Are Women with Sjögren's Syndrome Androgen-Deficient?

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ABSTRACT. ***Objective.*** We hypothesize that androgen deficiency is a critical etiologic factor in the pathogenesis of aqueous-deficient and evaporative dry eye in Sjögren's syndrome (SS). We investigated whether women with SS have a deficiency in total androgens. We also examined whether these patients have elevated serum concentrations of estrogens.

Methods. Blood was drawn from women with primary and secondary SS and age matched controls, and analyzed for steroid concentrations by gas and liquid chromatography-mass spectrometry.

Results. Our results show that women with SS are androgen-deficient. Concentrations of 5-androstene-3ß,17ß-diol (5-diol), dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), androsterone-glucuronide (ADT-G), and androstane-3α,17ß-diol-G (3α-diol-G) were all significantly reduced in SS sera relative to controls. In contrast, SS was not associated with significant alterations in the serum concentrations of testosterone, androstenedione, estrone, or 17ß-estradiol. These overall findings could not be attributed to the use of oral contraceptives or hormone replacement therapy, because the concentrations of 5-diol, DHEA, DHT, ADT-G and 3α-diol-G were also decreased in patients with SS compared to levels in control women who were not taking exogenous estrogens.

Conclusion. Our results show that women with SS are androgen-deficient. (J Rheumatol 2003; 30:2413–9)

Key Indexing Terms:
SJÖGREN'S SYNDROME ANDROGENS ESTROGENS DRY EYE

Sex steroid deficiency has been linked to the development and/or progression of a wide variety of clinical disorders, including insulin resistance, cardiovascular disease, obesity, osteoporosis, and certain cancers[1]. We hypothesize that sex steroid deficiency, particularly that of androgens, may also be a critical etiologic factor in the pathogenesis of dry eye syndromes. These syndromes, which afflict over 10 million Americans[2], are classified into 2 major types: aqueous-deficient and evaporative[3]. Aqueous-deficient dry eye is due to a lack of tear secretion by the lacrimal glands. An example is Sjögren's syndrome (SS), an autoimmune disease that occurs almost exclusively in women. This disorder is associated with extensive inflammation of lacrimal tissue, immune mediated destruction and/or dysfunction of acinar and ductal epithelial cells, and a precipitous decline in aqueous tear output[4,5]. SS may be either primary (i.e., no associated connective tissue disease) or secondary [e.g., patients with systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA)]. The second type of dry eye is called evaporative and is typically caused by meibomian gland dysfunction and lipid insufficiency, resulting in increased evaporation and decreased stability of the tear film[6]. This form of dry eye is also found in SS, as well as during menopause and aging[7-9]. It has been estimated that meibomian gland disease may be a contributing factor in over 60% of all patients with dry eye[10].

The rationale for our hypothesis concerning androgen deficiency and dry eye syndromes is 2-fold. First, androgens regulate multiple aspects of the lacrimal gland, including epithelial cell morphology, gene expression, protein synthesis, secretory processes, and immune activity[11]. Moreover, serum concentrations of testosterone are reportedly decreased in patients with SS[12], and this reduction may predispose to lacrimal gland dysfunction, reduced tear secretion, and dry eye. Consistent with this proposition is the finding that testosterone administration to female mouse models of SS (i.e., MRL/Mp-lpr/lpr and NZB/NZW F1)

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causes a dramatic suppression of the inflammation and a significant increase in the functional activity of lacrimal glands[5,13]. Similarly, androgen treatment has been reported to alleviate dry eye signs and symptoms, and stimulate tear flow, in patients with SS[14-16]. The second consideration is that androgens regulate meibomian gland function, enhance the quality and/or quantity of lipids produced by this tissue, and promote the formation of the tear film's lipid layer[17]. Conversely, androgen deficiency, such as occurs during menopause, aging, SS, complete androgen insensitivity syndrome (CAIS), and the use of anti-androgen therapy, is associated with meibomian gland dysfunction, altered lipid profiles in meibomian gland secretions, tear film instability, and evaporative dry eye[17]. Further, investigators have reported that low serum concentrations of testosterone are more prevalent in women with dry eye and correlate with the subjective severity of ocular symptoms[18].

