Serum Dehydroepiandrosterone, Dehydroepiandrosterone Sulfate, and Pregnenolone Sulfate Concentrations in Patients with Hyperthyroidism and Hypothyroidism

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Background: Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEA-S) have been suggested to have protective effects against cardiovascular disease, cancer, immune-modulated diseases, and aging. We examined serum concentrations of DHEA, DHEA-S, and pregnenolone sulfate (PREG-S) in patients with thyroid dysfunction.

Methods: Steroids extracted with methanol from serum sample were separated into an unconjugated fraction (DHEA) and a monosulfate fraction (DHEA-S and PREG-S), using a solid-phase extraction and an ion-exchange column. After separation of unconjugated steroids by HPLC, the DHEA concentration was measured by enzyme immunoassay. The monosulfate fraction was treated with arylsulfatase, and the freed steroids were separated by HPLC. The DHEA and PREG fractions were determined by gas chromatography–mass spectrometry, and the concentrations were converted into those of DHEA-S and PREG-S.

Results: Serum concentrations of DHEA, DHEA-S, and PREG-S were all significantly lower in patients with hypothyroidism (n = 24) than in age- and sex-matched healthy controls (n = 43). By contrast, in patients with hyperthyroidism (n = 22), serum DHEA-S and PREG-S concentrations were significantly higher, but the serum DHEA concentration was within the reference interval.

Serum concentrations of these three steroids correlated with serum concentrations of thyroid hormones in these patients. Serum albumin and sex hormone-binding globulin concentrations were not related to these changes in the concentration of steroids.

Conclusions: Serum concentrations of DHEA, DHEA-S, and PREG-S were decreased in hypothyroidism, whereas serum DHEA-S and PREG-S concentrations were increased but DHEA was normal in hyperthyroidism. Thyroid hormone may stimulate the synthesis of these steroids, and DHEA sulfotransferase might be increased in hyperthyroidism.

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The serum concentration of dehydroepiandrosterone sulfate (DHEA-S)[4] in adult men and women is higher than that of any other steroid except cholesterol; however, the physiological role of this adrenal steroid has long been unknown. Recently, it has been clarified that humans largely depend on peripheral tissues for androgen and estrogen formation from the adrenal precursor DHEA-S and DHEA *(1, 2)*. Moreover, it has been suggested that DHEA and DHEA-S have some protective effect against cardiovascular disease, obesity, hypercholesterolemia, cancer, Alzheimer disease, insulin-dependent diabetes mellitus, and other immune-modulated diseases *(3–7)*. Furthermore, DHEA and DHEA-S have been suggested to help slow the aging process and improve memory *(8, 9)*. More recently, DHEA and DHEA-S have been applied to the treatment of several disease conditions *(1, 2, 10)*. Con-

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[4] Nonstandard abbreviations: DHEA-S, dehydroepiandrosterone sulfate; PREG-S, pregnenolone sulfate; FT_4, free thyroxine; FT_3, free triiodothyronine; TSH, thyrotropin; EIA, enzyme immunoassay; ALP, alkaline phosphatase; SHBG, sex hormone-binding globulin; and PBS, phosphate-buffered saline.

sequently, the serum concentrations of DHEA and DHEA-S are important to the pathophysiology of various diseases.

The serum concentration of cholesterol is known to change markedly in patients with thyroid dysfunction (11), i.e., hypercholesterolemia is associated with hypothyroidism and hypocholesterolemia is associated with hyperthyroidism (12). Less is known, however, about the serum concentrations of DHEA and DHEA-S in patients with thyroid dysfunction. In this study, we examine serum DHEA, DHEA-S, and pregnenolone sulfate (PREG-S) concentrations in patients with hyperthyroidism and hypothyroidism and discuss the mechanism of change in the concentration of these steroids in thyroid dysfunction.

