Increased Estrogen Formation and Estrogen to Androgen Ratio in the Synovial Fluid of Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective***. It has been proposed that physiologic levels of estrogens stimulate immune responses whereas androgens suppress inflammatory reactions. Thus, prevalence of synovial androgens relative to estrogens would be favorable in rheumatoid arthritis (RA). We investigated synovial fluid (SF) concentrations of several estrogens and androgens and conversion products of the sex steroid precursor dehydroepiandrosterone (DHEA) in supernatants of mixed synoviocytes.

Methods. SF steroid concentrations were measured by high performance liquid chromotography and mass spectrometry in 12 patients with RA and 8 subjects with traumatic knee injury (noninflammatory controls). Conversion of DHEA to downstream hormones was measured by thin-layer chromatography and phosphorimaging detection in 3 patients with RA and 3 patients with osteoarthritis (OA).

Results. Overall, SF concentration of free estrogens tended to be higher in RA patients versus controls ($p < 0.06$). Molar ratio of free SF estrogens/free SF androgens was elevated in RA compared to controls (1.17 ± 0.32 vs 0.29 ± 0.08, without unit; $p = 0.017$). The free SF concentration of the precursor androstenedione was significantly higher in RA patients than in controls (104.6 ± 32.6 vs 30.4 ± 0.4 ng/ml; $p = 0.011$), and SF estrone — the aromatase conversion product of androstenedione — was also elevated in RA compared to controls (13.6 ± 2.6 vs 6.6 ± 0.8 ng/ml; $p = 0.035$). The biologically active estrogen derivatives, 16α-hydroxyestrone and 4-hydroxyestradiol, were both higher in RA compared to controls ($p = 0.085$ and $p = 0.044$, respectively). In mixed RA synoviocytes, DHEA conversion yielded high local levels of 17ß-estradiol (708 pmol/l = 0.193 ng/ml) compared to testosterone (88 pmol/l = 0.026 ng/ml).

Conclusion. SF levels of estrogens relative to androgens are significantly elevated, while those of androgens are markedly reduced, in patients with RA compared to controls. This imbalance is most probably due to increased aromatase activity. Thus, an available steroid precursor, such as DHEA, may be rapidly converted to proinflammatory estrogens in the synovial tissue, which may in turn stimulate the inflammatory process in patients with RA. (J Rheumatol 2003;30:2597–605)

Key Indexing Terms:
RHEUMATOID ARTHRITIS ESTROGEN ANDROGEN
DHEA SYNOVIAL FLUID SYNOVIOCYTES

Generally, estrogens, in physiological concentrations, serve to enhance immune responses and may act as important stimuli of human humoral immunity (as reviewed[1]). In patients with systemic inflammatory diseases, 17ß-estradiol enhances IgG and IgM production by peripheral blood mononuclear cells (PBMC)[2]. In patients with systemic lupus erythematosus (SLE), this may eventually lead to elevated levels of polyclonal IgG, including IgG anti-dsDNA, in PBMC, through the enhancement of B cell activity via interleukin 10 (IL-10)[3]. With respect to proinflammatory

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cytokines, such as IL-1, IL-6, and tumor necrosis factor (TNF), effects of 17ß-estradiol apparently show a bimodal behavior, whereby pharmacological concentrations (for experiments *in vitro*: 10^{-6} M ≅ 280 ng/ml) decrease while physiological concentrations (for experiments *in vitro*: 10^{-9} M ≅ 0.28 ng/ml) increase cytokine production[4-6]. Recently, it has been reported that estrogens enhance secretion of matrix metalloproteinases and IL-1ß-induced IL-6 production by human fibroblast-like synoviocytes in rheumatoid arthritis (RA)[7,8]. Further, there is evidence that anti-estrogens inhibit both the differentiation of synovial fluid (SF) macrophages into dendritic cells (DC) and the capacity of SF macrophage-derived DC to stimulate allogeneic T cells[9]. Taken together, the above data support the proinflammatory role of physiologic levels of estrogens in inflammatory diseases.