However, although these findings are consistent with our hypothesis concerning menopause, aging, CAIS, and the use of anti-androgen medication, they do not show a definitive link between androgen deficiency and dry eye syndromes in women with SS. The reason is that a previous study[12] focused primarily upon serum testosterone concentrations, which represent only a very small fraction of the total androgen pool in humans[1,19-25]. Indeed, it appears that the measurement of serum testosterone in women has little or no value except as an index of ovarian activity[19,21,23], and this tissue is not the principal origin of androgens (or estrogens) in women[19,21,23,25]. As shown in the emerging field of intracrinology, the majority of androgens (e.g., 75% before and 100% after menopause) in women are synthesized in peripheral tissues from adrenal sex steroid precursors [i.e., dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEA-S), and androstenedione][1,19,21-26]. Indeed, humans and primates are unique in possessing adrenal glands that secrete large amounts of DHEA and DHEA-S, which are then converted into potent androgens (e.g., testosterone, DHT) and estrogens by steroidogenic enzymes in peripheral sites and thereby permit target tissues to adjust the formation and metabolism of sex steroids to local requirements[21,25]. This situation contrasts sharply with that in lower mammals (e.g., mice, rats, guinea pigs), in which the ovaries and testes are the exclusive source of active sex steroids[1,21]. Further, the most valid and perhaps the only reliable estimate of the total androgen pool in humans is the serum concentration of the conjugated DHT metabolites, such as androsterone-glucuronide (ADT-G) and androstane-3α,17ß-diol-G (3α-diol-G), which reflects the total intracrine production and metabolism of androgens in peripheral tissues throughout the body[19,23,24]. These levels have not been measured in women with either primary or secondary SS.

To further test our hypothesis, we sought to determine whether women with SS have a deficiency in total androgens, as indicated by the serum concentrations of androgen precursors [i.e., DHEA, 5-androstene-3ß,17ß-diol (5-diol), and androstenedione], active androgens [i.e., testosterone and dihydrotestosterone (DHT)], and their metabolites (i.e., ADT-G and 3α-diol-G). For comparative purposes, we also examined whether these patients have elevated concentrations of circulating estrogens (i.e., estrone and 17ß-estradiol). The levels of potent estrogens are known to be increased in SLE[27], and may well predispose to the sex related incidence and development of SS[28,29].

MATERIALS AND METHODS

Human subjects. Women (age 53.1 ± 2.9 yrs) with primary SS (n = 10; ≥ 4 European criteria[30]) and secondary SS (n = 12 SLE; n = 9 RA) were recruited from outpatient clinics at Brigham and Women's Hospital and Tufts University School of Dental Medicine, as well as from the Sjögren's Syndrome Foundation (Jericho, NY, USA). Patients with primary SS met the criteria established by the European Study Group[30] whereas women with SLE[31] and RA[32] fulfilled the criteria set by the American College of Rheumatology. Age and sex matched controls (n = 26, age 51.7 ± 3.6 yrs), who had no history of autoimmune disease, were recruited from the Boston area and from The Harvard Cooperative Program on Aging (Boston, MA). After written informed consent was obtained, blood was drawn by phlebotomists in the Clinical Research Center at Brigham and Women's Hospital or Tufts University School of Dental Medicine. Samples were then processed for separation of serum and stored at –70°C until experimental analysis. These studies were approved by the Human Studies Committees of Brigham and Women's Hospital and Tufts University School of Dental Medicine, and were conducted in accordance with the Declaration of Helsinki.

