Measurements of serum-free thyroid hormone concentrations 
by ultrafiltration
— a comparison with equilibrium dialysis and mathematical calculation —

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An ultrafiltration method (UF) for measuring free thyroxine (FT$_4$) and free triiodothyronine
(FT$_3$) using the Diaflow YM membrane (Centricon-10) is described. The results are compared
with those by equilibrium dialysis (ED) and also by mathematical calculations derived
from T$_4$, T$_3$, and binding protein concentrations. The precision with the UF method was
excellent. The normal ranges of FT$_4$ and FT$_3$ by the three methods are all comparable. There
was a high degree of correlation of FT$_4$ or FT$_3$ results by UF with those by ED and by
calculation (r=0.940–0.974, n=161, P<0.001). FT$_4$ and FT$_3$ by all methods agreed well
for hyperthyroidism, hypothyroidism, and for patients with low T$_4$-binding globulin. The
mean FT$_3$ in pregnancy was lower than the normal value for all methods, and FT$_4$ concen-
trations by UF and calculation also decreased in late pregnancy. The mean FT$_4$ by UF
and ED in low T$_3$ syndrome were significantly higher than in the normal controls, while the
calculated FT$_4$ was lower. The FT$_3$ in low T$_3$ syndrome distributed normal to subnormal
in all methods. These results indicate that a) the UF method is a reliable reference method
for measuring FT$_4$ and FT$_3$ concentrations; b) the UF results agree well with those by ED
and also with theoretically derived values in subjects with thyroid diseases and TBG abnor-
malities; c) for patients with low T$_3$ syndrome, the FT$_4$ results obtained by UF and ED are
similarly discrepant from the calculated results, implying the existence of binding inhibitor(s)
which affect both UF and ED measurements.

Key words: Free thyroid hormones, Ultrafiltration, Equilibrium dialysis, Calculation,
Diagnostic aid, Thyroid function

INTRODUCTION

Circulating thyroid hormones, thyroxine (T$_4$) and triiodothyronine (T$_3$), are bound to the binding proteins,
T$_4$-binding globulin (TBG), T$_4$-binding prealbumin (TBPA), and albumin, and only very small
fractions of T$_4$ and T$_3$ are in the unbound (free) form in human serum.$^1$ Because free T$_4$(FT$_4$) and
free T$_3$(FT$_3$) are generally not affected by variations in T$_4$-binding proteins,$^{2,3}$ FT$_4$ and FT$_3$
concentrations reflect the thyroid function more closely than the total concentrations of T$_4$ and T$_3$.

The most widely used method for estimation of FT$_4$ and FT$_3$ is equilibrium dialysis (ED),$^{4,5}$ but this
method is time-consuming and not convenient for clinical use. To overcome this, a number of radio-
immunoassays (RIA) based on different principles have been developed for estimates of FT$_4$ and FT$_3$,
but results with these may be influenced by changes in serum proteins and by the effect of nonthyroidal
illnesses (NTI).$^6,7$

Ultrafiltration techniques have been developed in recent years for use with dialysis tubing or micro-
partition systems\(^8\)–\(^{12}\) and this technique is an alternative to ED and RIA. In the present study, we evaluate the ultrafiltration technique using Amicon YM Diaflow membrane for FT\(_4\) and FT\(_3\) measurements and compare the results with those by ED and mathematical calculations.

**MATERIALS AND METHODS**

**Patients**

Six groups of patients were studied: (I) 45 normal subjects who visited the hospital for routine health examinations, ages 38–68 years; (II) 17 hyperthyroid patients with supranormal T\(_4\) and T\(_3\); (III) 10 hypothyroid patients with subnormal T\(_4\) and (or) T\(_3\) and supranormal thyrotrpin; (IV) 5 patients with low TBG; (V) 40 pregnant women; (VI) 44 patients with NTI, including 9 patients with acute hepatitis associated with supranormal TBG, and 35 patients with low T\(_3\) syndrome. The etiological diagnoses of the low T\(_3\) syndrome include malignant neoplasia (22 cases), cerebral vascular disorders (four cases), rheumatoid arthritis (three cases), renal failure (two cases), cardiac failure (two cases), pulmonary failure (one case), and hepatic failure (one case). None of these patients were treated with heparin or aspirin. All serum samples were stored at \(-20^\circ\)C until assayed.

