ADRENAL PLASMA STEROID RELATIONS IN GLUCOCORTICOID-NAÏVE PREMENOPAUSAL RHEUMATOID ARTHRITIS PATIENTS DURING INSULIN-INDUCED HYPOGLYCEMIA TEST COMPARED TO MATCHED NORMAL CONTROL FEMALES

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Objective. Clinical and experimental data indicate the involvement of adrenal steroids in the complex of rheumatoid arthritis (RA) pathogenesis. A subtle adrenocortical hypocompetence has been suggested in a subset of glucocorticoid-naïve premenopausal females with RA.

Methods. The interrelations among adrenal steroids: cortisol (CORT), 17α-hydroxyprogesterone (17-OHP), androstenedione (ASD), dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) were evaluated in 15 glucocorticoid-naïve premenopausal females with RA and in 14 age- and body mass index- matched healthy females at basal and during insulin-induced hypoglycemia states. Spearman's correlations were used to analyze baseline plasma concentrations as well as areas under response curves of these steroids levels as assayed during the basal and/or insulin-induced hypoglycemia status.

Results. Six among 15 RA patients, but none of 14 controls had combined "lower" quartile range of basal cortisol (<431 nmol/l) and lower DHEAS (<2.79 μmol/l) levels, i.e., concentrations within the lowest quartiles of the control group (p=0.017). In all subjects combined, basal correlations were significantly positive between ASD and other steroids (CORT, 17OHP, DHEA, DHEAS). When patient and control groups were analyzed separately, the positive basal correlation between ASD and CORT was significant only in RA patients (p=0.030). In contrast, a positive basal correlation between ASD and DHEA was significant only in controls (p=0.004). When comparing the areas under response curves (AUCs), the correlation of ASD and CORT was significantly negative in RA (p=0.009), but positive in controls (RA vs control difference in Spearman's correlations, p=0.002). The correlation between AUCs of ASD and DHEA was strongly positive in controls (p=0.006), but not in RA (RA vs. control difference p=0.044).

Conclusions. The results suggest relative hypocompetence of adrenocortical function in premenopausal RA females. Different patterns of correlations of the adrenal steroids during basal vs. stimulatory testing suggested certain alterations in adrenal synthetic pathways or deficiencies in the dynamics of steroidogenesis in RA.

Key words: Rheumatoid arthritis – Insulin – Hypoglycemia – Adrenal steroids – Premenopausal women

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A substantial body of research indicates that hormonal factors may play a role in the complex physiopathogenesis of rheumatoid arthritis (RA) (Masi and Aldag 2005; Masi et al. 2005). Among those factors, major attention has been given to adrenal steroids, the end products of the hypothalamic-pituitary-adrenal (HPA) axis. Subtle differences in HPA axis function were found between normal subjects versus RA patients as well as pre-symptomatic (pre-RA) susceptibles in carefully controlled studies (Masi and Aldag 2005). The data support involvement of adrenal steroids in the predisposition and development of RA, particularly in women with premenopausal onset.

Substantial body of research indicates that hormonal factors may play a role in the complex pathogenesis of rheumatoid arthritis (RA) (Masi and Aldag 2005; Masi et al. 2005). Among those factors, major attention has been paid to adrenal steroids which are the end products of hypothalamic-pituitary-adrenal (HPA) axis. Subtle differences in HPA axis function were found between normal subjects versus RA patients as well as pre-symptomatic (pre-RA) susceptibles in carefully controlled studies (Masi and Aldag 2005). The data support the involvement of adrenal steroids in the predisposition and development of RA, particularly in women with premenopausal RA onset.

The HPA axis is a neuroendocrine system regulated by several negative feedback loops at different levels of action, i.e., the central nervous system, hypothalamus, pituitary, and at the primary adrenal target organ. Most of the physiological attention had previously been given to the *tropic* (stimulatory) aspects in the negative feedback signaling system (Watts 2005), while a little attention has been paid to the involvement of *trophic* (cell mass/competence) influences, which may particularly apply at the adrenal gland level (Masi et al. 1996; Lee et al. 2002). Specifically, adrenal glands of premenopausal onset RA patients or susceptibles may show a relatively deficient mass capacity of their endocrine function, particularly that of adrenal androgens (AA) production in the *zona reticularis* (ZR). The controlled data accumulated so far support only some subtle alterations in HPA axis function in RA patients, mainly at the adrenal level, and particularly in a subgroup of premenopausal onset women. Such interpretation is supported by consistent findings of lower levels of adrenal androgens, particularly these of dehydroepiandrosterone sulphate (DHEAS), in premenopausal onset RA vs. controls (Masi and Aldag 2005). Lower levels of cortisol (CORT), produced by the *zona fasciculata* (ZF), were also found in postmenopausal onset pre-RA susceptibles compared to matched control subjects (Masi and Chrousos 2003).

