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Steroid concentrations in atopic dermatitis patients: Reduced plasma DHEAS and increased cortisone levels

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DEAR EDITOR, atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease, which is characterized by a disrupted epidermal barrier function present both in affected skin and in non-affected skin (5). Mainly glucocorticosteroids were used in topical and systemic atopy treatments because of their potent anti-inflammatory effects (8), unfortunately with strong side effects (1). Other steroid hormones have also been reported to have supportive or detrimental effects on atopy (2, 12).

In this study we aimed to investigate how the levels of several steroids are altered in the plasma of AD-patients compared to healthy volunteers.

Plasma samples of 20 healthy volunteers and 20 AD-patients were collected at comparable day time, between 8-10 a.m. in order to minimise the influence of circadian regulation of hormones. In addition, AD-patients were not under treatment of oral glucocorticoids and topical corticosteroids for at least 5 days prior to blood sampling. SCORAD (SCORe Atopic Dermatitis) index of AD-patients was 35.2 (range 13-64) (11). The sterroid hormones were quantified with the commercially available AbsoluteIDQ® Stero17 kit from Biocrates that comprises 16 endogenous steroids (Table 1). 200-450 µL of human plasma were used for analysis. The HPLC-MS/MS analysis in multiple reaction monitoring mode (MRM) using a SCIEX API 4000 QTrap was preceeded by a sample preparation procedure based on solid phase extraction technique in a 96 well plate-format fundamental for pre-cleaning and pre-concentration of the target steroid hormones. For quantification of the steroid compounds, 7-point external calibration curves and 13 isotope-labeled internal standards were used.

Steroid hormones can be divided into five different categories; a) mineralocorticosteroids, b) glucocorticosteroids, c) androgens, d) estrogens and e)

progestagens. Mineralocorticoid, progestagen levels remained mainly unchanged in AD-patients vs healthy volunteers (table 1). From the glucocorticoids the level of cortisone was significantly increased (H: 66.2 ± 17.5 ng/ml, D: 79.7 ± 21.5 ng/ml, p=0.04) while the bioactive metabolite cortisol also displayed a slight tendency of increase (H: 327 ± 154 ng/ml, D: 407 ± 209 ng/ml, p=0.19). The plasma level of the androgen DHEAS was significantly decreased (H: 8857 ± 3918 ng/ml to D: 5187 ± 3362 ng/ml) in AD-patients. Regarding the levels of the estrogen estrone a tendency of decreased levels could be found in AD-patients (H: 0.33 ± 0.14 ng/ml, D: 0.25 ± 0.11 ng/ml, p=0.07). When comparing steroid concentrations in healthy male (HM) volunteers and male AD-patients (DM) (table 2A), the mineralocorticoid, estrogen and progestagen levels were comparable in HM vs DM, while for the androgen DHEAS levels (HM: 10141 ± 4758 ng/ml, DM: 5186 ± 3612 ng/ml) were significantly lower and DHEA levels just displayed a lower tendency (HM: 22.6 ± 7.4 ng/ml, DM: 15.4 ± 7.5 ng/ml, p=0.08) in the plasma of DM. Levels of the steroids mineralocorticoid, estrogens and progestagen in healthy female volunteers (HF) and female AD-patients (DF) (table 2B) were comparable between HF and DF, while the androgen DHEAS levels (HW: 8166 ± 3305 ng/ml, DF: 5188 ± 3323 ng/ml) were significantly lower in plasma of female AD-patients. The glucocorticoid levels of 11-deoxycortisol (HW: 0.47 ± 0.26 ng/ml, DF: 1.16 ± 0.81 ng/ml) were significantly higher and cortisone levels displayed just a higher tendency (HF: 66.0 ± 17.3 ng/ml, DF: 79.5 ± 17.4 ng/ml, p=0.07) in female AD-patients. Steroid concentration of DHT (M: 0.82 ± 0.41 ng/ml, F: 0.56 ± 0.29 ng/ml) and testosterone (M: 11.1 ± 8.39 ng/ml, F: 3.15 ± 4.94 ng/ml) were significantly lower in female individuals (F) vs male individuals (M) (table 2C), while ethiocolanolone levels (M: 0.37 ± 0.17 ng/ml, F: 0.58 ± 0.39 ng/ml) were significantly lower in male individuals. Mineralocorticoid, glucocorticoid, estrogen and progestagen levels were surprisingly comparable in woman and man.

