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Reduced carotenoid and retinoid concentrations and altered lycopene isomer ratio in plasma of atopic dermatitis patients

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Abstract

In the human organism various carotenoids are present of which, some are retinoid precursors. The bioactive derivatives of these retinoids are the retinoic acids, which can potently activate nuclear hormone receptors like the retinoic acid receptor and the retinoid X receptor. In our study using an HPLC analytical approach 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). We found that plasma levels of the carotenoids lutein (HV 198 ± 68 ng/ml, AD 158 ± 57 ng/ml), zeaxanthin (HV 350 ± 142 ng/ml, AD 236 ± 85) as well as the retinoids retinol (HV 216 ± 89 ng/ml, AD 167 ± 76 ng/ml) and all-*trans*-retinoic acid (HV 1.1 ± 0.6 ng/ml, AD 0.7 ± 0.5 ng/ml) were significantly lower in AD-patients, while lycopene, α-carotene and β-carotene levels were comparable. In addition the ratios of 13-*cis* vs. all-*trans* lycopene as well as 13-*cis* vs. all-*trans* retinoic acid were increased in the plasma of AD-patients indicating an AD-specific 13C-isomerisation. A positive correlation with SCORRAD was calculated with 13-*cis* vs. all-*trans* lycopene ratio, while a negative correlation was observed with zeaxanthin plasma levels. Based on our results we conclude that in the plasma of AD-patients various carotenoids and retinoids are at lower levels, while the ratio of lycopene isomers was also altered. The higher rate of lycopene and retinoic acid isomerisation products might be a consequence of AD or might result in an altered activation of nuclear hormone receptor signaling pathways and thus maybe partly be responsible for the AD-phenotype and additionally may represent a good plasma marker for AD.

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Introduction

Carotenoids and retinoids are considered to have beneficial effects in the prevention of many major diseases and play a crucial role in skin physiology and allergic responses (1). They regulate a variety of physiological processes, like proliferation, differentiation, immune regulation and epidermal barrier function (2). Several skin diseases have been associated with alterations of the retinoid metabolism and signaling (3). These retinoids include both natural forms of vitamin A, retinaldehyde and retinoic acid as well as synthetic retinol analogs. Regarding their source high vitamin A or high pro-vitamin A containing diet resulted in increased plasma levels of all-*trans* retinoic acid thus providing an important link between nutritional factors and retinoic acid signaling mediated pathways (4).

Carotenoids, a family of more than 600 compounds (1, 5, 6), are important micronutrients in the human diet and are also present in the human plasma (7). The mostly studied members such as β-carotene, lycopene, lutein and zeaxanthin are major dietary carotenoids. Amongst them β-carotene is known to have the highest provitamin A activity (5).

Lycopene, a red pigment mainly originating from tomatoes and tomato products, is found as all-*trans* isomer in the majority of the food sources (8) where besides the all-*trans* isomer (AT) the 9-*cis*, 13-*cis* and 15-*cis* isomers are the most predominant forms (9). On the other hand, it is also present in human and animal tissues but mainly as *cis*-isomers, of which the 5-*cis* isoform is the most frequent (10, 11).

Supporting the importance of retinoic acid in atopic dermatitis (AD) (patho)-physiology, in our previous study, we showed that retinoic acid levels are lower in the skin of AD-patients in comparison to healthy volunteers. Based on the observed alterations in retinoid transport, synthesis and concentrations we concluded that retinoid signaling pathways might contribute to AD pathogenesis (12-15).

In this study we aimed to address if the concentrations of carotenoids and retinoids especially of different lycopene and retinoic acid isomers, that are potential activators of the involved retinoic acid pathways also differ in the plasma of AD-patients.

Materials and methods

Study population

After informed consent and the approval of the local Ethics Committee of the University of Debrecen, Hungary, Medical and Health Science Center, peripheral blood was collected from 20 AD-patients (8 male, 12 female; mean age 20 years, range 15-32 years). A group of 20 healthy age-matched volunteers (6 males, 14 females, mean age 21 years, range 19-24 years) served as controls in this study. All AD patients fulfilled the diagnostic criteria established by Hanifin and Rajka (16). The severity and activity of the disease was determined by the SCORAD (SCORe Atopic Dermatitis) index (17) the mean SCORAD was 35.15 (range 13-64).

The same patients plasma were used like in the previous studies (18-20). In 8 of 20 patients the disease started in the first year of life, in 2 patients between ages 3-4, in 7 patients between 6-18 years and in 3 patients in adulthood. In patients' history 10 patients of 20 had rhinitis, 3 had asthma, and 3 had both rhinitis and asthma (table 1). Patients have not been treated with oral glucocorticosteroids, non-steroidal anti-inflammatory drugs or other systemic immunomodulatory agents for at least 4 weeks, also did not receive antihistamins and topical corticosteroids for at least 5 days prior to blood sampling. Patients were tested for plasma total IgE by ELISA (ADALTIS Italia S.p.A., Casalecchio di Reno, Italy) according to the manufacturer's instructions. The severity of the disease was determined with the SCORAD index (17). The rate and absolute count of eosinophils as in whole blood were determined by Advia 120 hematology analyzer (Siemens, Deerfield, USA), as part of the routine clinical investigation of the patients and calculated as percentage (EOS %). The eosinophils in the analyzer were identified based on their scatter properties and myeloperoxidase positivity.

