Serum Concentration of Androstenediol and Androstenediol Sulfate in Patients with Hyperthyroidism and Hypothyroidism

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Abstract. Androstenediol (5-androsten-3β, 17β-diol, ADIOL) and androstenediol 3-sulfate (ADIOLS) are active metabolites of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS), respectively, and have estrogenic activity and immunoregulatory function. We examined serum concentrations of ADIOL, ADIOLS, DHEA, DHEAS and pregnenolone sulfate (5-pregnen-3β-ol-20-one sulfate, PREGS) in patients with Graves’ thyrotoxicosis (male/female 9/14), hypothyroidism (11/20) and in normal controls (14/29). In hypothyroidism serum levels of all these steroids were significantly decreased in both genders. In hyperthyroidism, in contrast, serum levels of ADIOLS (male 1.49±0.69, female 0.64±0.31 μmol/l), DHEAS (male 7.43±3.91, female 5.13±2.03 μmol/l), and PREGS (male 1.13±0.58, female 1.07±0.85 μmol/l) were markedly increased, but serum concentrations of ADIOL and DEHA were not significantly different from controls (ADIOLS male 0.36±0.33, female 0.14±0.09 μmol/l; DHEAS male 2.88±1.70, female 1.86±1.03 μmol/l; PREGS male 0.18±0.12, female 0.11±0.08 μmol/l; ADIOL male 3.76±1.35, female 1.91±1.17 nmol/l; DHEA male 9.23±3.49, female 13.5±10.8 nmol/l). Serum concentrations of all these steroids correlated with the serum concentration of the thyroid hormones in these patients. Serum albumin and sex hormone-binding globulin concentrations were not related to these changes in the concentrations of steroids. These findings indicate that serum concentrations of ADIOLS, ADIOL, DHEAS, DHEA and PREGS were decreased in hypothyroidism, whereas serum ADIOLS, DHEAS and PREGS concentrations were increased but ADIOL and DHEA were normal in hyperthyroidism. Thyroid hormone may stimulate the synthesis of these steroids and sulfotransferase is speculated to be increased in hyperthyroidism. Increased ADIOLS might contribute to menstrual disturbances and gynecomastia in hyperthyroidism.

Key words: Androstenediol (ADIOL), ADIOL sulfate, Dehydroepiandrosterone (DHEA), DHEA sulfate, Thyroid dysfunction

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has been reported to be a more biologically active metabolite than dehydroepiandrosterone (DHEA). For example, ADIOL was at least 100-fold more effective in up-regulating systemic resistance against Coxsackie virus infection [7, 8].

Thyroid dysfunctions are predominantly found in female subjects and hyperthyroidism is induced by Graves' disease and hypothyroidism by Hashimoto's thyroiditis. Both these diseases are organ-specific autoimmune diseases and thus development of thyroid dysfunction may have important relations with changes in estrogenic activity and immunologic function. Therefore, it is suggested that there might be some changes in serum ADIOL in patients with thyroid dysfunction. We recently reported that serum concentrations of DHEA, DHEAS and pregnenolone sulfate (5-pregnen-3β-ol-20-one sulfate, PREGS), which are the precursors of ADIOL, were low in patients with hypothyroidism, and concentrations of DHEAS, but not DHEA, were high in patients with hyperthyroidism [9]. However, there has been no study on serum ADIOL in patients with thyroid disease.

In light of these considerations, we examined serum concentrations of ADIOL and ADIOLS in patients with thyroid dysfunction using a recently established sensitive enzyme immunoassay (EIA) for measurement of serum ADIOL [10].

**Subjects and Methods**

**Patients**

Serum samples were obtained from 23 untreated patients with hyperthyroidism due to Graves' disease, 31 untreated patients with hypothyroidism due to autoimmune thyroiditis, and 43 normal controls (Table 1). Venipuncture was performed from 0830 to 1100 h and informed consent was obtained from all patients. The mean age and gender distribution were not significantly different among these six groups (Table 1). Patients with Graves' hyperthyroidism had thyrotoxic symptoms and diagnosis was confirmed by high levels of free T₄ (FT₄) and free T₃ (FT₃), suppressed levels of TSH, and positive anti-TSH-receptor antibody and/or high radioactive iodine thyroid uptake. Patients with hypothyroid autoimmune thyroiditis had hypometabolic symptoms and diagnosis was confirmed by low levels of FT₄ and FT₃, increased levels of TSH, and a positive test for anti-thyroid microsomal and/or thyroglobulin antibodies.