In contrast, androgens suppress immune responses (as reviewed[10]). It has been observed that both physiological (for experiments *in vitro*: 10^{-8} M ≅ 2.9 ng/ml) and pharmacological (for experiments *in vitro*: 10^{-6} M ≅ 290 ng/ml) concentrations of testosterone inhibit IL-1ß secretion by PBMC from RA patients[11]. In addition, physiological concentrations of testosterone decrease IL-1 synthesis in primary cultured human synovial macrophages[12]. The biologically active androgen, dihydrotestosterone, has been shown to repress the expression and activity of human IL-6 gene promoter in human fibroblasts, thus supporting the concept of antiinflammatory/immunosuppressive effects of androgens[13]. Testosterone treatment does not appear to directly affect isolated B or T cells. However, a followup study of patients with SLE has revealed that testosterone suppresses both IgG anti-dsDNA antibody and total IgG production of PBMC[14]. Low serum and SF androgen levels have been observed in RA patients[15,16]. Further, androgen treatment of patients with RA led to significant improvement of RA joint disease[17]. In conclusion, a number of studies strongly support a crucial role for sex steroids, i.e., estrogens and androgens, in the modulation of immunoreactivity in patients with RA. In addition, some immunoreactive cells are also endowed with functional high affinity binding sites for estrogens and androgens[18,19] and hence should be considered as target cells for steroid hormones.

Since circulating precursors of androgens and estrogens, such as dehydroepiandrosterone (DHEA), can be converted along defined pathways to downstream hormones at the peripheral level (Figure 1), multiple secondary effects may well arise according to local levels and activity of steroid enzymes and the resulting metabolic products, regardless of plasma concentrations and bioavailability of individual hormones[20-23]. It is not known whether, under certain circumstances, macrophages or other peripheral immune cells are able to synthesize androgens or estrogens in adequate amounts. Thus, the mode of conversion and local levels of androgens and estrogens in the synovial microenvironment are of outstanding importance in patients with RA.

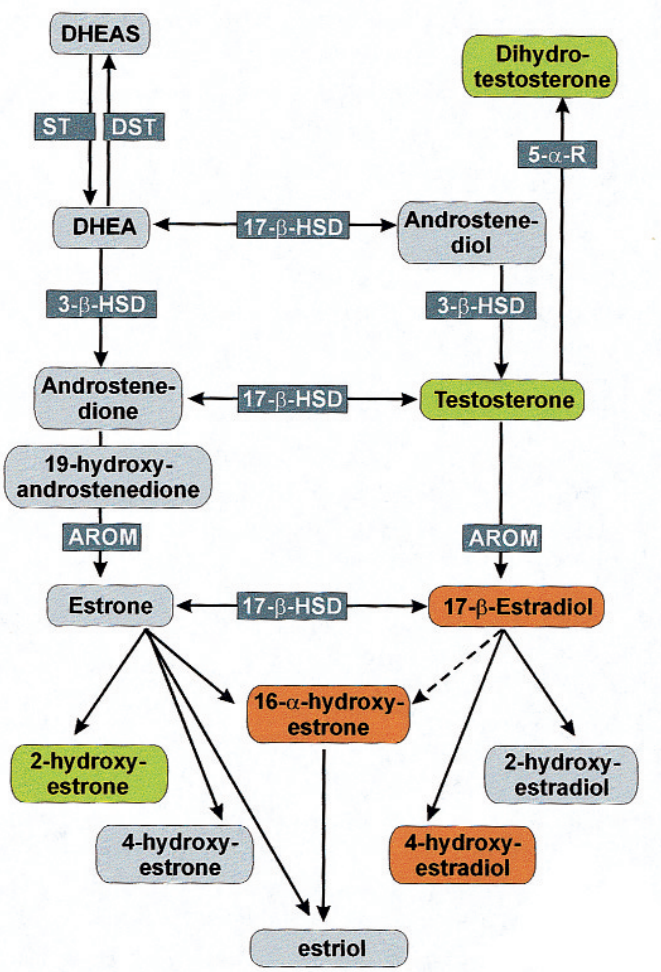

Figure 1. The biosynthesis of relevant steroid hormones. Precursor hormones such as DHEAS and DHEA can be converted to downstream androgens (androstenedione, testosterone) and estrogens (hormones below the AROM = aromatase enzyme). Steroid hormones in a green (red) area indicate supposed antiinflammatory (proinflammatory) activity. DHEA: dehydroepiandrosterone; DHEAS: DHEA sulfate. Enzymes: 3-ß-HSD: 3ß-hydroxysteroid dehydrogenase; 5-α-R: 5α-reductase; 17-ß-HSD: 17ß-hydroxysteroid dehydrogenase; DST: DHEA sulfatase; ST: sulfotransferase.

