

PAPER

[View Article Online](#)
[View Journal](#) | [View Issue](#)
Cite this: *Food Funct.*, 2022, **13**, 6534

An immune-shift induced by lycopene; from an eosinophil-dominant type towards an eosinophil/neutrophil-co-dominant type of airway inflammation†

 Attila Bácsi,^a Renáta Lucas,^b Máté István Sütő,^{a,c} Mónika Szklenár,^d Torsten Bohn^e and Ralph Rühl^{*b,d}

Lycopene as the main carotenoid from tomatoes is known to have beneficial effects on various inflammatory diseases. In mice, lycopene ameliorates asthma symptoms and in human asthmatic patients serum lycopene levels are reduced. To further investigate the immunomodulatory effect of lycopene, first, we used a ragweed pollen extract (RWE)-induced asthma model in mice. In a second approach, we established a RWE-induced asthma model in gerbils, because of a more human-like carotenoid absorption in these animals. In RWE-sensitized/RWE-challenged gerbils (C+) following a basal diet, mainly the number of eosinophils in the broncho-alveolar lavage (BAL) significantly increased, comparable to RWE-sensitized/PBS-challenged gerbils (C−). In RWE-sensitized/PBS-challenged gerbils with lycopene-supplementation (L−), an elevated number of mainly neutrophils, in addition to eosinophils, was detected compared to C−, whereas in RWE-sensitized/RWE-challenged animals with lycopene-supplementation (L+), mainly increased neutrophil numbers in BAL were detected compared to C+. Furthermore, using LC-MS, we determined an array of eicosanoids/docosanoids in the lungs and observed that 5-, 8-lipoxygenase (LOX) and cyclooxygenase (COX) pathways were significantly increased after intranasal RWE-challenge in sensitized mice and just by tendency in gerbils. In PBS- and RWE-challenged animals, lycopene-supplementation significantly raised COX-pathway metabolites. In conclusion, we found that lycopene-supplementation resulted in an increased inflammatory influx of neutrophils in combination with increased COX-pathway metabolites. This pro-inflammatory, pro-neutrophil activity induced by lycopene might be an important shift from allergic asthma towards an inflammatory symptomatic asthma type, though with the potential for resolution.

 Received 1st April 2022,
 Accepted 19th May 2022

DOI: 10.1039/d2fo00875k

rsc.li/food-function

Introduction

Allergic asthma is a chronic, inflammatory disorder of the airways. Various eicosanoids and docosanoids are strongly increased and are considered as markers of this pro-inflammatory disease.¹ In the focus are the leukotrienes B4 (LTB4) and

LTC4,^{2–6} as well as the prostaglandins E2 and D2,^{7–10} which are strongly upregulated in bronchoalveolar lavage samples or sputum of human asthmatics.^{2,5,6,11,12} These derivatives mainly originate from the cyclooxygenase-2 (COX-2), and 5-lipoxygenase (5-LOX) pathways.^{13,14}

Asthma incidence was described to be associated with carotenoid intake.^{15–18} Endogenous carotenoid blood levels of β-carotene, lutein, β-cryptoxanthin and lycopene were described to be lower in asthmatics.^{19–24} The major carotenoid in the western diet is lycopene, the red pigment originating from tomatoes and tomato based products in the food as well as in the human body.²⁵ Recent studies describe that especially lycopene supplementation can be beneficial for asthma.^{26–28} Lycopene was described as a potent antioxidant, while its nutritional and endogenous relevance for these antioxidant mediated effects are discussed controversially.²⁹ Novel studies point towards lycopene functioning as a precursor of various nuclear hormone receptor ligands, ranging from retinoic acid

^aDepartment of Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

^bLaboratory of Nutritional Bioactivation and Bioanalysis, Medical and Health Science Center, University of Debrecen, Hungary

^cDoctoral School of Molecular Cellular and Immune Biology, University of Debrecen, Debrecen, Hungary

