On the Albumin-Dependence of Measurements of Free Thyroxin. II. Patients with Non-Thyroidal Illness

Gyorgy Csako, Mark H. Zweig, Carol Benson, and Mark Rudder

We studied the relation between thyroxin-binding proteins and free thyroxin (FT₄) measurements by five radioimmunoassays (RIA) and an FT₄ index (FT₄I) in patients with non-thyroidal illness (NTI). The one-step FT₄ RIAs and the FT₄I frequently failed to identify the true FT₄ status (as determined by equilibrium dialysis) of NTI patients. In these patients, falsely low FT₄ results with one-step RIAs and FT₄I were associated with decreasing total T₃ and T₄ concentrations, which, furthermore, paralleled decreasing serum albumin concentrations. All NTI patients with "low T₃, low T₄ syndrome" had subnormal albumin concentration. The two-step RIAs and equilibrium dialysis showed normal FT₄ concentrations in most patients with NTI. However, sera from a subset of NTI patients with "low T₃ syndrome" gave above-normal FT₄ results with these methods. From their predictably poor performance in the presence of a subnormal albumin concentration, we conclude that the one-step FT₄ RIAs and FT₄I are inappropriate for testing the thyrometabolic status of NTI patients.

Additional Keyphrases: radioimmunoassay · thyroxin analog · free thyroxin index · equilibrium dialysis compared · clinical suitability · falsely low results

Currently available one-step radioimmunoassays (RIAs) for free thyroxin (FT₄), in which radiolabeled analogs of T₄ are used to decrease binding to serum TBG, are affected by increases in T₄ binding to albumin or variations in serum albumin concentration (1–7). Patients with non-thyroidal illness (NTI) often have spuriously low FT₄ concentrations by one-step methods, and it has been suggested that this may be ascribed to low albumin concentrations (5, 7). Previously, we reported on albumin dependence in three one-step (analog) methods, while we found that two two-step methods and equilibrium dialysis were not affected (8). We have now reclassified these same patients according to thyroid function and have specifically examined the relation between albumin and thyroxin-binding globulin (TBG) concentrations and the FT₄ concentrations determined by six FT₄ methods in those patients with NTI. Equilibrium dialysis was our reference method.

Materials and Methods

Subjects

Blood specimens were collected from a total of 183 adults at the Clinical Center, National Institutes of Health (8). The controls included 26 healthy volunteers (mainly laboratory workers) and 47 ambulatory patients with suspected or known thyroid disorders. The NTI patients included 45 critically ill patients from the Medical Intensive Care Unit (MICU); 27 patients who were in the immediate postoperative period after major surgery, almost exclusively for cancer (Surgical Intensive Care Unit, SICU); 14 patients who had just undergone open-heart surgery and, being seriously ill, were in the Cardiac Surgery Recovery Room (CSR); and 24 ambulatory patients, all in various stages of primary biliary cirrhosis (PBC).

FT₄ Assays

We measured FT₄ concentration by five different RIAs. Three were one-step (analog) methods that involved no blocking agents: Amerlex (Amerlex Corp., Arlington Heights, IL), Coat-a-Count (Diagnostic Products Corp., Los Angeles, CA), and GammaCoat one-step (Clinical Assays, Cambridge, MA). The other two were two-step noncompetitive sequential methods, GammaCoat two-step FT₄ (Clinical Assays, Cambridge, MA) and Spiria-FT₄ (International Immunoadsorbs Laboratories, IIL, Inc., Santa Clara, CA). Details of these techniques have been published previously (8).

The "true" FT₄ concentration was determined by the technique we used as our reference method, equilibrium dialysis, at Bio-Science Laboratories, Columbia, MD.

FT₄ was estimated indirectly by calculating an FT₄ index (FT₄I) from the ratio of total T₄ to TBG (multiplied by 10).

Reference ranges of FT₄ RIAs were those recommended by the manufacturers. Bio-Science's reference range was used for equilibrium dialysis. Because the number of observations was too small for a statistically-derived range, the reference range for FT₄I was arbitrarily defined by the minimum and maximum value observed in the 26 healthy volunteers. The FT₄ reference intervals are shown below in Figures 1 and 3.