In our recent controlled investigation of glucocorticoid-naïve premenopausal RA females, basal levels and hypoglycemia-stimulated responses of several adrenal steroids were studied (Imrich et al. 2005). When compared to age- and BMI-matched healthy females, RA patients had lower basal DHEAS levels and, unexpectedly, a tendency to higher stimulated CORT response. The aim of the present study was to further evaluate possible pathways related to the adrenal androgen hypofunction observed in glucocorticoid-naïve premenopausal females with RA. Utilizing the data obtained in our previous controlled investigation (Imrich et al. 2005), the relationships among baseline concentrations of selected adrenal steroids and responses of these steroids to hypoglycemia stress stimulation were compared in RA and healthy subjects.

Subjects and Methods

Patients. Fifteen females with low or moderated RA activity aged of 41.2 ± 1.5 years (mean±SEM) with body mass index (BMI) of 21.6 ± 1.1 kg/m^2 fulfilling the revised criteria of the American College of Rheumatology (ACR) for RA (Arnett et al. 1988) were recruited from the National Institute of Rheumatic Diseases in Piestany (Slovakia) and the 1st Clinic of Internal Medicine, Medical Faculty of Comenius University in Bratislava (Slovakia). Fourteen healthy females aged of 44 ± 2.8 years with BMI of 23 ± 1.1 served as controls and were matched to the patients on age- (± 5 years) and BMI- (± 3 kg/m^2) from volunteer laboratory staff of the Institute of Experimental Endocrinology, Slovak Academy of Sciences in Bratislava (Slovakia). All females studied had negative history of diabetes or impaired glucose tolerance and had regular menstrual cycles. During the past five years the patients had been treated with non-steroidal anti-inflammatory drugs (NSAIDs) and disease-modifying drugs (DMARDs), but none with glucocorticoids or other drugs known to interfere with the neuroendocrine function,. The last dose of medications was administered 24 hours prior to the investigations. All subjects signed written informed consent and the study was approved by the Ethics Committee of the National Institute of Rheumatic Diseases.

Investigation procedure was described elsewhere in detail (Imrich et al. 2005). Briefly, basal samples were taken at approximately 8:30 a.m. (time 0 min) from an

indwelling catheter inserted into the cubital vein, 30 min earlier. An intravenous bolus of insulin (0.1 IU per kg, Actrapid HM, Novo Nordisk A/S Bagsvaerd, Denmark) was administered at time zero. Further blood samples during the insulin-induced hypoglycemia test were collected into EDTA-filled tubes at the time points 15, 30, 45, 60 and 90 min. After centrifugation, plasma aliquots were stored at -25 °C until analyzed. Concentrations of CORT were assayed by immunoradiometric assay (IRMA). Levels of DHEAS, 17α-hydroxyprogesterone (17OHP), and androstenedione (ASD) were assayed by radioimmunoassay (RIA); and dehydroepiandrosterone (DHEA) by RIA after extraction in diethyl ether. All kits were manufactured by Immunotech (Prague, Czech Republic).

Areas under response curves (AUCs) of each hormone were calculated after the subtraction of basal value (time 0 min) from the concentrations in 0-90 min of the hypoglycemia test using the corresponding procedure in SigmaPlot 2001 (Systat Software Inc., Richmond, CA, USA) software. Concentrations of a given plasma steroid within the lowest quartile of the control group were considered "lower" quartile reference range values. Spearman's correlations and Fisher's exact tests were performed using SPSS 11.01 (SPSS Inc., Chicago, IL, USA) software. Differences between Spearman's correlations of RA vs. CN were tested by Fisher Z-transform using: http://www.fon.hum.uva.nl/Service/Statistics/Two_Correlations.html. All data are expressed as mean (±SEM). Two-tail significance was set at $p<0.05$, and no adjustment was made for multiple comparisons (Rothman 1990), due to the relatively small sample sizes.

