Serum androgen levels in hyperthyroid women

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Key words: Androgen levels, hyperthyroidism, 5α-reductase

Summary: Thyroid hormone is known to affect androgen metabolism, however, there are few studies in which alterations of androgen metabolism are simultaneously examined in patients with clinical thyroid disorders. In this study, we investigated the alterations of thyroid hormone and androgens before and during treatment in patients with hyperthyroidism. Fifteen female patients with hyperthyroidism due to Graves’ disease were studied. From these patients, blood samples were obtained before treatment and at 1 month (M), 2M, 3M, 4M and 6M after beginning of treatment. Concentrations of free T4 (FT4), free T3 (FT3), testosterone (T), androstenedione (Δ4A), dihydrotestosterone (DHT), 5α-androstane-3α, 17β-diol (3α-diol) and androstenedione (AD) and sex hormone binding globulin (SHBG) were measured by radioimmunoassays (RIAs) or immunometric assay (IRMAs). As normal controls, ten healthy women were also studied. Before treatment concentrations of DHT (mean ± SD: 3.35 ± 0.78 nmol/L), 3α-diol (0.78 ± 0.11 nmol/L), AD (6.73 ± 0.64 nmol/L) and SHBG (184.9 ± 68.1 nmol/L) were significantly elevated compared with those of normal controls. T and Δ4A levels were not significantly different from normal values. DHT/T ratio indicating 5α-reductase activity was 4.62 ± 2.55 and significantly higher than that in normal controls. At 2 months after beginning of treatment with anti-thyroid drugs, thyroid function (FT4 and FT3) became normal and at 3 months after beginning of treatment, DHT, 3α-diol, AD and DHT/T ratio decreased to normal range. SHBG level was gradually decreased, however still higher than that of the normal control group at 6 months after beginning of treatment. There was a time lag in recovery between serum androgen levels and SHBG level.

Introduction

It is documented that hyperthyroidism or hypothyroidism is associated with alterations in androgen metabolism (Hellman et al., 1959; Southren et al., 1974; Rosenfeld et al., 1976). Scanlon et al. (1988) reported that serum 5α-androstane-3α, 17β-diol glucuronide (3α-diol G) levels were increased in hyperthyroid women and reduced in hypothyroid women. To explain these results, it is postulated that thyroid hormones stimulate hepatic 5α-reductase activity. Ford et al. (1992) described that hyperthyroidism was associated with elevations of the total testosterone and sex hormone binding globulin (SHBG) levels. Serum SHBG levels are often elevated in patients with hyperthyroidism and in such patients the elevated SHBG levels are reduced after treatment (Olivo et al., 1970; Akande and Anderson, 1975; De Nayer et al., 1986; Sarne et al., 1988). The administration of large doses of triiodothyronine or of thyroxine to normal subjects has been found to increase serum SHBG to the levels of patients with endogenous hyperthyroidism (Ruder et al., 1971). However, few investigators have reported on the alterations of androgen metabolism after beginning of treatment in patients with actual thyroid disorders. We investigated the alterations of thyroid hormone and androgens before and after beginning of treatment in patients with hyperthyroidism.

Materials and methods

Subjects

Fifteen female patients with hyperthyroidism due to Graves’ disease (age: mean ± SD = 30.9 ± 7.9y) were studied. Hormonal findings are shown in Table 1. All patients had high levels of free T4 (FT4) (100 ± 34.4 pmol/L), free T3 (FT3) (28.4 ± 9.1 pmol/L) and TSH receptor antibody (TRAb) (54.8 ± 18.0%) and suppressed TSH levels (<0.1 μU/ml). Furthermore, they showed typical symptoms such as palpitation, sweating, hand tremor and ophthalmopathy. They were free of medications known to affect thyroid hormone and steroid hormone levels for at least three months. From these patients blood samples were obtained in the follicular phase before treatment and at 1 month
Table 1  Alterations of androgen levels and thyroid hormones in hyperthyroidism

