

Low Serum Levels of Sex Steroids Are Associated with Disease Characteristics in Primary Sjogren's Syndrome; Supplementation with Dehydroepiandrosterone Restores the Concentrations

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Context: Serum levels of the sex steroid prohormones dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) decline upon aging and are reduced in primary Sjogren's syndrome.

Objective: Our aim was to investigate: 1) effects of 50 mg oral DHEA/day on changes in serum levels of DHEA and 12 of its metabolites; 2) relationships between steroid levels and disease characteristics; and 3) whether these parameters were influenced by DHEA.

Design: Twenty-three postmenopausal women with primary Sjogren's syndrome and subnormal levels of DHEA-S were included in a randomized, 9-month, controlled, double blind crossover study. Liquid chromatography/mass spectrometry (MS)/MS and gas chromatography/MS were used to measure the sex steroids. Anti-SS-A/Ro and/or anti-SS-B/La, salivary gland focus score, salivary flow rates, dry mouth and eye symptoms, and routine laboratory tests were assessed.

Results: Baseline erythrocyte sedimentation rate was inversely correlated with testosterone (Testo), dihydrotestosterone, and DHEA-S ($r_s = -0.42, -0.45, \text{ and } -0.58$, respectively). Dry mouth symptoms correlated with low Testo and androstenedione, whereas dry eyes correlated with low estrogens, most strongly estrone ($r_s = -0.63$). Presence of anti-SS-A and/or anti-SS-B was independently associated with low estradiol (area under the receiver operating characteristic curve, 0.82). All metabolites increased during DHEA but not during placebo. The relative increases were less for estrogens and Testo compared to dihydrotestosterone and glucuronidated androgen metabolites. Dry mouth symptoms decreased during DHEA therapy.

Conclusions: Disease manifestations in primary Sjogren's syndrome were associated with low sex hormone levels, dry mouth symptoms with low androgens, and dry eyes with low estrogens. Exogenous DHEA was preferentially transformed into androgens rather than into estrogens. (*J Clin Endocrinol Metab* 94: 2044–2051, 2009)

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Abbreviations: ADT, Androsterone; ADT-G, ADT glucuronide; ALT, alanine aminotransferase; BMD, bone mineral density; DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; DHT, dihydrotestosterone; 5-DIOL, 5-androstene-3 β , 17 β -diol; 4-DIONE, androstenedione; DMARD, disease-modifying antirheumatic drugs; E₁, estrone; E₁-S, E₁ sulfate; E₂, 17 β -estradiol; ESR, erythrocyte sedimentation rate; 3G, androstane-3 α , 17 β -diol-3glucuronide; 17G, androstane-3 α , 17 β -diol-17glucuronide; GC, gas chromatography; Hb, hemoglobin; KCS, keratoconjunctivitis sicca; LC, liquid chromatography; MS, mass spectrometry; r_s , Spearman's correlation coefficient; Testo, testosterone; VAS, Visual Analog Scale; WBC, white blood cell.

PPrimary Sjogren's syndrome is an autoimmune disease described as a form of "epithelitis" or an exocrinopathy (1). Most of the patients develop their disease at the age of 40–50 yr, and the female:male ratio is about 9:1 (2). Salivary and lacrimal gland involvement is prominent and is associated with decreased production of saliva and tears, often resulting in severe symptoms of dryness in the mouth and eyes. In addition, the criteria of primary Sjogren's syndrome include immunological abnormalities demonstrated as lymphocyte and plasma cell infiltrates in labial gland biopsies and/or the presence of antibodies to SS-A/Ro and/or SS-B/La in serum (3).

Reduced serum concentration of dehydroepiandrosterone sulfate (DHEA-S), the precursor sex steroid hormone, has been described in rheumatoid arthritis (4, 5), systemic lupus erythematosus (6), as well as in Sjogren's (7–9) compared with healthy controls. There is evidence of hypofunctioning of the hypothalamic-pituitary-adrenal axis in women with primary Sjogren's syndrome, as shown by a selective failure to produce DHEA-S after stimulation of the hypothalamic-pituitary-adrenal axis with CRH (7).

Humans have adrenal glands that secrete large amounts of dehydroepiandrosterone (DHEA), and especially DHEA-S. DHEA-S *per se* has no effect, but after its conversion to DHEA in peripheral tissues, DHEA is intracellularly processed, yielding active androgens and/or estrogens. All the enzymes required to transform DHEA into androgens and/or estrogens are expressed in many peripheral target tissues to make the sex hormones locally (10). This field of endocrinology has been called intracrinology (10).

The metabolism of oral and percutaneously administered DHEA in women has recently been measured. The serum concentration of DHEA and nine to 11 of its metabolites was analyzed by liquid chromatography (LC)/mass spectrometry (MS)/MS and gas chromatography (GC)/MS. DHEA was preferentially transformed into androgens rather than into estrogens (11–13).

The role of the adrenal precursors, DHEA-S and DHEA, and the peripheral formation of active sex hormones is more important in women than in men because in men androgen secretion by the testes continues throughout life, whereas in women, estrogen secretion by the ovaries ceases at menopause (10).

Against this background, it can be hypothesized that the declining serum DHEA and DHEA-S during aging (14) in combination with even more suppressed levels of the hormones in primary Sjogren's syndrome (7) as well as decreased production of estrogens by the ovaries could be involved in the female predominance, age of onset, and/or disease course in patients with Sjogren's. We therefore believed that the primary Sjogren's syndrome patients might benefit from DHEA supplementation.

The aim of this randomized, double-blinded, controlled trial was to investigate the effects of oral administration of 50 mg DHEA/d on changes of concentrations in serum of DHEA and 12 of its sex-steroid metabolites in postmenopausal women with primary Sjogren's syndrome with subnormal baseline serum values of DHEA-S. In addition, we wanted to examine whether there was a significant relationship between the hormonal levels

and disease-related parameters and to find out whether these disease characteristics were influenced by DHEA therapy.