Results

Basal concentrations (mean±SEM) of all studied hormones and glucose were within normal range as well as the areas under response curve (AUC; 0-90) values in RA, CN, and in all subjects. No significant difference in basal glucose, CORT, 17OHP, ASD was observed between RA patients and controls. Basal DHEA concentration tended to be lower in RA patients (13.4 ± 1.19 nmol/l) compared to healthy controls (18.3 ± 2.54 nmol/l; $p=0.067$). However, the mean basal DHEAS level in RA patients (3.04 ± 0.37 μmol/l) was lower than that in controls (5.17 ± 0.98 μmol/l; $p<0.05$) as previously reported (Imrich et al. 2005). However, due to insufficient volume of plasma sample, basal ASD levels were measured only in 14 RA patients and in 10 controls.

During insulin-induced hypoglycemia, nadir mean plasma glucose concentrations decreased significantly ($p<0.001$), while the peak concentrations of CORT, 17OHP, ASD and DHEA significantly increased ($p<0.001$) uniformly in RA and control groups. AUC of cortisol ($CORT_{AUC\ 0\text{-}90}$) was higher in RA patients (31041 ± 4060 min x nmol/l) vs. controls (17459 ± 2871 min x nmol/l; $p<0.05$), while AUCs of 17OHP ($17OHP_{AUC\ 0\text{-}90}$), ASD ($ASD_{AUC\ 0\text{-}90}$) and DHEA ($DHEA_{AUC\ 0\text{-}90}$) did not differ between RA patients and controls (Imrich et al. 2005).

The number of subjects with lower quartile normal reference range of basal steroid levels, i.e., CORT <431 nmol/l, 17OHP <1.39 nmol/l, ASD <3.67 nmol/l, DHEA <11.7 nmol/l, and DHEAS <2.79 μmol/l versus the remainder of higher values is shown in Table 1. The greater number of RA patients with lower quartile DHEAS was nearly significant ($p=0.060$) compared to

Table 1

The numbers of RA patients and controls with basal concentration within/above lower quartile normal limit (LQL)*

	RA	CN	RA vs CN
Steroids	LQL/remainder	LQL/remainder	2 tail p
CORT	6/9	3/11	0.427
17OHP	3/12	3/11	1.0
ASD	3/11	2/8	1.0
DHEA	8/7	4/7	0.453
DHEAS	9/6	3/11	0.060
CORT&DHEAS	6/9	0/14	0.017

*Limits were based upon the normal frequency distributions as follows: CORT<431 nmol/L, 17OHP<1.39 nmol/L, ASD<3.67 nmol/L, DHEA<11.7 nmol/L or DHEAS<2.79 μmol/L. A p value was calculated using Fisher's Exact Test.