**Assays for serum steroid levels**

The detailed methods for preparation of serum samples and the serum calibrator using solid phase extraction, EIA for ADIOL and DHEA, high-performance liquid chromatography (HPLC) conditions, and gas chromatography-mass spectrometry (GC-MS) measurements, were as described previously [9-11]. Briefly, the steroids were extracted with methanol from 0.2 ml of serum sample or serum calibrator, then the extracts were applied to a solid-phase extraction and an ion exchange column to separate unconjugated fractions, ADIOL and DHEA, and sulfon-conjugated steroids, ADIOLS, DHEAS and PREGS. After further purification of the unconjugated steroids by HPLC, ADIOL and

<p>| Table 1. Age, gender and serum concentrations of FT₄, albumin and SHBG in patients with hyperthyroidism and hypothyroidism, and normal controls |
|---------------------------------|-----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Gender</th>
<th>Group</th>
<th>n</th>
<th>Age (yrs)</th>
<th>FT₄ (pmol/l)</th>
<th>Albumin (μmol/l)</th>
<th>SHBG (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Hyperthyroidism</td>
<td>9</td>
<td>43.1±15.2</td>
<td>45.6±9.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>695±47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>165±95&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hypothyroidism</td>
<td>11</td>
<td>49.6±8.9</td>
<td>2.8±2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>789±54</td>
<td>38±16</td>
</tr>
<tr>
<td></td>
<td>Normal controls</td>
<td>14</td>
<td>48.1±14.5</td>
<td>14.8±4.0</td>
<td>753±63</td>
<td>39±7</td>
</tr>
<tr>
<td>Female</td>
<td>Hyperthyroidism</td>
<td>14</td>
<td>37.9±14.2</td>
<td>57.2±24.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>614±65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>365±180&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Hypothyroidism</td>
<td>20</td>
<td>45.1±11.3</td>
<td>3.3±3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>741±73</td>
<td>74±31</td>
</tr>
<tr>
<td></td>
<td>Normal controls</td>
<td>29</td>
<td>42.5±12.9</td>
<td>14.5±1.5</td>
<td>703±71</td>
<td>70±31</td>
</tr>
</tbody>
</table>

SHBG, sex hormone-binding globulin. Values are expressed as mean±SD. <sup>a</sup>P<0.05; <sup>b</sup>P<0.01; <sup>c</sup>P<0.001 vs. normal controls in same gender.
DHEA were measured by EIA. The sensitivity of the assay for ADIOL and DHEA were 1.38 nmol/l and 0.13 nmol/l, respectively. The intra- and interassay coefficient of variations (CVs) for ADIOL were 6.7% and 11.5%, and those for DHEA were 5.6% and 8.9%, respectively. The recovery of DHEA and ADIOL was 92.4–102%. The sulfo-conjugated fraction which contains ADIOLS, DHEAS and PREGS were treated with arylsulfatase and applied to HPLC for purification of the freed steroids. After acylation of the steroids with heptafluorobutyric anhydride, these were measured by GC-MS. Selective ion monitoring was carried out at m/z 468 [M-214]+ for ADIOL, m/z 270 [M-214]+ for DHEA, m/z 298 [M-214]+ for PREG and m/z 314 [M-60]+ for ADIOL diacetate as an internal standard. The sensitivity of the GC-MS for ADIOL, DHEA and PREG was 0.008 pmol/l. The intra-assay CVs for ADIOLS, DHEAS and PREGS were less than 3.0%, and the interassay CVs for these steroids were less than 8.3%. The recovery of ADIOLS, DHEAS and PREGS was 84.9–98.9%.