Patients and Methods

Patients

Patients with primary Sjogren's syndrome between 18 and 80 yr of age were identified from registers in two rheumatology clinics in Western Sweden. In this report, only results of postmenopausal women without hormone replacement therapy are reported. Patients were invited to participate in a randomized, placebo-controlled, double-blind study with a crossover and washout design. One group received 50 mg DHEA orally every morning, and the other group received placebo for 4 months followed by a 1-month washout; thereafter a new 4-month DHEA/placebo period was initiated.

The University Pharmacy (Helsinki, Finland) produced, bottled, and labeled *ex tempore* 50 mg DHEA tablets and identical placebo tablets according to good manufacturing practice (GMP) standards. DHEA (C₁₉H₂₈O₂, European Commission no. 200-175-5) was produced by Diosynth B.V. (Akzo Nobel, TD Oss, The Netherlands) according to GMP standards.

Before inclusion in the trial, a radioimmunoassay was used for the quantitative determination of serum levels of DHEA-S (Diagnostic Products Corporation, Los Angeles, CA). Only women with serum levels of DHEA-S at least lower than the average levels in corresponding age ranges from a series of 81 healthy adult women (18–30 yr, 2.29 µg/ml; 31–50 yr, 1.47 µg/ml; 51–80 yr, 1.15 µg/ml) and with severe fatigue assessed by the Multidimensional Fatigue Inventory (MFI-20) (15, 16) with a score of at least 14 in general fatigue were included in the trial.

The mean serum value of DHEA-S in the 23 included women was 0.58 µg/ml ± 0.34 SD, median value was 0.52 µg/ml, and the minimum and maximum values were 0.052 and 1.22 µg/ml, respectively. Four women were no more than 50 yr old. The mean value of these four women was 0.86 µg/ml ± 0.40 SD, median value was 0.92 µg/ml, and the minimum and maximum values were 0.37 and 1.22 µg/ml, respectively. Nineteen women were at least 51 yr old. The mean value of these 19 women was 0.52 µg/ml ± 0.30 SD, median value was 0.48 µg/ml, and the minimum and maximum values were 0.052 and 1.14 µg/ml, respectively. Patients fulfilled the revised European criteria for primary Sjogren's syndrome (3). Patients with a history of breast cancer and/or uterine cancer, previous stroke or known diathesis for thrombosis, difficult acne, or a significant liver disease and patients with changes in treatment with disease-modifying antirheumatic drugs (DMARD) and/or changes in low-dose glucocorticosteroids taken for Sjogren's during the previous 3 months were excluded from the trial, as were patients taking more than 10 mg prednisolone per day.

Antibodies to SS-A/Ro and SS-B/La antigens had previously been tested at the Department of Clinical Immunology, Sahlgrenska University Hospital. Histological evaluations of the minor salivary glands were performed in the past, and the intensity of sialoadenitis was evaluated by the number of focal lymphocytic inflammation (foci) per 4 mm² of glandular tissue.

Biochemical analyses of blood samples

Venous blood samples were drawn between 0900 and 1100 h at baseline and at 4, 5, and 9 months and were stored at –20 C until the time of analysis. Erythrocyte sedimentation rate (ESR), C-reactive protein, hemoglobin (Hb), white blood cell (WBC) count, and alanine aminotransferase (ALT) were analyzed consecutively by routine laboratory techniques at the Department of Clinical Chemistry, Sahlgrenska University Hospital. The patients were instructed to take their study medication in the mornings but not to take it in the follow-up mornings.

GC/MS

The validated GC/MS system was used for the analysis of DHEA (limit of detection, 0.10 ng/ml), 5-androstene-3β, 17β-diol (5-DIOL)

(limit of detection, 30 pg/ml), androstenedione (4-DIONE) (limit of detection, 0.05 ng/ml), androsterone (ADT) (limit of detection, 20.00 pg/ml), testosterone (Testo) (limit of detection, 0.02 ng/ml), dihydrotestosterone (DHT) (limit of detection, 5.00 pg/ml), estrone (E_1) (limit of detection, 5.00 pg/ml), and 17 β -estradiol (E_2) (limit of detection, 1.00 pg/ml) by a 50% phenyl-methyl polysiloxane (DB-17HT) capillary column (30 m \times 0.25 mm internal diameter, 0.15 μ m film thickness) with helium as the carrier gas. The analytes and internal standard were detected using a HP5973 quadrupole mass spectrometer equipped with a chemical ionization source.

LC/MS

The validated LC/MS/MS system was used for the analysis of DHEA-S (limit of detection, 0.075 μ g/ml), E_1 sulfate (E_1 -S) (limit of detection, 0.075 ng/ml), ADT glucuronide (ADT-G) (limit of detection, 2.00 ng/ml), androstane-3 α , 17 β -diol-3glucuronide (3G) (limit of detection, 0.50 ng/ml), and androstane-3 α , 17 β -diol-17glucuronide (17G) (limit of detection, 0.50 ng/ml) by a method using TurboIonSpray.

The baseline values of DHEA-S measured by LC/MS/MS corresponded well with the serum levels of DHEA-S measured by radioimmunoassay before inclusion in the study ($r_s = 0.93$; $P < 0.001$). Baseline values of DHEA-S measured by LC/MS/MS were as follows: mean, 0.52 μ g/ml \pm 0.35 SD; median, 0.41 μ g/ml; minimum, 0.08 μ g/ml; maximum, 1.24 μ g/ml.

Collection of saliva

Saliva samples were collected by dentists at all visits. Unstimulated whole saliva was collected for 15 min by the draining method, and stimulated saliva was collected for 5 min after 1 min of prestimulation by paraffin chewing (17).