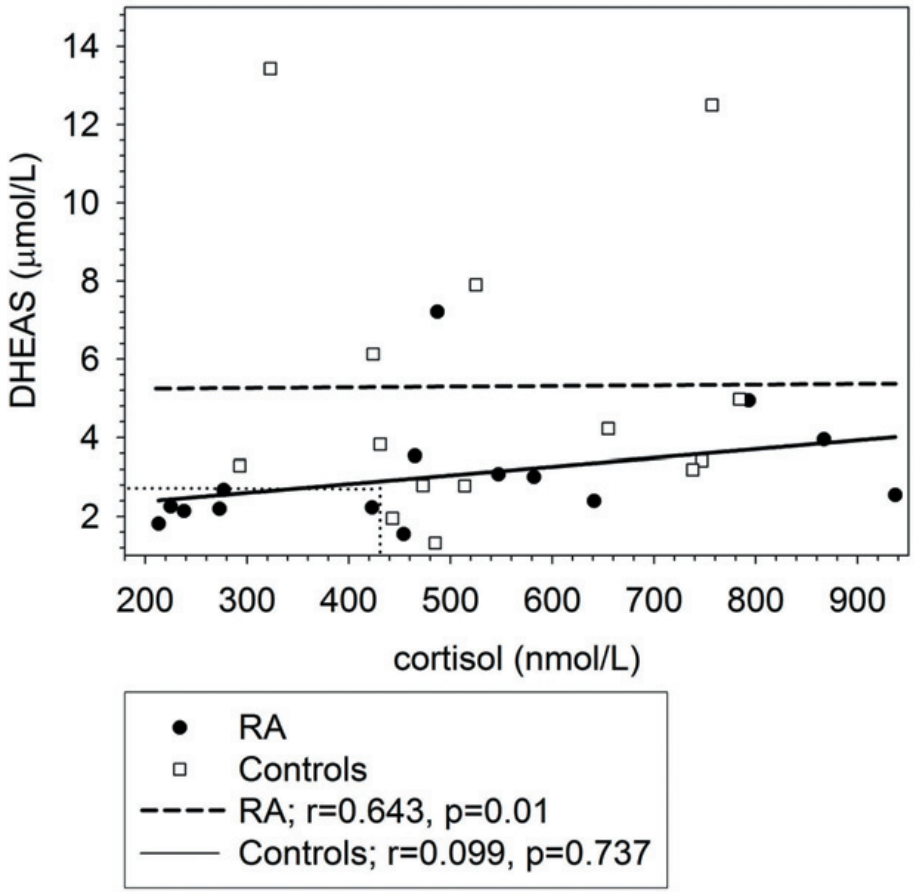

Fig 1 Scatter plot and regression lines of basal cortisol vs dehydroepiandrosterone sulphate (DHEAS) in 15 RA patients (black circles, solid line) and 14 healthy controls (white squares, dashed line). Six out of total 15 RA patients (as delineated by a dotted rectangle) but none of the controls had combined lower basal cortisol (< 431 nmol/L) and lower DHEAS (< 2.79 µmol/l). Black circles – RA patients (r = 0.643, p<0.01); squares – controls (r = 0.099; p = 0.757)

Table 2

Correlations between basal levels of ASD and other steroids (CORT, 17OHP, DHEA, DHEAS) in RA patients, healthy controls (CN), differences in RA vs CN correlations, and correlations in all subjects combined

ASD	RA			CN			Fisher's	All		
vs	r	P	N	r	P	N	p*	r	p	N
CORT	0.579	0.030	14	0.370	0.293	10	NS	0.531	0.008	24
17OHP	0.286	0.322	14	0.588	0.074	10	NS	0.467	0.021	24
DHEA	0.350	0.220	14	0.881	0.004	8	NS	0.606	0.003	22
DHEAS	0.266	0.358	14	0.564	0.090	10	NS	0.411	0.046	24

*NS = non-significant.

controls. Six (N=6) of fifteen (40 %) RA patients, but none of the 14 controls had combined lower CORT and DHEAS (Fig 1), suggesting a relative hypocompetence in achieving relatively higher combined basal levels in the glucocorticoid ZF and androgenic zona reticularis (ZR) pathways concomitantly.

Significant positive correlations were found between basal levels of androstenedione (ASD) and other steroids (CORT,

Table 3

Correlations between area under response curve of androstenedione (ASDAUC 0-90) and area under response curve of cortisol (CORTAUC 0-90), 17hydroxy progesterone (17OHPAUC 0-90) and (DHEAAUC 0-90) in RA patients, healthy controls (CN), differences in RA vs CN correlations, and correlations in all subjects combined

$ASD_{AUC\ 0\text{-}90}$	RA			CN			Fisher's	All		
vs	r	p	N	R	p	N	p	r	p	N
$CORT_{AUC\ 0\text{-}90}$	-0.670	0.009	14	0.545	0.083	11	0.002	0.030	0.887	25
$17OHP_{AUC\ 0\text{-}90}$	-0.134	0.648	14	0.427	0.190	11	NS*	0.214	0.305	25
$DHEA_{AUC\ 0\text{-}90}$	0.070	0.811	14	0.764	0.006	11	0.044	0.477	0.016	25

*NS = non-significant.

