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Dehydroepiandrosterone, ageing and immune activation

Maximilian Ledochowski[a], Christian Murr[b], Matthias Jäger[b], Dietmar Fuchs[b,*]

[a]*Department of Clinical Nutrition, Leopold-Franzens University, Innsbruck, Austria*
[b]*Institute of Medical Chemistry and Biochemistry, Leopold-Franzens University, and Ludwig Boltzmann Institute for AIDS Research, Innsbruck, Austria*

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Abstract

The age-related decline in dehydroepiandrosterone (DHEA) production is currently attracting attention because of its possible relevance to the etiology and management of a number of age-related clinical disorders. Various abnormalities of immune system function have been described in the elderly. Among them, increased concentrations of neopterin have been reported, which is produced by human monocytes/macrophages upon stimulation by interferon-γ. In order to examine the relation of serum DHEA to serum neopterin, we studied 281 otherwise healthy outpatients, who visited the physician's office for a medical health check-up. 10% presented with increased neopterin concentrations, 0.4% had increased DHEA sulfate (DHEAs) concentrations. DHEAs concentrations were significantly higher in patients with lower neopterin concentrations (Mann–Whitney test: $U = 4793$, $P < 0.0001$). There existed a rather strong inverse correlation between DHEAs concentrations and serum neopterin concentrations (Spearman's rank correlation: $r_s = -0.221$, $P < 0.0001$). The data support the concept that the decrease of DHEA with increasing age is related to immune system activation. Oxidative stress which accompanies immune response may diminish DHEA synthesis.

Keywords: Neopterin; Dehydroepiandrosterone; DHEA; Interferon-γ; Ageing

1. Introduction

Dehydroepiandrosterone (DHEA) and its sulfated metabolite DHEA sulfate (DHEAs) are hormones secreted by the adrenal cortex in response to adrenocorticotropin. DHEA is

* Corresponding author. Address: Institute of Medical Chemistry and Biochemistry, University of Innsbruck, Fritz Pregl Str. 3, A-6020 Innsbruck, Austria. Tel.: +43-512-507-3519; fax: +43-512-507-2865.
E-mail address: dietmar.fuchs@uibk.ac.at (D. Fuchs).

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Table 1
Baseline characteristics of the study subjects (n = 281 : 68 males and 213 females) (*n*, number of observations; DHEAS, dehydroepiandorsterone sulfate; BMI, body mass index)

Characteristics	First quartile	Median value	Third quartile	Range	Out of reference range[a]	
					Below *n* (%)	Above *n* (%)
Age (year)	38	47	57	15–91		
BMI (kg/m^2)	20.8	23.0	27.0	126–45.9	31 (11.0)	107 (38.1)
Neopterin (nmol/l)	4.3	5.2	6.4	< 2.0–31.2		28 (10.0)
DHEAS, μmol/l (μg/ml)	1.67 (0.6)	3.08 (1.1)	4.75 (1.8)	0.65–17.23 (0.2)–(6.4)	41 (14.6)	1 (0.4)

[a] Reference ranges: Neopterin, ≤13.5 nmol/l (<19 years). ≤8.7 nmol/l (19–75 years), ≤19 nmol/l (>75 years), DHEAS, 1.35–11.21 μmol/l BMI, 19–25 kg/m^2.

the most abundant adrenal steroid and has only mild androgenic properties. DHEAs is inactive and converted peripherally to DHEA, which exerts biological activity. Recently, it has been clarified that humans largely depend on peripheral tissues for androgen and estrogen formation from the adrenal precursor DHEA and DHEAs (Kroboth et al., 1999). The age-related decline in DHEA production is currently attracting attention because of its possible relevance to the etiology and management of a number of age-related clinical disorders (Williams, 2000; James et al., 1997). In addition to androgenic effects, DHEA has been shown to have immunmodulatory effects as well. Thus, application of DHEA proved as beneficial in patients suffering from systemic lupus erythematosus (SLE) (van Vollenhoven et al., 1998; Derksen, 1998).