Measurement of steroid hormone concentrations in sera. Serum steroid levels were measured as described by gas chromatography-mass spectrometry (DHEA, 5-diol, androstenedione, testosterone, DHT, estrone, and 17ß-estradiol) using electron impact or chemical ionization, and by liquid chromatography-tandem mass spectrometry using a turboionspray (ADT-G and 3α-diol-G)[33-35]. Briefly, steroids were extracted from serum by liquid-liquid and/or solid-phase extraction. Derivitization reactions were performed to improve chromatographic and detection response, with the exception of ADT-G. Interassay variations for these steroid measurements were as follows: DHEA 4.9%, 5-diol 5.9%, androstenedione 2.9%, testosterone 3.3%, DHT 1.9%, estrone 3.5%, 17ß-estradiol 4.1%, ADT-G 2.9%, and 3α-diol-G 3.7%. Repeat analyses of selected serum samples yielded identical results. Data were analyzed using the Mann-Whitney U test.

RESULTS

As shown in Figure 1, our results show that SS is associated with significant alterations in the circulating concentrations of sex steroids. Concentrations of DHEA, 5-diol, DHT, ADT-G, and 3α-diol-G were all significantly reduced in sera of patients with SS relative to those of controls. In contrast, SS was not associated with significant changes in the serum levels of androstenedione, testosterone, estrone, or 17ß-estradiol (Figure 2).

These alterations in sex steroid levels in SS could not be attributed to oral contraceptive use, hormone replacement therapy (HRT), concurrent Hashimoto's thyroiditis, liver or kidney disease, corticosteroid treatment, or statin medications. The concentrations of DHEA, 5-diol, DHT, ADT-G, and 3α-diol-G were also attenuated in SS patients (n = 15, age 54.5 ± 4.9 yrs), compared to control women (n = 24, age

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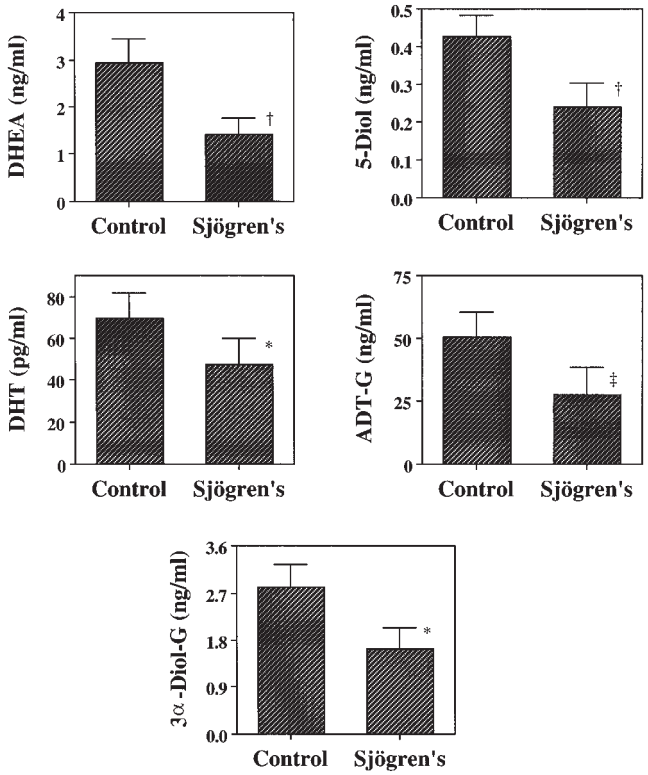

Figure 1. Influence of SS on serum concentrations of androgen precursors, hormones, and metabolites. Sera were obtained from women with primary or secondary SS (n = 31) and age matched controls (n = 26). Assay sensitivities for the steroids were as follows: DHEA 0.40 ng/ml, 5-diol 0.15 ng/ml, DHT 25 pg/ml, ADT-G 2.0 ng/ml, and 3α-diol-G 0.5 ng/ml. If levels were below the limit of assay quantitation, values were recorded as zero. Columns and bars show the mean ± SE. Significantly less than control: *p < 0.05; †p < 0.005; ‡p < 0.0005.

