CALCULATION OF AN ESTIMATE OF THYROXINE-BINDING GLOBULIN CAPACITY

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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 (T_3U) and the total serum thyroxine (T_4), 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 T_3U and a direct relationship between bound TBG and T_4. Total TBG should therefore be related to a summation of some function of $1/T_3U$ and T_4, 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 T_3U. 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 T_3U methods. Thus, the general equation describing TBG as a function of T_3U and T_4, where T_3U and T_4 are determined by specific methods, is:

$$TBG = a\left[\frac{1}{T_3U}\right]^m + b[T_4]^n + c. \qquad (1)$$

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

T_3U was performed using a surface-adsorbent technique (Tri-Tab®, Nuclear Medical Laboratories) and T_4 was measured using a competitive binding assay employing a surface adsorbent (Tetra-Tab®, Nuclear Medical Laboratories). Total TBG (as maximum binding capacity of T_4) 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 T_3U 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 T_3U using Tri-Tab is 35–45%. If the T_3U and T_4 terms are expressed as

Received March 24, 1975; revision accepted May 5, 1975.

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010 DIMENSIØN WØ(100),X(100),Y(100),AM(100),AN(100),A(100),
020&B(100),C(100),WC(100)
025 CØNTINUE
030 PRINT 10
040 10 FØRMAT(1X,"THIS PRØGRAM EVALUATES THE EQUATIØN"/
050&1X,"WC=A*X**M+B*Y**N+C, WHERE X AND Y ARE EXPERIMENTALLY"/
060&1X,"ØBSERVED VALUES AND A, M, B, N AND C ARE VARIABLE"/
070&1X,"CØEFFICIENTS. THE CØMPUTED VALUE WC IS CØMPARED TØ A"/
080&1X,"KNØWN VALUE WØ AND THRØUGH AN ITERATIVE PRØCESS THE"/
090&1X,"VARIABLES A, M, B, N, AND C ARE CHANGED IN ØRDER TØ"/
100&1X,"DETERMINE THE CØEFFICIENT VALUES WHICH YIELD THE MIN-"/
110&1X,"IMUM DIFFERENCE BETWEEN THE CØMPUTED AND ØBSERVED"/
120&1X,"VALUES."////)
130 PRINT 11
140 11 FØRMAT(1X,"READ IN A TWØ DIGIT FIXED PØINT NUMBER"/
150&1X,"CALLED NUM WHICH SPECIFIES  THE NUMBER ØF SETS ØF"/
160&1X,"ØBSERVED VALUES FØR WØ, X AND Y TØ BE EVALUATED."/)
170 READ 12, NUM
180 12 FØRMAT(I2)
190 PRINT 13
200 13 FØRMAT(1X,"READ IN THE SETS ØF ØBSERVED VALUES FØR"/
210&1X,"WØ, X AND Y IN 3F7.3 FØRMAT. EXAMPLE - "/
220&1X,"019.000001.034001.034"/)
230 DØ 38 I=1, NUM
240 38 READ 1, WØ(I),X(I),Y(I)
250 1 FØRMAT(3F7.3)
260 PRINT 14
270 14 FØRMAT(//1X,"READ IN FIVE F5.2 NUMBERS CALLED WIDTHA"/
280&1X,"WIDTHM, WIDTHB, WIDTHN, AND WIDTHC WHICH SPECIFY THE"/
290&1X,"INTERVAL WIDTHS FØR THE VARIABLE CØEFFICIENTS A, M,"/
300&1X,"B, N AND C RESPECTIVELY. EXAMPLE ØF AN INTERVAL WIDTH"/
310&1X,"FØR M MIGHT BE 00.25 AND FØR C, 05.00, ETC."/)
320 READ 23, WIDTHA, WIDTHM, WIDTHB,WIDTHN,WIDTHC
330 23 FØRMAT(5F5.2)
340 PRINT 15
350 15 FØRMAT(1X,"READ IN FIVE TWØ DIGIT FIXED PØINT NUMBERS"/
360&1X,"CALLED LIMA, LIMM, LIMB, LIMN AND LIMC WHICH SPECIFY"/
370&1X,"THE NUMBER ØF SUCCESSIVE INTERVALS TØ BE PRØCESSED FØR"/
380&1X,"EACH ØF THE VARIABLE CØEFFICIENTS A, M, B, N, AND C"/
390&1X,"RESPECTIVELY."/)
400 READ 16, LIMA,LIMM,LIMB,LIMN,LIMC
410 16 FØRMAT(5I2)
420 PRINT 17
430 17 FØRMAT(1X,"READ IN FIVE F5.2 NUMBERS CALLED A(1), AM(1)"/
440&1X,"B(1), AN(1) AND C(1) WHICH SPECIFY THE INITIAL VALUES"/
450&1X,"ØF EACH VARIABLE CØEFFICIENT A, M, B, N AND C RESPECT-"/
460&1X,"IVELY."/)
470 READ 18, A(1),AM(1),B(1),AN(1),C(1)
480 18 FØRMAT(5F5.2)
490 DØ 41 I=2,LIMA
500 41 A(I)=A(I-1)+WIDTHA
510 DØ 42 I=2,LIMM
520 42 AM(I)=AM(I-1)+WIDTHM
530 DØ 43 I=2,LIMB
540 43 B(I)=B(I-1)+WIDTHB
550 DØ 44 I=2,LIMN
560 44 AN(I)=AN(I-1)+WIDTHN
570 DØ 45 I=2,LIMC
580 45 C(I)=C(I-1)+WIDTHC
590 PRINT 66
600 66 FØRMAT(1X,"SUM ØF DIFF"/1X,"SQUARED",7X,"A",8X,
610&"M",8X,"B",8X,"N",8X,"C"/)
620 DØ 55 I=1, LIMA
630 DØ 54 J=1, LIMM
640 DØ 53 K=1, LIMB
650 DØ 52 L=1, LIMN
660 DØ 50 M=1,LIMC
670 SUMSQ =0.0
680 DØ 51 N=1,NUM
690 WC(N)=A(I)*X(N)**AM(J)+B(K)*Y(N)**AN(L)+C(M)
700 51 SUMSQ=(WC(N)-WØ(N))**2+SUMSQ
705 IF (SUMSQ .GT. 215.) GØ TØ 50
710 PRINT 99, SUMSQ, A(I), AM(J), B(K), AN(L), C(M)
720 99 FØRMAT(1X, F10.3, 5(3X, F6.2))
730 50 CØNTINUE
731 52 CØNTINUE
732 53 CØNTINUE
733 54 CØNTINUE
734 55 CØNTINUE
740 STØP
750 END
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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.