Materials and Methods

PATIENTS

Serum samples were obtained from 22 untreated patients with hyperthyroidism associated with Graves disease, 24 untreated patients with hypothyroidism attributable to autoimmune thyroiditis, and 43 healthy controls. Venipuncture was performed from 0830 to 1100, and informed consent was obtained from all patients. The mean age and sex distributions were not significantly different among these three groups (Table 1). Patients with Graves hyperthyroidism had thyrotoxic symptoms, and the diagnosis was confirmed by increased concentrations of free thyroxine (FT_4) and free triiodothyronine (FT_3), decreased thyrotropin (TSH), and positive TSH receptor antibody and/or high radioiodine thyroid uptake. Patients with hypothyroid autoimmune thyroiditis had hypometabolic symptoms, and the diagnosis was confirmed by decreased FT_4 and FT_3 concentrations, increased TSH, and a positive test for anti-thyroid microsomal and/or thyroglobulin antibodies.

CALIBRATOR AND SAMPLE PREPARATION

Stock solutions containing 1.20 mmol/L DHEA, DHEA-S, and PREG-S (Steraloids) in methanol were serially diluted with methanol; these were the methanol calibrators. To prepare serum steroid calibrators containing DHEA (0–521 nmol/L), DHEA-S (0–18.8 μmol/L), or PREG-S (0–8.54 μmol/L), the methanol calibrators were added into the tubes (0–0.10 nmol DHEA, 0–3.75 nmol DHEA-S, 0–1.71 nmol PREG-S) followed by 0.2 mL of steroid-free serum (13).

Separation of DHEA, DHEA-S, and PREG-S from serum samples was carried out as reported previously (14). Briefly, 0.2 mL of serum sample or serum calibrator was extracted three times with 1 mL of methanol. The obtained solvent was combined and evaporated under reduced pressure. The residue was applied to a solid-phase extraction column, Sep-Pak Vac tC18® (Waters), and an anion-exchange column, Accell Plus QMA® (Waters). Because the DHEA serum calibrators did not contain DHEA-S and PREG-S, the Accell Plus QMA was not used for the treatment of DHEA serum calibrators. After these treatments, the steroid glucuronides were excluded, and

the unconjugated fraction (DHEA) and monosulfate fraction (DHEA-S and PREG-S) were obtained.

DHEA ASSAY

The unconjugated fraction was evaporated under reduced pressure. The residue was dissolved in 0.3 mL of the HPLC mobile phase solvent, water-acetonitrile (70:30, by volume), and 0.25 mL of the sample was subjected to HPLC using a system (Waters) of two pumps (model 510), an ultraviolet detector (model 481) operating at 210 nm, an autosampler (model 710B), and a Wakosil-II 5C18-HG column [150 × 4.6 mm (i.d.); Wako Pure Chemical Industries]. The separations were performed at 40 °C and at a flow rate of 1 mL/min with a linear gradient of acetonitrile (2%/min) from 30% to 70% in water. The eluate between 15.2 and 17.7 min (DHEA retention time, 16.2 min) was collected as the DHEA fraction. The solvent was evaporated under reduced pressure, and the residue was dissolved in 0.25 mL of methanol. Aliquots of 0.1 mL of this solution were transferred into two tubes for duplication. After evaporation of the solution, the enzyme immunoassay was performed.

Anti-DHEA antiserum [raised in rabbits against DHEA 7-(O-carboxymethyl)oxime-bovine serum albumin; UCB-Bioproducts S.A.] was diluted (1:3200) with the assay buffer (0.07 mol/L phosphate buffer solution containing 2.5 g/L bovine serum albumin, pH 7.4). Alkaline phosphatase (ALP)-labeled DHEA was prepared by conjugation of DHEA 7-(O-carboxymethyl)oxime (Sigma) and ALP (EC 3.1.3.1, from calf intestine; Boehringer Mannheim) according to the carbodiimide method (15), and was diluted with the assay buffer containing 6 mL/L normal rabbit serum. To the tubes prepared as above, 0.5 mL of anti-DHEA antiserum and 0.1 mL of ALP-labeled DHEA were added. After the tubes stood at room temperature for 1 h, 0.1 mL of 30 mL/L second antibody was added, and the mixture was allowed to stand at 4 °C overnight. After bound/free separation, the enzyme activity of the resulting precipitate was measured using the Alkaline Phospha K-Test® kit (Wako) (15). The measurable range was 0.13–521 nmol/L. The overall recovery was 97.2% ± 4.8%. The intra- and interassay CVs for DHEA (mean, 4.24 nmol/L; n = 5) were 5.6% and 8.9%, respectively.