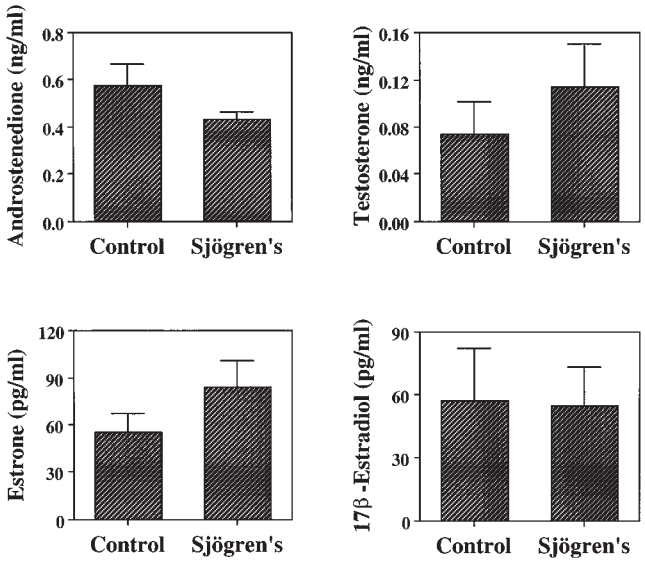

Figure 2. Influence of SS on serum concentrations of various androgens and estrogens. Sera were collected as described in the legend to Figure 1. Assay sensitivities for the steroids were as follows: androstenedione 0.25 ng/ml, testosterone 0.20 ng/ml, estrone 10 pg/ml, and 17ß-estradiol 5.0 pg/ml. If levels were below the limit of assay quantitation, values were recorded as zero.

52.5 ± 3.5 yrs) who were not taking exogenous estrogens (Figure 3). No differences between these subgroups were found in the levels of serum androstenedione, testosterone, estrone, or 17ß-estradiol (Figure 4). Moroever, only one patient had Hashimoto's thyroiditis and one other had glomerulonephritis, and exclusion of these data did not change the significance of the results. No patient or control had liver disease.

Our finding of androgen deficiency in women with SS could also not be explained by the use of corticosteroids. Evaluation of data from subjects not undergoing corticosteroid therapy showed that the serum levels of DHEA and ADT-G were significantly reduced ($p < 0.05$) in women with SS (n = 14, age 57.1 ± 4.3 yrs), compared to controls (n = 26, age 51.7 ± 3.4 yrs). The mean serum concentration of 3α-diol-G was also decreased by 32% ($p < 0.087$) in the SS group.

During the course of the study, 3 subjects were taking statin medications, including fluvastatin (control), simvastatin (primary SS), and lovastatin (secondary SS). Exclusion of these data from the analyses did not change the significance of any of the comparative results.

Lastly, it is unlikely that the androgen deficiency in SS could be explained by variations in sex steroid levels during the menstrual cycle. Menopause in the industrialized countries occurs at a median age of 51.4 years[36], and we analyzed data from individuals who were 55 years and older and who were not taking estrogen supplementation. The serum concentrations of DHEA, 5-diol, DHT, ADT-G, and 3α-diol-G were all still significantly ($p < 0.05$) decreased in women aged 55+ years with SS (n = 8, age 69.8 ± 3.2 yrs) relative to controls (n = 12, age 66.3 ± 2.7 yrs). The mean concentration of androstenedione was also attenuated by 76% ($p < 0.0993$) in patients with SS.

DISCUSSION

Our results demonstrate that women with Sjögren's syndrome are androgen-deficient. Thus, the serum concentrations of DHEA, 5-diol, DHT, ADT-G, and 3α-diol-G were significantly decreased in women with SS compared to controls. In contrast, SS was not associated with significant changes in the serum concentrations of testosterone, androstenedione, estrone, or 17ß-estradiol. These findings support our hypothesis that an androgen deficiency exists in SS. This deficit, in turn, may be a critical etiologic factor in the pathogenesis of aqueous-deficient and evaporative dry eye in this disorder.