Other assays

Sex hormone-binding globulin (SHBG) was measured by enzyme linked immunosorbent assay (ELISA). The serum samples were diluted to 500- or 1,000-fold with assay buffer as described elsewhere [9, 12]. The measurable range was 0.002–0.53 nmol/l. The recovery was 101.0 ± 3.1%. The intra- and interassay CVs were 4.3% and 5.4%, respectively. Serum albumin was measured by the “Dry Chemistry” method using DRI-CHEM 3000 (FUJIFILM). The slide used was ALB-P®. The serum concentrations of FT4, FT3 and TSH were measured with the commercial kits, AxSYM Free T4, Dainapack® (Dainabot), Mab-FreeT3® (Amerlex) and AxSYM TSH Dainapack® (Dainabot), respectively. Anti-TSH-receptor antibody was measured by radioreceptor assay using a TRAAb-II kit® (Cosmic). Anti-thyroid microsomal and thyroglobulin antibodies were measured with passive particle agglutination kits (Fuji Rebio).

Statistical analysis

Differences between two groups were statistically analyzed by the Mann-Whitney U test. To examine the relation between two hormones, the log-normal distributions of the hormone levels were confirmed by Kolmogorov-Smirnov tests, the regression lines were calculated by standard least-squares methods, and Pearson's correlation coefficients were determined. A P value below 0.05 was considered significant.

Results

Serum levels of ADIOLS, ADIOL, DHEA, DHEAS, DHA and PREGS in thyroid dysfunction

In hyperthyroidism, serum levels of ADIOLS, DHEAS and PREGS were significantly higher than age-matched normal controls but those of ADIOL and DHEA were normal in both males and females (Fig. 1). However, in hypothyroidism, the serum concentrations of all steroids studied were significantly lower than normal controls in both males and females.

Serum levels of ADIOLS in males were significantly higher than those in females in the normal, hyperthyroid and hypothyroid groups (Fig. 1). Serum ADIOL concentrations in males were also significantly higher than those in females in the normal and hypothyroid groups but not in the hyperthyroid group. There was no significant difference between male and female values of DHEAS, DHEA and PREGS in the three groups.

Serum levels of steroid binding proteins in thyroid dysfunction

Serum levels of steroids are affected by their binding proteins [13]. Thus, we examined the serum concentrations of the binding protein for C19 steroids, albumin and SHBG in patients with thyroid dysfunction and normal controls (Table 1). A significant decrease of serum albumin and a significant increase in serum SHBG compared with those in normal controls were observed in both genders with hyperthyroidism. However, the serum concentrations of these proteins in hypothyroidism were not different from those in the normal controls.
Fig. 1. Comparison of serum concentrations of ADIOLS (A), ADIOL (B), DHEAS (C), DHEA (D) and PREGS (E) among male and female patients with hyperthyroidism and hypothyroidism, and in age-matched normal controls. Values are the mean ± SD.

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; N.S., not significant vs. normal controls.

#, $P<0.05$; ##, $P<0.01$; ###, $P<0.001$ vs. female.
Relations between the serum levels of thyroid hormones and those of ADIOLS, ADIOL, DHEAS, DHEA or PREGS

The relations between the serum concentration of thyroid hormones and those of ADIOLS, ADIOL, DHEAS, DHEA or PREGS were also investigated. In both male and female groups, the distributions of FT₄, ADIOLS, ADIOL, DHEA and PREGS were confirmed to fit the log-normal distribution by Kolmogorov-Smirnov tests. Therefore, they were transformed to logarithmic scale, and linear correlations between log (FT₄), and log (ADIOLS), log (ADIOL), log (DHEAS), log (DHEA) or log (PREGS) were analyzed (Fig. 2). In both males and females, the concentrations of all steroids strongly correlated with that of serum FT₄. Similar findings were obtained between serum FT₃ and these steroids (data not shown). However, within the group of hyperthyroidism or hypothyroidism, no significant positive correlation was obtained between the concentrations of steroids and thyroid hormones.

Values of ADIOLS, ADIOL, DHEAS, DHEA or PREGS were not related with titers of anti-thyroid microsomal or thyroglobulin antibodies, or anti-TSH-receptor antibodies.