ASSAYS FOR DHEA-S AND PREG-S

The monosulfate fraction was hydrolyzed with arylsulfatase (EC 3.1.6.1, from Helix pomatia; Boehringer Mannheim) (14). The resulting samples were subjected to HPLC in the same manner as the above DHEA assay. The eluate was collected from 15.2 to 17.7 min (DHEA) and 20.5 to 23.0 min (PREG retention time, 21.5 min) and was combined in a tube. Androstenediol diacetate in methanol (0.11 nmol/sample) was added to each sample as an internal standard. After evaporation, derivatization was performed with 0.1 mL of heptafluorobutyric anhydride (GL Sciences) at 60 °C for 30 min. Under these conditions, no dehydrated or decomposed compounds were ob-

served. The reaction mixture was evaporated to dryness. The residue was dissolved in 0.4 mL of dichloromethane, and 2 μL of the sample was analyzed by gas chromatography–mass spectrometry *(14)*. Selective-ion monitoring was carried out at m/z 270 $[M - 214]^+$ for DHEA, m/z 298 $[M - 214]^+$ for PREG, and m/z 314 $[M - 60]^+$ for the internal standard. The concentrations obtained by gas chromatography–mass spectrometry were expressed as the values of serum steroid sulfates. The overall recoveries were 91.3% ± 4.4% for DHEA-S and 89.2% ± 4.3% for PREG-S, respectively. The measurable range for each steroid was as follows: DHEA-S, 0.01–18.8 μmol/L; PREG-S, 0.01–8.54 μmol/L. The intra- and interassay CVs for DHEA-S (mean, 1.36 μmol/L; n = 5) or PREG-S (mean, 0.63 μmol/L; n = 5) were 2.3% and 5.4%, and 2.1% and 3.1%, respectively.

OTHER ASSAYS

Sex hormone-binding globulin (SHBG). SHBG was measured by an ELISA sandwich assay according to the reported method *(16)* with minor modifications. Diluted serum samples (1:500 or 1:1000) and SHBG calibrators (0–0.53 nmol/L, purified from human serum; Calbiochem-Novabiochem) were diluted with 0.07 mol/L phosphate-buffered saline (PBS, pH 7.4) containing 5 g/L gelatin. The plates were incubated at room temperature for 3 h and then washed six times with 0.07 mol/L PBS containing 0.5 g/L Tween 20 (Bio-Rad). To each well, 0.1 mL of ALP-labeled anti-SHBG antibody diluted 1:50 with 0.07 mol/L PBS was added. The plates were left to stand at room temperature for 2 h, and then were washed six times with 0.3 mL of 25 mmol/L Tris-HCl, pH 7.4. The ALP activity in each well was measured using an Alkaline Phospha K-Test. The measurable range was 0.002–0.53 nmol/L. The recovery was 101.0% ± 3.1%. The intra- and interassay CVs (129 nmol/L; n = 5) were 4.3% and 5.4%, respectively.

