Free thyroxine concentrations in serum measured by equilibrium dialysis in chronic renal failure

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The serum concentration of free thyroxine (FT₄) is often low in patients with chronic renal failure (CRF) with low serum concentrations of triiodothyronine (T₃). We evaluated the serum FT₄ concentration by using both an equilibrium dialysis RIA kit (D-FT₄) and a labeled-antibody kit (M-FT₄) in two different groups of CRF patients, undergoing chronic hemodialysis (HD, n = 145) or not (non-HD, n = 30), and in a group of normal healthy subjects (n = 58). Thyroid peroxidase antibodies and thyroglobulin antibodies were not detected in any patient. Serum FT₄ concentrations (mean ± SD, pmol/L) by the D- and M-FT₄ assays were, respectively, 21.5 ± 4.6 and 16.6 ± 2.0 in the healthy subjects, 17.8 ± 4.3 and 13.9 ± 3.6 in the non-HD patients, and 16.9 ± 4.9 and 10.7 ± 1.9 in the HD patients. By the D-FT₄ assay, results for both CRF groups were significantly different from those for the healthy group (P < 0.01), as were the results for each pair of groups by the M-FT₄ assay (P < 0.01). FT₄ values were reported as being within the healthy reference range by D-FT₄ in 73 of 113 HD subjects who had low T₃, and low M-FT₄ values. Serum FT₄ concentrations measured by both assay kits showed a significant inverse correlation with the serum concentration of creatinine (P < 0.01), but the serum concentrations of sex-hormone-binding globulin did not differ significantly among the three groups. Our results indicate that the low FT₄ concentration measured by D-FT₄ in patients with CRF, particularly those on HD, probably reflects the actual, mild nonthyroidal illness of renal failure.

INDEXING TERMS: thyroid status • triiodothyronine • hemodialysis • immunoassay compared

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Nonthyroidal illness (NTI) is associated with many diverse effects on thyroid homeostasis.6 Serum concentrations of triiodothyronine (T₃) are usually decreased, and thyrotropin (TSH) concentrations are usually within the reference range [1, 2]. Serum thyroxine (T₄) concentrations are often, though variably, affected in the low-T₃ syndrome, whereas subsequently measured TSH concentrations are usually within the reference range but transiently slightly increased [3]. Thus, when assessing thyroid functions, the probability that the NTI is the cause of these findings is much greater than that of hypothyroidism.

Previous studies have shown that low serum concentrations of T₃ are often but not always accompanied by increased concentrations of “reverse T₃” (3,3',5'-triiodothyronine) [4, 5]. In mild forms of NTI, serum concentrations of T₄ and FT₄ tend to be higher than normal, whereas moderate forms of NTI or caloric deprivation will produce normal or decreased concentrations of serum T₄, with normal or high FT₄ concentrations [as measured by equilibrium dialysis (ED)]; in critical illness, serum concentrations of T₄ and FT₄ are often low. Despite the low serum T₄, however, FT₄ concentrations determined by ED are normal, increased, or only slightly decreased [6, 7]. Most commercially available FT₄ assay methods, which are not based on an ED methodology, measure low-normal or below-normal concentrations of FT₄ in NTI [8, 9]. Thus, the variations in serum FT₄ values in NTI seem to depend mostly on the assay methodology [10].

The serum FT₄ concentrations have also often been shown to be low in patients with chronic renal failure (CRF) [7, 11, 12]. However, the serum FT₄ concentrations measured in such patients by ED/RIA are usually slightly higher than those measured by other methods, especially in patients undergoing chronic hemodialysis (HD). One reason for this difference may be the presence of heparin, which is administered to patients before dialysis [13]. Thus, we evaluated the serum FT₄ concen-
trations measured with kits based on ED or on use of a monoclonal antibody in two groups of patients with CRF, one of which was undergoing chronic HD.

Materials and Methods

SUBJECTS
Participating in this study as a group of normal, healthy subjects (group I) were 32 men (ages 47.8 ± 11.6 years, mean ± SD) and 26 women (ages 46.5 ± 9.2 years). We also studied serum samples from 175 patients with CRF. Group II, CRF patients who were not undergoing HD (non-HD), consisted of 20 men (ages 64.4 ± 13.7 years) and 10 women (ages 64.6 ± 9.6 years). Group III, CRF patients who were undergoing chronic HD, comprised 87 men (ages 58.3 ± 13.6 years) and 58 women (ages 57.0 ± 10.7 years). The serum samples in patients from group III were collected from the three-way valve in the circular HD route within 2 min after 2000 IU of heparin was injected. All blood samples from groups II and III were collected during routine examination. Informed consent for evaluation of thyroid function was obtained from all subjects.

