

Sex Hormone Concentrations in Patients with Rheumatoid Arthritis Are Not Normalized During 12 Weeks of Anti-Tumor Necrosis Factor Therapy

RAINER H. STRAUB, PETER HÄRLE, FABIOLA ATZENI, CLAUDIA WEIDLER, MAURIZIO CUTOLO, and PIERCARLO SARZI-PUTTINI

ABSTRACT. Objective. Androgens such as dehydroepiandrosterone sulfate (DHEAS) and testosterone are markedly lower in postmenopausal women with rheumatoid arthritis (RA) than in controls. In contrast, compared to controls, serum levels of estrogens are normal or elevated in women with RA. Since tumor necrosis factor (TNF) alters production of these hormones, we investigated changes of these hormones during anti-TNF antibody (anti-TNF) therapy with adalimumab in longstanding RA. **Methods.** In this longitudinal anti-TNF therapy study in 13 patients with long-standing RA without prior prednisolone (7 infusions of anti-TNF: Week 0, 2, 4, 6, 8, 10, and 12), we measured serum concentrations of interleukin 6 (IL-6), androstenedione, DHEA, DHEAS, free testosterone, estrone, and 17 β -estradiol. Levels of these hormones in patients were compared to serum levels of 31 age and sex matched healthy controls.

Results. Upon treatment with anti-TNF, there was an impressive decrease of clinical markers of inflammation, erythrocyte sedimentation rate, and serum levels of IL-6. Serum levels of DHEAS and free testosterone were markedly lower at baseline in patients compared to controls, but this did not change during anti-TNF therapy. Serum levels of DHEA and 17 β -estradiol were significantly elevated in patients compared to controls, but similarly, anti-TNF therapy did not change initially increased levels. Molar ratios of hormones, which reflect hormone shifts via converting enzymes, showed typical alterations at baseline, but did not change markedly during anti-TNF therapy.

Conclusion. Longterm therapy with anti-TNF did not change altered serum levels of typical sex hormones in patients with RA, although baseline values were largely different. In patients with RA, this indicates that alterations of sex hormones and altered activity of respective converting enzymes are imprinted for a long-lasting period over at least 12 weeks. (J Rheumatol 2005;32:1253–7)

Key Indexing Terms:

RHEUMATOID ARTHRITIS
SEX HORMONES

ADALIMUMAB
ESTROGEN

TUMOR NECROSIS FACTOR
ANDROGEN

Since their discovery in the early 1990s¹, anti-tumor necrosis factor antibodies (anti-TNF) are now widely used as anti-inflammatory therapy in patients with rheumatoid arthritis (RA). The effect of anti-TNF therapy is most probably

direct neutralization of proinflammatory TNF. Recently, it was pointed out that androgens in RA might play an anti-inflammatory role, whereas estrogens, particularly 16-hydroxylated estrogens, may be proinflammatory and mitogenic². However, effects of anti-TNF therapy on these sex hormones are presently not known. Thus, we investigated the effects of longterm anti-TNF therapy on serum concentrations of typical sex hormones.

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R.H. Straub and C. Weidler are supported by a grant from the Deutsche Forschungsgemeinschaft (DFG Str 511/10-1). P. Sarzi-Puttini and F. Atenzi were supported by Abbott SpA, Campoverde, Italy.

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Accepted for publication March 1, 2005.

MATERIALS AND METHODS

Patients and blood samples. In this study of anti-TNF therapy with adalimumab (Abbott SpA, Campoverde di Aprilia, Italy), we included 13 postmenopausal Caucasian women with long-standing RA³. Patients were selected according to the inclusion criteria of the ReAct study^{4,5}. No patient had received parallel or prior (within 6 months before) prednisolone, and additionally they received stable methotrexate but no other immunosuppressives (Table 1). Patients received 40 mg adalimumab subcutaneously every other week. Adalimumab was infused on Week 0, 2, 4, 6, 8, 10, and 12. At baseline, Week 2, 6, and 12, patients were clinically investigated and blood was drawn between 8:00 AM and 9:00 AM. Efficacy assessments included American College of Rheumatology and European League of Associations Against Rheumatism response criteria, as described^{4,5}. The

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Table 1. Characteristics of patients and healthy controls. Data are given as means \pm SEM.