Other Techniques

Methods for total T₃, TBG, and albumin have been described elsewhere (8). Total T₃ was measured by the Quantimmune T₃ RIA (Bio-Rad Labs., Richmond, CA). Thyrotropin (TSH) was determined by the RIAphase HTSH Reagent System (Beckman Instruments, Inc., Fullerton, CA). Reference intervals for these methods are in Table 1.

Statistical Analysis

Deming-debiased regression, assuming equal errors in the x and y variables, was used for analysis of correlation between total T₃ or T₄ and albumin concentrations.

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1 Nonstandard abbreviations: CSRR, Cardiac Surgery Recovery Room; FT₄, free thyroxin; FT₄I, free thyroxin index (total T₄/TBG) × 10; IIL, International Immunoadsorbs Laboratories; MICU, Medical Intensive Care Unit; NTI, non-thyroidal illness; PBC, primary biliary cirrhosis; SICU, Surgical Intensive Care Unit; TBG, thyroxin-binding globulin; and TSH, thyrotropin (thyroid-stimulating hormone).
Table 1. Thyroid-Function Studies in Patients with NTI

<table>
<thead>
<tr>
<th></th>
<th>Total T₃, μg/L</th>
<th>Total T₄, μg/L</th>
<th>TSH, milli-</th>
<th>TBG, mg/L</th>
<th>Albumin, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers</td>
<td>1.55 ± 0.19</td>
<td>80 ± 15</td>
<td>4.3 ± 2.0</td>
<td>25 ± 2</td>
<td>41 ± 2</td>
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<tr>
<td>(n = 26)</td>
<td>(n = 26)</td>
<td>(n = 26)</td>
<td>(n = 26)</td>
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<tr>
<td>Patients with normal T₃ and normal T₄</td>
<td>1.48 ± 0.28</td>
<td>91 ± 17</td>
<td>4.3 ± 3.9</td>
<td>28 ± 6</td>
<td>39 ± 3</td>
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<tr>
<td>(n = 22)</td>
<td>(n = 22)</td>
<td>(n = 18)</td>
<td>(n = 22)</td>
<td>(n = 22)</td>
<td></td>
</tr>
<tr>
<td>Patients with &quot;low T₃&quot; syndrome</td>
<td>0.53 ± 0.28</td>
<td>69 ± 16</td>
<td>3.2 ± 1.5</td>
<td>21 ± 4</td>
<td>33 ± 6</td>
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<tr>
<td>(n = 37)</td>
<td>(n = 37)</td>
<td>(n = 37)</td>
<td>(n = 37)</td>
<td>(n = 36)</td>
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<tr>
<td>Patients with &quot;low T₃, low T₄&quot; syndrome</td>
<td>0.22 ± 0.20</td>
<td>37 ± 11</td>
<td>5.6 ± 0.0</td>
<td>18 ± 4</td>
<td>29 ± 4</td>
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<td>(n = 23)</td>
<td>(n = 23)</td>
<td>(n = 18)</td>
<td>(n = 23)</td>
<td>(n = 23)</td>
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</tr>
<tr>
<td>Reference interval</td>
<td>1.11-1.99</td>
<td>50-120</td>
<td>1-8</td>
<td>12-28</td>
<td>38-49</td>
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</table>

Mean ± SD.

Results

Thyroid Studies in NTI Patients Classified According to Total T₃ and T₄ Status

Based on the measured concentrations of total T₃ and T₄, NTI patients with abnormal thyroid test results are often classified into "low T₃," "low T₃, low T₄" ("euthyroid sick"), "high T₄," and "mixed" forms (9-11). Owing to the unavailability of some T₃ results, we could classify only 82 (75%) of our 110 NTI patients according to both T₃ and T₄ status. As expected, all healthy controls expressed a euthyroid pattern (100%). In turn, the great majority of the MICU (92%) and SICU (85%) patients and all of the CSRR patients (100%) presented with abnormally low values for total T₃. In addition to subnormal total T₃, 47% of MICU and 38% of SICU patients also had subnormal total T₄, but none of the CSRR patients had abnormally low values for total T₄. The ambulatory patients with PBC most commonly showed an euthyroid pattern (71%) and none was "low T₃, low T₄," but there were a few cases of "low T₃" (21%) and "high T₄" (17%) abnormalities. No "mixed" patterns occurred in our NTI patients.