We investigated local SF concentrations of several estrogens and androgens in relation to each other in patients with RA as compared to patients with traumatic knee joint effusion. Further, we investigated downstream conversion products of DHEA in mixed synoviocytes of patients with RA as compared to osteoarthritis (OA). The results of the 2 independent studies, *in vivo* and *in vitro*, should be carefully compared according to the very different situations.

MATERIALS AND METHODS

Patients and controls. For the SF studies, 12 Caucasian patients with early disease (7 women, 5 men) (duration of symptoms ± 24 months at entry) fulfilling the American College of Rheumatology criteria for RA[24] were

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included. The mean age of RA patients was 54.5 ± 3.5 years (range 40–66). All the patients were being treated only with nonsteroidal antiinflammatory drugs. None had received any oral or intraarticular corticosteroid therapy or slow acting antirheumatic drugs during the 4 months prior to the investigation, and no patient reported use of contraceptives, 1,25-dihydroxyvitamin D, or any hormone replacement therapy. In the SF study, 8 otherwise healthy patients (3 women, 5 men; mean age 55.6 ± 6.4 yrs) with traumatic knee joint effusion undergoing diagnostic arthroscopy were included as controls. The obtained SF ranged from 20 to 50 ml. All study subjects had normal liver, renal, prostate, and thyroid function and were within 20% of their ideal body weight. After collection, SF were frozen at –20°C until steroid extraction.

In the DHEA conversion study, early mixed synoviocyte cell cultures from 3 patients diagnosed with RA (mean age ± SEM: 59.2 ± 3.0 yrs) and 3 patients with OA (58.3 ± 4.1 yrs) with longterm disease (duration 4–14 yrs) were used. Patients underwent knee joint replacement surgery, and RA patients were treated with DMARD (only low dose MTX) and nonsteroidal antiinflammatory drugs, but no glucocorticoids.

Measurement of SF concentration of steroid hormones. Five milliliter aliquots of SF were extracted using 15 ml ethanol, vortexed, and then centrifuged at 2000 g for 10 min. The resulting pellet was extracted again with 4 ml of ethanol:acetone (50:50, v/v) to separate the conjugate from the free forms of steroids. The 2 extracts were combined, evaporated to dryness at 40°C under nitrogen stream, resuspended in 1 ml water-methanol solution (95:5, v/v), and applied for solid phase extraction, to C18 cartridge (Bond-Elut C18 500 mg/3 ml) in a Vac-Elut system SPS 24 (Analytichem International, Harbor City, CA, USA) previously rinsed with methanol and water, and washed with water-methanol (95:5, v/v). Two fractions were collected: in the first, the sulfate and glucosylate forms were eluted using 2 ml of water-methanol solution (60:40, v/v); in the second, the free forms of steroids were eluted using 3 ml of water-methanol solution (15:85, v/v) in presence of 50 µl of 0.1% ascorbic acid (p/v). The extracts were evaporated to dryness in a SVC100H Speed-Vac evaporator-concentrator (Savant Instruments Inc., Farmingdale, NY, USA) and then the fraction containing the conjugate steroids was solubilized in 1.970 ml of 0.2 M acetate buffer (pH 5.0) to which 30 µl of Glusulase (NEN, Dreieich, Germany) were added. After 18 h incubation at 37°C, the hydrolyzed sample was extracted using C18 cartridges and processed as above. Recovery percent values of some representative tritiated authentic steroids were between 92.2 and 95.6%. The dried extracts were stored at –20°C until reverse phase-high performance liquid chromatography (HPLC) analysis.

The HPLC system consisted of a Model LC-10 equipped with a model LC-10AD solvent delivery unit, a Model SPD-M10 photodiode array UV-VIS detector (Shimadzu Corporation, Tokyo, Japan), and an on-line Model 5100A Coulochem electrochemical detector with a Model 5010 analytical cell on-line (ESA, Bedford, MA, USA). Steroids were separated under isocratic conditions using a Spherisorb S5 ODS2 (Waters, Milford, MA, USA) column (4.6 × 250 mm) and acetonitrile: 0.025 M citric acid (40:60, v/v) as an optimized mobile phase at 1 ml/min flow rate[25,26]. Routine data validation and integration were achieved automatically by a 2 channel Class-VP 4.2 chromatography data station (Shimadzu).