	RA Patients, n = 13	Controls, n = 31
Age, yrs (range)	58.2 \pm 2.4 (48–73)	59.8 \pm 1.7 (46–75)
Female/male	13/0	31/0
Disease duration, yrs (range)	6.5 \pm 1.8 (0.5–20)	NA
Baseline erythrocyte sedimentation rate, mm/h	32.6 \pm 5.5	ND
Baseline C-reactive protein, mg/l	143.4 \pm 52.3	ND
Baseline serum IL-6, pg/ml	23.2 \pm 8.9**	2.1 \pm 0.2
Positive rheumatoid factor (%)	13 (100)	ND
Positive antinuclear antibodies (%)	0 (0)	ND
Baseline swollen joint score, points	9.3 \pm 0.6	NA
Baseline tender joint score, points	10.9 \pm 0.9	NA
Baseline pain visual analog scale, points*	56.6 \pm 5.2	NA
Additional therapy (%)		
Prednisolone	0 (0)	NA
Methotrexate,	13 (100)	NA
mean weekly MTX, mg	8.7 \pm 0.9	NA
NSAID (COX-1, COX-2)	12 (92)	NA
COX-2 inhibitors	5 (38)	NA

* Minimum = 0, maximum = 100. ** $p < 0.001$ vs controls. NA: not applicable; ND: not determined.

study was approved by the Ethics Committee of L. Sacco University Hospital. For comparison, 31 healthy postmenopausal women were included as controls.

Laboratory measures. Serum levels of dehydroepiandrosterone (DHEA; Diagnostic Systems Laboratory, Webster, TX, USA), DHEAS (IBL, Hamburg, Germany), androstenedione (ASD; IBL), free testosterone (IBL), 17 β -estradiol (IBL), and estrone (IBL) were measured by ELISA.

Statistical analysis. Group medians were compared by the nonparametric Mann-Whitney U test (SPSS). Change of a variable over time was analyzed by nonparametric Friedman test (SPSS). $p < 0.05$ was the significance level.

RESULTS

Clinical improvement under adalimumab therapy. Swollen joint scores, tender joint scores, and pain visual analog scale (VAS) measures decreased significantly from baseline to Week 12 (swollen joint score, 9.3 \pm 0.6 to 2.9 \pm 0.6; $p = 0.001$; tender joint score, 10.9 \pm 0.9 to 6.5 \pm 0.6; $p = 0.005$; pain VAS measure, 56.6 \pm 5.3 to 25.9 \pm 3.9; $p = 0.003$). Similarly to others' results⁵, this demonstrates significant reduction of clinically assessed systemic inflammation by adalimumab therapy.

Change of serum DHEA, DHEAS, and ASD. In these RA patients without prior glucocorticoid therapy, we observed increased serum levels of DHEA in comparison to controls (Figure 1A). However, anti-TNF therapy over 12 weeks did not lower serum levels (Figure 1A). Serum levels of DHEAS were significantly decreased in RA patients compared to controls and did not change during anti-TNF therapy (Figure 1B). The ratio of DHEAS/DHEA was significantly decreased in relation to controls, but it did not

increase during the observation period (Figure 1C). Figure 1D depicts serum levels of ASD, which were similar in RA patients and controls. In comparison to RA patients, controls had 5 times increased ASD in relation to DHEA, and findings did not change during anti-TNF therapy (Figure 1E).

Change of serum free testosterone in relation to ASD and DHEA. Serum free testosterone was largely lower in RA patients compared to controls, and did not change during anti-TNF therapy (Figure 2A). A similar decrease in patients compared to controls was observed for the ratio of free testosterone/ASD and free testosterone/DHEA and did not change during therapy (Figures 2B, 2C).