Questionnaires

Visual Analog Scale (VAS) was used to evaluate patients' self-perceived symptoms of dryness in mouth and eyes. The patients were asked: 1) How dry does your mouth feel most of the time (not dry at all, 0 mm; dry as a desert, 100 mm)? 2) How much discomfort do you feel in your mouth (no discomfort, 0 mm; worst possible discomfort, 100 mm)? 3) How dry do your eyes feel most of the time (not dry at all, 0 mm; very dry, 100 mm)? Pain during the preceding week (not at all, 0 mm; worst possible, 100 mm) and global health (very good, 0 mm; very bad, 100 mm) were assessed by VAS.

Ethical aspects

All patients gave informed written consent according to the Declaration of Helsinki. The study was approved by the Regional Ethics Committee.

Statistical analysis

Analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL). Descriptive statistics are presented as mean and SD values. Most of the data were not normally distributed. Spearman's rank correlation coefficients (r_s) were used to assess correlations. Logistic regression analysis was performed with antibodies to SS-A and/or SS-B as the dependent variable and metabolites significantly correlated to the presence of antibodies as covariates. A forward conditional method was used. A receiver operating characteristic curve was calculated with antibodies to SS-A and/or SS-B as the stated variable. Mann-Whitney U test was used for comparisons between groups, and Wilcoxon rank sum test was used for analyzing changes within groups. Analyses were done according to the intention to treat method. Fisher's exact test was used to compare categorical variables. All tests with a two-tailed P value < 0.05 were considered statistically significant.

Results

Patients

In Table 1, characteristics of the patients at baseline are displayed. Thirteen patients were randomized to initial DHEA treatment and 10 to initial placebo. There were no significant baseline differences between these groups concerning the variables in Table 1 or DHEA and its metabolites. At study entry, four (17%) patients were taking single DMARD, three hydroxychloroquine/chloroquine phosphate, and one cyclosporin A. One (4%) of the patients was on glucocorticosteroids and one (4%) on a combination with prednisolone and azathioprine. The mean dose of corticosteroids was 3 mg prednisolone per day. All the patients had ocular and oral symptoms of mucosal dryness. Eighteen of 20 women who had undergone minor salivary gland biopsy had focal sialoadenitis. Sixteen of 21 women who had been examined by an ophthalmologist had keratoconjunctivitis sicca (KCS). Ten women were able to produce unstimulated saliva, whereas 19 were able to produce stimulated saliva.

Two patients experienced adverse events resulting in discontinuation of the study drug: one woman due to increase in nightly calf cramps during DHEA, and the other to a suspected transitory ischemic attack during placebo. Other reported adverse events during the DHEA period were acne and increase in perspiration (one patient), increase in nightly dreams (one patient), depressiveness (one patient), dizziness (one patient), and hirsutism (one patient); and during the placebo period were gastric pain (one patient) and headache (one patient). The dropouts were followed until the end of the study, and the code was broken when all patients had completed the trial.

TABLE 1. Characteristics of 23 postmenopausal women with primary Sjogren's syndrome

Characteristics	
Age (yr)	60.7 \pm 8.6 (23)
Height (cm)	160.5 \pm 20.4 (23)
Weight (kg)	76.2 \pm 21.6 (23)
No. of years with SS diagnosis	6.0 \pm 5.8 (23)
No. of years since symptoms debuted	13.5 \pm 7.2 (17)
ESR (mm/h)	18.3 \pm 12.3 (23)
Hb (g/ml)	141.0 \pm 9.5 (23)
WBC (10^9 /liter)	6.1 \pm 1.5 (23)
ALT (μ cat/liter)	0.36 \pm 0.16 (23)
Antibodies to SS-A and/or SS-B (yes:no)	11:12 (23)
KCS (yes:no)	16:5 (21)
Focal sialoadenitis (yes:no)	18:2 (20)
Unstimulated salivary flow (yes:no)	10:12 (22)
Unstimulated salivary flow rate (ml/15 min)	0.98 \pm 0.90 (10)
Stimulated salivary flow (yes:no)	19:3 (22)
Stimulated salivary flow rate (ml/5 min)	2.41 \pm 2.51 (19)
Dry mouth symptoms (VAS, mm)	77.7 \pm 16.2 (23)
Dry mouth discomfort (VAS, mm)	70.0 \pm 22.7 (23)
Dry eye symptoms (VAS, mm)	70.0 \pm 18.6 (23)
Pain (VAS, mm)	58.3 \pm 21.4 (23)
Global health (VAS, mm)	47.8 \pm 20.6 (23)
DMARD (yes:no)	5:18 (23)
Prednisolone (yes:no)	2:21 (23)
T ₄ substitution (yes:no)	6:17 (23)

Values represent means \pm SD. Numbers of patients with available data are shown in parentheses.

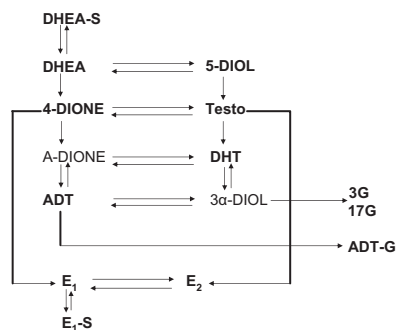


FIG. 1. A schematic presentation of DHEA and its metabolites. Results of the bolded metabolites are reported.

Associations between serum levels of sex steroids and disease-related variables at baseline

Figure 1 shows a schematic presentation of the metabolism of androgen precursors into bioactive androgens, estrogens, and glucuronidated androgen metabolites.