Plasma sample preparation

Peripheral blood was collected into EDTA-containing Vacutainer tubes (Bechton-Dickinson, Cedex, F) and transferred to a 15 ml falcon tube immediately after

collection and then centrifuged at room temperature, 2500 rpm for 15 min. Plasma was removed after centrifugation and kept on -80°C.

HPLC analysis for carotenoids

The plasma samples from 20 healthy volunteers and 20 samples from patients diagnosed with atopic dermatitis were obtained in Debrecen and transported, under appropriate conditions to Liverpool John Moores University, for analysis. Upon arrival the samples were stored at -80°C until they were processed.

Carotenoids and xanthophylls were extracted from plasma samples using the following method: 1.0 mL aliquot of patient's plasma was added to a glass flip-top squat vial and 1.0 mL of ethanol added. The sample was vortexed immediately for 2 seconds, prior to the addition of 1.5 mL diethyl ether. The sample was vortexed again for 2 seconds prior to the addition of 1.5 mL hexane. The sample was then vortexed a last time for 2 seconds and allowed to stand for partition. The top layer of the sample was then removed with a glass Pasteur pipette and dried down in a 4mL amber screw-top vial under oxygen free nitrogen. When dry the sample was resuspended in 100 µl tetrahydrofuran and 400 µL methanol and transferred to an amber 2 mL HPLC vial prior to injection.

All HPLC was performed on an Agilent 1100 series fully automated HPLC (Agilent Technologies UK Ltd. Berkshire, UK) with diode array detection. All samples were analysed by two separate systems using either a C18 or C30 column (VWR International Ltd, Lutterworth, UK). C18 column was used for estimating the concentration of carotenoids (β-carotene, lycopene, β-cryptoxanthin, lutein) (21). The C30 was used or determining the concentration of isomers for β-carotene and lycopene (22, 23). Analysis using a C18 reverse phase column: A sample volume of 50 µL was injected into the HPLC using an auto-sampler. The solvent system used was 66/22/10 acetonitrile/tetrahydrofuran/methanol (0.005% w/v ammonium acetate). The solvent mixture was delivered at a rate of 0.8 mL/min. Lycopene and its components were separated using a 5 µm Gemini (Phenomenex Macclesfield, UK) C18 reverse phase column (4.6 x 250 mm). Analysis using a C30 column: The

solvents system comprised of 50/40/10 MTBE/methanol/ethyl Acetate. This was delivered isocratically at a rate of 0.45 mL/min. The column was a YMC (VWR, Lutterworth, UK) C30 (5 µm 4.6 x 250 mm), and kept at a temperature of 40°C. The detection of the eluted compounds was by diode array screening between 300-600 nm and integration of each peak was performed using the Chemstation software (v10A) supplied by Agilent Technologies UK Ltd, Berkshire, UK.

HPLC MS-MS analysis for retinoids

Concentrations of 13CRA, ATRA and retinol were determined in human plasma samples by our high performance liquid chromatography mass spectrometry - mass spectrometry (HPLC MS-MS) method as described previously (24).

Statistics

The data are shown as mean and standard deviation based on n=20 samples. Statistical analysis was performed using Graph pad Prism using Pearson´s correlation analysis and significance analysis considering a *p* value of less than 0.05 significant. Further graphical evaluations were made using clustered image map (mixOmics, Web interface, (25)).

Results

A. Characterisation of the study cohort

These data were adapted from a previous manuscript using the same patient cohort (18-20). AD-patients and healthy volunteers did not differ in age and gender. Clinical markers of atopic dermatitis like SCORRAD, total IgE and percental amount of eosinophils from PBMCs were significantly increased in addition to increased protein concentrations of the TH1-marker IFNγ and the Th2 marker IL5 that were observed in the plasma of AD-patients (Table 1).

B. Carotenoid concentrations and lycopene isomers

Reduced concentrations of lutein and zeaxanthin in AD-patients

Lutein and zeaxanthin plasma levels were significantly decreased in the plasma of AD patients compared to healthy volunteers (from 198 ± 68 ng/ml to 158 ± 57 ng/ml, $p=0.04$ respectively from 350 ± 142 ng/ml to 236 ± 85 ng/ml, $p>0.01$). α-Carotene and β-carotene levels were comparable in healthy volunteers and AD-patients (Table 2A).

Altered lycopene isomer concentrations in healthy volunteers and AD-patients

Total lycopene levels were non-significantly lower in the plasma of AD-patients (from 281 ± 133 ng/ml to 249 ± 160 ng/ml). Individual concentrations of lycopene isomers, like all-*trans* (from 126 ± 15 ng/ml to 107 ± 74 ng/ml), 9-*cis* (from 25 ± 11 ng/ml to 21 ± 12 ng/ml), and 5-*cis* (from 94 ± 44 ng/ml to 80 ± 54 ng/ml), isomers showed also non-significant decrease in the plasma of AD-patients, while the concentration of 13-*cis* lycopene was non-significantly augmented compared to healthy volunteers from 37 ± 15 ng/ml to 40 ± 21 ng/ml (Table 2B).

