We focused on the investigation of lipids in the skin as well as in serum and PBMCs from atopic dermatitis patients vs non affected volunteers. When focusing on the skin we investigated affected skin vs non affected skin samples from AD patients vs the skin of healthy volunteers.

**We focused on three major lipid classes:**

**a) Various steroids in the serum and published the results in Brit. J. Nutr.**

Short report, 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.

**b) Carotenoids and retinoids in serum and published the results in Nutrients:**

**Abstract:** Carotenoids and retinoids are known to alter the allergic response with important physiological roles in the skin and the immune system. In the human organism various carotenoids are present, some of which are retinoid precursors. The bioactive derivatives of these retinoids are the retinoic acids, which can potently activate nuclear hormone receptors such as the retinoic acid receptor and the retinoid X receptor. In this study, we aimed to assess how plasma carotenoid and retinoid concentrations along with the ratio of their isomers are altered in atopic dermatitis (AD) patients (n = 20) compared to healthy volunteers (HV, n = 20). The study indicated that plasma levels of the carotenoids lutein (HV 198 ± 14 ng/mL, AD 158 ± 12 ng/mL, p = 0.02; all values in mean ± SEM), zeaxanthin (HV 349 ± 30 ng/mL, AD 236 ± 18 ng/mL, p ≤ 0.01), as well as the retinoids retinol (HV 216 ± 20 ng/mL, AD 167 ± 17 ng/mL, p = 0.04)

and all-trans-retinoic acid (HV 1.1 ± 0.1 ng/mL, AD 0.7 ± 0.1 ng/mL, p = 0.04) were significantly lower in the AD-patients, while lycopene isomers, α-carotene, and β-carotene levels were comparable to that determined in the healthy volunteers. In addition, the ratios of 13-cis- vs. all-trans-lycopene (HV 0.31 ± 0.01, AD 0.45 ± 0.07, p = 0.03) as well as 13-cis- vs. all-trans-retinoic acid (HV 1.4 ± 0.2, AD 2.6 ± 0.6, p = 0.03) were increased in the plasma of AD-patients indicating an AD-specific 13-cis-isomerisation. A positive correlation with SCORAD was calculated with 13-cis- vs. all-trans-lycopene ratio (r = 0.40, p = 0.01), while a negative correlation was observed with zeaxanthin plasma levels (r = −0.42, p = 0.01). Based on our results, we conclude that in the plasma of AD-patients various carotenoids and retinoids are present at lower concentrations, while the ratio of selected lycopene isomers also differed in the AD-patient group. An increase in plasma isomers of both lycopene and retinoic acid may cause an altered activation of nuclear hormone receptor signaling pathways and thus may be partly responsible for the AD-phenotype.

**c) Free fatty acids and eicosanoids / docosanoids in serum and skin and published the results in Experimental Dermatology.**

**Abstract:** Lipoxygenases (LOX) and cycloxygenase (COX) are the main enzymes for PUFA-metabolism to highly bio-active prostaglandins, leukotrienes, thromboxanes, lipoxines, resolvins and protectins. LOX and COX pathways are important for the regulation of pro-inflammatory or pro-resolving metabolite synthesis and metabolism for various inflammatory diseases such as atopic dermatitis (AD). In this study we determined PUFAs and PUFA-metabolites in serum as well as affected and non-affected skin samples from AD-patients and the dermal expression of various enzymes, binding proteins and receptors involved in these LOX and COX pathways. Decreased EPA and DHA levels in serum and reduced EPA level in affected and non-affected skin were found; in addition n3/n6-PUFA ratios were lower in affected and non-affected skin and serum. Mono-hydroxylated PUFA-metabolites of AA, EPA, DHA and the sum of AA, EPA and DHA metabolites were increased in affected and non-affected skin. COX1 and ALOX12B expression, COX and 12/15-LOX metabolites as well as various lipids, which are known to induce itch (12-HETE, LTB4, TXB2, PGE2 and PGF2) and the ratio of pro-inflammatory vs pro-resolving lipid mediators in non-affected and affected skin as well as in the serum of AD patients were increased, while n3/n6-PUFAs and metabolite ratios were lower in non-affected and affected AD-skin. Expression of COX1 and COX-metabolites were even higher in non-affected AD-skin. To conclude, 12/15-LOX and COX pathways were mainly upregulated, while n3/n6-PUFA and metabolite ratios were lower in AD-patients skin. All these parameters are a hallmark of a pro-inflammatory and non-resolving environment in affected and partly in non-affected skin of AD-patients.

**d) A ready set manuscript is additionally focusing on Vitamin D and RXR activators in serum plus atopic dermatitis specific cytokines.**

**Abstract:** Over the past decade several controversial studies described a relationship between topical or plasma / serum vitamin D levels and atopic diseases. Insufficient plasma vitamin D levels or even a vitamin D deficiency was associated with an increased incidence of atopic disease. Various studies

postulate that a higher dietary intake of vitamin D and a resulting insufficiency or deficiency of vitamin D may be related with an increased risk of developing atopic disease and especially atopic dermatitis (AD). Our goal is to deeper analyze the relationship between AD and plasma vitamin D levels as well as the endogenous retinoid-X receptor ligand 9-cis-13,14-dihydroretinoic acid (9CDHRA) as a ligand for crucial heterodimer binding partner of the vitamin D-receptor, which is activated by the active vitamin D derivative 1.25-dihydroxyvitamin D3 (1.25VD3). In our study, the vitamin D derivatives 25-hydroxyvitamin D3 (25VD3),1.25VD3 and 9CDHRA were determined in the plasma of healthy volunteers (HV, n=17-20) and AD-patients (AD, n=17-20) and no different levels were determined between the HV and AD groups regarding 1.25VD3, 25VD3 and 9CDHRA plasma levels. This initially indicates no direct correlation between plasma vitamin D and 9CDHRA levels with atopy. Further, we calculated correlations between plasma levels of 1.25VD3, 25VD3 and 9CDHRA with known AD-markers like amounts percentile amount of eosinophils in blood (EOS), plasma IgE and SCORAD. Surprisingly, we found various tendencies for negative correlation between the plasma 25VD3 levels and the AD-markers EOS and SCORAD in the AD-patients group, while 1.25VD3 and partly 9CDHRA positively correlated with IgE, EOS and SCORAD. This indicates that metabolic vitamin D bioactivation from 25VD3 to 1.25VD3 as well as the potential co-activation by the RXR-ligand may be an important trigger for atopy prevalence and severity.

e) We already created a half ready manuscript where we used a systems biology approach calculating a systematic connection between all lipids measured in manuscripts a-c plus the data from manuscript (d) with AD-markers and AD-specific cytokines.