Change of 17 β -estradiol and estrone in relation to precursor hormones. Serum levels of 17 β -estradiol were increased in RA patients compared to controls and did not change during therapy (Figure 3A). The ratio of 17 β -estradiol/free testosterone indicates a hormone shift via the aromatase complex. This ratio was largely higher in RA patients than in controls and did not change during therapy (Figure 3B). The ratios of 17 β -estradiol/DHEAS and 17 β -estradiol/ASD were higher in RA patients compared to controls (Figures 3D, 3E). Only the ratio 17 β -estradiol/DHEA was somewhat decreased during the observation period, but the mean values did not differ significantly from the ratio in controls (Figure 3C, compare with the solid horizontal line).

DISCUSSION

TNF is able to modulate adrenal hormone production and peripheral hormone conversion on very different levels in different cell types such as adrenocortical cells⁶. We expected to observe normalization of these hormones during 12 weeks of anti-TNF therapy. However, largely altered serum concentrations of adrenal androgen precursors and endpoint sex hormones did not change during 12 weeks of anti-TNF therapy.

In a recent study we indicated that aromatase activity was increased in mixed synovial cells of patients with RA compared to monocyte-derived macrophages of healthy controls⁷. Others have demonstrated that TNF and interleukin 6 stimulate aromatase activity⁸. A recent study in male patients with RA confirmed increased serum concentrations of 17 β -estradiol were positively correlated with erythrocyte sedimentation rate⁹. From these findings, it was likely that anti-TNF therapy should reduce 17 β -estradiol in relation to free testosterone and adrenal androgens. This is particularly true because disease activity, measured by joint scores, decreased markedly during adalimumab therapy.

Several reasons for this obvious discrepancy may exist: (1) The neutralizing capacity of anti-TNF therapy in the target tissue was inadequate to restore these hormones. (2) Anti-TNF antibodies do not neutralize TNF in the target tissue, but influence other important proinflammatory immune phenomena such as apoptosis¹⁰. (3) The amount of adrenal and sex hormones from noninflamed tissue, such as fat tis-