**Reagents and Apparatus**

*Tracer.* T\(_4\) labeled with \(^{125}\)I (T\(_4\) tracer) and T\(_3\) labeled with \(^{125}\)I (T\(_3\) tracer), both with a listed specific radioactivity of more than 1.2 mCi/\(\mu\)g dissolved in 75% ethanol, were obtained every four weeks from Amersham International plc. On receipt, the tracers were evaporated under nitrogen at 37°C, and human serum albumin (Sigma Chemical Company, T\(_4\) and T\(_3\) concentrations in the albumin were 0.08 \(\mu\)g/g, and 5.5 ng/g, respectively by RIA) in 0.063 M phosphate buffer (pH=7.4) in 0.05% saline was added to yield final concentrations of 1.0 to 1.2% of albumin and incubated for 20 min at 37°C in darkness. The mixture was dialyzed overnight at 4°C against 2.0 L of phosphate buffer to remove labeled contaminants of the tracers. This purification procedure was performed every two weeks. The paper chromatographic analysis (butanol: acetic acid: water, 78: 5: 17 v/v) showed that the dialyzed specimens of T\(_4\) and T\(_3\) tracers contained less than 1.0 % iodide. The tracer concentration for the assay was approximately 20 \(\mu\)Ci/ml, or 16.7 \(\mu\)g/L for both T\(_4\) and T\(_3\).

*Apparatus.* For the ultrafiltration, a Centricon centrifugal microconcentrator (Centricon-10, Amicon Corp., Lexington, Mass, USA) with a Diaflow YM-10 membrane and membrane support base, an O-ring, and a filtercup was used. This membrane has a relative molecular mass (Mr) cut-off of 10,000 daltons.

**Procedures**

*Ultrafiltration.* We added 50 \(\mu\)l of T\(_4\) tracer or 25 \(\mu\)l of T\(_3\) tracer to 100 \(\mu\)l of serum diluted with 650 \(\mu\)l (for FT\(_4\) assay) or 675 \(\mu\)l (for FT\(_3\) assay) of 0.15 M phosphate buffer (pH=7.4) in 0.05% saline. After mixing, the diluted sera were kept in the test tubes for 10 min at room temperature. A 700 \(\mu\)l portion of each serum sample was transferred to the Centricon-10 device, and incubated for a further 20 min at 37°C. It was centrifuged at 1,000 \(\times\)g for 40 min at 37°C. Operation instructions recommend the use of a fixed angle rotor for centrifugation, however a conventional swinging-bucket rotor suitable for obtaining a sufficient volume of filtrate was used. A 300 \(\mu\)l portion of filtrate was added to 1.7 ml of phosphate buffer and 1 ml of the carrier T\(_4\) and T\(_3\) solution. The radioactive T\(_4\) and T\(_3\) were separated by magnesium chloride precipitation techniques as described elsewhere.\(^5\),\(^14\) We determined the total radioactivity by counting a 24 \(\mu\)l aliquot of the original diluted serum samples. The recovery of T\(_4\) and T\(_3\) by MgCl\(_2\) precipitation were 95% and 83%, respectively. The free fractions of iodothyronines were calculated as follows:

\[
\text{CPM of labeled precipitate in 300 \(\mu\)l of filtrate} \times 100
\]
\[
\frac{\text{CPM of labeled T}\_4 \text{ or T}\_3 \text{ in 25 \(\mu\)l of serum} \times 12 \times 8}{8}
\]

where the 8 in the denominator is the dilution factor. For the FT\(_4\) assay, the results were corrected for the 83% yield during the MgCl\(_2\) precipitation. FT\(_4\) and FT\(_3\) concentrations were expressed as the products of total T\(_4\) (or T\(_3\)) and \(\%\)FT\(_4\) (or \(\%\)FT\(_3\)). By this procedure, 20 single samples can be assayed in less than three hours.