17OHP, DHEA, DHEAS) in all subjects combined (N = 22 or 24) (Table 2). Significant positive correlation was found between basal levels of ASD and CORT in 14 RA patients (p=0.030), but not in controls. In contrast, a significant positive correlation was found between basal ASD and DHEA only in the 8 controls (p=0.004), but not in RA. The testing for differences in basal steroid correlations between RA vs. controls (Table 2) did not show any significance.

Correlation between $ASD_{AUC\ 0\text{-}90}$ and $CORT_{AUC\ 0\text{-}90}$ was significantly (p=0.009) negative in RA, but positive in CN (RA vs CN difference p=0.002) (Table 3, Fig 2). Correlation between $ASD_{AUC\ 0\text{-}90}$ and $DHEA_{AUC\ 0\text{-}90}$ was

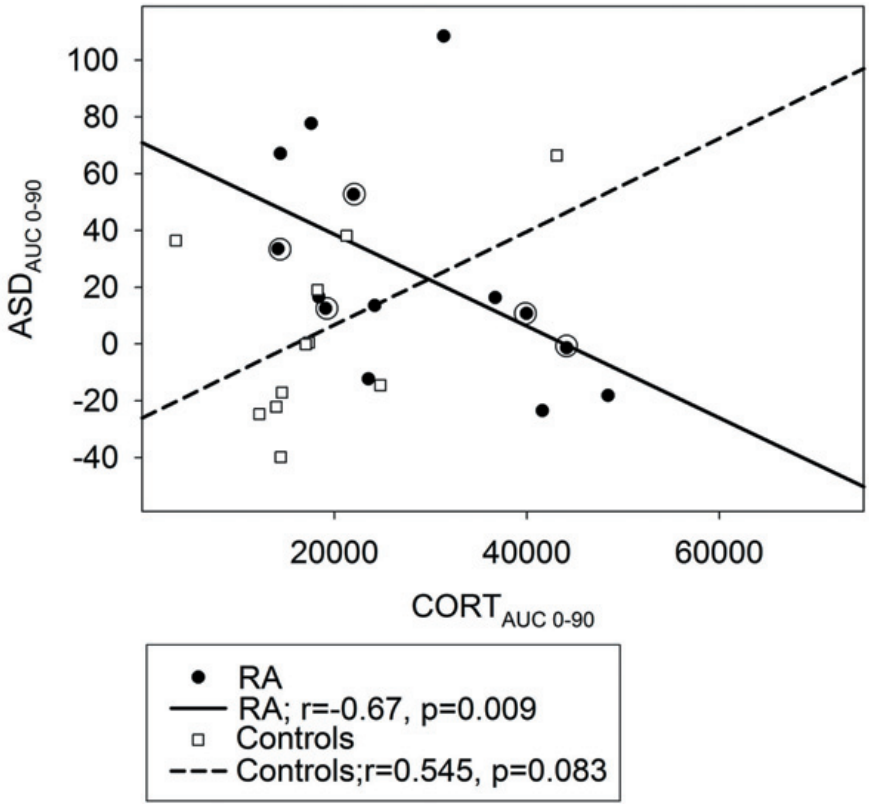

Fig. 2 Scatterplot and regression lines of area under response curve (AUC) of cortisol (CORT) vs AUC of androstenedione (ASD) in 14 RA patients (black circles, solid line) and 11 healthy controls (white squares, dashed line). AUCs are shown in nmol/L x 90 min. Five RA patients who had combined lower basal cortisol (< 431 nmol/l) and lower DHEAS (< 2.79 µmol/l) are shown with additional black circle.
Black circles – RA patients (r = -0.67, p=0.009); squares – controls (r = 0.545; p = 0.083)

significantly positive in CN (p=0.006), but not in RA (RA vs. CN difference p=0.044) (Table 3).

A significant positive correlation was found between basal CORT and DHEAS levels in RA patients (r=0.643, p=0.01, N=15), and combined subjects (r=0.429, p=0.020, N=29), but not in CN (r=0.099, p=0.737, N=14). No significant correlations between basal levels of steroids and their respective AUCs were found (data not shown).

No significant differences in product: precursor ratios of basal CORT to 17OHP and ASD to 17OHP as well as their respective incremental AUC ratios were found between RA and CN subjects (data not shown).