<table>
<thead>
<tr>
<th></th>
<th>T nmol/L</th>
<th>ΔA4A nmol/L</th>
<th>DHT nmol/L</th>
<th>3α-diol nmol/L</th>
<th>AD nmol/L</th>
<th>FT4 pmol/L</th>
<th>FT3 pmol/L</th>
<th>SHBG nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>before</td>
<td>0.82±0.21</td>
<td>3.19±0.76</td>
<td>3.35±0.78*</td>
<td>0.78±0.11*</td>
<td>6.73±0.64*</td>
<td>100 ±34.4</td>
<td>28.4±9.1</td>
<td>184.9±68.1</td>
</tr>
<tr>
<td>1M</td>
<td>0.90±0.22</td>
<td>3.31±0.58</td>
<td>2.86±0.58*</td>
<td>0.68±0.08*</td>
<td>6.01±0.74*</td>
<td>28.6±9.0</td>
<td>11.9±3.0</td>
<td>150.6±48.9</td>
</tr>
<tr>
<td>2M</td>
<td>0.88±0.15</td>
<td>3.50±0.40</td>
<td>2.21±0.52*</td>
<td>0.53±0.07*</td>
<td>5.05±0.86*</td>
<td>15.4±8.2</td>
<td>6.8±2.6</td>
<td>112.7±24.9</td>
</tr>
<tr>
<td>3M</td>
<td>0.86±0.17</td>
<td>3.34±0.38</td>
<td>1.39±0.43</td>
<td>0.41±0.06</td>
<td>3.87±0.45</td>
<td>11.9±8.8</td>
<td>6.3±2.1</td>
<td>82.3±25.4</td>
</tr>
<tr>
<td>4M</td>
<td>0.89±0.19</td>
<td>3.54±0.53</td>
<td>1.24±0.28</td>
<td>0.37±0.05</td>
<td>3.63±0.47</td>
<td>12.4±6.2</td>
<td>5.4±1.6</td>
<td>73.3±19.3</td>
</tr>
<tr>
<td>6M</td>
<td>0.95±0.21</td>
<td>3.61±0.52</td>
<td>1.30±0.26</td>
<td>0.40±0.05</td>
<td>3.58±0.35</td>
<td>14.3±3.3</td>
<td>5.5±1.2</td>
<td>65.1±11.5</td>
</tr>
</tbody>
</table>

Normal 0.77±0.26  3.59±1.09  1.04±0.39  0.40±0.07  3.42±0.83  16.4±3.9  5.8±1.0  50.9±12.0*

* p < 0.05 vs normal control

(M), 2M, 3M, 4M and 6M after beginning of treatment (Menstrual cycles of five patients were unclear). During two months treatment with anti-thyroid drugs, some cases showed suppressed TSH level (<0.1 μU/ml) and in others TSH level were detectable (≥0.1 μU/ml). In the study the calculation of mean TSH value is omitted. Concentrations of FT4, FT3, testosterone (T), androstenedione (ΔA4A), dihydrotestosterone (DHT), 5α-androstan-3α, 17β-diol (3α-diol) and androsterone (AD) were measured by radioimmunoassays (RIA). Sex hormone binding globulin (SHBG) was also determined by immunoradiometric assay (IRMA). Therapy for Graves’ disease was with methimazole (MMI) or propylthiouracil (PTU). As normal controls, ten healthy women (age: 34.2 ± 4.6y) were studied in the follicular phase. From our preliminary studies there was no difference in these steroid levels between follicular and luteal phase. Their hormonal findings are shown in Table 1. They had no thyroid illness in their past history and were physically well and medication-free. All had normal physical examinations and no goiter. Informed consent was obtained from all subjects.