Mixed synoviocytes and cell culture. Synovial tissue samples were obtained during surgery immediately after opening the knee joint capsule. Synovial cells were isolated by enzymatic digestion of fresh synovial tissue for 1–2 h at 37°C (Dispase Grade II, Boehringer, Mannheim, Germany). Approximately 5 to 6 × 10^5 viable cells were seeded in RPMI-1640 (Sigma, Deisenhofen, Germany), 10% fetal calf serum (Sigma), 1% penicillin/streptomycin (Gibco, Paisley, UK), 0.1% amphotericin B (Bristol-Myers Squibb, Munich, Germany) and cultured for 12–18 h (primary early-culture mixed synoviocytes) in a final volume of 3 ml in 6-well plates. The percentage of different types of synoviocytes was tested by specific antibodies against prolyl 4 hydroxylase (for the synoviocyte type B = fibroblasts; Calbiochem, Bad Soden, Germany; 37 ± 3% of cells were positive) and CD163 (synoviocyte type A = activated macrophages; Dako, Hamburg, Germany; 26 ± 3% of cells were positive). During culture, cells were kept in a humidified atmosphere with 5% CO_2 at a temperature of 37°C.

Incubation with radio-labeled DHEA and thin-layer chromatography (TLC) analysis. For analysis of synthesis of hormones downstream of DHEA, we used a described technique[22]. Briefly, mixed synoviocytes were incubated with 40 nCi [4-14C]DHEA (NEN, Dreieich, Germany) at a final concentration of 240 nM for 72 h. Steroids were extracted twice with 4 ml cold ethyl acetate. More than 99% of the total radioactivity was routinely recovered in the organic phase, which was lyophilized in a speed-vac concentrator. Lyophilized extracts were dissolved in 50 µl ethanol. Samples of 10 µl were spotted on silica gel 60 F254 TLC aluminum sheets (Merck, Darmstadt, Germany) and developed in 2 dimensions[27]. The first separation was done in toluol:methanol (90:10). After drying, the second development was done in chloroform:diethylether (50:50). Radioactivity on the TLC plates was quantified with a phosphorimaging system (FLA 3000, Fuji-Raytest, Straubenhardt, Germany). Spots were assigned only if their intensity was more than 2 standard deviations above the background level.

Statistical analysis. Group means were compared by nonparametrical Wilcoxon signed rank test (SPSS/PC, Advanced Statistics, V10.0.1, SPSS Inc., Chicago, IL, USA). After logarithmic transformation of data, Pearson linear correlation analysis was used to describe the relation between SF levels of: free estrone versus free 19-hydroxyandrostenedione, and free estrone versus androstenedione (using SPSS). $p < 0.05$ was the significance level.

RESULTS

Overall concentration of SF estrogens and androgens. Table 1 shows SF levels of both free and conjugated estrogens and androgens. Conjugated SF estrogens tended to be elevated in patients with RA compared to controls. The free SF androgen levels were much lower in RA patients than in controls, while a more pronounced decrease (3.5-fold) of conjugated androgen SF levels was also seen in RA compared to controls (Table 1). This was corroborated by the total sum of estrogens and androgens, respectively (Table 1). Separation of female and male patients demonstrated very similar results, but due to the low number of subjects in the different subgroups the mean values were not significantly different (Table 1, see data in parentheses).

Using molar ratios of SF levels of these hormones (Figure 2), RA patients showed a significantly elevated ratio of free SF estrogens/free SF androgens, which indicates a preponderance of the proinflammatory estrogens relative to androgens. Regarding conjugated steroids, RA patients tended to exhibit an increased ratio of conjugated SF estrogens/conjugated SF androgens (Figure 2).

Conversion of androgens to estrogens via the aromatase. As reported in Table 2, the SF levels of free androstenedione (precursor of estrone upstream of the aromatase, see Figure 1) were significantly increased in RA patients compared to controls. This was not the case for the intermediate metabolite 19-hydroxyandrostenedione (Table 2). Conversely, the SF levels of the aromatase conversion product estrone were significantly elevated in RA patients compared to controls (Table 2). However, in a correlation analysis, SF levels of estrone were positively correlated with those of 19-hydroxyandrostenedione in control subjects but negatively in

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Table 1. Overall synovial fluid (SF) levels of estrogens and androgens in patients with RA and in controls. The data in parentheses give the values of female and male subjects. SF levels are given as means ± SEM in ng/ml.