**Equilibrium Dialysis.** FT\(_4\) and FT\(_3\) in the 80-fold diluted sera were also measured by equilibrium dialysis with the tracer method and MgCl\(_2\) precipitation techniques as described elsewhere.\(^3\),\(^14\)

**Mathematical Calculation.** The calculation of free thyroid hormone concentrations was made from the following equation, according to Lécureuil et al.\(^15\):

\[
F^2K_{TBG}(1+C_{TBPA}(K_{STBPA+K_{STBPA}}) + C_{TBPA}(K_{TBPA+K_{TBPA}}) + C_{TBPA}(K_{TBPA}(K_{TBPA+K_{TBPA}})}} + C_{TBPA}(K_{TBPA+K_{TBPA}}) + C_{TBPA}(K_{TBPA}(K_{TBPA+K_{TBPA}}) + 1 - K_{TBG}T_{4(3)} = 0)
\]

where, F is the molar concentration of FT\(_4\) or FT\(_3\), C\(_{TBG}\), C\(_{TBPA}\), C\(_{TBPA}\) are the molar concentrations of

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Table 1 Binding constants used in the calculation

<table>
<thead>
<tr>
<th>Proteins</th>
<th>No. of sites</th>
<th>$K_{\text{asso}}$ (L/mol)</th>
<th>$T_4$</th>
<th>Ref. No</th>
<th>$T_3$</th>
<th>Ref. No</th>
</tr>
</thead>
<tbody>
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<td>TBG</td>
<td>2</td>
<td>$2.3 \times 10^{10}$</td>
<td>(16)</td>
<td></td>
<td>$1.15 \times 10^9$</td>
<td>(16)</td>
</tr>
<tr>
<td>TBPA</td>
<td>1</td>
<td>$1.5 \times 10^8$</td>
<td>(17)</td>
<td>§</td>
<td>$5.0 \times 10^6$</td>
<td>(17)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$1.5 \times 10^6$</td>
<td>(17)</td>
<td></td>
<td>$2.0 \times 10^6$</td>
<td>(18)</td>
</tr>
<tr>
<td>Albumin</td>
<td>1</td>
<td>$1.4 \times 10^6$</td>
<td>(19)</td>
<td></td>
<td>$1.0 \times 10^5$</td>
<td>(19)</td>
</tr>
<tr>
<td></td>
<td>2–6</td>
<td>$8.0 \times 10^4$</td>
<td>(20)</td>
<td></td>
<td>$7.0 \times 10^3$</td>
<td>(21)</td>
</tr>
</tbody>
</table>

§Within range quoted by various authors listed in Table 1 of paper from Princé and Ramsden.\(^{17}\)

TBG, TBPA and albumin, and $n$ is the number of binding sites of albumin. We selected values for the binding constants ($K$) that were within the wide ranges quoted by different authors (Table 1)\(^{16–21}\). The program was written for an M-20 (Olivetti, Japan) personal computer.

**Total $T_4$, $T_3$, TBG, TBPA and albumin.** Total $T_4$, $T_3$, and TBG were measured by RIA.\(^{14,22}\) Serum TBPA and albumin were measured by immunoturbidimetry.\(^{23}\) Albumin concentration in the ultrafiltrate was measured by sensitive RIA for urine analysis (Albumin RIA kit, Diagnostic Products Corporation, USA).

**Precision of Assay**

Intersay CV ($n=11$) were: $T_4$ 1.8%; $T_3$ 4.4%; TBG 7.9%; TBPA 4.7%; albumin 6.9%; $\%FT_4$ by E11.0%; $\%FT_3$ by E12.5% for normal control sera.

**Statistical Analysis**

Analysis was by least-square regression, and groups were compared by Student’s $t$-test.

**RESULTS**

In preliminary experiments, we used the albumin RIA kit for urine analysis (approximate sensitivity 0.1 mg/L) to detect protein leakage into the ultrafiltrates. Leakage was $0.00158 \pm 0.00036$% (mean ± S.D.) ($n=4$) albumin from normal sera during ultrafiltration at 37°C for 40 min. This suggests that the protein leakage through the membrane does not cause appreciable error in the FT4 and FT3 measurements.

During centrifugation at 1,000 $\times$ g, progressive increase in filtrate volume was seen from 10 through 60 min. The $\%FT_4$ and $\%FT_3$ for each centrifugation period were measured with 8-fold diluted sera, and all values were nearly constant (Fig. 1).

The binding of thyroid hormones to the Centricon-10 devices was examined. An 800 $\mu$L volume of 8-fold diluted sera enriched with tracers was centrifuged at 37°C for 40 min at 1,000 $\times$ g, and radioactivity bound to the device was counted after three times washing with water. The binding of $T_4$ was $0.18 \pm 0.014$% ($n=3$) and for $T_3$ it was $0.39 \pm 0.09$% ($n=3$) of total.

The effect of temperature during centrifugation on $\%FT_4$ and $\%FT_3$ was also examined, and there were progressive increases in both fractions from 4°C to 37°C.