Discussion

Thyroid function alters the metabolism of steroid hormones by changing their rates of secretion or production, serum steroid binding protein levels, and rate and pathway of catabolism [14-21]. In the present study, we found a marked increase of serum ADIOLS in hyperthyroidism, especially in male patients. Serum levels of steroid hormones are strongly influenced by binding proteins, SHBG and albumin. In general sulfated steroids, such as ethiocholanolone sulfate, androsterone sulfate and DHEAS, are primarily bound to albumin rather than SHBG in human plasma [22, 23]. The serum albumin concentration was significantly decreased in hyperthyroidism in the present study, and increased ADIOLS levels were thus not explained by the changes in binding proteins.

ADIOLS could be the most significant source of estrogenic activity, at least in tissues rich in steroid sulfatase, which is known to be the most widespread enzyme in the body [24]. The estrogenic potential of ADIOL is only approximately 1% that of estradiol [2]; however, the serum levels of ADIOLS are about 10,000-fold higher than that of estradiol. It was reported that estradiol 3-sulfate has similar activity as unconjugated estradiol in vitro [25]. Likewise, ADIOLS may also have similar estrogenic activity as ADIOL in vivo. Furthermore, ADIOL indirectly augments the activity of estradiol by inhibiting 17β-hydroxysteroid dehydrogenase and estrogen sulfotransferase [3]. In breast cancer, by liberating the sulfate using steroid sulfatase, not only ADIOL but also ADIOLS plays a role in the induction and promotion of cancer cells [4, 5].

The incidence of gynecomastia in male patients with hyperthyroidism has been reported at 20 to 40 percent [15]. The possible roles of abnormalities in circulating estrogen and in the balance between estrogen and androgen [14] or increased progesterone has been suggested [20], but the exact mechanism is not fully understood. A marked increase in ADIOLS might also have an important role for induction of gynecomastia. Unfortunately, none of patients had typical gynecomastia in this study. Moreover, menstrual disturbances frequently occur and reduced or absent midcycle LH peak has been reported in hyperthyroidism [16]. It has been suggested that high SHBG-associated hormonal disturbance has a possible relation to oligomenorrhea or amenorrhea [16]. The present findings suggest that enhanced estrogenic activity induced by increased ADIOLS may also contribute to menstrual disturbances in patients with hyperthyroidism. In this study, two of fourteen female patients with hyperthyroidism were postmenopausal and two patients had oligomenorrhea but the rest of the patients had regular menstruation. The serum level of ADIOLS in the two patients with oligomenorrhea was moderately high but it was difficult to obtain statistical significance due to small number of patients.

We found a significant correlation between serum thyroid hormone level and ADIOLS, ADIOL, DHEAS, DHEA or PREGS, suggesting that thyroid hormones regulate the serum levels of these steroids. Steroid hormones are synthesized from cholesterol in the adrenal glands and other organs. The rate-limiting process in steroidogenesis is the transport of free cholesterol through the cytosol to the inner
Fig. 2. Relation between serum FT₄ levels and serum concentrations of ADIOLS (A), ADIOL (B), DHEAS (C), DHEA (D) and PREGS (E) in male and female patients with hyperthyroidism, hypothyroidism and age-matched normal controls. Values were transformed to a logarithmic scale and Pearson's correlation coefficients (r) and the least-squares regression lines were calculated. ***, $P<0.001$. 
mitochondrial membrane, the site of the cholesterol side-chain cleavage enzyme (cytochrome P-450SCC, P450\text{_{SCC}}), which cleaves the side chain from C-21 of cholesterol. This cleavage precedes the biosynthesis of all steroid hormones including ADIOLS ADIOL, DHEAS, and DHEA. A significant reduction in the activity of P450\text{_{SCC}} in the rat adrenal cortex was observed by thyroidectomy [26]. Therefore, the present findings of lower levels of ADIOLS, ADIOL, DHEAS, DHEA and PREGS in patients with hypothyroidism could be explained by the decreased adrenal steroidogenesis due to decreased thyroid hormones. In addition, this mechanism would contribute to the hypercholesterolemia in patients with hypothyroidism, although the metabolic rate of cholesterol was generally decreased in those patients [27, 28].