Albumin, FT_4, FT_3, and TSH. Serum albumin was measured by the "Dry Chemistry" method using DRI-CHEM 3000 (FUJIFILM). The slide used was ALB-P®. The serum concentrations of FT_4, FT_3, and TSH were measured with the commercial kits, AxSYM Free T₄ Dainapack® (Dainabott), Mab-FreeT₃® (Amerlex), and AxSYM TSH Dainapack (Dainabott), respectively. Anti-TSH receptor antibody was measured by radioreceptor assay using a TRAb-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 analyzed statistically by the Mann–Whitney *U*-test. To examine the relationship between two hormones, the log-normal distributions of the hormone concentrations were confirmed by Kolmogorov–Smirnov tests, the regression lines were calculated by standard least-squares methods, the ellipses of constant distance that represent 95% bivariate tolerance regions were calculated based on assumption of an underlying bivariate parametric distribution, and Pearson correlation coefficients were determined. $P < 0.05$ was considered significant.

Results

DHEA AND DHEA-S IN THYROID DYSFUNCTION

Serum concentrations of DHEA and DHEA-S were measured in patients with hyperthyroidism and hypothyroidism and compared with those of age- and sex-matched healthy controls (Table 1). In hyperthyroidism, the serum DHEA-S concentration was significantly increased, but DHEA was normal. In hypothyroidism, the serum concentrations of both DHEA and DHEA-S were significantly decreased. Individual values of DHEA and DHEA-S are shown in Fig. 1. DHEA values were widely distributed even in the controls. Interestingly, the serum concentration of PREG-S, the precursor of DHEA and DHEA-S, was increased in hyperthyroidism and decreased in hypothyroidism.

We examined serum concentrations of albumin, a binding protein of DHEA and DHEA-S, and SHBG, a weak binding protein of DHEA. Serum albumin was decreased in hyperthyroidism, and serum SHBG was increased in hyperthyroidism (Table 1).

Because DHEA and DHEA-S concentrations are gender-dependent, we analyzed these data using only female or male subjects, but the results were the same as above.

THYROID HORMONE AND DHEA, DHEA-S, OR PREG-S

To elucidate the mechanism of change in the steroid hormone concentrations, the relationships between the serum concentrations of thyroid hormones and those of DHEA, DHEA-S, and PREG-S were studied. The distributions of FT_4, DHEA, DHEA-S, and PREG-S in total (including hyperthyroidism, hypothyroidism, and controls) were confirmed to fit the log-normal distribution by Kolmogorov–Smirnov tests. Therefore, they were transformed to logarithmic scale, and linear correlations of log

Table 1. Age, sex, and serum concentrations of DHEA, DHEA-S, and PREG-S in patients with hyperthyroidism and hypothyroidism, and healthy controls.

Group	No. examined	Age,[a] years	Sex, M/F	DHEA,[a] nmol/L	DHEA-S,[a] μmol/L	PREG-S,[a] μmol/L	Albumin,[a] μmol/L	SHBG,[a] nmol/L
Hyperthyroidism	22	40.8 ± 14.3	9/13	12.3 ± 10.5	5.91 ± 3.07[b]	0.901 ± 0.553[b]	652 ± 64[b]	274 ± 172[b]
Hypothyroidism	24	47.9 ± 11.7	8/16	4.1 ± 5.5[b]	1.35 ± 1.07[b]	0.058 ± 0.040[b]	757 ± 69	61 ± 32
Healthy controls	43	44.3 ± 13.5	14/29	12.1 ± 9.2	2.19 ± 1.36	0.133 ± 0.097	720 ± 72	60 ± 29