When all NTI patients were combined, almost half (45%) of the classifiable patients had "low T₃ syndrome," while the rest were distributed equally between the "euthyroid" (27%) and "low T₃, low T₄" (28%) patterns (Table 1). For comparison, data of healthy volunteers were also included. The mean TBG concentration was within normal limits in each group (Table 1). Except for albumin, the euthyroid volunteers and "euthyroid" patients with NTI were essentially identical in the mean concentration of various analytes (Table 1). The albumin concentration was more often subnormal in the "euthyroid" patient group than in the volunteers, resulting in a borderline-low mean value (Table 1). The "low T₃" and "low T₃, low T₄" patients revealed increasingly greater numbers of subnormal albumin concentrations. In fact, all "low T₃, low T₄" patients had abnormally low serum albumin concentrations.

Euthyroid volunteers and NTI patients with normal T₃ and T₄ had similar FT₄ results (Table 2 and Figure 1). In turn, 24 to 30% of "low T₃" and 61 to 74% of the "low T₃, low T₄" patients had subnormal FT₄ results with the one-step techniques and 57% of the "low T₃, low T₄" patients were "hypothyroid" according to the FT₄ index (Table 2). None of the "low T₃" and "low T₃, low T₄" syndrome patients had subnormal FT₄ concentrations with the "reference method," equilibrium dialysis (Bio-Science) or with the two-step technique of Clinical Assays (Table 2 and Figure 1). Moreover, none of the "low T₃" and only one (4%) of the "low T₃, low T₄" patients had a subnormal result with the other two-step FT₄ method (ILL) (Table 2 and Figure 1). On the other hand, several patients with "low T₃ syndrome" exhibited abnormally high FT₄ concentration with the two-step RIA methods and equilibrium dialysis: 35, 16, and 46%, respectively (Figure 1). The same patients were responsible for the high or borderline-high FT₄ concentrations as measured with all three techniques. Although most of these patients were hospitalized for serious medical illnesses (MICU), some were from the heart-surgery intensive-care unit (CSRR), and some had just had surgery for cancer (SICU).

The TBG concentration is frequently found to be above normal in patients with chronic active hepatic disease (12). This is why we included patients with PBC in our study dealing with the possible influence of thyroxin-binding proteins on FT₄ measurements. However, when analyzed separately, in accord with our previous observation on a large group of subjects (8), we found no correlation between TBG concentrations and FT₄ concentrations as determined by any of the techniques in these patients (data not shown).

Table 2. Percentage (and Number) of NTI Patients with Subnormal Values for FT₄ and Thyroid Hormone Binding-Protein Concentration

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</thead>
<tbody>
<tr>
<td>Healthy volunteers</td>
<td>0% (0/26)</td>
<td>0% (0/26)</td>
<td>0%</td>
<td>0% (0/26)</td>
<td>0% (0/26)</td>
<td>0%</td>
<td>0% (0/26)</td>
</tr>
<tr>
<td>Patients with normal T₃ and T₄</td>
<td>4% (1/22)</td>
<td>4% (1/22)</td>
<td>0%</td>
<td>0% (0/22)</td>
<td>0% (0/22)</td>
<td>0%</td>
<td>0% (0/22)</td>
</tr>
<tr>
<td>Patients with &quot;low T₃ syndrome&quot;</td>
<td>24% (9/37)</td>
<td>30% (10/37)</td>
<td>0%</td>
<td>0% (0/37)</td>
<td>0% (0/37)</td>
<td>0%</td>
<td>3% (1/37)</td>
</tr>
<tr>
<td>Patients with &quot;low T₃, low T₄ syndrome&quot;</td>
<td>61% (14/23)</td>
<td>65% (17/23)</td>
<td>0%</td>
<td>4% (0/23)</td>
<td>0% (0/23)</td>
<td>57%</td>
<td>7% (13/23)</td>
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Correlation of Total $T_3$ and $T_4$ with Albumin Concentration in Patients with NTI

Because the serum albumin concentration was progressively lower in NTI patients who had low $T_3$ and low $T_4$ concentrations, we further analyzed this relationship. Figure 2A shows that in the controls (healthy volunteers and thyroid patients) the results for albumin were scattered within a narrow range, exhibiting no correlation with $T_3$ and only a borderline significant correlation with $T_3$. In turn, with the wide scattering of albumin concentrations a highly significant correlation ($p<0.0001$) was seen in NTI patients for both $T_4$ and $T_3$ (all patients who had either $T_3$ or $T_4$ measured were included in this analysis) (Figure 2B).