Various abnormalities of immune system function have been described in the elderly. Among them, increased concentrations of neopterin have been reported in older-aged people (Reibnegger et al., 1988; Solichova et al., 1999). Large amounts of neopterin are released from human monocytes/macrophages preferentially upon stimulation with interferon-γ (IFN-γ) (Fuchs et al., 1993), a typical Th1-type cytokine. In humans, increased concentrations of neopterin in serum and urine have been found in viral infections including human immunodeficiency virus (HIV) type 1, various malignant disorders, autoimmune diseases and during allograft rejection episodes (Fahey et al., 1990; Reibnegger et al., 1991; Fuchs et al., 1993; Samsonov et al., 1995; Murr et al., 1999a). Significant associations between enhanced neopterin and IFN-γ production have been obtained also in patients (Fuchs et al., 1989), and monitoring of neopterin concentrations has turned out to be a sensitive and useful marker for monitoring the activation of cellular (= Th1-type) immune response (Fuchs et al., 1993).

The present study was designed to examine the possible relation of serum DHEAs to serum neopterin levels in a population of healthy individuals.

2. Materials and methods

2.1. Subjects

Two hundred and eighty one otherwise healthy outpatients, 68 males and 213 females, who visited the physician's office for a medical health check-up, but not feeling sick, were studied. Patients' age varied from 15 to 91 years (median age: 47 years, Table 1).

With exception of contraceptives in some females, all patients were without any medications. Patients' body mass was classified by body mass indices (BMI) as described earlier (James, 1984). Accordingly, the majority of 143 (51%) showed normal weight (BMI 19–25 kg/m^2), whereas 107 subjects (38%) were overweight (BMI above 25 kg/m^2) and 31 (11%) were underweight (BMI below 19 kg/m^2).

2.2. Blood collections and measurements

Blood samples were drawn after an overnight fast. The blood was allowed to clot at room temperature, and serum was obtained by centrifugation at $1500 \times g$ for 15 min. All analyses were performed within one day after blood collection. Serum DHEAs was measured by a competitive immunoassay with an IMMULITE 2000 Analyzer and a

Table 2
Spearman's rank correlations of investigated characteristics

	n	Spearman's rank correlation coefficient		
		Value	95% confidence interval	*P*-value
Neopterin versus DHEAS	281	−0.221	−0.333–−0.103	0.0002
Neopterin versus age	281	0.286	0.172–0.393	< 0.0001
DHEAS versus age	281	−0.541	−0.621–−0.450	< 0.001
DHEAS versus sex	281	−0.247	−0.357–−0.131	< 0.001
DHEAS versus BMI	281	−0.026	−0.146–0.095	n.s

commercial kit (Immulite® 2000 DHEA-SO_4, Diagnostic Products Corporation, Los Angeles, CA), with a sensitivity of 0.038 μmol/l DHEAs and a total coefficient of variation ranging from 7.9 to 13.0%. The laboratory's normal range for adults being 1.35–11.21 μmol/l (0.5–4.15 μg/ml). Serum neopterin was measured by a commercially available ELISA (IMMUtest Neopterin, BRAHMS Diagnostica, Berlin, Germany) with a sensitivity of 1 nmol/l neopterin and an interassay coefficient of variation ranging from 4.7 to 8.5%. Upper limits of the normal (95th percentiles) for neopterin concentrations depend on age ranging from 8.7 nmol/l (19–75 years) to 13.5 nmol/l (below 19 years) and 19.0 nmol/l (above 75 years) as described earlier (Fuchs et al., 1993).

2.3. Statistical analysis

Correlation between variables were assessed by the non-parametric Spearman's rank correlation technique, since the distributions of observed values were generally non-Gaussian. Differences of distributions of laboratory variables among patient groups were tested for significance by the non-parametric Mann–Whitney test. The effect of the three factors serum neopterin concentrations, age and sex on serum DHEAs concentrations was assessed by a three-way analysis of variance (ANOVA). Thereby the factors neopterin and age were dichotomized by the third quartile point of the observed distribution (for values see Table 1). Since variances in the eight subgroups formed on the basis of neopterin, age and sex were different, a logarithmic transformation of DHEAs concentrations was done before analysis. The success of transformation was confirmed by Bartlett's test for equal variances (test statistic = 6.61; not significant) as implemented in the program GRAPHPAD PRISM (GraphPad Software, San Diego, CA). To determine whether the response of DHEAs concentrations to one factor depends on the level of the second factor or the third factor, the interaction terms were also tested for significance. ANOVA was calculated by the program BMDP2V (BMDP Statistical Software, 1990 edition, University of California Press).

