Increased Urinary Thyroxine Sulfate Excretion in Thyroxine Therapy

WEN-SHENG HUANG, SHIOU-CHI CHERNG, CHAO-HUNG WANG*, BING-FU SHIH*, SHI-WEN KUO**, AND SING-YUNG WU***

Thyroid Laboratory, Department of Nuclear Medicine, Tri-Service General Hospital and National Defense Medical Center, Taipei, *Departments of Medicine and Nuclear Medicine, Mackay Memorial Hospital, Taipei, **Thyroid Laboratory, Division of Endocrinology and Metabolism, Department of Medicine, Tri-Service General Hospital and National Defense Medical Center, Taipei, Taiwan 100, ROC, and ***Nuclear Medicine and Medical Services, Veterans Administration Medical Center, Long Beach, CA 90822, USA

Abstract. Although increased thyroxine sulfate (T4S) levels have recently been detected in fetal serum and amniotic fluid, changes in patients in a high thyroxine (T4) state remain unclarified. This study was conducted to determine the changes in T4S in thyroid hormone regulation in women receiving suppressive T4 therapy. With a highly sensitive and specific radioimmunoassay, we measured the serum and urinary concentrations of T4S in 16 premenopausal women with benign nodular goiter before and after three months administration of T4 (3.2 µg/kg/day). Serum levels of other thyroid hormones were also measured. Significant increases in mean serum T4 levels post-treatment (11.1 vs. 6.6 µg/dL pre-treatment; *P<0.01) were found, although only low T4S levels were detectable in serum both pre- and post-T4 treatment. The mean urinary or creatinine corrected urinary T4S values post-treatment were significantly increased (20 ng/dL or 396 ng/g creatinine vs. 12 ng/dL or 174 ng/g creatinine pre-treatment, *P<0.01). There was a significant correlation between increased creatinine-corrected urine T4S and increased serum free T4. Our results indicate that the sulfation of T4 may be related to the regulation of thyroid hormone metabolism in T4-treated subjects with relative hyperthyroxinemia.

Key words: Thyroxine sulfate, High T4 state (Endocrine Journal 44: 467-472, 1997)

EARLIER studies in adult humans and rats have demonstrated the ability to maintain circulating 3,3',5-triiodothyronine (T3) at nearly normal levels with either thyroxine (T4) deficiency or excess [1, 2], but the homeostatic mechanism in man is different from that found in the rat [3]. The majority of circulating T3 in man appears to be derived from peripheral tissue T4 conversion rather than direct thyroid secretion [2]. In normal conditions, about 80% of total T4 is metabolized by way of monodeiodination into either bioactive T3 or biologically inert 3,3',5'-triiodothyronine (rT3), and the remaining 20% is thought to be metabolized by alternate pathways [4]. Metabolism of thyroid hormone (TH) by alternate pathways is augmented in some pathophysiological situations such as intrauterine life, caloric deprivation, nonthyroidal illnesses (NTIs), in high T4 states in humans and administration of 6-propyl-2-thiouracil (PTU) or iopanoic acid (IOP) in rats and humans [5-9].

Thyrosulfocojugation is one of the major alternate pathways of TH metabolism and is catalyzed by phenol sulfotransferase in the cytosolic fractions of several tissue types of humans and rats, and mainly in the liver [10, 11]. In contrast to

Received: October 7, 1996
Accepted: February 25, 1997
Correspondence to: Dr. Wen-sheng HUANG, Department of Nuclear Medicine, Tri-Service General Hospital, 8, Sec. 3, Ting-chou Road, Taipei, Taiwan 100 R. O. C.
glucuronidated iodothyronines, which mainly contribute to the entero-hepatic circulation of TH, sulfoconjugation participates in human systemic TH metabolism and may play a role in response to physiological requirements [12]. Available data from studies in rats indicate that sulfation of TH results in the inactivation of these hormones and enhancement of their excretion in urine and bile [13].

A recent observation in a human study has shown that the majority of normal T3 disposal occurs via T3 sulfate (T3S) formation [14], but little work regarding the effect of a high T4 state on the changes in thyroxine sulfate (T4S) concentrations in biologic fluids of adult humans has been reported. In the present study of 16 patients on suppressive T4 therapy, we measured the serum levels and urinary excretion of T4S by a T4S radioimmunoassay (RIA) [7]. To address the relationship between T4S and other TH, correlation studies were also performed.