Correction of $T_4$ Results for Albumin in Patients with NTI

That the subnormal $T_4$ results by analog RIA methods and $FT_4$ are indeed related to low albumin concentrations in serum of patients with NTI was confirmed by correcting for albumin. Using correlations previously established with these techniques on a large number of patients (17), we normalized the $FT_4$ concentrations for each technique for albumin (assuming a value of 41 g/L, the mean for healthy subjects) in healthy volunteers as controls and in NTI patients with normal values for $T_3$ and $T_4$, "low $T_3$," and "low $T_3$, low $T_4$ syndrome" (Figure 3). The $FT_4$ results (mean and proportion abnormal) with all techniques were essentially unchanged by correcting for albumin in healthy
volunteers and in NTI patients with normal values for T₃ and T₄ (Figures 1 and 3). In contrast, after correction for albumin, almost all of the formerly low FT₄ concentrations with the analog RIAs became normal in NTI patients with "low T₃" and "low T₃, low T₄ syndrome" (Figures 1 and 3). Normalization for albumin was somewhat less effective for the FT₄₁ and corrected only half of the cases from subnormal to normal in patients with "low T₃, low T₄ syndrome" (Figures 1 and 3). Except for a few up or down movements, NTI patients with "low T₃ syndrome" who previously had high FT₄₁ concentration with the two-step RIA methods and equilibrium dialysis (Figure 1) remained high (in 35, 32, and 40% of the cases, respectively) after the correction for albumin (Figure 3), indicating that these high FT₄₁ results are independent of the serum albumin concentration.

As a second control in this study, normalization of FT₄₁ concentrations for albumin in thyroidal-illness patients did not result in significant changes for any method (data not shown).

Discussion

Our results show that the low albumin concentrations observed in NTI patients may be accompanied by falsely low FT₄₁ as determined by one-step RIA methods, especially in those patients having low T₃ and (or) T₄ concentrations. Owing to differences in the patient populations studied, reports on NTI patients differ widely in the percentage of patients exhibiting low total T₃ and (or) T₄ (5, 13–17). We found that in our institution approximately half of the patients in the MICU or SICU had only low T₃, whereas the other half had both low T₃ and low T₄. Surprisingly, all patients after cardiac surgery (CSRR) had low T₃ but none showed low T₄. Grouping these NTI patients according to their combined total-T₃ and -T₄ status resulted in far more homogeneous populations for the study of various FT₄₁ RIAs. This homogeneity is mostly attributable to the fact that, according to our data, there is a highly significant correlation between total T₃ or T₄ and albumin concentrations in NTI patients. Consequently, NTI patients with low T₃ and low T₄ all had subnormal albumin concentrations, providing an optimal group for the study of possible albumin-dependence of FT₄₁ measurements.

In accord with several reports (1–7), previously we observed albumin-dependence of FT₄₁ measurements with the analog (one-step) RIAs and a FT₄₁ method (8). The present data indicate that this albumin-dependence is responsible
Fig. 3. Scattergram of FT₄ results obtained by one-step (analog) RIA methods (Amersham, Diagnostic Products, Clinical Assays), two-step RIA techniques (Clinical Assays, III), equilibrium dialysis (Bio-Science), and by calculating an index [(FT4/TBG) x 10] after correction for serum albumin concentration (assuming normal = 41 g/L).

The solid and (for FT₄) broken horizontal lines indicate limits of reference ranges (see Materials and Methods). "M" indicates a break in the scale above 6.

for the falsely low FT₄ results with analog techniques in patients with NTI. Because the low FT₄ results with these techniques were closely associated with low albumin and low total T₃ and T₄ concentrations, it is likely that determination of total T₃ and T₄ or of albumin alone can be used to predict the likelihood of obtaining falsely low FT₄ results with analog RIAs (as contrasted to the "true" value obtained with equilibrium dialysis) in NTI patients.