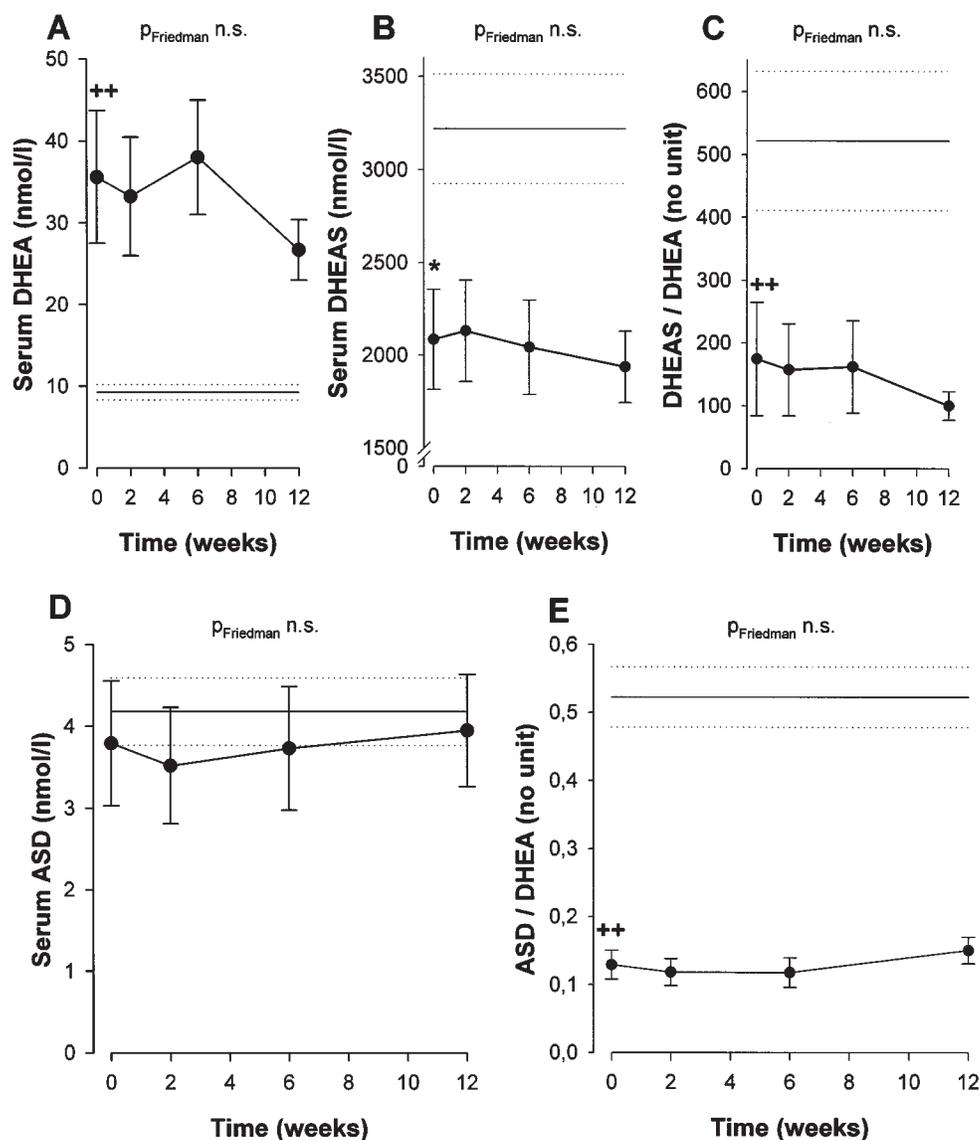


Figure 1. Course of adrenal androgens during 12 weeks of anti-TNF antibody therapy in RA patients. Baseline values are given as timepoint 0. Graph shows serum levels of DHEA (A), DHEAS (B), ratio of DHEAS/DHEA (C), androstenedione (ASD) (D), and ratio of ASD/DHEA (E). Data are given as means \pm SEM. p value for Friedman test is given. Broken lines indicate the mean (SEM) of healthy controls. *p = 0.02, **p < 0.001, comparison of baseline values of RA patients vs controls.

sue, may be much higher than expected, which would unmask the effects of anti-TNF induced restoration of local hormone secretion in inflamed tissue. (4) The hormonal dissociation with an increase of 17 β -estradiol in relation to androgens is imprinted for a considerably longer time than 12 weeks, as described¹¹. (5) Under consideration of strongly inflamed synovial tissue, TNF is not the sole modulator of altered hormone production. (6) Hormonal changes may be imprinted for a long time before the outbreak of RA, as described for serum DHEAS¹². These questions remain unresolved at the moment, and future studies are needed to

determine the most important factors for elevated estrogen and low levels of tissue testosterone.

ACKNOWLEDGMENT

We thank Angelika Gräber and Melanie Grünbeck for excellent technical assistance.

REFERENCES

1. Elliott MJ, Maini RN, Feldmann M, et al. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum* 1993;36:1681-90.
2. Cutolo M, Sulli A, Pizzorni C, Cravotto C, Straub RH.