The deiodination of $T_4$ and $T_3$ tracers during the 20 min incubation at 37°C was examined by paper chromatographic analysis using 8-fold diluted sera. With $T_4$ tracer containing 0.5% iodide, or $T_3$ containing 0.2% iodide added to sera, the iodide contamination of $T_4$ tracer after 20 min of incubation was $0.63 \pm 0.12$% ($n=5$) and for $T_3$ tracer, $0.48 \pm 0.11$% ($n=5$).

The effect of serum dilution of $\%FT_4$ and $\%FT_3$ is shown (Fig. 2). The dilution of sera from normal subjects, hyperthyroidism and pregnancy patients showed an initial decline (approximately 30%) up to 8-fold dilution. Further dilution had little effect on both $\%FT_4$ and $\%FT_3$. In the serum from low $T_3$ syndrome subjects, there was a progressive decline of $\%FT_4$ (48.3%) and $\%FT_3$ (35.3%) at 8-fold dilution and they declined further thereafter.
FT₃ concentrations of the pregnant women in the 2nd and 3rd trimester were significantly lower than the mean value for normal controls, whether calculated or measured by UF, but not by ED although the mean FT₄ by ED was lower than for normal controls. The FT₃ values in the pregnant women in all trimesters were significantly lower than in the normal controls with all the methods. The individual values for FT₄ and FT₃ showed that both measured and calculated FT₄ in pregnancy distributed essentially within the normal ranges, while the FT₃ values were subnormal in 5 of 39 for UF, and in 7 of 39 for ED.

The mean FT₄ values in NTI with high TBG were normal in all methods employed, but the FT₃ values in this group were significantly lower than the normal values by calculation and measured with UF but not by ED. The measured FT₄ concentration for low T₃ syndrome was significantly higher than that of normal controls, whereas the calculated value was significantly lower. Individual FT₄ also showed that the measured FT₃ values distributed from subnormal to supranormal, in contrast to the normal-to-subsnormal distribution of the calculated FT₄ values in NTI. The FT₃ concentrations, both measured and calculated, distributed from normal to subsnormal in NTI, and the mean values in the low T₃ syndrome were significantly lower than the corresponding normal values.

Table 3 summarizes the regression analysis of the results obtained by the three methods for FT₄ and FT₃ measurements. When all data were analyzed together, the best correlations were between UF and ED methods for FT₄ and FT₃ (r = 0.974, and 0.972, respectively). The calculated FT₄ or FT₃ has a similar correlation with the UF and ED methods. When each subgroup was subjected to separate statistical analysis, there was poor correlation between UF and ED for FT₃ in normal subjects and in pregnant women, and between ED and calculations for FT₃ in normal subjects and in pregnant women. The calculated FT₄ and FT₃ values showed good correlations with both ED and UF methods for all groups analyzed.

**DISCUSSION**

 Determination of free thyroid hormone concentrations using the Amicon ultrafiltration device with a Diaflow YM membrane offers several advantages. This method is rapid, reproducible, and technically simple. A minimal serum albumin leak will not produce appreciable errors in the measurements. The %FT₄ and %FT₃ values are unchanged throughout the centrifugation. In addition, binding of tracers to the device and deiodination of tracers are minimal.

Fig. 2 Effect of serum dilution on %FT₄ (A) and %FT₃ (B) by ultrafiltration method in sera from normal subjects, hyperthyroid patients, pregnant women, and patients with low T₃ syndrome.

The intra-assay precision (CV) of the UF method was 2.2% for %FT₄ (n = 5), and 3.1% for %FT₃ (n = 5), and the inter-assay CV was 6.8% for %FT₄ (n = 10) and 12.6% for %FT₃ (n = 10) for samples in the normal range.

The mean values (±S.D.) for measured and calculated free thyroid hormones, together with the values for the various parameters we used in our computation, are shown in Table 2, and the individual values in low TBG, pregnancy, and NTI are also shown in Fig. 3 and Fig. 4. The normal ranges of FT₄ were 0.95 × 10⁻¹¹ to 2.4 × 10⁻¹¹ mol/L for UF, 10⁻¹¹ to 2.1 × 10⁻¹¹ mol/L for ED, and 0.95 × 10⁻¹¹ to 2.2 × 10⁻¹¹ mol/L for calculated FT₄. The normal ranges of FT₃ concentrations were 2.1 × 10⁻¹² to 4.6 × 10⁻¹² mol/L for UF, 2.1 × 10⁻¹² to 5.0 × 10⁻¹² mol/L for ED, and 2.4 × 10⁻¹² to 5.0 × 10⁻¹² mol/L for calculated FT₃. The measured and calculated normal ranges for FT₄ and FT₃ were not significantly different.