^dPaprika Bioanalytics BT, Mezőgazdász utca 62, H-4002 Debrecen, Hungary.
 E-mail: ralphruehl@web.de; Tel: +36 30 2330 501

^eNutrition and Health Research Group, Department of Precision Health, Luxembourg Institute of Health, Strassen, Luxembourg

† Electronic supplementary information (ESI) available. See DOI: <https://doi.org/10.1039/d2fo00875k>

receptor (RAR), the retinoid X receptor (RXR) and the peroxisome proliferator-activated receptor (PPAR).^{30–34} Unfortunately, no specific ligand was identified until now and was only recently postulated.³³

The aim of our study was to investigate the effect of lycopene-supplementation in an appropriate animal model, with similar carotenoid metabolism and nutri-kinetics as in humans, the Mongolian gerbil.³⁵ Unfortunately, the mostly used animal model for asthma is the mouse, with carotenoid metabolism hardly comparable to humans.³⁶ Initially, we compared these two animal models, using ragweed pollen extract (RWE) for sensitization and challenge,^{37,38} and then further investigated supplemental lycopene effects on inflammatory cell influx, pro-inflammatory and pro-resolving eicosanoids/docosanoids and antioxidant response.

Materials and methods

Animal experiments

Mice. Experiments were carried out on 8-week-old female Balb/c mice (Charles River, Vac, H). Animals were randomly divided into two groups (6 mice each). Care and handling of animals followed the Helsinki Declaration, European Union regulations and adhered to the guidelines of the Committee for Research and Ethical Issues of IASP. All animal study protocols were approved by the Animal Care and Protection Committee at the University of Debrecen (#7/2011/DE MAB). Animals were maintained in the pathogen-free animal facility of the University of Debrecen.

Sensitization and intranasal challenge of mice. All mice were sensitized with 2 intraperitoneal administrations (on days 0 and 4) of 150 µg per injection endotoxin-free ragweed (*Ambrosia artemisiifolia*) pollen extract (RWE, Greer Laboratories, Lenoir, NC, USA), combined in a 3 : 1 ratio with alum adjuvant (Pierce Laboratories, Rockford, IL, USA) as previously described.³⁷ On day 11, mice were challenged intranasally with 100 µg RWE dissolved in 60 µl of phosphate buffered saline (PBS, PAA Laboratories, Pasching, A), or with same volumes of PBS as a vehicle. On day 14, mice were euthanized and allergic inflammation was evaluated.

Mongolian gerbils. Female Mongolian gerbils (*Meriones unguiculatus*) were also used as experimental animals (Charles River, Budapest, H). The gerbils were sorted into four groups 12 weeks after birth ($n = 6$ per group). The body weight of the animals was measured and the average weight was used for calculating dosages during the dietetic and experimental treatments. The grouping was based on the specific diet and intranasal challenge.

Dietary supplementation. Two groups of Mongolian gerbils ($n = 6$ per group) were treated by oral gavage with Cremophore EL solution (25%) as control groups. The other two groups ($n = 6$ per group) were supplemented with purified lycopene (a gift from CONESA, Badajoz, ES) solubilized in Cremophor EL solution (25%). The dosages of the solutions were calculated based on the average body weight of the experimental animals,

which was 55 g, and was measured one day before the first gavage treatment. The lycopene dosage was 100 mg per kg bw. The dietary supplementations of the gerbils were administered as lycopene or control administration on every second day, within a period of two weeks.

Sensitization and intranasal challenge of gerbils. The experimental animals were sensitized on the second and the sixth day with intraperitoneal injections of endotoxin-free ragweed extract (320 µg RWE per animal), mixed with alum adjuvant (Pierce Laboratories) in a 3 : 1 ratio. On day 13, parallel groups of animals were treated with RWE solution (240 µg in 120 µl PBS) intranasally. The control groups receiving the base diet and the lycopene rich diet fed animals were challenged intranasally with 120 µl PBS per animal by treatment.