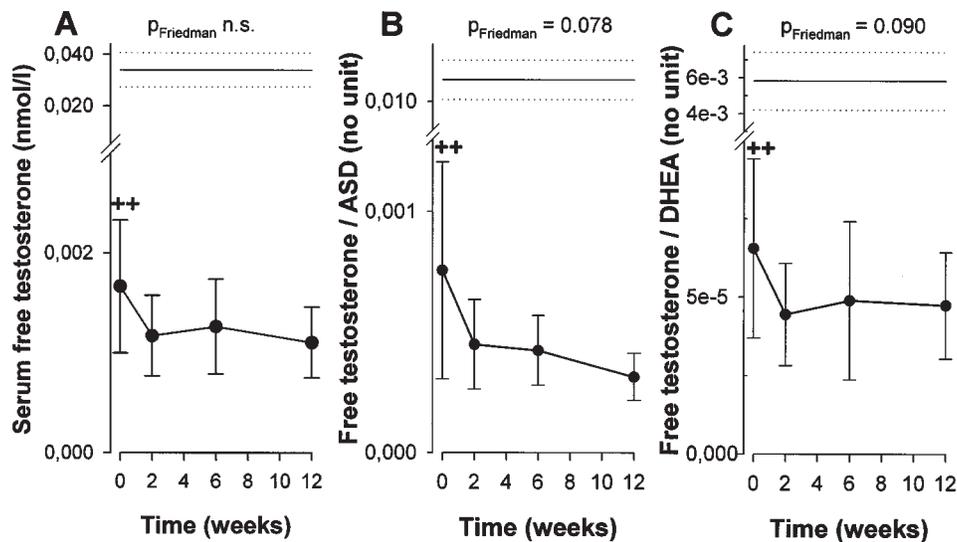


Figure 2. Course of free testosterone during 12 weeks of anti-TNF antibody therapy in RA patients. Baseline values are given as timepoint 0. Graph shows serum levels of free testosterone (A), ratio of free testosterone/androstenedione (ASD) (B), and ratio of free testosterone/DHEA (C). Data are given as means \pm SEM. p value for Friedman test is given. Broken lines indicate mean (SEM) of healthy controls. $^{**}p < 0.001$ for comparison of baseline values of RA patients vs controls.

- Hypothalamic-pituitary-adrenocortical and gonadal functions in rheumatoid arthritis. *Ann NY Acad Sci* 2003;992:107-17.
- Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
 - Atenzi F, Sarzi-Puttini P, Capsoni F, et al. Autoantibodies profile of rheumatoid arthritis patients during treatment with adalimumab [abstract]. *Ann Rheum Dis* 2004;63 Suppl:298.
 - Burmester GR, Monteagudo Saez I, Malaise M, Canas da Silva J, Webber DG, Kupper H. Efficacy and safety of adalimumab (Humira) in European clinical practice: The ReAct Trial [abstract]. *Ann Rheum Dis* 2004;63 Suppl:90.
 - Jäättelä M, Ilvesmaki V, Voutilainen R, Stenman UH, Saksela E. Tumor necrosis factor as a potent inhibitor of adrenocorticotropin-induced cortisol production and steroidogenic P450 enzyme gene expression in cultured human fetal adrenal cells. *Endocrinology* 1991;128:623-9.
 - Castagnetta LA, Carruba G, Granata OM, et al. Increased estrogen formation and estrogen to androgen ratio in the synovial fluid of patients with rheumatoid arthritis. *J Rheumatol* 2003;30:2597-605.
 - Macdiarmid F, Wang D, Duncan LJ, Purohit A, Ghilchick MW, Reed MJ. Stimulation of aromatase activity in breast fibroblasts by tumor necrosis factor alpha. *Mol Cell Endocrinol* 1994;106:17-21.
 - Tengstrand B, Carlstrom K, Fellander-Tsai L, Hafstrom I. Abnormal levels of serum dehydroepiandrosterone, estrone, and estradiol in men with rheumatoid arthritis: high correlation between serum estradiol and current degree of inflammation. *J Rheumatol* 2003;30:2338-43.
 - Van den Brande JM, Braat H, van den Brink GR, et al. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. *Gastroenterology* 2003;124:1774-85.
 - Gordon D, Beastall GH, Thomson JA, Sturrock RD. Prolonged hypogonadism in male patients with rheumatoid arthritis during flares in disease activity. *Br J Rheumatol* 1988;27:440-4.
 - Masi AT, Aldag JC, Chatterton RT, Adams RF, Kitabchi AE. Adrenal androgen and glucocorticoid dissociation in premenopausal rheumatoid arthritis: a significant correlate or precursor to onset? *Z Rheumatol* 2000;59 Suppl 2:54-61.

