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

Renata Lucas¹,², Johanna Mihály⁴, Gordon M. Lowe³, Daniel L. Graham³, Monika Szklenar⁴, Andrea Szegedi², Daniel Töröcsik², Ralph Rühl¹,⁴

¹ Department of Biochemistry and Molecular Biology, University of Debrecen;
² Department of Dermatology, Faculty of Medicine, University of Debrecen;
³ School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF. UK;
⁴ Paprika Bioanalytics BT, Debrecen, Hungary.

Corresponding author:
Dr. Ralph Rühl
Paprika Bioanalytics BT
Mezőgazdász utca 62
H-4002 Debrecen
Tel: +36-30 2330 501
E-mail: ralphruehl@web.de
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.
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).
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 zeaxanthin 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 atopers. 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
This project was funded by the OTKA project OTKA K109362 (RR), OTKA K 108421 (AS) and GINOP-2.3.2-15-2016-00005 (DT).
**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).

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

<table>
<thead>
<tr>
<th></th>
<th>Healthy volunteers</th>
<th>AD-patients</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>lutein</td>
<td>198 ± 68</td>
<td>158 ± 57</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>zeaxanthin</td>
<td>350 ± 142</td>
<td>236 ± 85</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>α-carotene</td>
<td>171 ± 91</td>
<td>149 ± 102</td>
<td>0.23</td>
</tr>
<tr>
<td>β-carotene</td>
<td>494 ± 345</td>
<td>395 ± 290</td>
<td>0.17</td>
</tr>
</tbody>
</table>

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

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>lycopene (sum)</td>
<td>281 ± 133</td>
<td>249 ± 160</td>
<td>0.47</td>
</tr>
<tr>
<td>lycopene (all-trans)</td>
<td>126 ± 15</td>
<td>107 ± 74</td>
<td>0.41</td>
</tr>
<tr>
<td>lycopene (13-cis)</td>
<td>37 ± 15</td>
<td>40 ± 21</td>
<td>0.57</td>
</tr>
<tr>
<td>lycopene (9-cis)</td>
<td>25 ± 11</td>
<td>21 ± 12</td>
<td>0.34</td>
</tr>
<tr>
<td>lycopene (5-cis)</td>
<td>94 ± 44</td>
<td>80 ± 54</td>
<td>0.38</td>
</tr>
</tbody>
</table>

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

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>lycopene (all-trans)</td>
<td>44 ± 4</td>
<td>42 ± 6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>lycopene (13-cis)</td>
<td>13 ± 2</td>
<td>17 ± 7</td>
<td>0.02</td>
</tr>
<tr>
<td>lycopene (9-cis)</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td>0.20</td>
</tr>
<tr>
<td>lycopene (5-cis)</td>
<td>34 ± 2</td>
<td>32 ± 5</td>
<td>0.17</td>
</tr>
</tbody>
</table>

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

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>13-cis / all-trans lycopene</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.3</td>
<td>0.04</td>
</tr>
<tr>
<td>5-cis / all-trans lycopene</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>0.40</td>
</tr>
</tbody>
</table>
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-\textit{trans} retinoic acid, 13CRA - 13-\textit{cis} retinoic acid, ROL – retinol. * - numbers in bold letters indicate significance.

<table>
<thead>
<tr>
<th></th>
<th>Healthy volunteers (n=20) in ng/ml</th>
<th>AD-patients (n=20) in ng/ml</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATRA</td>
<td>1.1 ± 0.6</td>
<td>0.7 ± 0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>13CRA</td>
<td>1.2 ± 0.5</td>
<td>1.0 ± 0.6</td>
<td>0.08</td>
</tr>
<tr>
<td>ROL</td>
<td>216 ± 89</td>
<td>167 ± 76</td>
<td>0.01</td>
</tr>
</tbody>
</table>

B. Ratio of the plasma levels of retinoic acid isomers 13CRA / ATRA.

<table>
<thead>
<tr>
<th></th>
<th>13CRA / ATRA</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.4 ± 0.8</td>
<td>2.6 ± 2.7</td>
</tr>
</tbody>
</table>
**Figure legend:**

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

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


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.

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

Dermatovener Suppl (Stockh) 92: 44.

the European Task Force on Atopic Dermatitis. Dermatology 186: 23.

Steroid concentrations in patients with atopic dermatitis: reduced plasma
dehydropiandrosterone 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

Reduced lipoxygenase and cyclooxygenase mediated signaling in PBMC of atopic
dermatitis patients. Prostaglandins Other Lipid Mediat 107: 35.

1999. Carotenoid composition and antioxidant potential in subfractions of human low-

lycopene isomers are major intermediates in the oxidative degradation of lycopene by


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