Discussion

One of the questions addressed in the present study of glucocorticoid-naïve premenopausal RA females was whether or not the decreased production of DHEAS, as indicated by its lower basal plasma levels, might be attributed to decreased stimulation induced response capacity of the adrenal glands. In the present analyses, we were able to identify 40 % of RA subjects with combined relatively lower basal CORT (ZF product) and lower DHEAS (ZR product), quantitatively the two most abundant adrenal steroids from their respective zones. In addition, a positive correlation between basal CORT and DHEAS was unexpectedly observed in RA. In line with others (Haning et al. 1981; Masi et al. 1999), no significant relationship was found between basal CORT and DHEAS in healthy premenopausal females. The results might suggest decreased basal combined adrenal function in a subset of RA patients that involves both the glucocorticoid-producing ZF and the androgen-producing ZR. The majority of evidence suggests relative AA hypofunction in premenopausal onset RA females, both before and following onset of clinical disease, possibly related to lower ZR sulfotransferase activity in RA (Masi et al. 1996; Masi et al. 1999; Masi and Aldag 2005; Masi et al. 2005). However, controlled study of postmenopausal female and male pre-RA susceptibles revealed small minorities with low CORT levels (Masi and Chrousos 2003; Masi et al. 2005).

Because of its relatively long plasma half-life and large pool in peripheral circulation, DHEAS plasma levels have been considered as a reliable indicator of AA function over longer periods of time (Masi et al. 1999). DHEAS plasma levels may, at least in part, also reflect trophic factors, i.e., the mass capacity or cellular competence of the adrenals, particularly of the ZR, which is the site of AA production. In the present analyses, we explored relationships between the basal DHEAS plasma concentrations and glucocorticoid-producing ZF of the adrenals, at basal status (Fig 1) and during strong HPA stimulation. A significant correlation between basal CORT and DHEAS was found in RA patients. On the other hand, baseline DHEAS and AUC of CORT did not correlate in either RA patients or healthy controls. In general, other authors failed to find any significant relationship between baseline levels of adrenal steroids including DHEAS and their responses to ACTH administration in a group of patients with hirsutism and healthy women (Siegel et al. 1990) or in other patients with hyperandrogenism (Azziz et al. 1990). Results suggest negligible relationship between stimulated ZF mass/functional capacity and basal ZR functional pool levels. Accordingly, basal DHEAS levels can be considered a rather reliable marker of only the androgen-producing zone of the adrenal glands.

The relatively broad distribution range of CORT AUC was similar in those RA subjects who had combined lower baseline CORT and DHEAS (additional black circle in Fig 2), as in the remaining 9 RA patients. This observation diminishes the likelihood that decreased basal mass/functional capacity of the ZR directly diminishes glucocorticoid responsiveness under stimulation. The latter is determined predominantly by the first, rate-limiting step of steroid synthesis, regulated by steroidogenic acute regulatory protein (Miller 2002) and by the ZF capacity. An explanation for the subset of 6 RA patients with combined lowest quartile CORT and DHEAS basal levels (Fig 1) is presently undetermined. One possibility is heterogeneity in these premenopausal cases, with the 6 having major basal hypofunction and the remaining 9 normal function (Fig. 1). Another possibility is that stimulation factors, e.g. hypothalamic-pituitary activity, combined with changes in both glucocorticoid and androgen producing capacity in the adrenals are to a certain extent co-incidental or overlapping alterations.

During the hypoglycemia test, other factors such as stress stimulus intensity or fast negative feedback of CORT, also can control intensity of adrenal stimulation (Dorin et al. 1996; Smith et al. 2003). Such mechanisms might have also affected the stimulated CORT secretion. Rapid feedback controls may have obscured relationships between baseline HPA setting and the stimulated capacity of the CORT producing ZF of the adrenals (Dorin et al. 1996). The observed tendency for higher CORT responses to hypoglycemia in RA compared to