All-*trans* lycopene and 13-*cis* lycopene %-amounts of lycopene isomers show significant alterations in AD-patients compared to healthy volunteers

The all-*trans* lycopene %-amounts decreased significantly in AD-patients (42 ± 6 ng/ml), compared to healthy volunteers (44 ± 4 ng/ml), while the 13-*cis* lycopene ratio has been significantly increased (from 13 ± 2 ng/ml to 17 ± 7 ng/ml). 9-*cis* lycopene and 5-*cis* lycopene did not show significant alterations in their %-amounts (Table 2C).

Calculated %-ratios of selected lycopene isomers in healthy volunteers and AD-patients

13-*cis* / all-*trans* lycopene %-ratio has been significantly increased in AD patients compared to healthy volunteers from 0.3 ± 0.1 to 0.4 ± 0.3 (p=0.04), while no alteration could be observed in the 5-*cis* / all-*trans* lycopene ratio (from 0.8 ± 0.2 to 0.8 ± 0.1) (Table 2D).

C. Retinoid concentration and ratios of retinoic acid isomerisation

Reduced retinoic acid and retinol concentrations in AD-patients compared to healthy volunteers

All-*trans* retinoic acid concentrations were significantly lower in the plasma of AD-patients (0.7 ± 0.5 ng/ml) compared to healthy volunteers (1.1 ± 0.6 ng/ml), while 13-*cis* retinoic acid concentrations were non-significantly lower (from 1.2 ± 0.5 ng/ml to 1.0 ± 0.6 ng/ml). Retinol concentrations were also significantly decreased in plasma of AD-patients compared to healthy volunteers (from 216 ± 89 ng/ml to 167 ± 76 ng/ml). Our results showed that both ATRA and 13CRA were present in a lower concentration in the plasma of atopic individuals (Table 2B).

Ratio of plasma levels of retinoic acid isomers 13CRA/ATRA

The 13CRA/ATRA ratio was significantly increased in the plasma of AD-patients from 1.3 ± 0.8 to 2.6 ± 2.7 (p=0.03) (Table 3B).

D. Correlation analysis

Zeaxanthin levels negatively and 13-*cis* / all-*trans* lycopene ratios positively correlate to clinical AD-markers

Clustered image map analysis visualize results in a distance matrix analysis of carotenoids (zeaxanthin, lutein, α-carotene, β-carotene and lycopene), retinoids (13-*cis* , all-*trans* retinoic acid and retinol), ratios (13-*cis* / all-*trans* lycopene, 5-*cis* / all-*trans* lycopene and 13-*cis* / all-*trans* retinoic acid) and percentile amounts of carotenoids (all-*trans,* 13-*cis,* 9-*cis,* 5-*cis* lycopene) with clinical atopy markers like number of eosinophils as percentage from PBMCs (EOS), plasma total IgE levels in kilounits per liter (IgE) and SCORRAD (20). Pearson´s correlation analysis determined significant negative correlation of plasma zeaxanthin levels and positive correlation of 13-*cis* / all-*trans* lycopene ratios with SCORRAD. No positive or negative correlation with plasma levels, calculated percentages and ratios were found with the clinical AD-markers number of eosinophils as percentage from PBMCs (EOS) or plasma total IgE levels in kilounits per liter (IgE).

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Discussion

Atopic dermatitis (AD) is a common chronic inflammatory skin disease, showing structural abnormalities of the epidermal barrier, moreover it is characterized by increased IgE secretion and Th2 response, which contributes to the pathophysiology of the disease (26). Vitamin A and retinoid derivatives play a pivotal role in cutaneous physiology, and various skin diseases have been associated with altered retinoid metabolism and signaling (3, 27). Previously we have demonstrated that in skin biopsy samples of AD-patients compared to healthy volunteers (12) as well as in skin samples from a mouse model of allergen induced dermatitis (14), the retinoid transport, synthesis, signaling, homeostasis and concentrations are severely altered.

Naturally occurring forms of vitamin A and other synthetic retinoid analogues are mainly present in all-*trans* configuration form, but *cis*-isomers also have relevant biological roles, maintaining essential physiological processes in the human organism, such as vision, cellular growth and differentiation, reproduction, normal growth and development, healthy immune system and healthy skin and barrier functions (28, 29). Retinoic acid exists in three major stereoisomeric forms: all-*trans* retinoic acid (ATRA), 9-*cis* retinoic acid (9CRA) and 13-*cis* retinoic acid (13CRA, also known as isotretinoin) (30). While ATRA binds only to retinoic acid receptors (RARs), 9CRA can bind to both RARs and retinoid X receptor (RXRs) and recently the real endogenous RXR ligand 9-*cis*-13,14-dihydroretinoic acid was identified by our group (31, 32) as well as its nutritional precursors presenting a new vitamin A-pathway, named Vitamin A5 (33). On the other hand 13CRA does not bind specifically to RXRs and has a lower affinity to RARs then ATRA or 9CRA (34). Increased retinoic acid (RA) concentrations have been shown also to increase retinoic acid signaling (35), while there is no correlation to serum levels of the pro-vitamin A β-carotene (Supplementary Figure 1). 13CRA is considered to be a non active form of the biologically active ATRA and it is generated endogenously, non-enzymatically by spontaneous isomerization from ATRA (36), or enzymatically by means of a novel identified enzyme 13-*cis* specific isomerohydrolase, which generates exclusively 13-*cis* retinol, a precursor of 13CRA (35). In addition a reduced non enzymatic or enzymatic isomerization back to the all-