Because none of the currently available T₄ derivatives satisfies the theoretical requirement (i.e., no binding to T₄-binding proteins in serum) for a reliable one-step analog method, the Berlin symposium of thyroid experts questioned the suitability of these techniques for clinical use (18). Investigations with second-generation FT₄ analog assays (e.g., Amerlex-M FT₄) which allegedly circumvent interference from albumin binding by the use of selective blockers, are no more encouraging than the original procedures (e.g., Amerlex FT₄) (6, 19). Owing to the widespread use of one-step (analog) RIA methods, the chances of obtaining falsely decreased FT₄ results in subjects with decreased albumin concentration (or with decreased T₄ affinity of albumin) and falsely high FT₄ results in patients with increased albumin concentration (or with increased T₄ binding affinity due to an anomalous albumin or to a T₄-autoantibody) appear at present great in the United States. In a nationwide proficiency testing (Ligand Assay—Series 1, Set K-B, July 1986 Survey; College of American Pathologists, Chicago, IL), of 412 laboratories that participated in the FT₄ survey with specimen K-4, 405 (98%) were identified by the type of method used. Of these, 322 laboratories (79%) utilized one-
step (analog) methods for FT4 measurement [Amersham (Amerlex-M), 69; Clinical Assays direct FT4, 93; Diagnostic Products (Coat-a-Count), 76; ciba-Corning "Magic," 57; Becton-Dickinson, 21; and ciba-Corning single-step, six]. Only 68 laboratories (17%) utilized two-step FT4 RIA (Clinical Assays two-step, 62; Med. & Scientific Designs, six). Fifteen laboratories (4%) used a rate-based RIA with labeled T4 (ciba-Corning Diagn. CRP). None was listed as using equilibrium dialysis for FT4 measurement.

In our study of NTI patients, the two-step FT4 techniques gave results similar to those by equilibrium dialysis. Consistent with their usually normal TSH concentrations, most NTI patients also had normal FT4 concentrations as measured by these techniques. Even severely ill patients with markedly decreased albumin, total T4, and total T3 concentrations showed normal FT4 concentration with two-step RIAs and equilibrium dialysis. Furthermore, the FT4 concentration was normal with the one-step analog methods after correction for albumin. Of interest is that correction for the albumin effect also reduced the decrease seen by the analog methods in FT4 during pregnancy, resulting in closer agreement with the dialysis method (6).

A subgroup of our NTI patients with "low T3 syndrome" showed, however, increased FT4 concentration with the two-step techniques and equilibrium dialysis (but not with the analog methods). Previous reports have indicated that in vitro addition of heparin causes a profound increase in FT4 as measured by equilibrium dialysis or the two-step RIA of Clinical Assays (20, 21) but decreased the results for apparent FT4 by one-step RIAs (22). Because heparin liberates fatty acids (20–22) that inhibit the carrier protein binding of T4 (20), it was surmised that free fatty acids are responsible for the unexpectedly high or low (depending on the method) FT4 concentrations (20, 22). By releasing fatty acids, prolonged fasting in critically ill patients may mimic the heparin effect. Because we have no data on free fatty acids, we do not know if the increased FT4 results with two-step techniques and equilibrium dialysis were etiologically related to unusually high concentrations of free fatty acids in some of our NTI patients with "low T3 syndrome."

In conclusion: our results show a positive correlation between decreased serum albumin, total T3 and T4, and spuriously low FT4 concentrations by one-step (analog) RIAs in NTI patients. Although some of the one-step FT4 RIAs now contain "specific" albumin blockers, except for a single case report (23), the albumin effect was reported to persist with the modified kits in NTI patients (19), pregnancy (6), and after in vitro addition of albumin to the serum (6). One-step (analog) FT4 RIAs (with or without albumin blockers) therefore can be used only with caution in patients with altered albumin concentration or increased T4 binding to albumin. Because the serum albumin concentration is often decreased in patients with NTI, samples from them are especially susceptible to albumin interference in the one-step (analog) FT4 methods.

The manufacturers of the FT4 assay kits examined here generously provided us with materials, information, and advice, for which we are grateful, as we also are to Dr. Chris Pappas for making available sera from patients with primary biliary cirrhosis.

References

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