Clinical profiles of the CRF patients are given in Table 1. Groups II and III showed no differences of serum hemoglobin, total albumin, total cholesterol concentrations, or age, but their serum concentrations of urea nitrogen and creatinine differed significantly (P < 0.01). Patients with positive (detectable) thyroid antibodies, e.g., antibodies to thyroid peroxidase or to thyroglobulin, were excluded from the study.

BIOCHEMICAL METHODS
Serum obtained from subjects was kept at −20°C until use. Albumin, hemoglobin, cholesterol, urea nitrogen, and creatinine were measured with the respective methods: Selatestam M ALB (Hitachikasei, Tokyo, Japan), SLS-Hb (Toa Medical Electronics, Kobe, Japan), Enzymatic DAOS (Nipponshoji, Osaka, Japan), Urease UV (Yatoron, Tokyo, Japan), and Creatininase F-DAOS (Wakojyunyaku, Osaka, Japan). Nonesterified unsaturated fatty acids (NEFA) were measured by ACS ASOD method (Wakojyunyaku). The concentrations of serum FT4 were determined by ED with a model FT4 kit (D-FT4) from Nichols Institute (obtained from Nihon Medi+Physics, Japan) [14–16] and by use of a labeled antibody assay [Amerlex-MAB FT4 kit (M-FT4), Johnson & Johnson Clinical Diagnostics, Bucks, UK] [17, 18]. T3 was quantified with a SPAC T3 RIA kit (Daiichi Radioisotope Lab., Tokyo, Japan), TSH with a TSH-Riabeads II IRMA (Dainabot, Tokyo, Japan), and sex-hormone-binding globulin (SHBG) with an SHBG IRMA (Orion Diagnostica, Espoo, Finland).

To determine the intraassay precision of D-FT4, we assayed two serum pools from different individuals. For the mean D-FT4 concentrations of 15.1 and 36.9 pmol/L, the CVs were 11.9% and 6.54%, respectively. The interassay precision of D-FT4 was similarly studied with one assay per pool per day on 10 different days. The mean serum FT4 measured was 15.0 and 37.8 pmol/L, with CVs of 12.1% and 11.3%, respectively. To determine intrassay and interassay precision for the M-FT4 assay, we measured two serum pools from other individuals. The mean intraassay M-FT4 concentrations (and CVs) were 20.6 and 48.5 pmol/L (1.75% and 1.6%), and interassay means were 16.5 and 49.0 pmol/L (1.3% and 2.1%).

STATISTICAL ANALYSIS
For statistical analysis we used ANOVA or Student’s t-test and the Mann–Whitney U-test. Linear regression was performed by the method of least squares, and the correlation coefficient was determined by the method of Pearson.

Results
As shown in Table 2, the serum FT4 concentrations measured by D-FT4 in either group II or III were significantly different from those in group I (P < 0.01), whereas those measured by M-FT4 differed significantly between all groups (P < 0.01), as shown in Fig. 1 and Table 2. Mean serum TSH concentrations in group II differed significantly (P < 0.05) from those in group III (Table 2).

<table>
<thead>
<tr>
<th>Table 2. Mean ± SD serum FT4 concentrations measured by ED (D-FT4) and labeled antibody assay (M-FT4) and concentrations of T3 and TSH.</th>
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<tr>
<td><strong>FT4, pmol/L</strong></td>
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<td><strong>Group I</strong></td>
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<td>Total</td>
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<td>Women</td>
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*a* Significantly different (P < 0.01) from *b* group I or *c* group II results.
*b* Significantly different (P < 0.05) from group I results.
*c* Significantly different from the results for the men in this group; *d* P < 0.01.
*p* P < 0.05.
Eighteen of 30 non-HD patients and 131 of 145 HD patients had low T₃ concentrations. Six of 11 non-HD patients with low T₃ and low M-FT₄ concentrations showed normal FT₄ results by D-FT₄. Of 113 HD patients with low T₃ and low M-FT₄ concentrations, 73 showed FT₄ values by D-FT₄ that were within the healthy reference range.

As measured by both D-FT₄ and M-FT₄ assay kits, serum FT₄ concentrations were not correlated with the serum albumin concentrations in group II and group III but did significantly correlate inversely with the creatinine concentrations (P < 0.01 for results by each kit), as shown in Fig. 2.

Serum SHBG concentrations (nmol/L) were 30.59 ± 12.92 for men and 62.10 ± 28.22 for women in group I; 50.28 ± 20.94 and 68.52 ± 28.40, respectively, in group II; and 30.58 ± 14.83 and 54.78 ± 25.35, respectively, in group III. The differences among the three groups were not significant.