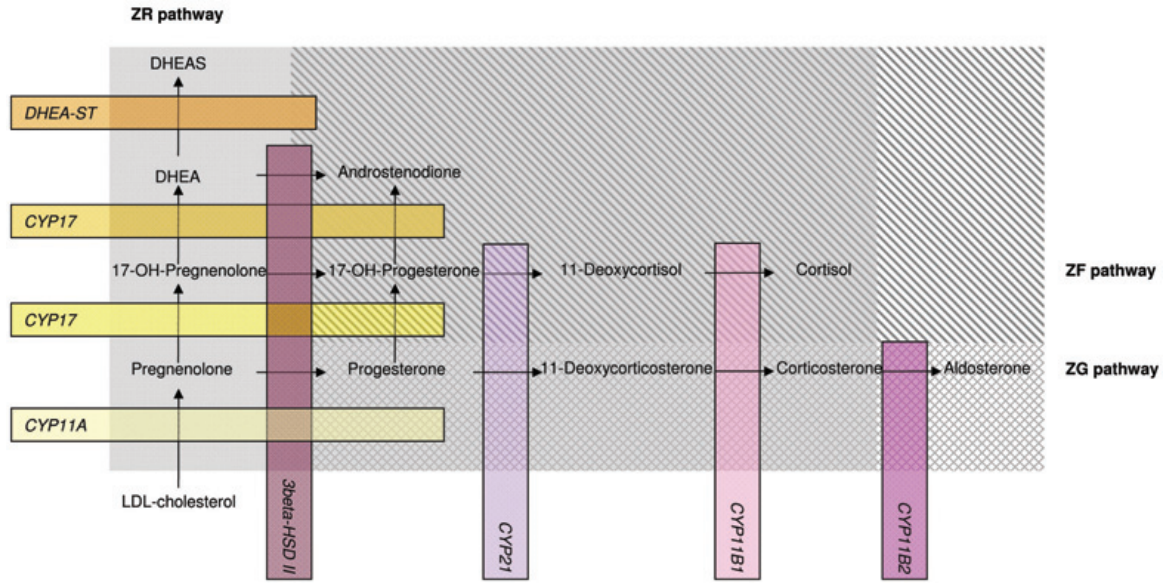

Fig. 3 Steroidogenesis pathways in the adrenal glands. With minor exception, the zona reticularis (ZR) pathway is shown as the unlined gray background, the zona fasciculata (ZF) pathway as oblique-lined background, and the zona glomerulosa (ZG) pathway as the diamond-lined background. Androstenedione is mainly produced in ZR, but lesser amounts are normally produced in the ZF. Aldosterone is entirely specific to ZG, but DHEAS, cortisol, and corticosterone are relatively specific to their respective zones. Expression of respective enzymes that differentiate the zonas are shown as horizontal and vertical bars. The lower basal plasma DHEAS levels in premenopausal RA females is inferred due to specific ZR pathway hypofunction. In RA compared to control subjects, the proportion of androstenedione produced by ZF might be greater than that produced by ZR, due to relative ZR hypocompetence in cases. The negative correlation of CORT and ASD in RA subjects under stimulation also suggests an insufficiency of combined ZF and ZR steroidogenic pathways in the cases.
CYP11A – cytochrome P450 cholesterol side-chain cleavage; CYP17 - cytochrome P450 17alpha-hydroxylase/17,20 lyase; 3beta-HSD II – 3beta hydroxysteroid dehydrogenase; CYP21 – 21 hydroxylase; CYP11B1 – 11beta-hydroxylase; CYP11B2 – aldosterone synthase; DHEA-ST – dehydroepiandrosterone sulphotransferase.

healthy controls resembles "chronic stress-like" adrenal responsiveness, perhaps reflecting a higher allostatic load of the chronic disease in RA patients (Korte et al. 2005). In this context, the hypoglycemic test can assess more fully the HPA axis than ACTH stimulation.

Baseline concentrations of AA steroids were reported to be strongly correlated in females with normal menstrual cycles (Landgren et al. 1977; Haning et al. 1981; Bonney et al. 1984), as was found between ASD and DHEA levels in the controls (Table 2). Significant positive correlations were reported also among baseline levels of AA and CORT (Landgren et al. 1977), but not between DHEAS and CORT (Haning et al. 1981). Our results are in agreement with latter reports showing comparable coefficients in the study groups for correlations among baseline adrenal steroid levels (Table 2).