Our experimental observations are consistent with the findings of other investigators with regard to circulating androgen levels in primary SS, SLE, and RA. Primary SS is associated with a significant decrease in the serum level of

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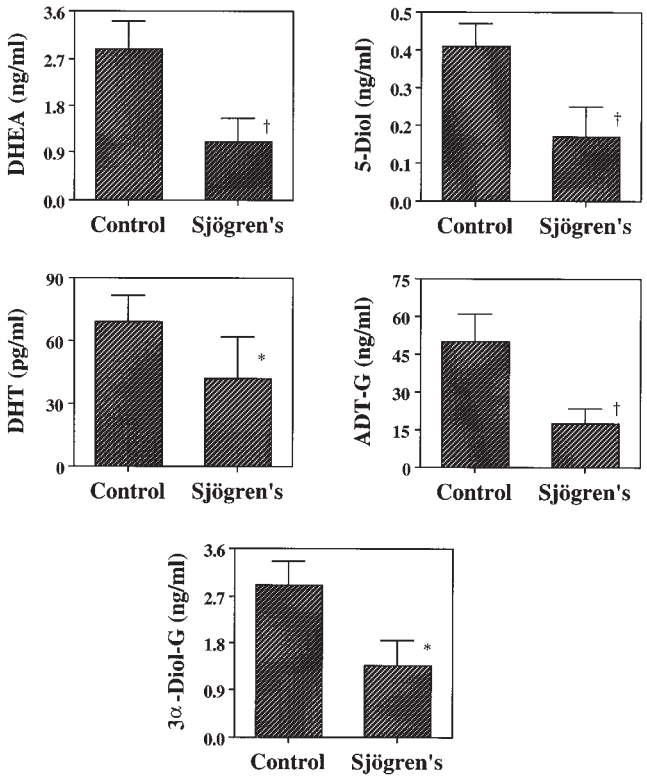

Figure 3. Effect of SS on serum levels of androgen precursors, hormones, and metabolites in women not taking exogenous estrogens. Sera were obtained from women with SS (n = 15) and age matched controls (n = 24) and processed for steroid analyses. Significantly less than control: *$p < 0.05$; †$p < 0.005$.

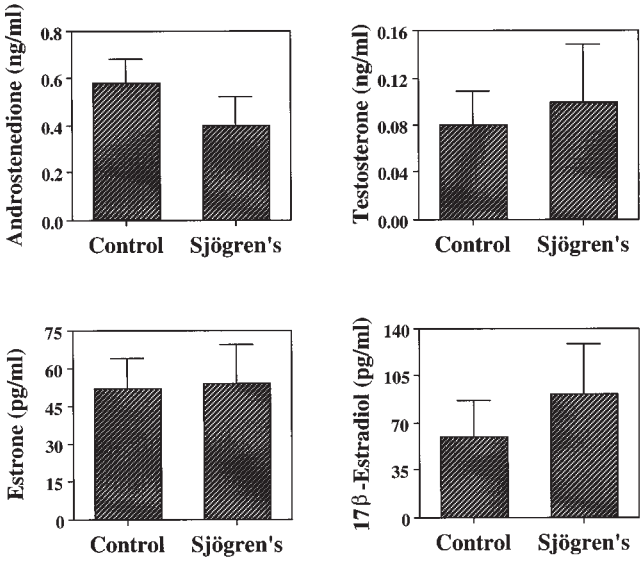

Figure 4. Influence of SS on serum concentrations of various androgens and estrogens in women not taking exogenous estrogens. Sera were processed as described in the legend to Figure 3.