In Table 2, the Spearman’s correlation coefficient values between the sex steroids and some disease-related and demographic data are indicated. ESR was moderately inversely correlated with serum Testo and DHT and more strongly with DHEA-S ($r_s = -0.58$; $P < 0.01$; Fig. 2). The presence of antibodies to SS-A/Ro or SS-B/La antigens or both was connected with low levels of most of the studied steroids, including the glucuronidated androgen metabolites. KCS was associated with low levels of 4-DIONE and Testo. Aspects of sicca symptoms in the mouth were associated with low Testo and 4-DIONE levels, whereas sicca symptoms of the eyes were associated with low levels of all measured estrogens, most strongly with E_1 ($r_s = -0.63$; $P < 0.01$; Fig. 2).

A multiple logistic regression analysis was performed to find out the most important hormones associated with the presence of SS-A and/or SS-B antibodies. Covariates were the significant variables in Table 2, and the dependent variable was SS-A and/or SS-B antibodies (yes or no). After multiple logistic regression analyses, only E_2 remained independently significantly associ-

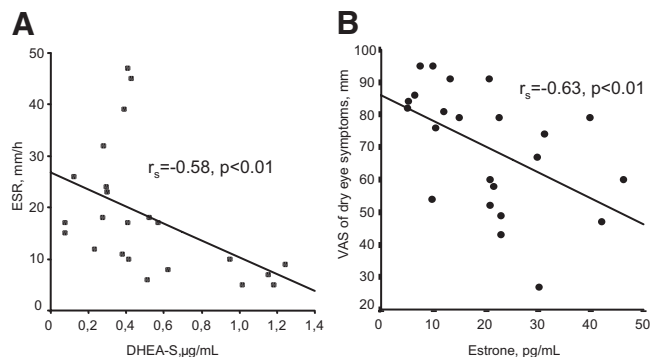


FIG. 2. Scatter diagrams of DHEA-S and ESR (A) and E_1 and dry eye symptoms assessed by VAS (B); 0 mm indicates not dry at all, and 100 mm indicates very dry. The regression lines are inserted, and Spearman’s correlation coefficients (r_s) are given.

ated with the antibodies. Area under the receiver operating characteristic curve for E_2 was 0.82 (95% confidence interval, 0.64–0.99).

The intensity of inflammation in the minor salivary glands (number of foci score/4 mm²) was correlated to the sex steroids. No significant associations were found, except a correlation of borderline significance to 17G ($r_s = -0.42$; $P = 0.07$). There was a borderline significance between the number of the foci and the degree of discomfort in the mouth ($r_s = 0.42$; $P = 0.07$). High intensity of salivary gland inflammation was associated with the presence of SS-A and/or SS-B antibodies ($r_s = 0.47$; $P = 0.035$).

Associations between changes in sex steroids

Table 3 shows the correlations between the alterations in serum concentrations of DHEA and DHEA-S and changes in the other sex steroids during the 4-month treatment with DHEA. The strongest correlations were found between DHEA and the precursor hormones 5-DIOL, 4-DIONE, ADT, and glucuronidated androgen metabolites.

TABLE 2. Spearman’s correlation coefficients (r_s) at baseline between demographic and disease-related variables and serum levels of DHEA-S, DHEA, androgens, estrogens, and glucuronidated androgen metabolites in 23 postmenopausal women with primary Sjogren’s syndrome

	DHEA	DHEA-S	5-DIOL	4-DIONE	ADT	Testo	DHT	E_1	E_1 -S	E_2	ADT-G	3G	17G
Weight	0.22	0.02	0.29	0.31	0.09	0.39	0.12	0.26	0.43^a	0.59^b	0.21	0.32	0.50^a
ESR	-0.36	-0.58^b	-0.34	-0.38	-0.34	-0.42^a	-0.45^a	-0.11	-0.22	-0.10	-0.13	-0.22	-0.18
SS-A and/or SS-B	-0.41	-0.39	-0.50^a	-0.49^a	-0.46^a	-0.51^a	-0.25	-0.42^a	-0.55^b	-0.55^b	-0.45^a	-0.56^b	-0.54^b
KCS	-0.36	-0.30	-0.20	-0.55^a	-0.15	-0.45^a	-0.13	-0.39	-0.36	-0.26	-0.22	0.06	-0.10
Dry mouth symptoms	-0.04	-0.06	-0.08	-0.36	-0.07	-0.42^a	-0.17	-0.24	-0.09	-0.10	0.05	-0.07	-0.04
Dry mouth discomfort	-0.16	-0.07	-0.18	-0.44^a	-0.28	-0.32	-0.29	-0.36	-0.14	-0.18	-0.22	-0.12	-0.07
Dry eye symptoms	-0.04	-0.01	-0.10	-0.32	-0.12	-0.30	-0.22	-0.63^b	-0.47^a	-0.47^a	-0.11	-0.29	-0.06
T ₄ substitution	-0.37	-0.48^a	-0.40	-0.37	-0.25	-0.54^b	-0.39	0.45^a	-0.38	-0.33	-0.30	-0.28	-0.34

Significant correlations are in *boldface*.

^a $P < 0.05$.

^b $P < 0.01$.

TABLE 3. Spearman's correlation coefficients (r_s) between the 4-month changes in serum levels of DHEA-S and DHEA and changes in the other hormones during treatment with DHEA in 23 postmenopausal women with primary Sjogren's syndrome

	Δ DHEA	Δ DHEA-S	Δ 5-DIOL	Δ 4-DIONE	Δ ADT	Δ Testo	Δ DHT	Δ E ₁	Δ E ₁ -S	Δ E ₂	Δ ADT-G	Δ 3G	Δ 17G
Δ DHEA	1.00	0.56^b	0.73^c	0.69^c	0.66^b	0.40	0.44^a	0.41	0.48^a	0.38	0.62^b	0.58^b	0.46^a
Δ DHEA-S	0.56^b	1.00	0.53^b	0.50^a	0.36	0.30	0.17	0.44^a	0.69^c	0.36	0.44^a	0.45^a	0.26

Significant correlations are in *boldface*.
^a $P < 0.05$.
^b $P < 0.01$.
^c $P < 0.001$.