	RA, n = 12 (female, n = 7; male, n = 5)	Control Subjects, n = 8 (female, n = 3; male, n = 5)
Estrogens, ng/ml		
Free	517 ± 90 (549 ± 126; 473 ± 137)	444 ± 75 (635 ± 101; 328 ± 63)
Conjugated	2974 ± 1046 (3428 ± 1770; 2338 ± 686)	1528 ± 191 (1553 ± 455; 1513 ± 200)
Total	3491 ± 1117 (3976 ± 1873; 2812 ± 818)	1972 ± 219 (2188 ± 475; 1842 ± 236)
Androgens, ng/ml		
Free	672 ± 124* (763 ± 191; 545 ± 136)	6047 ± 4431# (13250 ± 11862#; 1725 ± 563)
Conjugated	605 ± 86 (559 ± 66; 670 ± 194)	2141 ± 751 (1136 ± 720; 2745 ± 1087)
Total	1277 ± 148* (1322 ± 174; 1215 ± 280)	8188 ± 4282# (14386 ± 11533#; 4469 ± 1489)

* $p < 0.05$ for the difference vs control subjects. # The high variation is due to one outlier in the group of female control subjects with a very high level of free dehydroepiandrosterone (25269 ng/ml) and free epiandrosterone (11250 ng/ml). The outlier was kept in the analysis because more than one hormone in the same woman was increased.

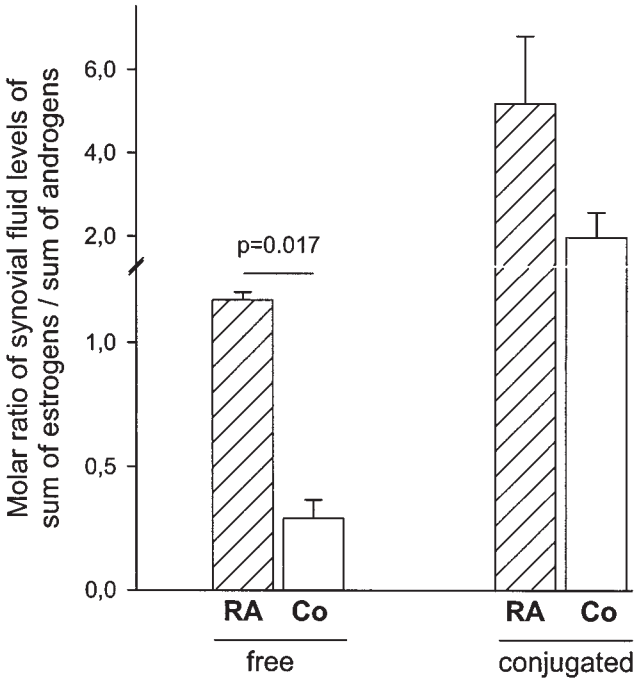

Figure 2. Molar ratio of synovial fluid levels of estrogens divided by androgens. The left (right) bars show free (conjugated) hormones. Shaded and white bars give mean molar ratios ± SEM of patients with rheumatoid arthritis (RA) and control subjects (Co), respectively.

Table 2. Conversion of androstenedione to estrone via the aromatase complex. Synovial fluid levels are given as means ± SEM.

	RA, n = 12	Control Subjects, n = 8
Free androstenedione, ng/ml	104.6 ± 32.6**	30.4 ± 0.4
Free 19OH-androstenedione, ng/ml	51.7 ± 9.1	54.1 ± 15.2
Free estrone, ng/ml	13.6 ± 2.6*	6.6 ± 0.82

* $p = 0.035$; ** $p = 0.011$ for the difference vs control subjects.

patients with RA (Figure 3). This indicates that activity of aromatase is upregulated in patients with RA but not in control subjects. In both RA patients and controls, such a correlation was not observed between androstenedione and estrone (RA: R = 0.477, p = 0.117; controls: R = 0.275, p = 0.509), suggesting that 19-hydroxyandrostenedione is the important intermediate metabolite between androstenedione and estrone (see Figure 1). Interestingly, SF free DHEA levels were markedly lower in RA patients compared to controls (37.3 ± 7.3 vs 3524.3 ± 3108.7 ng/ml; p = 0.007: data not shown).