Materials and Methods

Patients

A group of 16 premenopausal women aged 28 to 42 (mean 34) years with benign nodular goiter participated in the present study. All of them were euthyroid, with no evidence of renal, hepatic or other endocrinological diseases. All were normally active outpatients and were not taking medication with a known effect on thyroid or renal function. Written instructions for correct urine collection were supplied to all patients. Urine samples were collected after the first voided morning specimen and blood samples were drawn at the same time.

Patients were then instructed to take T4 (3.2 ± 0.6 µg/kg/day) for 3 months. At the end of the study, while the patients were still taking T4, the same sampling procedure was performed. All patients gave their informed consent as part of a protocol approved by the Ethics Committee of the Hospital. All serum and urine specimens were stored at −70°C until the completion of the study and then analyzed at the same time for concentrations of various TH parameters.

Serum and urine sample preparations

Serum and urine samples obtained pre- and post-treatment were extracted with 2 volumes of 95% ethanol before the assay. Preliminary experiments showed that the extraction efficiency of T4S in serum and urine exceeded 95%. Final values for T4S content were not corrected for recovery efficiency.

T4S RIA

T4S, both radiolabeled and unlabeled, was prepared by the method described by Eelkman-Rooda and coworkers [15]. The RIA employed an anti-L-T4S antibody (Wu091) obtained from rabbits immunized with L-T4S-bovine serum albumin conjugate [7]. In a final dilution of 1:20,000, anti-T4S antibody bound to about 40% of a trace amount (5 pg) of [I-125]-T4S in 0.075 M barbital buffer (pH 8.6) containing 0.1% sodium azide and 0.125% normal rabbit serum. Ethanol did not inhibit the binding of [I-125]-T4S to the antibody up to a final concentration of 22%. We therefore employed ethanol (63%) extracts of sera and urine to measure T4S concentrations. The final ethanol concentration in the assay was 19%.

The T4S RIA was modified for measurement of serum and urine ethanol extracts, as described previously [7]. Briefly, it was carried out in duplicate in 10 x 75 mm glass tubes. RIA tubes contained 0.053 M barbital buffer (pH 8.6), 0.07% sodium azide, 0.088% normal rabbit serum, 19% ethanol and either an unknown or a standard amount of unlabeled T4S (1 to 1000 pg), diluted antiserum, and approximately 10,000 cpm [I-125]-T4S in a final volume of 1 mL. The tubes were thoroughly mixed and incubated at 4°C overnight. A sufficient amount of the titered second antibody was then added. The tubes were mixed, incubated at 4°C overnight, and centrifuged at 2,000 x g for 20 min. Supernatants were aspirated, and the radioactivity in the precipitates was quantified in an LKB 1272 Clinigamma γ-counter (Wallac, Turku, Finland). Nonspecific binding (determined in tubes without added antiserum) was < 5% and was corrected for. Standard curve plots, and other calculations were carried out by means of pre-set programs in the γ-counter. The lower limit of detection for T4S was 2 ng/dL in a 300 µL ethanol extract. Cross-reactivities were, T4, < 0.002%; rT3,
Increased Urinary T₄S in T₄-Treated Patients

< 0.002%; T₃, 0.002%; rT₃S, 9.9% and T₃S, 2.0%.

Hormone assays and urine creatinine measurements

Serum concentrations of total T₄, T₃ and free T₄ (FT₄) (Diagnostic Products Corp. Los Angeles, CA, USA) and rT₃ (Serono Diagnostic Inc. Milano, Italy) were all determined in whole sera by RIAs with commercial kits. Normal ranges were 4.5–12.5 µg/dL for T₄; 86–187 ng/dL for T₃; 0.8–2.0 ng/dL for FT₄; and 9–35 ng/dL for rT₃. The serum levels of TSH were also determined in whole sera by an ultrasensitive immunoradiometric assay (IRMA) (Diagnostic Products Corp. Los Angeles, CA, USA), with normal ranges of 0.3 to 5.0 mU/L. The analytical sensitivity of the TSH assay was 0.03 mU/L. Interassay and intraassay coefficients of variation for each assay were as follows: 6.1% and 4.1% for T₄; 5.8% and 4.5% for T₃; 7% and 5% for FT₄; 7.5% and 6.4% for rT₃; 3.0% and 1.5% for TSH. Urinary creatinine was measured in a Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, NY, USA).

Sources of materials

3,3',5,5'-tetraiodothyronine (T₄) was purchased from Henning-Berlin Co. (Berlin, Germany). [I-125]-T₄ (SA:1500 µCi/µg) was obtained from New England Nuclear Co. (Boston, MA, USA). Goat antirabbit γ-globulin (second antibody) was purchased from Calbiochem Co. (San Diego, CA, USA). N,N-dimethyl formamide was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chlorosulfonic acid, 99%, was purchased from Merck Co. (Schuchardt, Germany). The thyroxine tablets (Eltroxin) were purchased from Glaxo Operations Ltd. (Greenford, England).