trans configuration maybe an alternative reason of this altered isomer-distribution occurring in serum of atopic dermatitis patients (37, 38). This retinoid- and carotenoid-isomerization is still a highly controversial topic and multiple mechanisms may occur.

In our AD-patients lutein and zeaxanthin concentrations were significantly lower in the plasma compared to healthy volunteers and zeaxanthin negatively correlates to the atopy marker SCORRAD as visualized in figure 1. This let us postulate potential positive effects of food rich in zeaxanthin on atopy and zeaxantin as a negative serum marker for atopy. α-Carotene, β-carotene and lycopene concentrations were not altered in atopic patients, which is partly in agreement with a previous study in atopic children (39, 40) and due to increased oxidative stress present in chronic AD (41). Unfortunately carotenoid levels in human serum have a high variability due to altered individual nutrition, chronic inflammatory background and genetic background (40, 42-44).

All-*trans*, 9-*cis* and 5-*cis* lycopene-isomer levels were non-significantly lower, while 13-*cis* lycopene concentrations were higher in the plasma of AD-patients. When looking at the calculated %-amounts of lycopene isomers we found significantly decreased amounts of all-*trans* lycopene and significantly increased amounts of 13-*cis* lycopene in plasma of AD-patients compared to healthy volunteers. 9-*cis* Lycopene and 5-*cis* lycopene ratios remained on a comparable level in atopic subjects. Lycopene even slightly correlated with eosinophil numbers, which overlaps with its pro-inflammatory (45) and RAR-activating potential (46-48) found in animals. The 13-*cis* / all-*trans* lycopene %-ratio was significantly higher in AD-patients and also positively correlated with the clinical AD-marker SCORRAD.

Carotene oxygenases may cleave lycopene to yield retinoid-like derivatives (46, 48, 49). Retinoids have been shown to play important roles in skin homeostasis and signaling and alterations of retinoid signaling are related to several skin diseases and malignancies mediated by RARα, RARγ, RXR and PPARδ-mediated signaling. In our study ATRA and ROL levels were significantly lower in AD-patients, while 13CRA also

showed a decreasing tendency in plasma of atopics. Ratios of the plasma levels of retinoic acid isomers 13CRA / ATRA were also significantly increased in AD-patients. This altered retinoid concentrations in the skin might lead to altered receptor pathway activation resulting in skin abnormalities.

We postulate that specific differences in retinoid or carotenoid isomerization via an enzymatic or non-enzymatic specific 13-*cis*-isomerisation in AD might be a consequence of chronic AD. These 13-*cis* / all-*trans* isomer ratios may have a still unknown biological meaning and may serve as biological relevant priming factor for the AD-phenotype. Markers of 13-*cis*-isomerisation like the 13-*cis* / all-*trans* lycopene %-ratio positively correlate with SCORRAD and may serve as a serum biomarker for AD.

Acknowledgment

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Table 1: Clinical, immunological and basic demographic data from healthy volunteers and AD-patients. * - $p < 0.05$. Total IgE – plasma total IgE levels in kilounits (KU) / L; EOS % - number of eosinophils as percentage from PBMCs. This table is adapted from Mihaly et al. 2013 (20).

	healthy volunteers	**AD-patients**	Sign.
Age in years	21 ± 1	20 ± 5	-
Gender	70% female	60% female	-
SCORRAD	0 ± 0	35.2 ± 16.9	*
total IgE (KU /L)	32 ± 9.2	2941 ± 1134	*
EOS %	2.5 ± 0	7.3 ± 1.2	*
IFNγ (pg/ml)	0.84 ± 0.58	3.09 ± 0.93	*
IL5 (pg/ml)	1.81 ± 0.99	9.40 ± 1.55	*

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Table 2:

A. Carotenoid concentrations in plasma of healthy volunteers and AD-patients in ng/ml. Data are shown as mean and standard deviation based on n=20 samples. * - numbers in bold letters indicate significance.