DHEA-S, but not of testosterone or androstenedione[37]. Similarly, SLE and RA are accompanied by a significant reduction in circulating levels of DHEA[38-44], DHEA-S[38,39,43-46], and DHT[38,42], whereas the concentrations of testosterone and androstenedione may be decreased, increased, or unchanged[38,39,42,43,47]. It is important that a primary focus of these previous investigations was on the levels of circulating DHEA and DHEA-S, which are precursors for potent androgens and estrogens. In contrast, our study evaluated the serum concentrations of a number of androgens, including ADT-G and 3α-diol-G. These glucuronidated DHT metabolites reflect the overall intracrine production and metabolism of androgens in peripheral tissues and appear to be the most reliable measure of the total androgen pool in humans[19,23,24,48].

Interestingly, androgen deficiency may also contribute to the sex related prevalence of many autoimmune diseases. Thus, the incidence and/or severity of a number of autoimmune disorders are far greater in women compared to men[49]. This sexual dichotomy, in turn, has been linked in part to the differential actions of sex steroids on the immune system[50-52]. Estrogens often promote, whereas androgens frequently reduce, the progression of autoimmune sequelae[5,50-58]. Consequently, androgen insufficiency may predispose to the development of certain autoimmune states. This may explain why androgen therapy has been successful in ameliorating various signs and symptoms in animal models of SLE, thyroiditis, polyarthritis, autoimmune hemolytic anemia, and myasthenia gravis, as well as in humans with SLE and RA[5,14,16,50-52,59-62].

The androgen deficiency in SS could not be attributed to the influence of oral contraceptive use, HRT, menstrual cycle fluctuations, corticosteroid therapy, or statin treatment. This finding is significant, because estrogens and statins may decrease concentrations of precursor and/or active androgens[40,63], and the menstrual cycle is associated with variations in the concentrations of serum androgens[64]. In addition, corticosteroid administration is known to reduce circulating levels of DHEA[40] and testosterone[42,46,65,66]. It is possible in our investigation that corticosteroid therapy may have contributed to the attenuated levels of 5-diol and DHT in the SS group. The reason is that the serum concentrations of these hormones were analogous in the subsets of patients and controls who were not taking exogenous glucocorticoids.

The mechanism(s) by which a systemic androgen deficiency occurs in primary and secondary SS may involve genetic factors[37] and/or, as in RA, an adrenal gland abnormality in the elaboration of androgen precursors[44,67]. Another possibility is that an altered metabolism of sex steroids may occur in peripheral tissues. For example, in female patients with SLE both the oxidation of testosterone and the 16α-hydroxylation of estrone are increased, leading to an attenuated androgen/estrogen ratio and an enhanced

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amount of circulating and potent estrogen metabolites[27,53,68,69]. Although serum levels of estrone and 17ß-estradiol are not elevated in patients with SS, the concentrations of corresponding metabolites have yet to be measured. These analyses would be of particular interest, because estrogens may be involved in the etiology, progression, and/or amplification of SS[28,29]. These hormones may also enhance the polyclonal B cell activation, autoantibody formation, and tissue abnormalities encountered in this disorder[28,29,70].

The possibility exists that even greater deficiencies in androgen content may occur in specific tissues in SS. For example, elevated levels of proinflammatory cytokines (e.g., interleukins 1 and 6 and tumor necrosis factor-α) may disrupt the normal activity of steroidogenic enzymes and promote the aromatization of testosterone to 17ß-estradiol[71–73]. Considering that expression of such cytokines is increased in the lacrimal gland and inflamed lids (i.e., meibomian gland environment) in SS[74-76], and that the lacrimal and meibomian glands contain mRNA encoding the enzymes responsible for the intracrine synthesis and metabolism of active sex steroids[77], cytokine action may suppress the local formation of androgens. Further, these cytokines may attenuate the expression of androgen receptor mRNA[78] and interfere with certain androgenic effects[79]. Consequently, the influence of androgens may be significantly compromised in these ocular tissues, predisposing to lacrimal and meibomian gland dysfunction and the associated aqueous-deficient and evaporative dry eye.

Overall, our findings demonstrate that women with Sjögren's syndrome are androgen-deficient. This deficit may contribute significantly to the development of dry eye syndrome in this disorder.

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