The effect of DHEA substitution on sex steroids

Table 4 shows the serum concentrations of the different hormones and metabolites during the DHEA and placebo periods. All sex steroids were highly significantly increased by DHEA treatment. The percentage increases in estrogens were smaller in comparison with the increase in precursor hormones, DHT, and androgen metabolites. The median increases during the DHEA treatment period were: DHEA, 260%; DHEA-S, 900%; 5-DIOL, 340%; 4-DIONE, 180%; ADT, 770%; Testo, 100%; DHT, 470%; E₁, 80%; E₁-S, 70%; E₂, 70%; ADT-G, 1780%; 3G, 1220%; and 17G, 790%.

The effect of DHEA substitution on disease-related variables

The symptoms of dryness in the mouth decreased significantly ($P < 0.05$) by 14% during the DHEA period. When the differences during DHEA and during placebo were compared, a tendency of larger reduction during DHEA treatment was found ($P = 0.07$). The discomfort in the mouth also decreased during DHEA therapy, although not significantly ($P = 0.055$; Table 4).

Discussion

In this study, we have examined whether there was any association between the precursor hormone DHEA and 12 of its metabolites and disease-specific and inflammation-related characteristics in postmenopausal women with primary Sjogren's syndrome. Interestingly, we found (to the best of our knowledge) for the first time that disease characteristics were associated with low levels of sex steroids. We found that ESR was significantly inversely correlated with DHEA-S, Testo, and DHT. Almost 50% of the women in our study had antibodies to SS-A and/or SS-B. To have at least one of these autoantibodies was significantly associated with lower serum levels of both androgens and estrogens. The multiple logistic regression analysis with SS-A and/or SS-B antibodies as the dependent variable revealed that low E₂ was independently connected with the presence of the antibody/antibodies.

The patients' subjective experience of dryness in the mouth was inversely connected with serum levels of Testo and 4-DIONE. These results are interesting because previous animal studies have indicated that androgens have profound effects on the murine submandibular glands (18-20). Also, it was recently discovered that androgen deprivation in salivary glands in Sjogren's syndrome patients leads to low salivary levels of DHEA and low levels of DHEA-regulated salivary cysteine-rich secretory protein-3 (9).

Concerning the eyes, androgens appear to modulate lipid production and gene expression in mouse and/or rabbit meibomian glands, and antiandrogen therapy in men is associated with meibomian gland disease (21). We found that KCS was associated with both low 4-DIONE and Testo, but the patients' self-perceived dryness of the eyes was exclusively related to low levels of all of the estrogens measured, most strongly to low E₁, the most abundant active estrogen in postmenopausal women. In support of the significance of this finding, another study showed that

TABLE 4. The effects of DHEA and placebo treatment on serum levels of androgen precursors, androgens, estrogens, and glucuronidated androgen metabolites and on disease-related variables in 23 postmenopausal women with primary Sjogren's syndrome

Sex steroids and disease-related variables	Month 0	Month 4	Differences (4–0)
DHEA-S ($\mu\text{g/ml}$)			
DHEA	0.52 \pm 0.37	4.12 \pm 1.55	3.59 \pm 1.49 ^{c,f}
Placebo	0.52 \pm 0.35	0.51 \pm 0.31	-0.011 \pm 0.13
DHEA (ng/ml)			
DHEA	1.59 \pm 1.12	5.45 \pm 2.20	3.86 \pm 1.87 ^{c,f}
Placebo	1.67 \pm 1.38	1.70 \pm 1.25	0.024 \pm 0.57
5-DIOL (pg/ml)			
DHEA	228.2 \pm 149.7	841.5 \pm 274.3	613.3 \pm 263.8 ^{c,f}
Placebo	228.6 \pm 144.9	260.4 \pm 197.3	31.8 \pm 72.6
4-DIONE (ng/ml)			
DHEA	0.37 \pm 0.25	0.94 \pm 0.39	0.58 \pm 0.29 ^{c,f}
Placebo	0.39 \pm 0.27	0.37 \pm 0.22	-0.01 \pm 0.13
ADT (pg/ml)			
DHEA	115.0 \pm 87.5	868.2 \pm 496.8	753.1 \pm 496.1 ^{c,f}
Placebo	112.3 \pm 88.6	107.6 \pm 86.2	-4.71 \pm 25.5
Testo (ng/ml)			
DHEA	0.16 \pm 0.10	0.34 \pm 0.14	0.18 \pm 0.11 ^{c,f}
Placebo	0.16 \pm 0.09	0.17 \pm 0.12	0.02 \pm 0.04
DHT (pg/ml)			
DHEA	38.1 \pm 32.6	178.5 \pm 81.4	140.4 \pm 75.5 ^{c,f}
Placebo	37.6 \pm 31.1	38.8 \pm 35.6	1.17 \pm 5.89
E ₁ (pg/ml)			
DHEA	20.3 \pm 12.1	32.7 \pm 14.0	12.4 \pm 12.1 ^{b,f}
Placebo	20.1 \pm 10.2	21.6 \pm 11.6	1.5 \pm 5.4
E ₁ -S (ng/ml)			
DHEA	0.23 \pm 0.20	0.38 \pm 0.31	0.14 \pm 0.17 ^{b,f}
Placebo	0.21 \pm 0.17	0.24 \pm 0.20	0.032 \pm 0.074
E ₂ (pg/ml)			
DHEA	4.21 \pm 3.05	11.64 \pm 15.65	7.43 \pm 15.38 ^{a,f}
Placebo	4.96 \pm 3.79	5.82 \pm 5.42	0.87 \pm 5.65
ADT-G (ng/ml)			
DHEA	16.8 \pm 20.5	266.9 \pm 196.3	250.1 \pm 186.1 ^{c,f}
Placebo	18.6 \pm 25.8	17.3 \pm 22.0	-1.29 \pm 4.32
3G (ng/ml)			
DHEA	0.68 \pm 0.36	8.79 \pm 4.77	8.11 \pm 4.73 ^{c,f}
Placebo	0.71 \pm 0.41	0.69 \pm 0.36	-0.011 \pm 0.14
17G (ng/ml)			
DHEA	0.64 \pm 0.33	5.80 \pm 3.76	5.17 \pm 3.69 ^{c,f}
Placebo	0.65 \pm 0.38	0.63 \pm 0.39	-0.029 \pm 0.13
ESR (mm/h)			
DHEA	18.3 \pm 11.8	20.0 \pm 13.6	1.7 \pm 6.7
Placebo	18.0 \pm 11.8	19.5 \pm 11.0	1.4 \pm 3.7 ^d
Hb (g/ml)			
DHEA	140.9 \pm 9.1	142.1 \pm 11.4	1.2 \pm 5.9
Placebo	143.6 \pm 9.0	142.1 \pm 8.6	-1.5 \pm 5.6
WBC ($10^9/\text{liter}$)			
DHEA	6.1 \pm 1.7	6.8 \pm 2.3	0.67 \pm 1.2 ^{a,e}
Placebo	6.5 \pm 1.8	6.5 \pm 2.1	0.04 \pm 1.3
ALT ($\mu\text{cat/liter}$)			
DHEA	0.38 \pm 0.16	0.31 \pm 0.16	-0.06 \pm 0.13 ^d
Placebo	0.38 \pm 0.18	0.36 \pm 0.16	-0.03 \pm 0.13
Dry mouth symptoms (VAS, mm)			
DHEA	77.7 \pm 16.6	67.1 \pm 23.0	-10.6 \pm 21.1 ^d
Placebo	74.6 \pm 18.3	72.9 \pm 19.3	-1.9 \pm 12.6