Statistical analysis

The data in various groups are presented as the mean ± SEM. The mean values obtained before and after treatment were compared by paired t-test. Significance was defined as P<0.05. Relationships between urinary T₄S and serum TH were tested by linear regression analysis. Those pairs for which the corresponding TSH values were less than the cut-off values of detection, i.e. < 0.06 mU/L, were not taken into account in the linear regression analysis.

Results

Serum T₃, T₄, rT₃ and FT₄ concentrations

Serum T₃, T₄, rT₃ and FT₄ levels pre- and post-treatment are given in Table 1. Serum T₃ concentrations did not change significantly after treatment (134 ± 11 vs. 115 ± 6 ng/dL). The mean serum T₄ and FT₄ concentrations were significantly higher post-treatment although both were still within normal limits (11.1 ± 1.3 vs. 6.6 ± 1.6 µg/dL, P<0.01 and 2.1 ± 0.2 vs. 1.2 ± 0.1 ng/dL, P<0.01 respectively). The TSH values were significantly lower post-treatment (0.12 ± 0.02 vs. 0.69 ± 0.17 mU/L, P<0.01) and were below the normal range. In view of the serum TH changes, subclinical or tissue hyperthyroidism was strongly suspected.

T₄S concentrations in serum and urine

No significant increase in serum T₄S concentrations was found after treatment. In

Table 1. Changes in thyroid hormone levels before and after thyroxine therapy in premenopausal women with nodular goiter

<table>
<thead>
<tr>
<th></th>
<th>Normal ranges</th>
<th>Before therapy</th>
<th>After therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum T₃ (ng/dL)</td>
<td>(86–187)</td>
<td>115 ± 6</td>
<td>134 ± 11</td>
</tr>
<tr>
<td>T₄ (µg/dL)</td>
<td>(4.5–12.5)</td>
<td>6.6 ± 1.6</td>
<td>11.1 ± 1.3*a</td>
</tr>
<tr>
<td>rT₃ (ng/dL)</td>
<td>(9–35)</td>
<td>21.1 ± 2.1</td>
<td>38.8 ± 3.2*a</td>
</tr>
<tr>
<td>Free T₄ (ng/dL)</td>
<td>(0.8–2.0)</td>
<td>1.2 ± 0.1</td>
<td>2.1 ± 0.2*a</td>
</tr>
<tr>
<td>TSH (mU/L)</td>
<td>(0.3–5)</td>
<td>0.69 ± 0.17</td>
<td>0.12 ± 0.02*a</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. * P<0.01 compared to before thyroxine therapy.
contrast to serum levels, relatively large amounts of T4S existed in urine. The urinary levels of T4S were also significantly increased after treatment (20 ± 3.1 vs. 12 ± 1.4 ng/dL or 396 ± 46 vs. 174 ± 36 ng/g creatinine, P<0.01 respectively) (Table 2).

Table 2. Changes in sulfated thyroxine levels in serum and urine before and after thyroxine therapy in premenopausal women with nodular goiter

<table>
<thead>
<tr>
<th></th>
<th>Before therapy</th>
<th>After therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4S (ng/dL)</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4S (ng/dL)</td>
<td>12 ± 1.4</td>
<td>20 ± 3.1*</td>
</tr>
<tr>
<td>Urine (c)</td>
<td>174 ± 36</td>
<td>396 ± 46*</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. * P<0.01 compared to before therapy groups. Urine (c), creatinine corrected urine in ng/g creatinine.

Correlation of urinary T4S and serum thyroid hormones

Table 3 shows the correlations of urinary T4S values and the corresponding serum TSH, T4, and FT4. The closest correlation was found between urinary T4S levels and serum FT4 (r=0.64, P<0.01) (Fig. 1).

Table 3. Correlations between serum thyroid hormone profiles and creatinine corrected urine sulfated T4 (T4Uc) levels

<table>
<thead>
<tr>
<th></th>
<th>Serum TSH (mU/L)</th>
<th>Serum T4 (ng/dL)</th>
<th>Serum FT4 (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T4SUc</td>
<td>r</td>
<td>0.59</td>
<td>0.55</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.05</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

* T4SUc: creatinine corrected urine sulfated T4 in ng/g creatinine. Serum TSH levels were measured with highly sensitive IRMA kits. Those less than the clinical detection limit (< 0.06 mU/L) were not taken into account.