	Healthy volunteers	AD-patients	Significance
lutein	198 ± 68	158 ± 57	**<0.01**
zeaxanthin	350 ± 142	236 ± 85	**<0.01**
α-carotene	171 ± 91	149 ± 102	0.23
β-carotene	494 ± 345	395 ± 290	0.17

B. Total sums and concentration of lycopene isomers in plasma of healthy volunteers and AD-patients in ng/ml.

lycopene (sum)	281 ± 133	249 ± 160	0.47
lycopene (all-*trans*)	126 ± 15	107 ± 74	0.41
lycopene (13-*cis*)	37 ± 15	40 ± 21	0.57
lycopene (9-*cis*)	25 ± 11	21 ± 12	0.34
lycopene (5-*cis*)	94 ± 44	80 ± 54	0.38

C. Calculated %-amounts of lycopene isomers in plasma of healthy volunteers and AD-patients.

lycopene (all-*trans*)	44 ± 4	42 ± 6	**<0.05**
lycopene (13-*cis*)	13 ± 2	17 ± 7	**0.02**
lycopene (9-*cis*)	9 ± 1	9 ± 1	0.20
lycopene (5-*cis*)	34 ± 2	32 ± 5	0.17

D. Calculated %-ratios of selected lycopene isomers in plasma of healthy volunteers and AD-patients.

13-*cis* / all-*trans* lycopene	0.3 ± 0.1	0.4 ± 0.3	**0.04**
5-*cis* / all-*trans* lycopene	0.8 ± 0.2	0.8 ± 0.1	0.40

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Table 3:

A. Retinoic acid and retinol concentrations in human plasma from healthy volunteers as well as AD-patients. Data are shown as mean and standard deviation based on n=20 samples. ATRA - all-*trans* retinoic acid, 13CRA - 13-*cis* retinoic acid, ROL – retinol. * - numbers in bold letters indicate significance.

	Healthy volunteers (n=20) in ng/ml	AD-patients (n=20) in ng/ml	Significance
ATRA	1.1 ± 0.6	0.7 ± 0.5	**0.01**
13CRA	1.2 ± 0.5	1.0 ± 0.6	0.08
ROL	216 ± 89	167 ± 76	**0.01**
B. Ratio of the plasma levels of retinoic acid isomers 13CRA / ATRA.			
13CRA / ATRA	1.4 ± 0.8	2.6 ± 2.7	**0.03**