(Continued)

TABLE 4. Continued

Sex steroids and disease-related variables	Month 0	Month 4	Differences (4–0)
Dry mouth discomfort (VAS, mm)			
DHEA	69.8 \pm 20.1	62.3 \pm 21.4	-7.6 \pm 23.4
Placebo	64.1 \pm 20.5	62.8 \pm 19.8	-0.3 \pm 16.2
Dry eye symptoms (VAS, mm)			
DHEA	67.6 \pm 23.1	69.4 \pm 19.0	1.9 \pm 17.4
Placebo	71.3 \pm 16.8	68.1 \pm 19.2	-3.1 \pm 11.8

Values represent means \pm SD.^a $P < 0.05$ for the comparison with change during placebo.^b $P < 0.01$ for the comparison with change during placebo.^c $P < 0.001$ for the comparison with change during placebo.^d $P < 0.05$ for the comparisons with baseline.^e $P < 0.01$ for the comparisons with baseline.^f $P < 0.001$ for the comparisons with baseline.

treatment of menopausal KCS with topical E₂ ophthalmic drops improved ocular symptoms and Schirmer's test results (22). Accordingly, the importance of estrogen is also worthy in Sjogren's, and a study evaluating the effects of topical E₂ ophthalmic drops in primary Sjogren's syndrome is encouraged; a trial of systemic low-dose estrogen might also be interesting.

DHEA and all the measured 12 metabolites increased significantly during the 4-month treatment with 50 mg DHEA/day orally, whereas the serum levels of the steroids remained stable during placebo. It is well known that treatment with glucocorticosteroids suppresses the secretion of adrenal androgens. However, only two women in this study were treated with low-dose prednisolone, and if these two women were excluded the effects of DHEA on sex steroids remained similar. Labrie *et al.* (11–13) have previously measured the bioavailability and metabolism of oral and percutaneous DHEA administration in healthy postmenopausal women. In the study of oral DHEA treatment, 12 healthy 60- to 70-yr-old women received two capsules of 50 mg DHEA in the morning for 14 d. The baseline mean hormonal levels in that study were DHEA, 2.3 ng/ml; DHEA-S, 0.4 $\mu\text{g/ml}$; 5-DIOL, 0.31 ng/ml; 4-DIONE, 0.6 ng/ml; Testo, 0.38 ng/ml; and ADT-G, 14 ng/ml (12) in comparison to the postmenopausal women with primary Sjogren's syndrome in our study (mean age, 60.7 \pm 8.6 yr), demonstrating lower hormonal levels except for DHEA-S and ADT-G (DHEA, 1.6 ng/ml; DHEA-S, 0.5 $\mu\text{g/ml}$; 5-DIOL, 0.23 ng/ml; 4-DIONE, 0.37 ng/ml; Testo, 0.16 ng/ml; and ADT-G, 17 ng/ml). The three estrogens measured and Testo increased significantly by oral DHEA administration in our study, but the percentage increase was markedly lower compared with the percentage increase of the other androgens and metabolites, in line with the findings of Labrie *et al.* (12).

In another trial, changes in metabolites were assessed after 12-month percutaneous administration of 3 g of a 0.3% DHEA emulsion (18 mg) (13). Compared with the percutaneous ad-

ministration, our oral treatment resulted in higher metabolite elevations. DHEA was increased 203% and 5-DIOL 178% by the DHEA emulsion compared with 260 and 340% elevation, respectively, in our study. Again, the increases observed for the estrogens were less marked and not significant by the percutaneous therapy: E₁, 30%; E₁-S, 20%; and E₂, 17% (13) compared with the increases in our trial of 80, 70, and 70%, respectively.