[a] Mean ± SD.
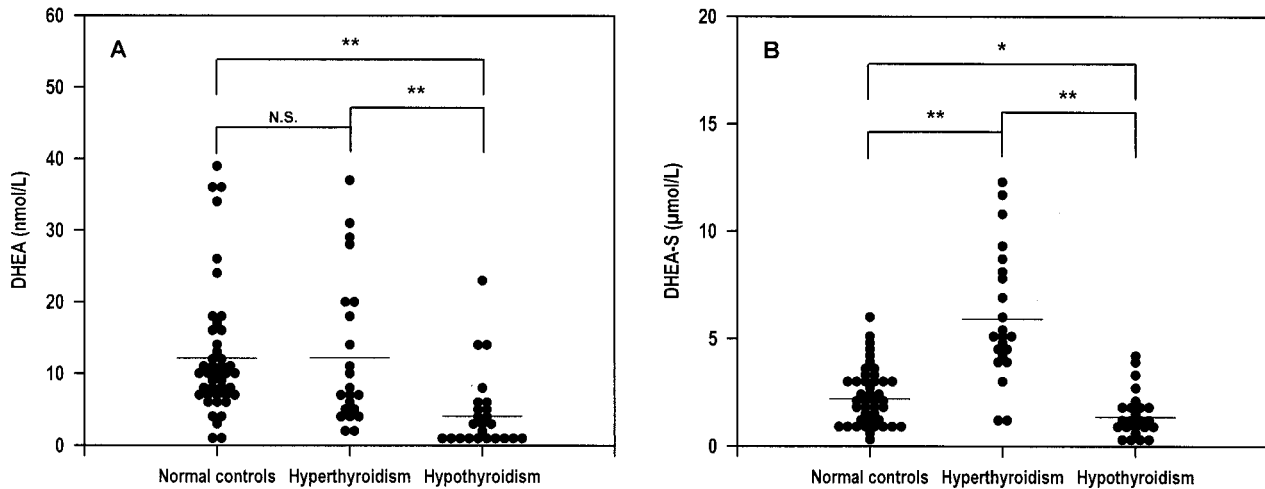[b] Significantly different from healthy controls at $P < 0.001$.

Fig. 1. Serum concentrations of DHEA (*A*) and DHEA-S (*B*) in patients with hyperthyroidism and hypothyroidism, and in healthy controls (*Normal controls*).

Hoizontal bars represent the mean values. ∗, significantly different at $P <0.01$; ∗∗, significantly different at $P <0.001$. *N.S.*, not significant.

(DHEA), log (DHEA-S), and log (PREG-S) with log (FT_4) were analyzed (Fig. 2). The serum concentrations of these steroids correlated with that of serum FT_4. The correlation was strongest between FT_4 and PREG-S (Fig. 2C). Similar results were obtained between serum FT_3 and these steroids (data are not shown).

Values of DHEA, DHEA-S, or PREG-S were not related to titers of anti-thyroid microsomal or thyroglobulin antibodies, or anti-TSH receptor antibodies.

RELATIONSHIP BETWEEN DHEA AND DHEA-S
Because DHEA and DHEA-S are interconvertible and DHEA was not increased in hyperthyroidism, we examined the relationship between DHEA and DHEA-S in patients and controls. The distributions of DHEA and DHEA-S for each (hyperthyroid, hypothyroid, and control) group were confirmed to fit the log-normal distribution by Kolmogorov–Smirnov tests. Therefore, they were transformed to logarithmic scale, and linear correlations

of log (DHEA) and log (DHEA-S) were analyzed (Fig. 3). The two steroids were correlated in controls and patients with hypothyroidism. However, there was no correlation between the two in hyperthyroidism. The ratio (nmol/nmol) of DHEA-S to DHEA in controls was 181, and that in hyperthyroidism was increased to 480, indicating that DHEA-S is more predominant in hyperthyroidism.