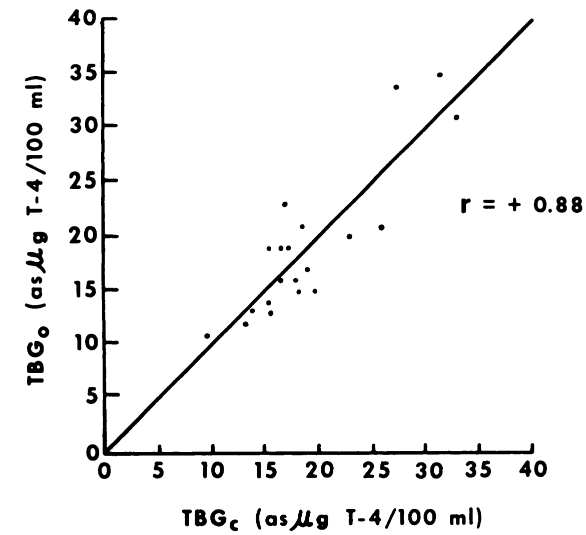

FIG. 2. Regression of calculated and observed TBG. Least-squares estimating equation is line of identity. Data used for calculating TBG were from our laboratory.

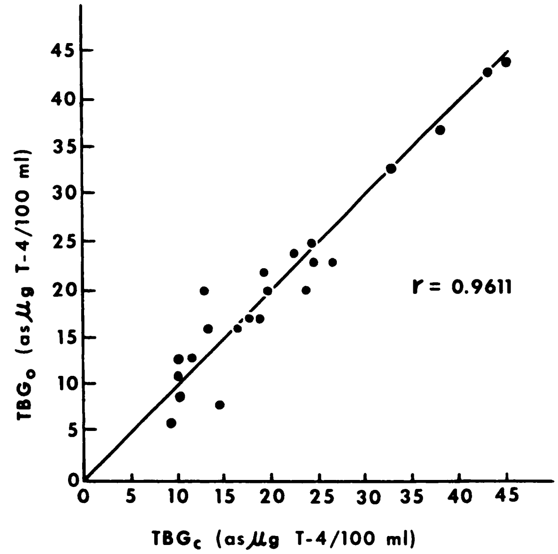

FIG. 3. Regression of calculated and observed TBG. Least-squares estimating equation is line of identity. Data used for calculating TBG were from Nuclear Medical Laboratories.

fractions of the midnormal values for the specific method (in order to increase uniformity among various assay methods), the equation becomes:

$$TBG = a\left[\frac{T_3U_{mid}}{T_3U}\right]^m + b\left[\frac{T_4}{T_{4\ mid}}\right]^n + c. \quad (2)$$

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 TBG_c, a calculated TBG. The difference between the observed TBG (TBG_o) and the TBG_c for each patient was computed, and the sum of the squares of the differences ($TBG_c - TBG_o$) 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:

$$X = T_3U_{mid}/T_3U$$
$$Y = T_4/T_{4\ mid}$$
$$WC = TBG_c$$
$$WO = TBG_o.$$

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 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_{mid} and 7.81 µg/100 ml for $T_{4\,mid}$, the preliminary equation determined by the iterative technique was:

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

The equation relating TBG_o and TBG_c using these parameters was:

$$TBG_o = 0.9815\ (TBG_c) + 0.33.$$

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:

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

Using this equation to calculate TBG, the mean difference between the 20 pairs of values TBG_o and TBG_c was 0.0 ± 3.3 (1 s.d.). Figure 2 shows the correlation between TBG_o and TBG_c for each of the 20 patients; the correlation coefficient $r = 0.88$. The equation for predicting TBG_o from TBG_c was the line of identity:

$$TBG_o = 1.00\,(TBG_c) + 0.00.$$

From 23 sets of data supplied by Nuclear Medical Laboratories, and using their laboratory values of 40% for T_3U_{mid} and 8.00 μg/100 ml for T_{4mid}, the final equation resulting was:

$$TBG_c = 15.35\left(\frac{40}{T_3U}\right)^{1.5} + 14.96\left(\frac{T_4}{8.00}\right)^{0.5} - 9.70.$$

Using this equation to calculate TBG, the mean difference between the 23 pairs of values TBG_o and TBG_c was 0.0 ± 3.1 (1 s.d.). Figure 3 shows the correlation between TBG_o and TBG_c for each of the 23 patients; the correlation coefficient $r = 0.96$ and the equation for predicting TBG_o from TBG_c 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

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