During oral administration of 50 mg DHEA/d in our trial, the serum concentrations of DHEA and 4-DIONE increased to levels within 5th and 95th percentiles of the concentration in 30- to 35-yr-old women. 5-DIOL, Testo, and DHT reached the upper or just above the upper limit, whereas DHEA-S, ADT-G, 3G, and 17G increased to concentrations much above levels in 30- to 35-yr-old women. These reference values are given in the article by Labrie *et al.* (13). The prominent elevations of DHEA-S and androgen glucuronide conjugates indicate that DHEA administered orally is metabolized through the gastrointestinal tract and/or first passage through the liver. This marked increase is not found when DHEA is metabolized after the percutaneous route of administration (12). Concerning the estrogens, oral treatment with 50 mg DHEA/d increased serum concentrations of E₁ and E₁-S to levels within the 5th and 95th percentiles of the concentration in 55- to 65-yr-old women and E₂ to concentrations just above levels in 55- to 65-yr-old women (13). According to the increase in sex steroids, it seems that supplementation with 50 mg DHEA/d orally results in physiological or slightly supra-physiological steroid hormone levels. However, a limitation with our study is the lack of a control group consisting of healthy women enabling direct comparisons between women with and without primary Sjogren's syndrome. Clinical trials of treatment with DHEA in both pharmacological and substitution doses have been conducted. In systemic lupus erythematosus, 20–30 mg DHEA/d improved mental well-being (23); treatment with 200 mg DHEA/d reduced disease activity (24), had a corticosteroid sparing effect (25), and increased bone mineral density (BMD) in postmenopausal women (26). Increase in BMD by DHEA has also been reported in older men and women (27), and the glucuronidated androgen metabolites 3G and 17G were found to be stronger predictors of BMD than Testo and DHT in elderly men (28). Recently, Hartkamp *et al.* (29) reported that 200 mg DHEA/d and placebo reduced fatigue and improved well-being in Sjogren's, suggesting possibilities of cognitive behavioral interventions. Pillemer *et al.* (30) found in a pilot trial of women with Sjogren's that dry mouth symptoms improved significantly during 24 wk of oral DHEA 200 mg/d.

We also demonstrate a significant reduction in dry mouth symptoms during DHEA in accordance with previous results. However, no significant increase in salivary flow rate was observed, a finding that might be explained by the limited number of patients who were able to secrete saliva. The sicca symptoms in the eyes did not improve by DHEA, which might be associated with our findings demonstrating inverse correlations between sicca symptoms in the eyes and the estrogens, which increased only moderately by DHEA substitution. One may also speculate that the treatment period of 4 months, with a physiological dose of DHEA, may not be long enough to improve disease outcome measures in patients with such a long-standing disease.

In conclusion, we show in this study of postmenopausal women with primary Sjogren's syndrome, for the first time, that low levels of sex steroids are associated with several disease characteristics including presence of anti-SS-A and/or SS-B, KCS, self-perceived symptoms of dryness in mouth and eyes, and ESR. It is known that women with primary Sjogren's syndrome are androgen-deficient and supplementation with 50 mg DHEA restored the hormonal levels to the normal range or slightly above in premenopausal healthy women. However, DHEA-S, ADT-G, 3G, and 17G increased markedly, indicating metabolism through the gastrointestinal tract and/or first passage through the liver. The sicca symptoms in the mouth decreased during 4-month DHEA treatment. We encourage a larger and long-term randomized controlled trial with DHEA substitution to be able to evaluate potential beneficial effects of DHEA in primary Sjogren's syndrome.

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References

1. Manoussakis MN, Moutsopoulos HM 2000 Sjogren's syndrome: autoimmune epithelitis. *Baillieres Best Pract Res Clin Rheumatol* 14:73–95
2. Talal N 1987 Overview of Sjogren's syndrome. *J Dent Res* 66:672–674
3. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, Daniels TE, Fox PC, Fox RI, Kassan SS, Pillemer SR, Talal N, Weisman MH 2002 Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 61:554–558
4. Masi AT 1995 Sex hormones and rheumatoid arthritis: cause or effect relationships in a complex pathophysiology? *Clin Exp Rheumatol* 13:227–240
5. Masi AT, Josipovic DB, Jefferson WE 1984 Low adrenal androgenic-anabolic steroids in women with rheumatoid arthritis (RA): gas-liquid chromatographic studies of RA patients and matched normal control women indicating decreased 11-deoxy-17-ketosteroid excretion. *Semin Arthritis Rheum* 14:1–23
6. Straub RH, Zeuner M, Antoniou E, Schölmerich J, Lang B 1996 Dehydroepiandrosterone sulfate is positively correlated with soluble interleukin 2 receptor and soluble intercellular adhesion molecule in systemic lupus erythematosus. *J Rheumatol* 23:856–861
7. Valtysdottir ST, Wide L, Hällgren R 2001 Low serum dehydroepiandrosterone sulfate in women with primary Sjogren's syndrome as an isolated sign of impaired HPA axis function. *J Rheumatol* 28:1259–1265
8. Sullivan DA, Bélanger A, Cermak JM, Bérubé R, Papas AS, Sullivan RM, Yamagami H, Dana MR, Labrie F 2003 Are women with Sjogren's syndrome androgen-deficient? *J Rheumatol* 30:2413–2419
9. Laine M, Porola P, Udby L, Kjeldsen L, Cowland JB, Borregaard N, Hietanen J, Ståhle M, Pihakari A, Kontinen YT 2007 Low salivary dehydroepiandrosterone and androgen-regulated cysteine-rich secretory protein 3 levels in Sjogren's syndrome. *Arthritis Rheum* 56:2575–2584