Laboratory assays

T, Δ4A and AD levels were determined by a modification of RIA methods as previously described (Ueshiba et al., 1991; Ueshiba et al., 1996). DHT was measured by RIA using rabbit antiserum to DHT-11α-succinate-bovine serum albumin (BSA). The cross-reactivity of the antiserum with other steroids was: 58% with T, 10% with Δ4A, 0.03% with progesterone, 0.02% with dehydroepiandrosterone and less than 0.01% with cortisol, corticosterone and estradiol. Eight ml of diethyl ether was added to 0.1 ml of serum and the mixture was shaken using a vortex mixer. The ether layer was transferred into another tube and evaporated under a stream of nitrogen. The dried residue was redissolved in methanol-water (60:40) and injected into a modification of high-performance liquid chromatography (HPLC) system as previously described (Ueshiba et al., 1991). DHT was clearly separated from T and Δ4A by this HPLC system. The DHT fraction was collected and evaporated at 40°C in a centrifugal concentrator. The dried residue was subjected to RIA. Radiolabeled [1,2-3H] DHT (40–60 Ci/mmol) was purchased from NEN (Boston, MA, USA). Sample or standard, tritium-labeled DHT and antibody to DHT (suitably diluted with a 1g/L solution of γ-globulin (bovine, Cohn Fraction II Sigma Chemical Co., St. Louis, MO, U.S.A.) in isotonic saline) were incubated at 4°C overnight (16–20 h). Two hundred μl of 5 g/L dextran-coated charcoal suspension [500 mg of dextran T70 (Pharmacia Fine Chemicals, Piscataway, NJ, U.S.A.) and 500 mg of charcoal (Sigma Chemical Co.) in 100 ml of isotonic saline, stored at 4°C] were added to the mixture, briefly vortex-mixed, incubated at 4°C for 15 min, and centrifuged for 15 min at 4°C and 1000 x g. The supernatant was transferred into a counting vial, mixed with 6 ml of the aqueous counting scintillant (ACS II Amersham, Arlington Heights, IL, U.S.A.) and the radioactivity was measured. The intra- and interassay coefficients of variation did not exceed 7% and 10%, respectively.

3α-diol was measured by RIA using rabbit antiserum to 3α-diol-17-succinate-BSA. The cross-reactivity of the antiserum with other steroids was: DHT, 1.6%, 5α-androstan-3α, 17β-dione, 1.48%, 5α-androstan-3β, 17β-diol, 1.2%, 5α-androstan-3α, 17β-dione, 0.6%, T, 0.2%, pregnenolone, 0.02%, progesterone, 17α-hydroxyprogesterone, corticosterone, cortisol and estradiol, 0.1%. The assay procedure was identical to that of DHT without HPLC purification. [9,11-3H]3α-diol (40–60 Ci/mmol, NEN) was used as tracer. The intra- and interassay coefficients of variation were below 8% and 11%, respectively. FT4, FT3, TSH, TRAb and SHBG were measured by RIAs or IRMA using commercially available kits [Amersham, Daiichi Radioisotope Labs. (Japan), Cosmic Co. (Japan) and Orion Diagnostica (Finland)]. In our hands, the intra- and interassay coefficients of variation did not exceed 10% in any assay.

Statistical analysis

All data are expressed as the mean ± SD. Statistical analysis was performed using Student’s t-test. P < 0.05 was used to define statistical significance.
Table 2  DHT/T ratio as an index of 5α-reductase activity

<table>
<thead>
<tr>
<th></th>
<th>Hyperthyroidism</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>before</td>
<td>4.62±2.55*</td>
<td></td>
</tr>
<tr>
<td>1M</td>
<td>3.34±0.95*</td>
<td></td>
</tr>
<tr>
<td>2M</td>
<td>2.38±0.74*</td>
<td>1.41±0.52</td>
</tr>
<tr>
<td>3M</td>
<td>1.63±0.53</td>
<td></td>
</tr>
<tr>
<td>4M</td>
<td>1.45±0.44</td>
<td></td>
</tr>
<tr>
<td>6M</td>
<td>1.40±0.25</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 vs normal control

Results

Androgen levels before treatment (Table 1)

In the hyperthyroid group before treatment, concentrations of DHT (3.35 ± 0.78 nmol/L), 3α-diol (0.78 ± 0.11 nmol/L), AD (6.73 ± 0.64 nmol/L) and SHBG (184.9 ± 68.1 nmol/L) were significantly lower than those of normal control group (DHT: 1.04 ± 0.39 nmol/L, 3α-diol: 0.40 ± 0.07 nmol/L, AD: 3.42 ± 0.83 nmol/L and SHBG: 50.9 ± 12.0 nmol/L). However, T (0.82 ± 0.21 nmol/L) and Δ4A (3.19 ± 0.76 nmol/L) levels were not significantly different from normal (T: 0.77 ± 0.26 nmol/L and Δ4A: 3.59 ± 1.09 nmol/L).