Inversely, the steroidogenesis in hyperthyroidism would be activated by thyroid hormone. This possibility is supported by the findings that treatment with triiodothyronine in some patients caused an increase in androsterone production [29-31]. Although different enzymes participate in the biosynthesis of androsterone, and it is unknown which step in the metabolic pathway from cholesterol to androsterone is activated by thyroid hormones, the present findings of high levels of ADIOLS, DHEAS and PREGS in patients with hyperthyroidism could be explained by the hypothesis that the P450\text{_{SCC}} is activated in hyperthyroidism [32]. Increases in these enzyme activities may partly be related to hypocholesterolemia in hyperthyroidism [33].

However, it is difficult to explain why there was no difference in the ADIOL and DHEA concentrations between hyperthyroidism and controls. The serum levels of these steroids are reflected by their metabolic clearance rate (MCR). Although there is no report on MCR of ADIOL or DHEA in hyperthyroidism, the MCR of ADIOL is about 1,300 L/24 hr in normal males and about 860 L/24 hr in normal females [34]. Similar findings for the MCR of ADIOL have also been reported: 580 L/24 hr in normal females [35] and 750 L/24 hr in postmenopausal females [36]. For DHEA, the MCR ranged between 1,500–2,000 L/24 hr in both normal males and females [35, 37–39]. However, the MCR of DHEAS was approximately 12–15 L/24 hr in normal males and females [39–41]. These findings show that unconjugated steroids are cleared rapidly from the blood. Furthermore, in the hyperthyroid condition, the rate of the clearance of DHEA and ADIOL would be increased more than in the euthyroid condition, so that the blood levels of these steroids could not be elevated.

Other than the production rate, there are at least two conceivable factors that could change the MCR. First, the changes in the serum levels of steroid binding protein would reflect the MCR. The major binding protein for ADIOL in serum is SHBG and that for DHEA is albumin, and about 60% of ADIOL binds to SHBG and about 90% of serum DHEA binds to albumin [42]. Although we found a significant increase in SHBG and a significant decrease in albumin in hyperthyroidism, there was no significant difference in the ADIOL and DHEA concentrations between hyperthyroidism and controls as mentioned above. These findings indicated that the changes in the serum levels of ADIOL and DHEA in hyperthyroidism appear unlikely due to the changes of the binding protein.

Second, the conversion rate from ADIOL or DHEA to ADIOLS or DHEAS, respectively, might be increased in hyperthyroidism due to activated sulfotransferase. In normal adults DHEAS is predominantly converted to DHEA [37], and DHEAS circulates at 200- to 500-fold higher concentrations than DHEA. Recently, Hansen et al. reported increased sulfatation of orbital glycosaminoglycans in patients with Graves’ ophthalmopathy [43]. The affected skin of pretibial myxedema of Graves’ disease also contained high amounts of sulfated glycosaminoglycans [44]. More recently, Dunn et al. reported that thyroid hormones could modulate hydroxysteroid sulfotransferase gene expression in rat liver [45]. Thus, it can be suggested that some kinds of sulfotransferases are activated in hyperthyroidism and sulfated chemicals may have an important role for the expression of various clinical characteristics in Graves’ hyperthyroidism, including ophthalmopathy and dermopathy.

Increased levels of ADIOLS and DHEAS, and normal concentrations of ADIOL and DHEA might be explained by activated sulfotransferase of these steroids in hyperthyroidism.

ADIOL has been suggested to have immunoregulatory activity and enhances resistance to infection in animal experiment [46, 47]. Furthermore, ADIOL may down-regulate the Th2 immune re-
response [48] and augment the Th1 immune reaction [49]. Patients with Graves' disease have been reported to have both activated Th1 and Th2 immune reactions [50]. Increased ADIOLS may down-regulate the Th2 immune reaction and have a homeostatic protective role for self-perpetuation of hyperthyroidism induced by stimulating anti-TSH-receptor antibody.

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