10. Labrie F, Luu-The V, Bélanger A, Lin SX, Simard J, Pelletier G, Labrie C 2005 Is dehydroepiandrosterone a hormone? *J Endocrinol* 187:169–196
11. Labrie F, Bélanger A, Bélanger P, Bérubé R, Martel C, Cusan L, Gomez J, Candas B, Chaussade V, Castiel I, Deloche C, Leclaire J 2007 Metabolism of DHEA in postmenopausal women following percutaneous administration. *J Steroid Biochem Mol Biol* 103:178–188
12. Labrie F, Bélanger A, Labrie C, Candas B, Cusan L, Gomez JL 2007 Bioavailability and metabolism of oral and percutaneous dehydroepiandrosterone in postmenopausal women. *J Steroid Biochem Mol Biol* 107:57–69
13. Labrie F, Cusan L, Gomez JL, Martel C, Bérubé R, Bélanger P, Chaussade V, Deloche C, Leclaire J 2008 Changes in serum DHEA and eleven of its metabolites during 12-month percutaneous administration of DHEA. *J Steroid Biochem Mol Biol* 110:1–9
14. Dessein PH, Joffe BI, Stanwix AE, Moomal Z 2001 Hyposecretion of the adrenal androgen dehydroepiandrosterone sulfate and its relation to clinical variables in inflammatory arthritis. *Arthritis Res* 3:183–188
15. Smets EM, Garssen B, Bonke B, De Haes JC 1995 The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J Psychosom Res* 39:315–325
16. Fürst CJ, Ahsberg E 2001 Dimensions of fatigue during radiotherapy. An application of the Multidimensional Fatigue Inventory. *Support Care Cancer* 9:355–360
17. Dawes C 1987 Physiological factors affecting salivary flow rate, oral sugar clearance, and the sensation of dry mouth in man. *J Dent Res* 66(Spec no.): 648–653
18. Islander U, Hasséus B, Erlandsson MC, Jochems C, Skrtic SM, Lindberg M, Gustafsson JA, Ohlsson C, Carlsten H 2005 Estren promotes androgen phenotypes in primary lymphoid organs and submandibular glands. *BMC Immunol* 6:16
19. Treister NS, Richards SM, Rowley P, Jensen RV, Sullivan DA 2006 Influence of testosterone on gene expression in the ovariectomized mouse submandibular gland. *Eur J Oral Sci* 114:328–336
20. Treister NS, Richards SM, Suzuki T, Jensen RV, Sullivan DA 2005 Influence of androgens on gene expression in the BALB/c mouse submandibular gland. *J Dent Res* 84:1187–1192
21. Sullivan DA, Sullivan BD, Evans JE, Schirra F, Yamagami H, Liu M, Richards SM, Suzuki T, Schaumberg DA, Sullivan RM, Dana MR 2002 Androgen deficiency, Meibomian gland dysfunction, and evaporative dry eye. *Ann NY Acad Sci* 966:211–222
22. Sator MO, Joura EA, Golaszewski T, Gruber D, Frigo P, Metka M, Hommer A, Huber JC 1998 Treatment of menopausal keratoconjunctivitis sicca with topical oestradiol. *Br J Obstet Gynaecol* 105:100–102
23. Nordmark G, Bengtsson C, Larsson A, Karlsson FA, Sturfelt G, Rönnblom L 2005 Effects of dehydroepiandrosterone supplement on health-related quality of life in glucocorticoid treated female patients with systemic lupus erythematosus. *Autoimmunity* 38:531–540
24. Petri MA, Mease PJ, Merrill JT, Lahita RG, Iannini MJ, Yocum DE, Ginzler EM, Katz RS, Gluck OS, Genovese MC, Van Vollenhoven R, Kalunian KC, Manzi S, Greenwald MW, Buyon JP, Olsen NJ, Schiff MH, Kavanaugh AF, Caldwell JR, Ramsey-Goldman R, St. Clair EW, Goldman AL, Egan RM, Polisson RP, Moder KG, Rothfield NF, Spencer RT, Hobbs K, Fessler BJ, Calabrese LH, Moreland LW, Cohen SB, Quarles BJ, Strand V, Gurwith M, Schwartz KE 2004 Effects of prasterone on disease activity and symptoms in women with active systemic lupus erythematosus. *Arthritis Rheum* 50:2858–2868
25. Petri MA, Lahita RG, Van Vollenhoven RF, Merrill JT, Schiff M, Ginzler EM, Strand V, Kunz A, Gorelick KJ, Schwartz KE 2002 Effects of prasterone on corticosteroid requirements of women with systemic lupus erythematosus: a double-blind, randomized, placebo-controlled trial. *Arthritis Rheum* 46:1820–1829
26. Hartkamp A, Geenen R, Godaert GL, Bijl M, Bijlsma JW, Derksen RH 2004 The effect of dehydroepiandrosterone on lumbar spine bone mineral density in patients with quiescent systemic lupus erythematosus. *Arthritis Rheum* 50: 3591–3595
27. Jankowski CM, Gozansky WS, Schwartz RS, Dahl DJ, Kittelson JM, Scott SM, Van Pelt RE, Kohrt WM 2006 Effects of dehydroepiandrosterone replacement therapy on bone mineral density in older adults: a randomized, controlled trial. *J Clin Endocrinol Metab* 91:2986–2993
28. Vandenput L, Labrie F, Mellström D, Swanson C, Knutsson T, Pecker R, Ljunggren O, Orwoll E, Eriksson AL, Damber JE, Ohlsson C 2007 Serum levels of specific glucuronidated androgen metabolites predict BMD and prostate volume in elderly men. *J Bone Miner Res* 22:220–227
29. Hartkamp A, Geenen R, Godaert GL, Bootsma H, Kruize AA, Bijlsma JW, Derksen RH 2008 Effect of dehydroepiandrosterone administration on fatigue, well-being, and functioning in women with primary Sjogren syndrome: a randomised controlled trial. *Ann Rheum Dis* 67:91–97
30. Pillemer SR, Brennan MT, Sankar V, Leakan RA, Smith JA, Grisius M, Ligier S, Radfar L, Kok MR, Kingman A, Fox PC 2004 Pilot clinical trial of dehydroepiandrosterone (DHEA) versus placebo for Sjogren's syndrome. *Arthritis Rheum* 51:601–604