In summary in this study we determined that 2 out of 16 steroids were significantly different in healthy volunteers vs AD-patients. Cortisone, which is higher in AD-patients plasma, is a direct precursor of the bioactive corticosteroid cortisol, which just displays a higher tendency and is known for its potent anti-inflammatory effects

(8). These increased levels of corticosteroids levels in AD-patients plasma may mean that there is an increased formation of anti-inflammatory steroids as a feedback mechanism present in AD-patients which comparably has also been found for precursors of anti-inflammatory eicosanoids (11). In addition a tendency of reduced levels of the anti-inflammatory ERα ligand estrone (6) was found in AD-patients. DHEA is a precursor of testosterone, its levels just display a lower tendency in male AD-patients, while its sulfonation metabolite DHEAS is lower in male and female AD-patients and decreased DHEAS levels were previously found in atopic allergy and chronic urticaria (8-9). In acne patients increased levels of DHEAS were found (3) and further impaired sebum secretion may be the outcome of this decreased DHEAS levels and may even could contribute to AD-phenotype (4). Furthermore DHEA(S) application affects production and secretion of Th1 and Th2 cytokines showing an immunomodulatory effect during allergic sensitization and allergic responses (9-10). The DHEA sulfonation is mediated by dehydroepiandrosterone sulfotransferase (SULT2A1), which is a direct vitamin D receptor target gene (7) and thereby suggests reduced vitamin D-signaling during atopy (13).

We conclude that altered steroid levels in the plasma of AD-patients indicate altered vitamin D signaling (based on reduced DHEA sulfonation) and increased feedback for anti-inflammatory signaling (increased levels of cortisone) present in AD-patients.

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Table 1. Concentration of mineralocorticoids, glucocorticoids, androgens, estrogens and progestagen in healthy volunteers vs AD-patients. SD - standard deviation. Data are shown as mean and standard deviation based on n=20 samples. Using a student ttest a *p* value of less than 0.05 was considered significant.

Steroid concentrations in healthy volunteers (H, n=20) and AD-patients (D, n=20) in ng/ml.

	Healthy volunteers mean ± SD		**AD-patients mean ± SD**		**Significance**
Mineralocorticoids					
11-deoxycorticosterone	0.76	± 0.39	65.5	± 200	0.17
aldosterone	0.51	± 0.39	0.27	± 0.41	0.06
Glucocorticoids					
11-deoxycortisol	0.79	± 0.78	0.98	± 0.65	0.45
cortisone	66.2	± 17.5	**79.7**	**± 21.5**	**0.04**
corticosterone	13.4	± 16.0	10.1	± 8.09	0.45
cortisol	327	± 154	407	± 209	0.19
Androgens					
androstenedione	4.74	± 1.89	4.28	± 1.73	0.44
androsterone	1.40	± 0.70	1.11	± 0.48	0.13
DHEA	21.8	± 9.38	21.2	± 14.4	0.95
DHEAS	8857	± 3918	**5187**	**± 3362**	**<0.01**
DHT	0.71	± 0.35	0.61	± 0.38	0.34
etiocholanolone	0.54	± 0.43	0.45	± 0.22	0.40
testosterone	6.63	± 8.31	6.04	± 7.05	0.72
Estrogens					
E1 (estrone)	0.33	± 0.14	0.25	± 0.11	0.07
E2 (estradiol)	0.58	± 0.46	0.43	± 0.21	0.22
Progestagen					
progesterone	2.02	± 6.04	2.53	± 9.14	0.87

Table 2: Concentration of mineralocorticoids, glucocorticoids, androgens, estrogens and progestagen in (A.) healthy male volunteers (HM) vs male AD-patients (DM), (B.) healthy female volunteers (HF) vs female AD-patients (DF) as well as (C.) female individuals (F, n=24) vs male individuals (M, n=16). SD - standard deviation.