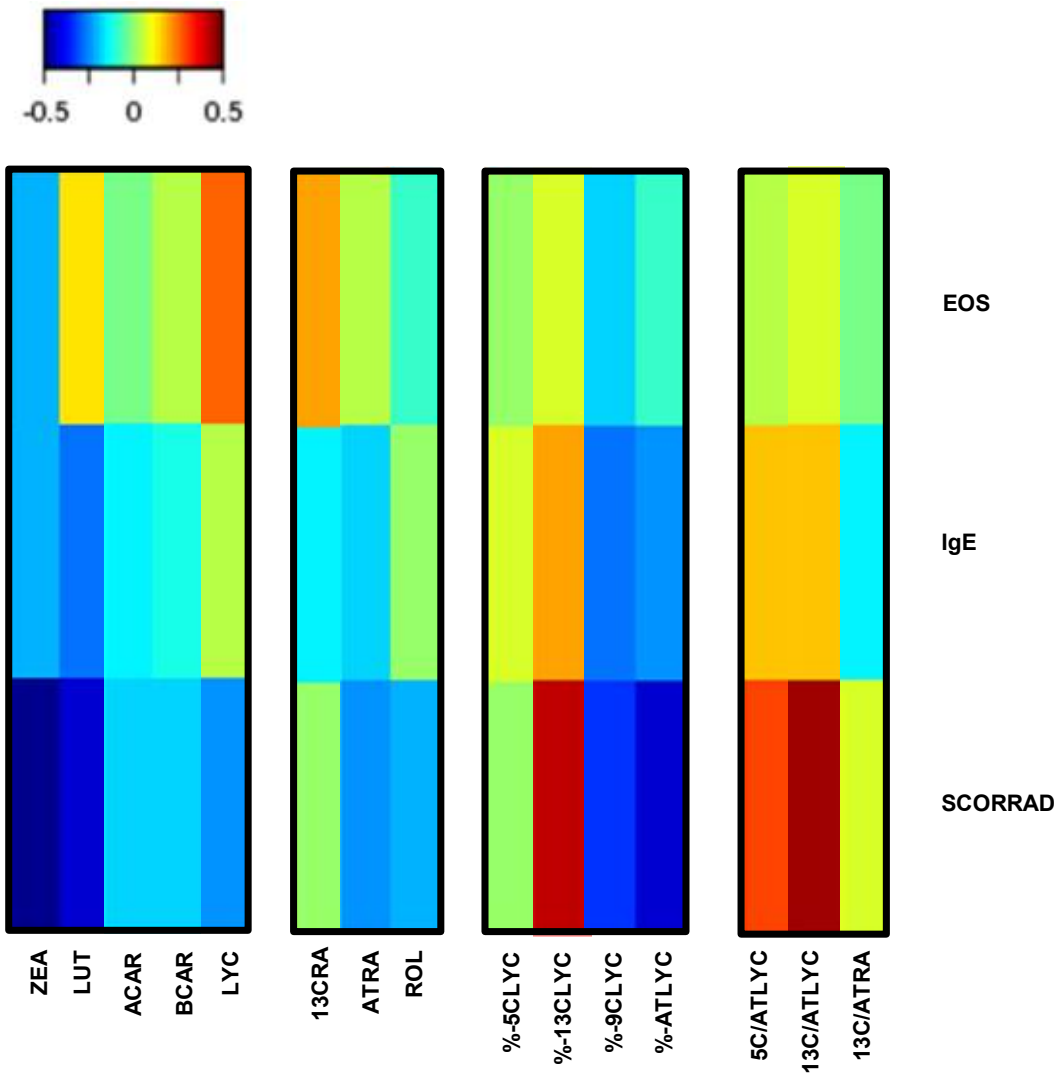
394 **Figure legend:**

395

396 **Figure 1:** Visualisation analysis using Clustered Image Maps (CIM) based on a
397 hierarchical clustering simultaneously applied on the clinical AD markers like number
398 of eosinophils as percentage from PBMCs (EOS), plasma total IgE levels in kilounits
399 per liter (IgE) and SCORRAD with zeaxanthin (ZEA), lutein (LUT), β-carotene
400 (BCAR), α-carotene (ACAR), sum of lycopene (LYC), retinol (ROL), all-*trans* retinoic
401 acid (ATRA), 13-*cis* retinoic acid (13CRA) levels and calculated %-amounts and ratios
402 of retinoids and carotenoids from tables 1 and 2 in a real-valued similarity matrix.
403 The matrix is graphically represented as a 2-dimensional coloured image, where each
404 entry of the matrix is coloured on the basis of its value, and where the rows and
405 columns are reordered according to a hierarchical clustering.

406

407 **Figure 1:**



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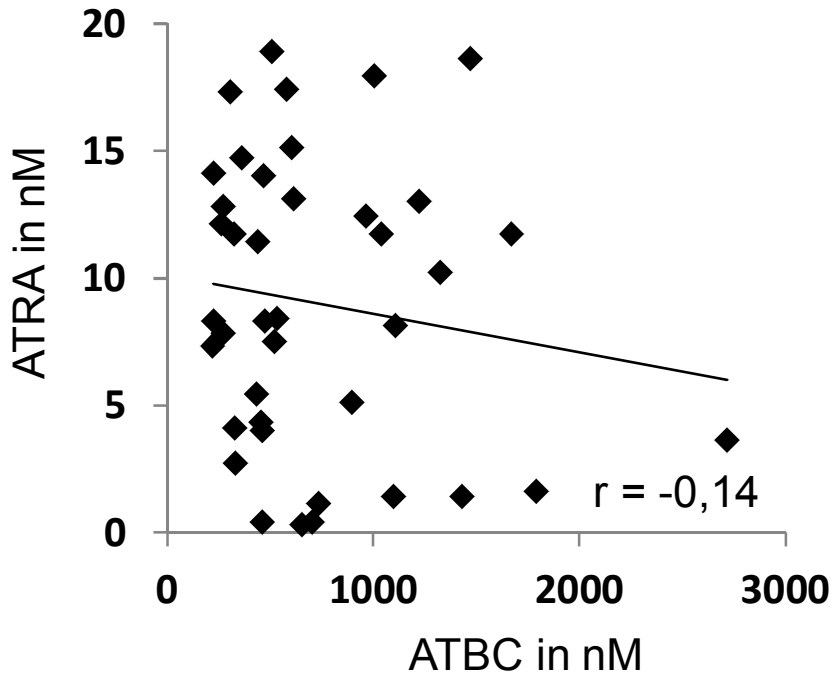
411 **Supplementary Figure1:**

412 Direct correlation based on serum all-trans-β-carotene (ATβC) to all-trans-retinoic
413 acid levels in healthy volunteers and AD-patients (n=40)

414



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References

1. Rühl, R. 2007. Effects of dietary retinoids and carotenoids on immune development. Proc Nutr Soc **66:** 458.
2. Elias, P. M. 2007. The skin barrier as an innate immune element. Semin Immunopathol **29:** 3.
3. Roos, T. C., Jugert, F. K., Merk, H. F., and Bickers, D. R. 1998. Retinoid metabolism in the skin. Pharmacol Rev **50:** 315.
4. Rühl, R., Bub, A., and Watzl, B. 2008. Modulation of plasma all-trans retinoic acid concentrations by the consumption of carotenoid-rich vegetables. Nutrition **24:** 1224.
5. Krinsky, N. I. 2002. Possible biologic mechanisms for a protective role of xanthophylls. J Nutr **132:** 540S.
6. Bohn, T., Desmarchelier, C., Dragsted, L. O., Nielsen, C. S., Stahl, W., Ruhl, R., Keijer, J., and Borel, P. 2017. Host-related factors explaining interindividual variability of carotenoid bioavailability and tissue concentrations in humans. Mol Nutr Food Res **61**.
7. Rühl, R., Sczech, R., Landes, N., Pfluger, P., Kluth, D., and Schweigert, F. J. 2004. Carotenoids and their metabolites are naturally occurring activators of gene expression via the pregnane X receptor. Eur J Nutr **43:** 336.
8. Boileau, T. W., Boileau, A. C., and Erdman, J. W., Jr. 2002. Bioavailability of all-trans and cis-isomers of lycopene. Exp Biol Med (Maywood) **227:** 914.
9. Boileau, T. W., Clinton, S. K., and Erdman, J. W., Jr. 2000. Tissue lycopene concentrations and isomer patterns are affected by androgen status and dietary lycopene concentration in male F344 rats. J Nutr **130:** 1613.
10. Stahl, W., Schwarz, W., Sundquist, A. R., and Sies, H. 1992. cis-trans isomers of lycopene and beta-carotene in human serum and tissues. Arch Biochem Biophys **294:** 173.
11. Erdman, J. W., Jr. 2005. How do nutritional and hormonal status modify the bioavailability, uptake, and distribution of different isomers of lycopene? J Nutr **135:** 2046S.
12. Mihaly, J., Gamlieli, A., Worm, M., and Rühl, R. 2011. Decreased retinoid concentration and retinoid signalling pathways in human atopic dermatitis. Exp Dermatol **20:** 326.