Discussion
Recent progress in understanding the physiological roles of DHEA and DHEA-S has advanced the study of serum DHEA and DHEA-S in various diseases. However, little attention has been paid to patients with thyroid disease. In 1980, Bassi et al. *(17)* reported lower DHEA-S concentrations in hypothyroid patients but found no relationship between DHEA-S and total T_4. They did not examine DHEA. Three years later, Földes et al. *(18)* reported decreased DHEA and DHEA-S in hypothyroid patients and increased DHEA-S, but not DHEA, in patients with Graves
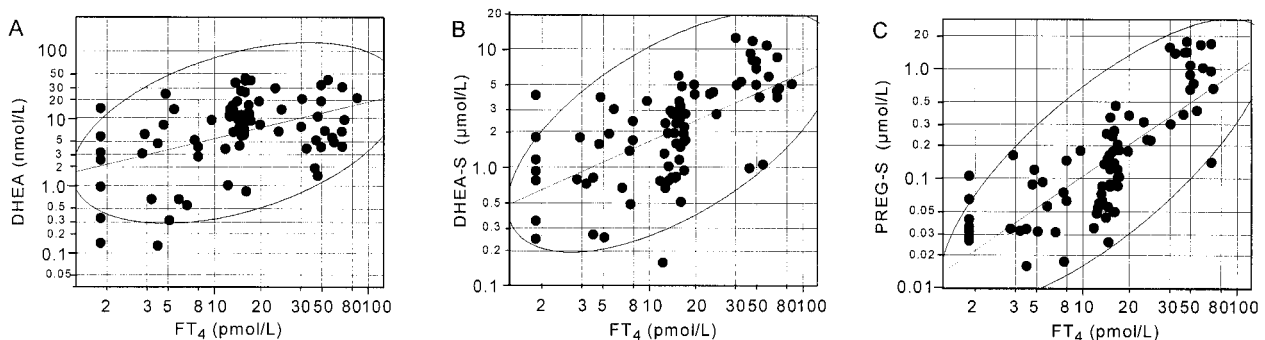

Fig. 2. Correlation between serum concentrations of FT_4 and DHEA (*A*), DHEA-S (*B*), or PREG-S (*C*) in patients with hyperthyroidism, hypothyroidism, and healthy controls.

Data were transformed into logarithmic scale, and Pearson correlation coefficients, 95% bivariate tolerance regions, and the least-squares regression lines were calculated. The *ellipses* represent 95% bivariate tolerance regions. (*A*), $r = 0.465$; $P <0.001$; (*B*), $r = 0.640$; $P <0.001$; (*C*), $r = 0.795$; $P <0.001$.
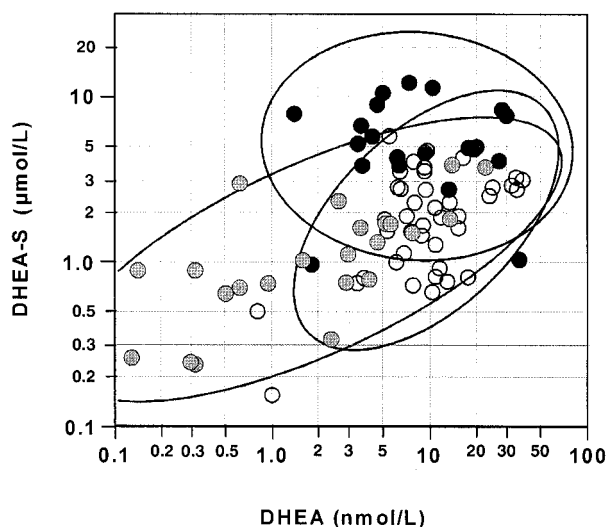
Fig. 3. Relationship between DHEA and DHEA-S in healthy controls (○), patients with hyperthyroidism (●), and patients with hypothyroidism (◉).

Data were transformed into the logarithmic scale, and Pearson correlation coefficients and 95% bivariate tolerance regions were calculated. Correlations: for controls, $r = 0.546$; $P < 0.05$; for patients with hyperthyroidism, $r = -0.073$; not significant; for patients with hypothyroidism, $r = 0.701$; $P < 0.001$.

hyperthyroidism. Curiously, the same group later reported normal or slightly decreased concentrations of DHEA-S in patients with hyperthyroidism *(19)*, although they selected patients with toxic nodular goiter for the study.

In the present study, we confirmed the decrease of serum DHEA and DHEA-S in hypothyroid patients. In the patients with Graves hyperthyroidism, serum DHEA-S was increased but serum DHEA was normal, similar to the earlier report by Földes at al. *(18)*. We also found that serum PREG-S was increased in hyperthyroidism and decreased in hypothyroidism.