SF levels of free estrogen metabolites. The estrogen metabolites 16α-hydroxyestrone and 4 hydroxyestradiol have been reported to be powerful estrogens in consideration of their strong mitogenic activity[28-30]. In contrast, 2-hydroxyestrone apparently counteracts these estrogens[31-33]. In our studies, SF levels of the powerful 4 hydroxyestradiol were significantly increased in RA patients compared to controls, while no significant differences of 2-hydroxyestrone SF levels could be detected between RA patients and controls (Table 3). Concerning 16α-hydroxyestrone, this powerful estrogen tended to be elevated in RA patients with respect to control subjects (Table 3).

Conversion of the precursor DHEA into downstream androgens and estrogens. Metabolic experiments carried out on early-mixed synoviocyte cell cultures revealed elevated conversion to androstenediol (the direct downstream metabolite, Figure 1) and high levels of 17ß-estradiol (Figure 4A, 4B). Supernatant levels of androstenedione and testosterone were markedly lower (Figure 4A) compared to our recent experimental work using blood monocyte-derived macrophages of healthy subjects (approximately 1000 pmol/l = 0.29 ng/ml and 3000 pmol/l = 0.87 ng/ml, respectively[22]). In contrast, both supernatant levels of estrone and 17ß-estradiol were markedly elevated as compared to experiments with monocyte-derived macrophages of healthy subjects (approximately 30 pmol/l = 0.008 ng/ml and 75 pmol/l = 0.020 ng/ml, respectively[22]).

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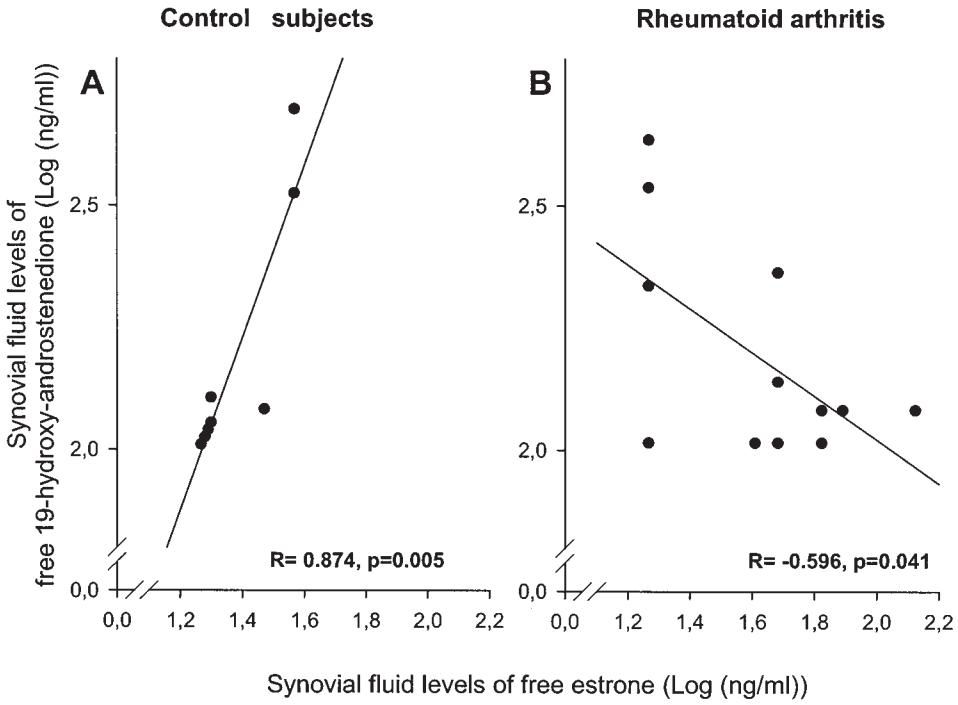

Figure 3. Correlation of synovial fluid levels of estrone and 19-hydroxyandrostenedione. Data for (A) control subjects and (B) patients with RA are given. Linear regression line, the respective Pearson correlation coefficient R, and its p value are given. Data are expressed as log (ng/ml).

Table 3. Levels of free estrogen metabolites in synovial fluid (SF). SF levels are given as means ± SEM.

	RA, n = 12	Control Subjects, n = 8
Free 16α-hydroxyestrone, ng/ml	28.8 ± 6.5*	12.5 ± 4.4
Free 4-hydroxyestradiol, ng/ml	203.3 ± 67.8**	46.5 ± 20.3
Free 2-hydroxyestrone, ng/ml	80.4 ± 34.5	46.8 ± 20.8

* p = 0.085; ** p = 0.044 for the difference vs control subjects.