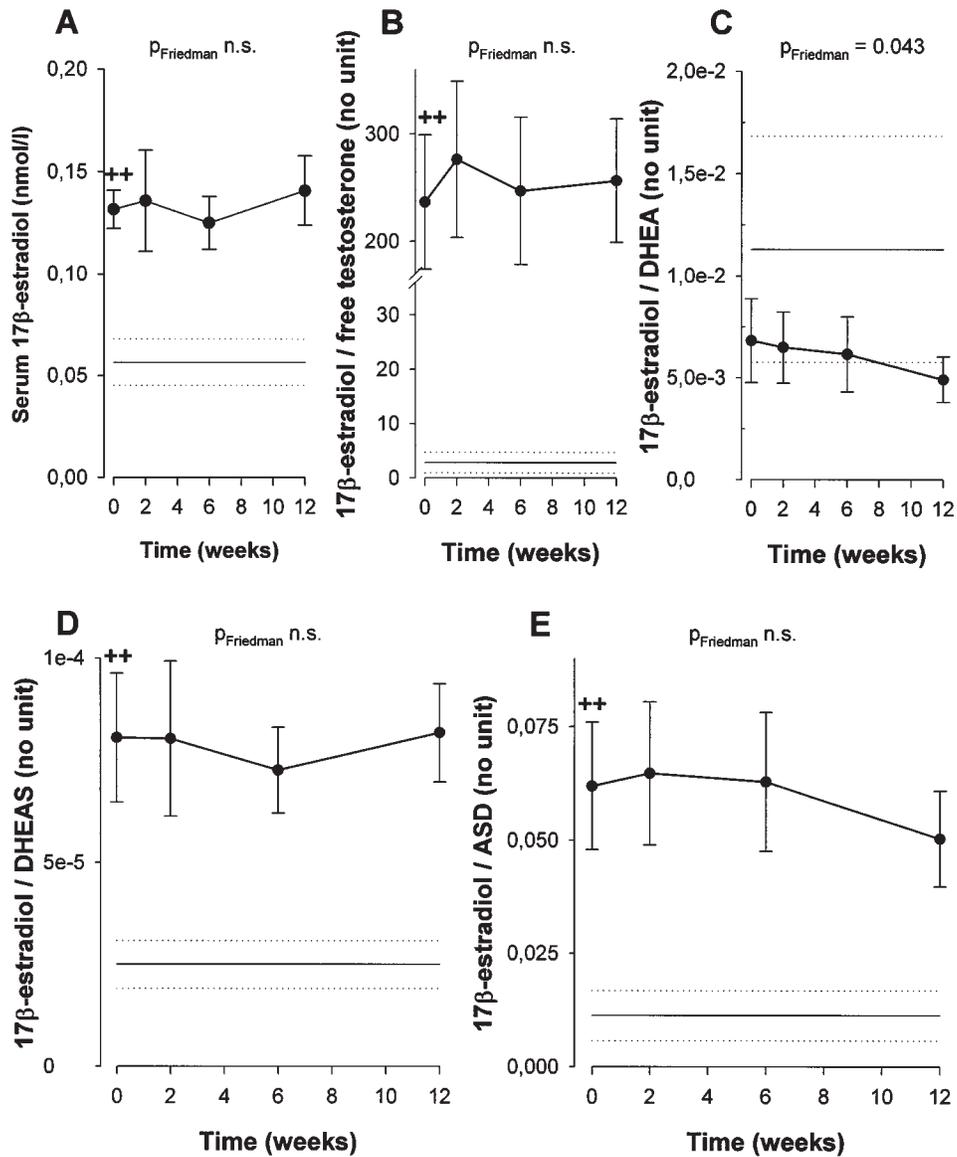


Figure 3. Course of 17 β -estradiol in relation to other hormones during 12 weeks of anti-TNF antibody therapy in RA patients. Baseline values are given as timepoint 0. Graph shows serum levels of 17 β -estradiol (A), ratio of 17 β -estradiol/free testosterone (B), 17 β -estradiol/DHEA (C), 17 β -estradiol/DHEA sulfate (DHEAS) (D), and 17 β -estradiol/androstenedione (ASD) (E). Data are given as means \pm SEM. p value for Friedman test is given. Broken lines indicate mean (SEM) of healthy controls. $^{++}p = 0.001$ for comparison of baseline values of RA patients vs controls.