED VALUES FOR W0, X AND Y TO BE EVALUATED'/'
170 READ 12, NUM
180 12 FORMAT(12)
190 PRINT 13
200 13 FORMAT(IX,"READ IN THE SETS OF OBSERVED VALUES FOR'
210 IX,'W0, X AND Y IN 3F7.3 FORMAT. EXAMPLE -"/
220 IX,'019.00001.034001.034''")
230 D0 38 =1, NUM
240 38 READ 1, W(I),X(I),Y(I)
250 1 FORMAT(3F7.3)
260 PRINT 14
270 14 FORMAT(//IX,"READ IN FIVE F5.2 NUMBERS CALLED WIDTHA")
280 IX,'WIDTH, WIDTHB, WIDTHN, AND WIDTHC WHICH SPECIFY THE'
290 IX,'INTERVAL WIDTHS FOR THE VARIABLE COEFFICIENTS A, M'
300 IX,'B, N AND C RESPECTIVELY. EXAMPLE OF AN INTERVAL WIDTH'
310 IX,'0.00005 0.00025 AND 0.00050, ETC'/'
320 READ 23, WIDTHA, WIDTHB, WIDTHN, WIDTHC
330 23 FORMAT(5F5.2)
340 PRINT 15
350 15 FORMAT(IX,"READ IN FIVE TWO DIGIT FIXED POINT NUMBERS'
360 IX,'CALLED LIMA, LIMB, LINN AND LIMC WHICH SPECIFY'
370 IX,'THE NUMBER OF SUCCESSIVE INTERVALS TO BE PROCESSED FOR'
380 IX,'EACH OF THE VARIABLE COEFFICIENTS A, M, B, N AND C'
390 IX,'RESPECTIVELY'/'
400 READ 16, LIMA,LIMB,LINN,LIMN,LIMC
410 16 FORMAT(5I2)
420 PRINT 17
430 17 FORMAT(IX,"READ IN FIVE F5.2 NUMBERS CALLED A(I),
440 IX,A(I) AND C(I) WHICH SPECIFY THE INITIAL VALUES'
450 IX,'OF EACH VARIABLE COEFFICIENT A, M, B, N AND C RESPECT'/'
460 IX,'IVELY'/'
470 READ 18, A(I),AM(I),B(I),AN(I),C(I)
480 18 FORMAT(5F5.2)
490 D0 41 =1,LIMA
500 41 A(I)=AM(I-1)+WIDTHA
510 D0 42 =2,LIMB
520 42 AM(I)=AM(I-1)+WIDTHM
530 D0 43 =2,LIMC
540 43 B(I)=B(I-1)+WIDTHB
550 D0 44 =2,LINN
560 44 AN(I)=AN(I-1)+WIDTHN
570 D0 45 =2,LIMC
580 45 C(I)=C(I-1)+WIDTHC
590 PRINT 66
620 D0 55 =1,LIMA
630 D0 54 =1,LIMB
640 D0 53 =1,LIMC
650 D0 52 =1,LINN
660 D0 50 =1,LIMC
670 SUMSQ =0.0
680 D0 51 =1,NUM
690 VCN=AM(I)+XN**AN(I)+B(K)+Y(N)**AN(L)+C(H)
700 51 SUMSQ=VCCN-W0**2+SUMSQ
705 IF (SUMSQ .GT. 215.) GO TO 50
710 PRINT 99, SUMSQ, A(I),AM(J),B(K),AN(L),C(H)
720 99 FORMAT(IX,F10.3,F5(3X,F6.2))
730 50 CONTINUE
731 52 CONTINUE
732 53 CONTINUE
733 54 CONTINUE
734 55 CONTINUE
735 STOP
750 END

FIG. 1. FORTRAN program to compute, by iteration of coefficients and exponents, values of TBG from patient data and to calculate sum of squares of differences between observed and calculated TBG.
fractions of the midnormal values for the specific method (in order to increase uniformity among various assay methods), the equation becomes:

$$\text{TBG} = a \left[ \frac{T_3U_{\text{mid}}}{T_4U} \right]^m + b \left[ \frac{T_4}{T_{4 \text{ mid}}} \right]^n + c. \quad (2)$$

Data from our laboratory were used to demonstrate the explicit methods employed. The $T_3U$, $T_4$, and TBG values were obtained on serum samples of 20 patients with a variety of disorders known to affect all three tests. The variables $a$, $m$, $b$, $n$, and $c$ were initialized to some arbitrary values to compute $\text{TBG}_c$, a calculated TBG. The difference between the observed TBG ($\text{TBG}_o$) and the $\text{TBG}_c$ for each patient was computed, and the sum of the squares of the differences ($\text{TBG}_o - \text{TBG}_c$) for each patient for the preselected values assigned to the five parameters was determined. Following an iterative scheme, the entire process was repeated for various assigned values of the parameters. The sums of squares were compared to find a minimum value and the iterative process was repeated until satisfactory convergence was obtained.