DHEA has been reported to enhance immune function, and some patients with autoimmune disease have decreased serum DHEA and DHEA-S concentrations *(2)*. Reported autoimmune diseases include rheumatoid arthritis *(20)* and systemic lupus erythematosus *(21)*, but these are generalized, not organ specific. Patients with pemphigoid/pemphigus, one of the organ-specific autoimmune diseases, are also reported to have decreased serum DHEA-S concentrations *(22)*. In animal experiments, DHEA-S retarded the onset of autoimmune disease *(23)*. It is speculated that abnormalities of DHEA or DHEA-S might have some pathological relation to autoimmune disease. Both Graves disease and autoimmune thyroiditis are representative of organ-specific autoimmune disease, but serum DHEA or DHEA-S concentrations in these patients were not consistently decreased. Thus, DHEA and DHEA-S may not have important roles in autoimmune thyroid disease.

We found a significant correlation between the serum thyroid hormone concentration and DHEA or DHEA-S,

suggesting that thyroid hormone regulates serum concentration of DHEA and DHEA-S. Steroid hormones are synthesized from cholesterol in the adrenal glands and other organs. The side chain of cholesterol at C-17/C-20 is cleaved by cholesterol side-chain cleavage enzyme (cytochrome $P-450_{SCC}$), producing PREG. This rate-determining step precedes the biosynthesis of all steroid hormones, including DHEA and DHEA-S. A significant reduction in the activity of $P-450_{SCC}$ in rat adrenal cortex-mix was detected by thyroidectomy *(24)*. Therefore, the significantly lower concentrations of DHEA, DHEA-S, and PREG-S in patients with hypothyroidism could be explained by the decreased adrenal steroidogenesis induced by the low concentration of thyroid hormone. Additionally, this mechanism would contribute to the hypercholesterolemia in patients with hypothyroidism, although the metabolic rate of cholesterol principally decreased in those patients *(25, 26)*.

Inversely, the steroidogenesis in hyperthyroidism could be activated by thyroid hormone. This possibility is supported by reports that treatment with T_3 in some patients caused an increase of androsterone production *(27–29)*. 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 hormone, the increased DHEA-S and PREG-S concentrations in the patients with hyperthyroidism are explained by the hypothesis that $P-450_{SCC}$, 17α-hydroxylase, $C_{17,20}$-lyase, and sulfotransferase are activated in hyperthyroidism. However, that there was no difference in the DHEA concentrations between patients with hyperthyroidism and controls is harder to explain. It is possible that the rate of conversion from DHEA to DHEA-S is increased in hyperthyroidism because of activated sulfotransferase.

As for the evaluation of serum concentrations of steroid hormones, their binding proteins may influence them when extreme changes in binding proteins are observed. Therefore, we measured albumin and SHBG. Only a small portion of serum DHEA binds to SHBG, and ~90% of this steroid binds weakly to albumin *(30)*. Likewise, >90% of serum DHEA-S binds weakly to albumin, but DHEA-S does not bind to SHBG *(9, 31, 32)*. In this study, we found that only slight perturbation occurs as a result of thyroid function. Small changes in these binding proteins may not affect the serum concentration of DHEA and DHEA-S.

DHEA is rapidly cleared from the blood at a rate of ~2000 L/day, whereas DHEA-S has a clearance of ~13 L/day *(33)*. Thus, DHEA has a short half-life of 1–3 h, whereas the half-life of DHEA-S is 10–20 h *(34)*. In the hyperthyroid condition, the rate of clearance of blood constituents would be increased more than in the euthyroid condition. These differences might partly explain the normal DHEA and increased DHEA-S in hyperthyroidism.

In conclusion, considering these results, one should be careful when interpreting the serum concentrations of DHEA and DHEA-S in patients with thyroid dysfunction.

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