This may suggest that DHEA metabolism led to significantly increased estrogen formation and a corresponding withdrawal of androgens in mixed synoviocytes of patients with RA compared to OA.

DISCUSSION

Patients with chronic inflammatory diseases, such as RA, exhibit relatively normal serum levels of estrogens[34], whereas androgen serum levels are remarkably low[35-43]. Two different explanations for the estrogen/androgen dissociation are possible: (1) an inflammation-induced reduction of gonadotrophin-stimulated gonadal steroidogenesis, which affects androgens more than estrogens, and/or (2) an elevated conversion of androgens to estrogens at the peripheral level (epithelial cells, osteoblasts, fat cells, macrophages) with a resulting reduction of circulating androgens and maintenance of serum estrogens. First, secretion of gonadotrophin follicle-stimulating hormone and luteinizing hormone seems to be significantly elevated in patients with RA[37,44,45]. It is thought that this is a compensatory response to low serum levels of sex hormones. Gonadotrophins should, however, enhance serum levels of both androgens and estrogens, which would not explain the dissociation of their serum levels in RA patients. Thus, it seems more likely that a local alteration of sex hormone production in inflammatory diseases leads to a preponderance of estrogens relative to androgens. TNF and IL-1 are important proinflammatory cytokines in RA, and some reports have demonstrated that both cytokines can inhibit central steroidogenesis in Leydig and granulosa cells[46-48]; moreover, both cytokines inhibit aromatase activity in granulosa cells of mammals (Figure 1)[47,49-51]. These mechanisms would lead to an overall reduction of sex hormones and a preponderance of androgens relative to estrogens, contrary to the situation observed in patients with RA. Thus, in RA patients, the increase of synovial estrogen formation at the expense of androgens may be relevant for the observed dissociation of these hormones.

Proinflammatory cytokines such as TNF, IL-1ß, and IL-6 have been found to stimulate the activity of the aromatase enzyme complex in peripheral cells (epithelial cells, osteoblasts, fat cells, macrophages), leading to accelerated conversion of androgens to downstream estrogens (Figure 1)[22,52-54]. Accordingly, in tissue rich in macrophages a significant correlation was found between the aromatase activity

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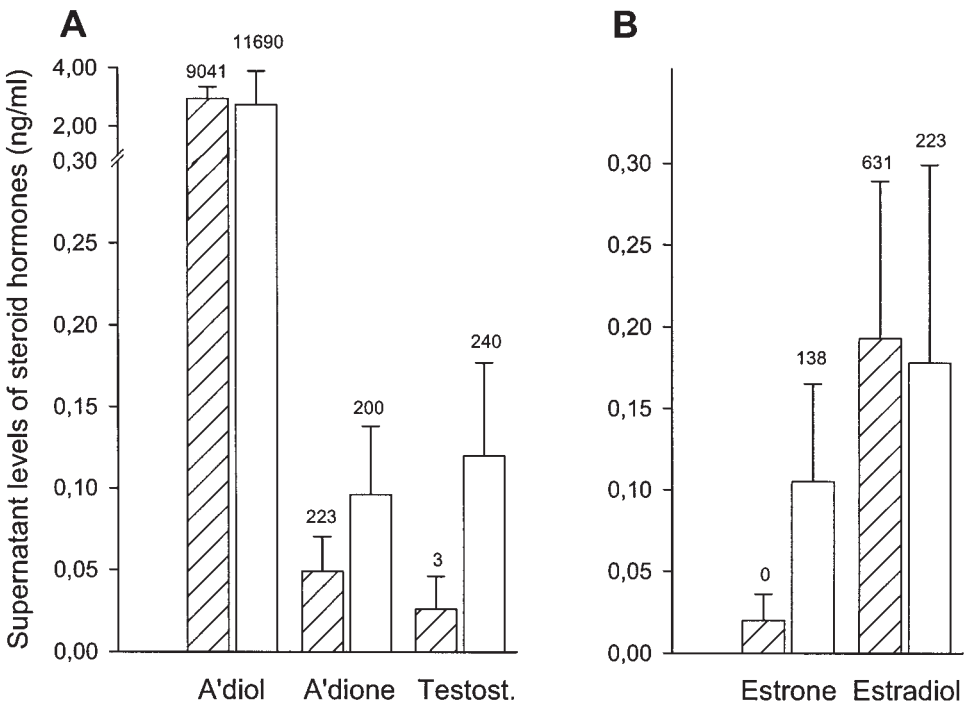