Discussion

In the present study, we observed a significant increase in urinary excretion of T4S in patients after T4 treatment, although only low T4S values were detectable in serum both pre- and post-T4 treatment. We also demonstrated a close correlation between urinary T4S and the corresponding serum T4, FT4 and TSH values. The correlation between creatinine corrected urine T4S and serum FT4 values were the most consistent. Our data suggest that substrate availability may play an important role in the changes in urinary T4S. These results are consistent with our earlier report [7] and that of Nicoloff and LoPresti [9].

The T4 levels after treatment in the present study were significantly higher than those before treatment. Moreover, TSH levels after T4 treatment were suppressed below the cut-off values after treatment (0.12 ± 0.02 mU/L; normal range: 0.3–5.0 mU/L), suggesting that the patients were borderline hyperthyroid due to high levels of T4 supplement. Notably, the mean serum T3 value in patients during treatment remained relatively stable compared to that before treatment (115 vs. 134 ng/dL, P>0.05). This is consistent with the phenomenon of "autoregulation" proposed by Nicoloff and LoPresti to describe the protection of human tissue from exposure to excessive amounts of T3 [9]. In this model, as serum T4 values increase, the efficiency of T4 to T3 conversion decreases [9], leading to a discrepancy between rates of T4 degradation and the formation of monodeiodinated derivates in patients treated with increasing doses.
INCREASED URINARY T₄S IN T₄-TREATED PATIENTS

of T₄ [16]. A similar result was also reported by Inada et al. [17].

Available evidence from kinetic studies indicates that metabolism of TH in patients in a high T₄ state involves a T₄ disposal accounting gap [9], indicating that some products are made intracellularly and are degraded or leave cells for disposal elsewhere, without equilibrating with the plasma pool. Our present data, along with earlier studies, show that sulfonation of TH in man may be an important pathway contributing to the accounting gap [7, 9, 14].

T₄S is an intermediate metabolite of TH metabolism, and is formed on the tissue level in response to changes in the T₄ concentration, as found in fetal sheep [18]. In a human study, an inverse relationship between the efficiency of conversion of T₄ to T₃, and the production of sulfated metabolites was also noted [16]. Notably, this regulatory metabolism appears to respond instantly [16, 17]. It is therefore plausible to speculate that the changes in T₄S may provide a clue that reflects the optimal T₄ level in human tissues.

Although many factors affect the metabolism of thyrosulfon conjugates, activities of tissue phenol sulfotransferase (PSTs), monodeiodinase and thyroid status appear to be the most important determinants [10–12, 19, 20].

Sulfation of TH is catalysed by multiple PST isoenzymes in human tissues [10, 11]. Although T₄ is not thought to be a good substrate for sulfation in vitro [12], Sato et al. showed accumulation of T₄S in primary cultures of rat hepatocytes, while type I monodeionase (MDI) was inhibited or saturated at a high T₄ concentration [21]. In the current study, the subjects were actually in high T₄ and relatively hyperthyroid states after treatment. The latter, as observed in rats [22], is rapidly converted to reverse T₃ sulfate (rT₃S) due to a remarkable decrease in Km and increases in V_max values in inner ring deiodination, as shown in rats [22]. A significant increase in creatinine corrected urinary rT₃S was also observed in our subjects after T₄ treatment (647 ± 121 vs. 215 ± 42 ng/g Cr before treatment; P<0.01) (unpublished data). Although the extent of T₄S contribution to the measurement of urinary rT₃S is still unknown, increased urinary excretion of T₄ and its analogs appears to be an alternative metabolic step in "autoregulation" and may be partially responsible for the T₄ disposal gap in high T₄ states. Clearly, more kinetic work on T₄S metabolism and excretion is needed to obtain more accurate quantitative data regarding this issue.

In conclusion, the present study serves as a preliminary report to rationalize the relationship between urinary excretion of T₄S and serum concentrations of FT₄ in T₄ treated subjects with relative hyperthyroxinemia, a commonly encountered clinical situation. The study of creatinine-corrected urine concentrations of T₄S therefore appears to be a supplementary method for the evaluation of subclinical tissue changes that result from overzealous T₄ treatment.

Acknowledgments

The authors thank Mr. Gin-Cherng Pong, Ms. Mei-Rong Chang and Ms. Melissa Jodan for their able technical assistance. This work has been supported by the National Science Council (R. O. C.) Grant NSC-85–2331-B016–134 and Tri-Service General Hospital Research Grant TSGH-C86–41.
References