3. Results

Table 1 reports characteristics of the study subjects. Notably, 10% of the studied patients showed elevated neopterin concentrations, but only 1 patient (0.4%) had elevated DHEAs concentrations. Correlations between the investigated variables assessed by

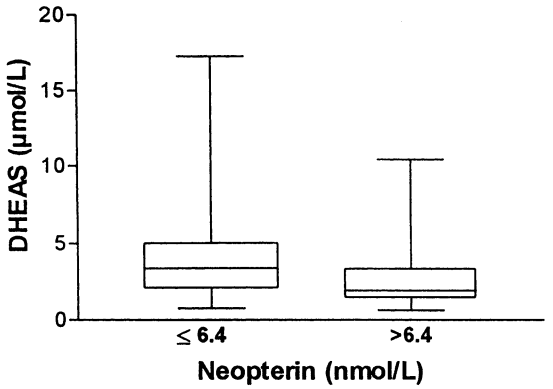

Fig. 1. Box and Whiskers plots of DHEAs concentrations of patients with different neopterin concentrations. The box extends from the 25th percentile to the 75th percentile, with a horizontal line at the median (50th percentile), bars showing the range of the data. The median DHEAs concentration of the group with serum neopterin above 6.4 nmol/l is statistically significantly lower ($P < 0.0001$) compared to the group with neopterin concentrations equal or below 6.4 nmol/l.

Spearman's rank correlation coefficients are shown in Table 2. There was a strong correlation ($P < 0.0001$) of age versus neopterin concentrations and an inverse correlation ($P < 0.0001$) of age versus DHEAs concentrations. Similarly, a weaker inverse correlation was found between neopterin concentrations and DHEAs concentrations ($P = 0.0002$). There was no significant correlation between DHEAs concentrations and BMI.

As shown in Fig. 1, patients with neopterin concentrations above 6.4 nmol/l, the 3rd quartile of the distribution, had statistically highly significant (Mann–Whitney test, $U = 4793$, $P < 0.0001$) lower DHEAs concentrations (median: 1.94 µmol/l) than those with neopterin concentrations equal or less than 6.4 nmol/l (median DHEAs: 3.40 µmol/l). Fig. 2 shows that the males showed higher DHEAs concentrations (median: 4.02 µmol/l) than females (median: 2.75 µmol/l; Mann–Whitney test, $U = 4829$, $P < 0.0001$).

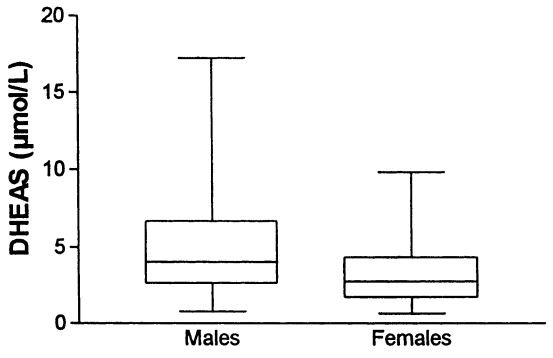

Fig. 2. Box and Whiskers plots of serum DHEAs concentrations of males and females. The box extends from the 25th percentile to the 75th percentile, with a horizontal line at the median (50th percentile), bars showing the range of the data. The median DHEAs concentration of the male group is statistically significantly higher ($P < 0.0001$) compared to the female group.

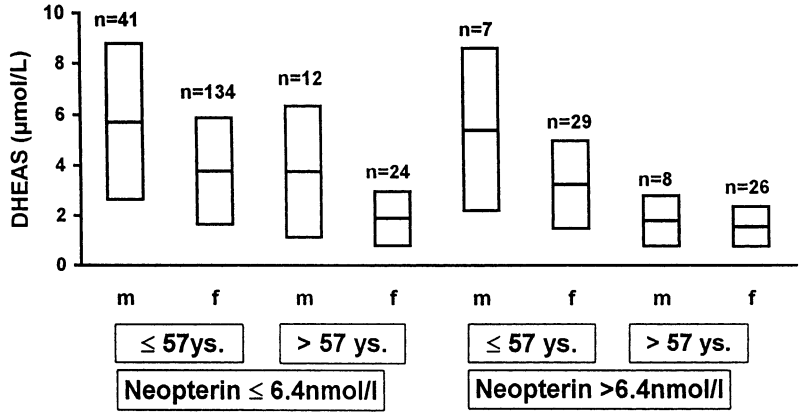

Fig. 3. Box and Whiskers plots of DHEAs concentrations of patients with different serum neopterin concentrations, age (below or equal 57 years, above 57 years; the two groups are defined according to the 3rd quartile of age distribution = 57 years) and sex ('m' indicates males, 'f' females). The box extends from the mean ± one standard deviation, with a horizontal line at the mean.