Compared with the values for normal controls, both FT₄ and FT₃ by these methods were significantly higher in hyperthyroidism and lower in hypothyroidism. Both mean values for FT₄ and FT₃ by these methods were similar to the corresponding normal controls in the low TBG group. The mean
Table 2 Values for calculated and measured serum free thyroid hormone concentrations in various groups

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<th>Normal (n=17)</th>
<th>Hyperthyroidism (n=10)</th>
<th>Hypothyroidism (n=17)</th>
<th>Low TBG (n=5)</th>
<th>Pregnancy First trimester (n=11)</th>
<th>Second trimester (n=13)</th>
<th>Third trimester (n=16)</th>
<th>Nonthyroidal illnesses with high TBG (n=9)</th>
<th>Low T3 (n=35)</th>
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<tr>
<td>T4 (10⁻⁹ mol/L)</td>
<td>118a</td>
<td>287a</td>
<td>44a</td>
<td>60a</td>
<td>144a</td>
<td>174a</td>
<td>167a</td>
<td>153a</td>
<td>82a</td>
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<tr>
<td></td>
<td>(20)</td>
<td>(74)</td>
<td>(21)</td>
<td>(11)</td>
<td>(40)</td>
<td>(26)</td>
<td>(37)</td>
<td>(27)</td>
<td>(29)</td>
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<tr>
<td>T3 (10⁻⁹ mol/L)</td>
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<td>6.31a</td>
<td>1.09a</td>
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<td>2.09a</td>
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<td>TBG (10⁻⁷ mol/L)</td>
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<td>57.1a</td>
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<td>(10.2)</td>
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<td>Albumin (10⁻⁶ mol/L)</td>
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<td>585</td>
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<td>(69)</td>
<td>(69)</td>
<td>(83)</td>
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<td>(52)</td>
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<tr>
<td>FT₄ (10⁻¹¹ mol/L)</td>
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<tr>
<td>UF</td>
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<td>7.58a</td>
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<td>(0.34)</td>
<td>(0.48)</td>
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<td>(0.04)</td>
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<td>(0.24)</td>
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<td>UF</td>
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<td>(0.77)</td>
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<td>(0.63)</td>
<td>(0.21)</td>
<td>(0.31)</td>
<td>(0.63)</td>
<td>(0.48)</td>
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</tbody>
</table>

§Mean (S.D.) is shown. *a* significantly different from normal control by p<0.001, 0.01, or 0.05.

Fig. 3 Individual values for FT₄ concentrations as measured by ultrafiltration (UF), equilibrium dialysis (ED), and by mathematical calculation (calc.) in low TBG, pregnancy, and nonthyroidal illnesses. Data for pregnancy include all FT₄ values in Table 2. Arrows indicate FT₄ value above 3.5 x 10⁻¹¹ mol/L in UF and ED methods.

Fig. 4 Individual values for FT₃ concentrations as measured by ultrafiltration (UF), equilibrium dialysis (ED), and mathematical calculation (calc.) in low TBG, pregnancy, and nonthyroidal illnesses. Pregnancy includes first (11), second (13), and third (16) trimester pregnant women.
## Table 3  Correlations between free thyroid hormone concentrations as measured by ultrafiltration, equilibrium dialysis, and mathematical calculation

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=45)</th>
<th>Hyperthyroidism (n=17)</th>
<th>Hypothyroidism (n=10)</th>
<th>Pregnancy (n=40)</th>
<th>Nonthyroidal illnesses (n=44)</th>
<th>Total (n=161)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrafiltration VS.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equilibrium dialysis</td>
<td>FT₄</td>
<td>0.705</td>
<td>0.963</td>
<td>0.971</td>
<td>0.732</td>
<td>0.758</td>
</tr>
<tr>
<td></td>
<td>FT₃</td>
<td>0.378</td>
<td>0.933</td>
<td>0.830</td>
<td>0.473</td>
<td>0.737</td>
</tr>
<tr>
<td>Ultrafiltration VS.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculation</td>
<td>FT₄</td>
<td>0.680</td>
<td>0.872</td>
<td>0.954</td>
<td>0.778</td>
<td>0.656</td>
</tr>
<tr>
<td></td>
<td>FT₃</td>
<td>0.640</td>
<td>0.930</td>
<td>0.915</td>
<td>0.820</td>
<td>0.774</td>
</tr>
<tr>
<td>Equilibrium dialysis VS.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculation</td>
<td>FT₄</td>
<td>0.620</td>
<td>0.912</td>
<td>0.903</td>
<td>0.792</td>
<td>0.419</td>
</tr>
<tr>
<td></td>
<td>FT₃</td>
<td>0.471</td>
<td>0.899</td>
<td>0.878</td>
<td>0.420</td>
<td>0.800</td>
</tr>
</tbody>
</table>