13. Gericke, J., Ittensohn, J., Mihaly, J., Alvarez, S., Alvarez, R., Töröcsik, D., de Lera, A. R., and Rühl, R. 2013. Regulation of retinoid-mediated signaling involved in skin homeostasis by RAR and RXR agonists/antagonists in mouse skin. PLoS ONE **8:** e62643.

14. Gericke, J., Ittensohn, J., Mihaly, J., Dubrac, S., and Rühl, R. 2013. Allergen-induced dermatitis causes alterations in cutaneous retinoid-mediated signaling in mice. PLoS One **8:** e71244.

15. Biswas, R., Chakraborti, G., Mukherjee, K., Bhattacharjee, D., Mallick, S., and Biswas, T. 2018. Retinol Levels in Serum and Chronic Skin Lesions of Atopic Dermatitis. Indian J Dermatol **63:** 251.

16. Hanifin, J., and Rajka, G. 1980. Diagnostic features of atopic dermatitis. Acta Dermatovener Suppl (Stockh) **92:** 44.

17. 1993. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology **186:** 23.

18. Mihaly, J., Sonntag, D., Krebiehl, G., Szegedi, A., Torocsik, D., and Rühl, R. 2015. Steroid concentrations in patients with atopic dermatitis: reduced plasma dehydroepiandrosterone sulfate and increased cortisone levels. Br J Dermatol **172:** 285.

19. Mihaly, J., Marosvolgyi, T., Szegedi, A., Koroskenyi, K., Lucas, R., Torocsik, D., Garcia, A. L., Decsi, T., and Rühl, R. 2014. Increased FADS2-Derived n-6 PUFAs and Reduced n-3 PUFAs in Plasma of Atopic Dermatitis Patients. Skin Pharmacol Physiol **27:** 242.

20. Mihaly, J., Gericke, J., Torocsik, D., Gaspar, K., Szegedi, A., and Rühl, R. 2013. Reduced lipoxygenase and cyclooxygenase mediated signaling in PBMC of atopic dermatitis patients. Prostaglandins Other Lipid Mediat **107:** 35.

21. Lowe, G. M., Bilton, R. F., Davies, I. G., Ford, T. C., Billington, D., and Young, A. J. 1999. Carotenoid composition and antioxidant potential in subfractions of human low-density lipoprotein. Ann Clin Biochem **36 (Pt 3):** 323.

22. Graham, D. L., Carail, M., Caris-Veyrat, C., and Lowe, G. M. 2012. (13Z)- and (9Z)-lycopene isomers are major intermediates in the oxidative degradation of lycopene by cigarette smoke and Sin-1. Free Radic Res **46:** 891.

23. Graham, D. L., Carail, M., Caris-Veyrat, C., and Lowe, G. M. 2010. Cigarette smoke and human plasma lycopene depletion. Food Chem Toxicol **48:** 2413.

24. Rühl, R. 2006. Method to determine 4-oxo-retinoic acids, retinoic acids and retinol in serum and cell extracts by liquid chromatography/diode-array detection atmospheric pressure chemical ionisation tandem mass spectrometry. Rapid Commun Mass Spectrom **20:** 2497.

25. Le Cao, K. A., Gonzalez, I., and Dejean, S. 2009. integrOmics: an R package to unravel relationships between two omics datasets. Bioinformatics **25:** 2855.

26. Leung, D. Y. 2013. New insights into atopic dermatitis: role of skin barrier and immune dysregulation. Allergol Int **62:** 151.

27. Heise, R., Mey, J., Neis, M. M., Marquardt, Y., Joussen, S., Ott, H., Wiederholt, T., Kurschat, P., Megahed, M., Bickers, D. R., Merk, H. F., and Baron, J. M. 2006. Skin retinoid concentrations are modulated by CYP26AI expression restricted to basal keratinocytes in normal human skin and differentiated 3D skin models. J Invest Dermatol **126:** 2473.