To assess the in vitro effect of heparin on the FT₄ concentrations in our collected samples that had been drawn within 2 min after injection of 2000 IU of heparin, we measured FT₄ by D-FT₄ and M-FT₄ and NEFA in five HD patients just before, and 2, 10, and 60 min after heparin injection. All 20 samples for each analyte/kit combination were determined with use of the same calibration curve for that kit. Serum D-FT₄ and NEFA in samples collected 10 min after heparin injection were significantly different from those in samples taken before or 2 min after injection (P < 0.05), but values in samples collected 2 min after injection were not substantially different from those taken before the heparin administration (Fig. 3). No effect on serum M-FT₄ concentrations was seen at any of the four sampling times (Fig. 3).

Discussion

Serum FT₄ concentrations have been shown to be low in patients with CRF [7, 19–21]. Our results also indicated that serum FT₄ concentrations measured by either D-FT₄ or M-FT₄ were lower in CRF patients than in healthy controls. Among the
group II patients, 27% and 50% had FT₄ concentrations below
the normal reference range as measured by D-FT₄ and M-FT₄,
respectively, compared with 32% and 86% of the group III
patients. Serum binding inhibitors such as furanic acid and
3-carboxy-4 methyl-5-propyl-2-furan propanoic acid in uremic
patients, which could alter the FT₄ fraction, may account for the
divergent results between the two assays [22]. The additional
albumin in the M-FT₄ assay buffers, not included in the D-FT₄
assay buffers, is capable of sequestering NEFA produced in vitro
as well as other interfering substances already present in serum.
This would result in lower FT₄ in the M-FT₄ assay than in the
D-FT₄ assay [17, 18] and would account for the difference in
response of the two assays to additional NEFA. Because the
ratio of mean FT₄ in group II (non-HD) to that in the healthy
control group is virtually identical by M-FT₄ and D-FT₄—
0.835 and 0.830, respectively—the decrease in FT₄ during CRF
may be real and may be measured equivalently by either method
even though the CV of D-FT₄ is greater than that of M-FT₄.
However, when we excluded the samples with great disparities
between D- and M-FT₄ assay estimates (high D-FT₄ — low
M-FT₄ >11.25 pmol/L; n = 30), in which 13 measurable
samples gave slightly high NEFA concentrations, the ratio of
mean FT₄ in group III (HD) to that of the healthy control group
measured by D-FT₄ (0.703) was higher than that measured by
M-FT₄ (0.643) (data not shown).

The serum FT₄ concentrations measured by ED/RIA are
usually slightly higher than those by other measurements,
especially in patients undergoing chronic HD. Heparin is
known to activate the lipoprotein lipases in blood, causing in
vitro production of NEFAs that could entirely explain apparent
increases in FT₄ through their effects on bound T₄ displace-
ment [13, 23, 24]. A recent report has demonstrated that a low
dose of intravenous heparin (5.6 IU) as well as a standard dose
of subcutaneous heparin could cause in vitro generation of
NEFA that would artifically increase the FT₄ concentrations
measured by ED [25]. To investigate the effect of heparin, we
measured FT₄ by D-FT₄ and M-FT₄ and NEFA concentrations
at four different times before and after injection of 2000 IU of
heparin into patients on HD. Although the observed increase in
NEFA concentrations in the relatively few samples measured 2
min postheparin was not enough to be significantly different
from the values in the preheparin group, this increase was
accompanied by an equivalent very small nonsignificant increase
in D-FT₄ estimates. In some subjects, higher than usual concen-
trations of NEFA produced in vitro may partly account for the
much wider range in D-FT₄ concentrations postheparin, which
is not seen in the M-FT₄ results. Therefore, one must
consider possible in vitro effects of heparin when evaluating the
FT₄ concentrations measured in group III samples, even in
samples drawn as soon as 2 min after heparin injection.

Midgley et al. reported a significant correlation between the
serum albumin concentration and the FT₄ concentration mea-
sured by M-FT₄ [26]. Because we found no correlation between
serum albumin concentrations and the FT₄ results, measure-
ments in our assay were not influenced by albumin concen-
trations, even though the latter were greater than those studied in
the other report [26]. However, serum FT₄ concentrations were
significantly inversely correlated with the serum creatinine
concentrations, probably indicating that serum FT₄ concen-
trations do reflect the severity of the disease as previously reported
[21].

Oxygen consumption and T₃ nuclear binding have been
reported to be higher in leukocytes from patients with NTI than
in those from healthy controls [27], as have higher concen-
trations of T₃ receptor mRNA in liver [28]. Because the serum
SHBG concentrations in groups II and III did not differ
significantly from those in group I, we speculate that the thyroid
hormone-protective function in tissues such as the liver is
normal despite the low circulating concentrations of FT₄.

In conclusion, our results indicate that the low FT₄ values
measured by D-FT₄ in patients with CRF, particularly those
undergoing chronic HD, may in part reflect the actual, mild
nonthyroidal illness of renal failure. However, we need to
consider the different distributions of FT₄ results postheparin,
which are not reflected in the preheparin distributions.

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