Pregnancy includes all data listed in Table 2. The total is all the subjects in Table 2, including patients with low TBG (n=5). All values for correlation coefficients in this table are statistically significant (p<0.01).

During the procedures, these points must be added to the evaluations in previous reports.11–13

The normal ranges for FT₄ and FT₃ concentrations by the UF method in the present study were somewhat lower than previously shown with undiluted sera.9,10 This is presumably due to the effect of serum dilution, as the data showed serum dilution to produce a lowering of free thyroid hormone fractions, as previously shown in theoretical and experimental studies.17,24–26

There are reports where the free thyroid hormone concentrations by the UF method were compared with the ED method,11–18 however no reports have included a comparison with mathematical calculations. The mathematical model for the interaction between thyroid hormones and their transport proteins in sera has been proposed by several investigators.15,17,27,28 The calculated values for FT₄ and FT₃ varied considerably, possibly because of different values for affinity constants of the binding proteins employed in the calculations.27,28 The model and calculation method by Lécureuil et al.15 which we used here, is simpler than that by Princé and Ramsden.17 The affinity constants used here can still be considered acceptable, as the measured and calculated normal free thyroid hormone concentrations were quite similar. The values for both methods also correlated well with each other in all the combined data, and also in the various groups analyzed separately.

The calculated and measured free thyroid hormone concentrations agree well with each other and are predictable in hyperthyroidism, hypothyroidism, and in patients with low TBG levels. The FT₄ concentrations decrease as pregnancy progresses in the calculated values as well as in the UF measured values but not in the ED-measured FT₄. The mean FT₃ values are also lower than the normal values in pregnancy for all methods. The reason for the discrepancy with the FT₃ results in late pregnancy as measured by UF and ED is unknown, but large variation in the ED method may be involved because the interassay CV for ED is larger than that for UF. Although the reduced FT₄ and FT₃ levels in late pregnancy were established theoretically,29 there is disagreement with previous investigations on the UF measured FT₄ and FT₃ values.3 Our data agree with Shannon et al.,12 Weeke et al.,10 and Lee et al.,30 but not with Faber et al.,9 Sophianopoulos et al.,11 and Wang et al.,13 The FT₄ and FT₃ concentrations by the present UF method agree well with the calculated values, and it is apparent that this method may be useful in evaluating the thyroid functional status with altered TBG concentrations.

We found discrepancies between measured and calculated concentrations of FT₄ but not for FT₃ in low T₃ syndrome. The FT₄ as measured by UF and ED were both significantly higher than the normal values, while the calculated values were conversely lower. Similar discrepancies were reported by Brown-Grant et al. using a different model for FT₄ calculations and the ED method.27 One of the reasons for this discrepancy may be due to the presence of compound(s) that interfere with hormone-protein interaction.31 The progressive decline of FT₄ and FT₃ by serum dilution in the low T₃ syndrome are in accord with the findings by Nelson et al.26 and Weeke et al.10 and may be interpreted to mean that the serum from this syndrome may contain such an inhibitor(s). Chopra et al. have shown that free fatty acid is a likely candidate for the inhibitor, and this inhibitor affects the T₃ binding stronger than the T₄ binding.31 Although the role of free fatty acid has been discounted recently,32 our agreement between calculated and measured FT₃ in the low T₃ syndrome, unlike the FT₄, may be consistent with

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Chopra et al. Our data also indicate that the inhibitor may be equally effective with ED and UF systems. However, we cannot exclude other possibilities such as an alteration of binding characteristics of TBG, or the presence of a different type of TBG in the sera of low T3 syndrome.

The principles of the UF method have been well established, and we describe a method for measurements of FT4 and FT3 which is a reliable and useful reference method for evaluation of thyroid functional status in clinical and experimental studies.

REFERENCES