In RA patients, a negative correlation between AUCs of CORT and ASD was unexpectedly observed (Table 3, Fig 2). The finding supports a concept of relative adrenal hypocompetence or subtle pathway shifts in activity of steroid producing enzymes, probably favoring production of adrenal glucocorticoids in RA (Herrmann et al. 2002; Masi and Aldag 2005). The results of present analyses underline the importance of correlational analyses among steroids in detecting subtle changes of adrenal function. Both CORT and ASD production are dependent upon the combined actions of 3β-hydroxysteroid-dehydrogenase (3β-HSD) and of 17α-hydroxylase/17, 20-lyase cytochrome P450 (P450c17), as outlined in Fig 3. These enzymes are co-expressed in the ZF and ZR, but 3beta-HSD expression is weak in ZR of the adult adrenals (Sasano et al. 1989; Mapes et al. 1999; Suzuki et al. 2000). Furthermore, presence of other important co-factors for AA synthesis, such as cytochrome b5, is restricted to ZR (Mapes et al. 1999). Thus, the conversion to Δ_4-steroids is facilitated

in the ZF. On the other hand, the 17,20 lyase action of human P450c17 is about 30-fold greater with Δ_5-substrates than with Δ_4-substrates (Miller 2002). The latter enzyme activities would markedly facilitate synthesis of DHEA than ASD from their respective precursors, i.e., 17-OH pregnenolone and 17OHP (Fig 3). Thus, CORT and ASD might be competing more directly for the 17OHP precursor in RA patients, after the 3β-HSD step in the ZF (Fig 3). It is currently thought that virtually all of the Δ_4-ASD normally derives from the action of 3β-HSD on Δ_5-DHEA, and that only insignificant quantities derive from 17OH progesterone (Miller 2002). However, due to ZR insufficiency in RA, the proportion of ASD produced by ZF might be relatively higher (Fig. 3).

According to the concept of adrenal hypocompetence in RA from either mass or other factors, a greater relative ZF (CORT) vs. ZR (DHEAS) steroidogenesis may reflect preferential ACTH feedback production of GCs that maintain essential energy homeostasis, compared to relatively less important AA functions. Additionally, a subtle relative decrease of P450c17 may be present in ZF of RA patients, particularly the Δ_5-17,20-lyase function in relation to 3β-HSD, thereby facilitating CORT production at the expense of ASD production during ACTH stimulation. This possibility is suggested by the opposite trend in correlations between AUCs of CORT vs AUCs of ASD in RA vs CN (Table 3, Fig 2).

The common substrate precursor for ASD and the CORT pathway 17OHP (Fig 3) may help to rationalize our correlational findings in premenopausal RA. Under basal conditions, fully sufficient 17OHP substrate may be available to synthesize normal levels of physiologically controlled CORT as well as the competing ASD product in both subject groups. Assuming sufficient 17OHP substrate levels, the competing products would be expected to be positively correlated. However, under maximal stimulation and consumption of the common 17OHP substrate, a relative deficiency in RA cases, could explain the negative correlation between AUCs of CORT and ASD. In contrast, the correlation would be expected to remain positive in normal females with competent ZF function and amply sufficient 17OHP levels, permitting full CORT and ASD synthesis. However, our analyses of the product: precursor ratios of ASD: 17-OHP and CORT: 17-OHP under basal and stimulation states did not show any significant differences in RA vs. CN subjects.

In conclusion, combined lower baseline CORT and DHEAS levels were found in 40 percent of glucocorticoid-naïve premenopausal females with RA, but none in control. This finding may reflect either a relative adrenal cortical insufficiency of both ZF and ZR in a subset of RA or relatively separate alterations of HPA control and function in these zones. The negative correlation between AUCs of CORT and ASD in RA suggest a subtle imbalance or hypocompetence in the differential production capacity of these steroids or a more complex steroidogenic alteration of the glucocorticoid-producing ZF. The lower basal DHEAS levels in RA vs. CN subjects likely reflects a mass/functional hypocompetence of the ZR in a subset of patients with premenopausal onset of disease (Masi et al. 1996; Masi et al. 1999; Imrich et al. 2005; Masi and Aldag 2005; Masi et al. 2005). However, further investigation is needed to test the expanded hypothesis of relative adrenal ZF hypocompetence among a subset of premenopausal RA patients. The latter alteration follows from the evidence of combined lower basal ZF and ZR steroid levels as well as an inability to maintain a positive correlation of CORT and ASD responsiveness under maximal adrenal stimulation.

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