To test the relationship between serum neopterin concentrations, DHEAs concentrations and the two basic characteristics age and sex, the effect of neopterin concentrations, age and sex on serum DHEAs concentrations was calculated by three-way ANOVA. Thereby the factors neopterin and age were dichotomized by the third quartile point of the observed distribution. Untransformed mean values and standard deviations of the formed subgroups are shown in Fig. 3. All the three factors, namely neopterin concentrations ($F = 5.93$; $P = 0.0155$), age ($F = 49.97$; $P < 0.0001$) and sex ($F = 14.20$; $P = 0.0002$) showed an effect on DHEAs concentrations, whereas all the interaction terms (neopterin vs. age, neopterin vs. sex, sex vs. age, neopterin vs. age vs. sex) were statistically not significant. This indicates that neopterin concentrations, age and sex are influencing DHEAS concentrations independently as interactions between these factors are negligible.

4. Discussion

The significant inverse correlation of DHEAs concentrations with age and the higher DHEAs concentrations in males compared to females in our study are in good agreement with today's understanding of the course of physiological DHEA production in humans. (Kroboth et al., 1999; Williams, 2000; James et al., 1997). In agreement with several other studies (for review see Williams, 2000), no relationship between DHEAs concentrations and BMI was found. Notably, the fact that only 0.4% of the studied population showed elevated DHEAs concentrations, but 10% elevated neopterin concentrations, further underlines the inverse correlation between DHEAs and neopterin concentrations. With increasing age DHEAs levels decrease while neopterin production increases. A positive correlation was found between neopterin concentrations and patients' age, which agrees

well with several earlier studies (Reibnegger et al., 1988; Solichova et al., 1999; Diamondstone et al., 1994; Weiss et al., 1994; Ledochowski et al., 1999). And the data are in line with an increased stimulation of the immune system and cytokine production with age.

Recently, it was speculated that oxidative stress might play a role in decreased plasma levels of DHEAs of patients with chronic heart failure by inhibiting DHEA synthesis (Moriyama et al., 2000). Correlations between neopterin and advanced oxidation products were found in patients with diabetic nephropathy, the latter compounds representing a reliable indicator for the degree of oxidant-mediated protein-damage (Abou Deya et al., 1998). Likewise, in nonagerians and in demented patients an inverse relationship has been found between neopterin concentrations and antioxidant α-tocopherol (Sattler et al., 1999; Solichova et al., 1999). As monocytes/macrophages produce increased amounts of reactive oxygen species in parallel to neopterin, the assumption that neopterin may be an indicator for increased oxidative stress seems to be probable (Murr et al., 1999b). Thus, increasing oxidative stress indicated by increasing neopterin levels with age might be responsible for a decrease of DHEA synthesis with increasing age.

By three-way ANOVA, in our study, DHEAs values were statistically significantly influenced by neopterin values, patients' age and sex, whereas interaction terms were not significant. This indicates an independent influence of patient's age and sex and especially of neopterin values on DHEAS concentrations and further favors the assumption that immunoactivation could influence DHEA production. The consequence of this immunoactivation is not necessarily a higher immunocompetence of the older individual. In fact, it was shown that preferentially in patients with signs of a chronic activated immune response functional deficiency of cellular immunity develops (Fuchs et al., 1989). As increasing age is accompanied by lower DHEAs levels, a contribution of relative DHEAs deficiency for immunoactivation cannot be excluded. In HIV-1 infection, which is accompanied by increased neopterin production with disease progression (Fahey et al., 1990; Fuchs et al., 1993), low DHEAs levels are found as well, low DHEAs levels even being predictive for subsequent progression to AIDS (Jacobson et al., 1991). Nevertheless, application of DHEA in patients with symptomatic HIV disease did not markedly improve, e.g. CD4 counts, but lead to a decrease of neopterin levels (Dyner et al., 1993). In patients with SLE serum neopterin levels correlate well with the activity of the disease (Samsonov et al., 1995). As several studies of patients with SLE documented low levels of DHEAs, DHEA was applied and proved as clinically beneficial (van Vollenhoven et al., 1998; Derksen, 1998). Although neopterin levels were not documented in these studies, one might speculate that DHEA which shows immunomodulatory properties, might influence neopterin levels in such patients. Further studies will be necessary to investigate such situations.

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