28. Vahlquist, A. 1999. What are natural retinoids? Dermatology **199 Suppl 1:** 3.

29. D'Ambrosio, D. N., Clugston, R. D., and Blaner, W. S. 2011. Vitamin A metabolism: an update. Nutrients **3:** 63.

30. Blomhoff, R., and Blomhoff, H. K. 2006. Overview of retinoid metabolism and function. J Neurobiol **66:** 606.

31. Rühl, R., Krzyzosiak, A., Niewiadomska-Cimicka, A., Rochel, N., Szeles, L., Vaz, B., Wietrzych-Schindler, M., Alvarez, S., Szklenar, M., Nagy, L., de Lera, A. R., and Krezel, W. 2015. 9-cis-13,14-dihydroretinoic acid is an endogenous retinoid acting as RXR ligand in mice. PLoS Genet **11:** e1005213.

32. de Lera, A. R., Krezel, W., and Rühl, R. 2016. An Endogenous Mammalian Retinoid X Receptor Ligand, At Last! ChemMedChem **11:** 1027.

33. Krezel, W., Rühl, R., and de Lera, A. R. 2018. Precursor compounds for providing retinoids of their vitamin A5 pathway and uses thereof.

34. Idres, N., Marill, J., Flexor, M. A., and Chabot, G. G. 2002. Activation of retinoic acid receptor-dependent transcription by all-trans-retinoic acid metabolites and isomers. J Biol Chem **277:** 31491.

35. Takahashi, Y., Moiseyev, G., Nikolaeva, O., and Ma, J. X. 2012. Identification of the key residues determining the product specificity of isomerohydrolase. Biochemistry **51:** 4217.

515 36. Kane, M. A., Folias, A. E., Wang, C., and Napoli, J. L. 2008. Quantitative profiling of
516 endogenous retinoic acid in vivo and in vitro by tandem mass spectrometry. Anal
517 Chem **80:** 1702.
518 37. McBee, J. K., Van Hooser, J. P., Jang, G. F., and Palczewski, K. 2001. Isomerization
519 of 11-cis-retinoids to all-trans-retinoids in vitro and in vivo. J Biol Chem **276:** 48483.
520 38. Redmond, T. M., Poliakov, E., Kuo, S., Chander, P., and Gentleman, S. 2010. RPE65,
521 visual cycle retinol isomerase, is not inherently 11-cis-specific: support for a
522 carbocation mechanism of retinol isomerization. J Biol Chem **285:** 1919.
523 39. Grüber, C., Taner, C., Mihaly, J., Matricardi, P. M., Wahn, U., and Rühl, R. 2012.
524 Serum retinoic acid and atopy among children of different ethnic origin living in
525 Germany. J Pediatr Gastroenterol Nutr **54:** 558.
526 40. Rühl, R., Taner, C., Schweigert, F. J., Wahn, U., and Gruber, C. 2010. Serum
527 carotenoids and atopy among children of different ethnic origin living in Germany.
528 Pediatr Allergy Immunol **21:** 1072.
529 41. Antille, C., Sorg, O., Lubbe, J., and Saurat, J. H. 2002. Decreased oxidative state in
530 non-lesional skin of atopic dermatitis. Dermatology **204:** 69.
531 42. Wood, L. G., Garg, M. L., Blake, R. J., Garcia-Caraballo, S., and Gibson, P. G. 2005.
532 Airway and circulating levels of carotenoids in asthma and healthy controls. J Am
533 Coll Nutr **24:** 448.
534 43. Canfield, L. M., Clandinin, M. T., Davies, D. P., Fernandez, M. C., Jackson, J.,
535 Hawkes, J., Goldman, W. J., Pramuk, K., Reyes, H., Sablan, B., Sonobe, T., and Bo,
536 X. 2003. Multinational study of major breast milk carotenoids of healthy mothers. Eur
537 J Nutr **42:** 133.
538 44. Borel, P. 2011. Genetic variations involved in interindividual variability in carotenoid
539 status. Mol Nutr Food Res **56:** 228.
540 45. Bohn, T., Planchon, S., Leclercq, C. C., Renaut, J., Mihaly, J., Beke, G., and Ruhl, R.
541 2018. Proteomic responses of carotenoid and retinol administration to Mongolian
542 gerbils. Food Funct.
543 46. Aydemir, G., Carlsen, H., Blomhoff, R., and Rühl, R. 2012. Lycopene induces
544 Retinoic Acid Receptor transcriptional activation in mice. Mol Nutr Food Res **56:** 702.
545 47. Aydemir, G., Kasiri, Y., Bartok, E. M., Birta, E., Frohlich, K., Bohm, V., Mihaly, J.,
546 and Rühl, R. 2016. Lycopene supplementation restores vitamin A deficiency in mice
547 and possesses thereby partial pro-vitamin A activity transmitted via RAR-signaling.
548 Mol Nutr Food Res.

549 48. Aydemir, G., Kasiri, Y., Birta, E., Beke, G., Garcia, A. L., Bartok, E. M., and Rühl, R.
550 2013. Lycopene-derived bioactive retinoic acid receptors/retinoid-X receptors-
551 activating metabolites may be relevant for lycopene's anti-cancer potential. Mol Nutr
552 Food Res **57:** 739.
553 49. von Lintig, J., Hessel, S., Isken, A., Kiefer, C., Lampert, J. M., Voolstra, O., and Vogt,
554 K. 2005. Towards a better understanding of carotenoid metabolism in animals.
555 Biochim Biophys Acta **1740:** 122.
556