Figure 4. Conversion of DHEA to downstream steroid hormones in mixed synoviocytes of patients with RA (shaded bars) and OA (white bars). Synovial tissue was received during knee joint replacement surgery and mixed synoviocytes were separated by an enzymatic digestion method (see Materials and Methods). After incubation of mixed synoviocytes with radiolabeled DHEA for 72 h, supernatant was collected to measure (A) androstenediol (A'diol), androstenedione (A'dione), testosterone (Testost), and (B) estrone and estradiol. Data for 3 patients in each group are given as mean ± SEM in ng/ml; the median in pmol/l is given above the error bars (for comparison with Schmidt M, *et al*[22]).

and IL-6 production, resulting in lower androgen and higher estrogen levels (Figure 1)[55]. In this respect, peripheral cells may behave in a very different way compared to gonadal cells. Our study confirms locally elevated aromatase activity in patients with RA, as mirrored by high levels of SF free estrone being related to increased SF free 19-hydroxyandrostenedione. This indicates the presence of a strong conversion of 19 hydroxyandrostenedione to estrone via the aromatase complex in RA patients but not in control subjects. Further, the 17ß-hydroxysteroid dehydrogenase, which converts less active estrogens to the biologically powerful 17ß-estradiol (Figure 1), is also stimulated by proinflammatory cytokines such as TNF and IL-6[56-58]. In our study, we have demonstrated that SF concentration of free estrogens is significantly elevated in relation to free androgens, thus reversing the estrogen to androgen ratio, and that the precursor DHEA is strongly converted to estrogens by local effector cells. In comparison to previous data on DHEA conversion in macrophages in vitro[22], we observed more than 10 times lower (higher) supernatant levels of androgens (estrogens) in synoviocytes of RA patients and, to a lesser extent, of OA patients as compared to supernatant levels of blood monocyte-derived macrophages of healthy subjects[22].

Since estrogens may play a proinflammatory role in chronic inflammatory diseases[1-3,7-9], the preponderance of these hormones at the local inflammatory site may be detrimental. With either free or conjugated estrogens being biologically active, this could account for an enhanced immune reactivity, which may be a permanent condition in patients with RA. Moreover, the increased local estrogen production implies a corresponding decrease of androgen levels in the synovial microenvironment, leading to a reduction in androgenic antiinflammatory action. One could speculate that measurement of a molar ratio of SF estrogens/SF androgens may be a helpful diagnostic tool for the assessment of the local inflammatory situation. In addition, experimental data from both *in vitro* and *in vivo* experiments show that local effector cells are equipped with key enzyme activities for estrogen formation, particularly for the maintenance of high levels of biologically active estrogens, such as 16α-hydroxyestrone and 4 hydroxyestradiol, having mitogenic and proliferative effects. The reason for the significant production of 16α-hydroxyestrone and 4 hydroxyestradiol observed in RA patients is not yet clear, although it could simply be a consequence of the increased estrogen production and metabolic rates. In conclusion, there is a strong

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indication that a proinflammatory situation becomes imprinted at a certain time in the course of RA.

DHEA has been used as an antiinflammatory agent in patients with SLE[59-61] and inflammatory bowel disease[62]. This has led to the recommendation of the combined use of glucocorticoids and DHEA for the treatment of RA patients[63,64]. However, a Dutch group has reported no measurable beneficial effect of DHEA treatment in patients with RA[65,66]. From the present point of view, DHEA treatment of RA patients may increase the local levels of both androgens and estrogens so that only temporary beneficial effects could be achieved. Nevertheless, concomitant treatment of DHEA together with glucocorticoids may lead to positive effects in non-inflamed areas of the body in RA patients, particularly for bone and arteries. Further, parallel use of an aromatase inhibitor together with DHEA may be much more antiinflammatory.

In conclusion, our study clearly indicates that local SF levels of proinflammatory estrogens are significantly elevated relative to androgens in patients with RA as compared to controls. This is most probably due to increased aromatase activity. Available steroid precursors, such as DHEA, are rapidly converted to proinflammatory estrogens in cultured synoviocytes. This evidence may provide a basis to develop an alternative hormonal therapy for RA patients, using strategies designed to achieve a reduction of estrogen formation (e.g., aromatase inhibitors), eventually leading to an overall reduction of estrogens in the synovial microenvironment in RA.

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