Androgen levels during treatment (Table 1)

Along with the treatment with anti-thyroid drugs, FT4 and FT3 of the hyperthyroid group were decreased to normal range. SHBG level was gradually decreased, but still higher than that of the normal control group at 6 months after beginning of treatment. Meanwhile, DHT, 3α-diol and AD levels, which were elevated before treatment, were similar to normal at 3 months after beginning of treatment and maintained the same levels. There was a time lag in recovery between serum androgen levels and SHBG level. T and Δ4A levels did not change significantly after beginning of treatment and were not different from those of normal control group.

DHT/T ratio (Table 2)

In the hyperthyroid group before treatment, the DHT/T ratio, indicating 5α-reductase activity, was 4.62 ± 2.55 and significantly higher than that in the normal control group (1.41 ± 0.52). At 1 or 2 months after beginning of treatment, this ratio was still higher than that in the normal control group. However, at 3 months after beginning of treatment this ratio decreased to normal range and remained there for 6 months suggesting that it took 3 months until 5α-reductase activity normalized.

Discussion

Androgen metabolism in thyroid disorders was reported by several investigators, however little information is available concerning the relationship between improvement of thyroid function and change of androgen levels in the same patient. Ford et al. (1992) investigated serum levels of total testosterone in the hyperthyroid and euthyroid state in 11 female patients with Graves' disease and reported that total testosterone concentration was significantly higher during hyperthyroidism than during euthyroidism. However, this alteration occurred only in the reference range of total blood testosterone levels, and it seems that total testosterone levels are not necessarily higher in Graves' disease when compared with age-matched normal control subjects.

We investigated androgen metabolism in 8 female patients with hypothyroidism due to Hashimoto's thyroiditis (Ueshiba et al., 1993). Serum levels of DHT, 3α-diol, AD and DHT/T ratio were decreased, while T and Δ4A levels were normal. These data suggest that 5α-reductase activity was low in hypothyroidism. After 6 months treatment with levo-thyroxine, 5α-reductase activity normalized.

In this study, androgen levels in 15 female patients with hyperthyroidism was examined and contrary to hypothyroidism DHT, 3α-diol, AD and DHT/T ratio were all increased before treatment. T and Δ4A levels showed no significant difference compared with those in normal control group. These data suggest that 5α-reductase activity was increased under the influence of high blood levels of thyroid hormones. In parallel with the recovery of thyroid function, 5α-reductase activity was improved. 5α-reductase is a microsomal, NADPH-dependent enzyme catalyzing the 5α-reduction of T to DHT. From biochemical analysis of 5α-reductase present in human skin-derived fibroblasts, two 5α-reductase isoenzymes called Type I and Type II were shown to exist (Andersson, 1993). Biochemical and pharmacological evidence suggests that the Type I exists in various tissues such as ovary, adrenal gland, brain, liver and kidney, whereas the Type II is the predominant 5α-reductase in androgen target tissues such as prostate and epididymis (Normington and Russel, 1992). Therefore, thyroid hormone may affect Type I 5α-reductase.

Other investigators suggested that thyroid hormone caused an increase in hepatic 5α-reductase activity in a study of 5 hyperthyroid and 5 hypothyroid female patients (Scanlon et al., 1988).

However, little is known concerning the detailed mechanism of thyroid hormone action on 5α-reductase. While thyroid hormone affects generalized metabolism, it is possible that 5α-reductase present in ovary, adrenal gland and kidney except liver are also increased. We need further study to elucidate the role of thyroid hormone for the activity of 5α-reductase.

In this study before treatment, serum SHBG level was decreased. At 6 months after beginning of treatment, FT4, FT3 and androgen levels were within the normal range, however serum SHBH was still higher.
There was a time lag in recovery between serum androgen levels and SHBG level. It is suggested that the influence of thyroid hormone exist even though FT4 and FT3 are normalized.

In conclusion, we showed DHT, 3α-diol, AD, DHT/T ratio and SHBG were increased in patients with hyperthyroidism and suggested that 5α-reductase activity was increased under the influence of high blood levels of thyroid hormones. There was a time lag in recovery between serum androgen levels and SHBG level.

References

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Ectopic gene expression rare in cases of thyroid cancer. et al., theo Cusick et al.