A. Steroid concentrations in healthy male volunteers (HM, n=7) and male AD-patients (DM, n=9) in ng/ml.

	Healthy male mean ± SD		**Male AD-patients mean ± SD**		**Significance**
Mineralocorticoids					
11-deoxycorticosterone	0.93	± 0.31	124	± 290	0.28
aldosterone	0.36	± 0.31	0.21	± 0.17	0.24
Glucocorticoids					
11-deoxycortisol	1.38	± 1.03	0.75	± 0.27	0.10
cortisone	66.6	± 19.2	79.9	± 26.7	0.29
corticosterone	16.4	± 18.4	8.72	± 7.00	0.27
cortisol	332	± 168	389	± 264	0.63
Androgens					
androstenedione	4.17	± 1.34	3.86	± 1.08	0.61
androsterone	1.39	± 0.69	1.07	± 0.55	0.32
DHEA	22.6	± 7.44	15.4	± 7.53	0.08
DHEAS	10141	± 4758	**5186**	**± 3612**	**0.03**
DHT	0.90	± 0.25	0.76	± 0.50	0.52
etiocholanolone	0.33	± 0.15	0.40	± 0.19	0.41
testosterone	14.1	± 7.50	8.81	± 8.73	0.23
Estrogens					
E1 (estrone)	0.29	± 0.12	0.25	± 0.07	0.42
E2 (estradiol)	0.45	± 0.19	0.50	± 0.29	0.70
Progestagen					
progesterone	0.35	± 0.19	0.58	± 0.97	0.56

B. Steroid concentrations in healthy female volunteers (HF, n=13) and female AD-patients (DF, n=11) in ng/ml.

	Healthy female		**Female AD-patients**		**Significance**
Mineralocorticoids					
11-deoxycorticosterone	0.67	± 0.41	17.7	± 56.5	0.29
aldosterone	0.59	± 0.40	0.32	± 0.54	0.18
Glucocorticoids					
11-deoxycortisol	0.47	± 0.26	**1.16**	**± 0.81**	**0.01**
cortisone	66.0	± 17.3	79.5	± 17.4	0.07
corticosterone	11.8	± 14.5	11.1	± 9.08	0.89
cortisol	323	± 147	421	± 164	0.14
Androgens					
androstenedione	5.05	± 2.04	4.62	± 2.11	0.62
androsterone	1.40	± 0.70	1.15	± 0.43	0.31
DHEA	21.3	± 11.3	25.9	± 17.2	0.45
DHEAS	8166	± 3305	**5188**	**± 3323**	**0.04**
DHT	0.61	± 0.35	0.49	± 0.20	0.31
etiocholanolone	0.65	± 0.48	0.49	± 0.25	0.34
testosterone	2.63	± 5.36	3.76	± 4.56	0.59
Estrogens					
E1 (estrone)	0.35	± 0.15	0.26	± 0.14	0.12
E2 (estradiol)	0.65	± 0.53	0.38	± 0.11	0.11
Progestagen					
progesterone	2.92	± 7.24	4.14	± 12.3	0.77

C. Steroid concentration distribution in female individuals (F, n=24) vs male individuals (M, n=16) in ng/ml.

	Male individuals		**Female individuals**		**Significance**
Mineralocorticoids					
11-deoxycorticosterone	70.2	± 221	8.45	± 38.2	0.19
aldosterone	0.28	± 0.24	0.47	± 0.48	0.15
Glucocorticoids					
11-deoxycortisol	1.03	± 0.76	0.79	± 0.67	0.30
cortisone	74	± 24	72.2	± 18.3	0.78
corticosterone	12.1	± 13.3	11.5	± 12.0	0.89
cortisol	364	± 222	368	± 159	0.94

Androgens					
androstenedione	3.99	± 1.17	4.85	± 2.04	0.14
androsterone	1.21	± 0.62	1.29	± 0.59	0.70
DHEA	18.6	± 8.11	23.4	± 14.2	0.22
DHEAS	7354	± 4739	6801	± 3578	0.68
DHT	0.82	± 0.41	**0.56**	**± 0.29**	**0.02**
etiocholanolone	0.37	± 0.17	**0.58**	**± 0.39**	<**0.05**
testosterone	11.1	± 8.39	**3.15**	**± 4.94**	<**0.01**
Estrogens					
E1 (estrone)	0.27	± 0.09	0.31	± 0.15	0.36
E2 (estradiol)	0.48	± 0.25	0.53	± 0.41	0.67
Progestagen					
progesterone	0.48	± 0.73	3.48	± 9.68	0.23

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