A FORTRAN computer program (Fig. 1) was employed to evaluate the equation. In the program the following symbolism was used:

- $X = \frac{T_3U_{\text{mid}}}{T_4U}$
- $Y = \frac{T_4}{T_{4 \text{ mid}}}$
- $WC = \text{TBG}_c$
- $WO = \text{TBG}_o$

The program includes provision for the arbitrary selection of a value for the sum of squares such that only values less than this are printed. The output was inspected for the minimum value of sum of squares and for the corresponding values of the coefficients and exponents. Iterations were then made around these values until convergence resulted.

Final determination of the coefficients $a$, $b$, and $c$ was made by determining the regression equation on the preliminary value of $\text{TBG}_c$ as calculated using the values given by the iterative technique and modifying these preliminary values by the coefficients of the regression equations. Identical methods were employed in devising the regression equation for the data supplied by Nuclear Medical Laboratories.

**RESULTS**

From 20 sets of results from our laboratory, and using our laboratory values of 30% for $T_3U_{\text{mid}}$ and 7.81 $\mu g/100$ ml for $T_{4 \text{ mid}}$, the preliminary equation determined by the iterative technique was:

$$\text{TBG}_c = 14 \left( \frac{30}{T_3U} \right)^{1.5} + 15 \left( \frac{T_4}{7.81} \right)^{0.4} - 14.6.$$  

The equation relating $\text{TBG}_o$ and $\text{TBG}_c$ using these parameters was:

$$\text{TBG}_o = 0.9815 \text{ (TBG}_c) + 0.33.$$  

Accordingly, the values for $a$, $b$, and $c$ were modified by multiplying by 0.9815 and adding 0.33 to the value of $c$. The final equation resulting was:

$$\text{TBG}_c = 13.74 \left( \frac{30}{T_3U} \right)^{1.5} + 14.72 \left( \frac{T_4}{7.81} \right)^{0.4} - 14.00.$$  

Using this equation to calculate TBG, the mean difference between the 20 pairs of values $\text{TBG}_c$ and $\text{TBG}_o$ was $0.0 \pm 3.3$ (1 s.d.). Figure 2 shows the
correlation between TBGα and TBGε for each of the 20 patients; the correlation coefficient \( r = 0.88 \). The equation for predicting TBGα from TBGε was the line of identity:

\[
TBG_\alpha = 1.00 \times (TBG_\epsilon) + 0.00.
\]

From 23 sets of data supplied by Nuclear Medical Laboratories, and using their laboratory values of 40\% for \( T_3U_{mid} \) and 8.00 \( \mu g/100 \text{ ml} \) for \( T_4_{mid} \), the final equation resulting was:

\[
TBG_\epsilon = 15.35 \left( \frac{40}{T_3U} \right)^{1.5} + 14.96 \left( \frac{T_4}{8.00} \right)^{0.8} - 9.70.
\]

Using this equation to calculate TBG, the mean difference between the 23 pairs of values TBGα and TBGε was 0.0 \( \pm 3.1 \) (1 s.d.). Figure 3 shows the correlation between TBGα and TBGε for each of the 23 patients; the correlation coefficient \( r = 0.96 \) and the equation for predicting TBGα from TBGε was also the line of identity.

**DISCUSSION**

The results clearly demonstrate that a relatively accurate estimate of the TBG may be computed from the \( T_3U \) and \( T_4 \).

The method employed may be adapted to the specific analytic technique used in each laboratory to compute the values of the coefficients and exponents of the general equation, enabling estimations of TBG from the \( T_3U \) and \( T_4 \). While the estimated value really reflects the binding capacities of all the thyroxine-binding proteins, we have chosen to call it a TBG estimate since TBG is the binding protein of major significance. The estimated TBG is valid over a wide range of TBG values and provides a valuable tool for interpreting the serum \( T_4 \) and \( T_3U \) in states in which these are affected by binding-protein changes. In addition, the TBG estimate is a useful screening test for abnormalities of TBG (or other binding proteins); thus, an abnormal TBG estimate would indicate which patients should be studied by costlier but direct assays of TBG.

The method described herein provides a means of utilizing two routine chemical determinations for calculating the concentration of a physiologically important protein, which is important to know for proper interpretation of thyroid function studies and for correct determination of the clinical state.

**REFERENCES**