Evaluation of allergic inflammation. Inflammatory cell infiltration into the airways was assessed by the analysis of the bronchoalveolar lavage (BAL) fluid at 72 h after intranasal allergen challenge as previously described.³⁸ To collect BAL fluid samples, animals were euthanized with ether and their tracheas were cannulated. Lavage was performed with 2 aliquots of 0.7 ml of ice-cold PBS. The BAL fluid samples were centrifuged (400g for 10 min at 4 °C), the supernatants were then removed and stored at –80 °C for further analysis. Total cell counts in the BAL fluid were determined from an aliquot of the cell suspension. Eosinophils, neutrophils, lymphocytes and macrophages on Wright-Giemsa-stained cyto-centrifuge preparations were enumerated by counting at least 400 cells.

After the lavage, lungs were removed and either immediately frozen at –70 °C until the HPLC-ESI-MS-MS analysis, or fixed for histology by inflating with formalin as previously described.³⁷ The formalin-fixed lungs were embedded in paraffin, sectioned at a thickness of 4 µm, and stained with hematoxylin and eosin. Stained sections were analyzed by using a DP21-CU 2-megapixel digital color camera (Olympus America, Center Valley, PA, USA) mounted on a motorized IX-81 inverted microscope (Olympus).

HPLC-ESI-MS-MS analysis of free fatty acids, eicosanoids and docosanoids in the lungs

The previously described procedure for the HPLC-ESI-MS-MS method was followed, with minor modifications.^{39,40}

Sample preparation. The entire analytical sample preparation procedure is based on an established method used for retinoid quantification. In summary, to 50 mg of lung tissue, 150 µl acetonitrile was added and the lung samples were cut with scissors into small pieces on ice. These mixtures were shaken for 3 min, the precipitated protein was centrifuged at 15 700g (4 °C, 6 min), 130 µL of the resulting supernatant was spiked with 10 µl isotope supernatant mix, evaporated in Eppendorf reaction vials at 30 °C for ~60 min, until the sample volume was ~10 µl. The Eppendorf concentrator was flushed with argon to prevent degradation of eicosanoids and docosanoids. The dried extract was resuspended in approximately 25 µl of HPLC solvent A (64.3% water (water, Chromasolv Plus from Sigma-Aldrich, H) and 35.5% acetonitrile (Merck KGaA, D) and 0.2% formic acid (Fluka, H)) to

yield 35 μl , then vortexed (15 s), shaken (3 min) and transferred into micro-injection inserts vials (Waters, H). These glass vials with the 35 μl extract were transferred into brown screw top vials with PTFE/silicone septa sample (Waters, H) and placed in the pre-cooled (15 $^{\circ}\text{C}$) autosampler of the Waters 2695XE separation module.

Chromatographic system. The HPLC system consisted of a Waters 2695XE separation module (Waters, H) including a gradient pump, autosampler, degasser and a heated column compartment. An MS–MS detector with an ESI ionizing option was used (Micromass Quattro Ultima PT from Waters, UK) as a detector. The system was controlled *via* the MassLynx software (Waters, H).

HPLC conditions. The eluents were degassed in the Waters 2695XE separation module prior to mixing, then passed through an in-line filter (1–2 μm ; Knauer, D) before reaching the analytical column (LiChroCART, 125 $\times$ 2 mm; Superspher 100, RP-18, endcapped) from Merck KGaA (D) embedded in the column compartment. A multilinear gradient was formed from solvent A (see above) and solvent B (methanol; Merck KGaA D). The gradient consisted of the following steps: 0.0 min 20% B, 3.0 min 20% B, 5.0 min 60% B, 15.0 min 100% B, 15.9 min 100% B and 16.0 min 5% B. The flow rate was adjusted to 0.4 ml min^{-1} and the column was heated to 40 $^{\circ}\text{C}$. From the same biological extract, 10 μl for each HPLC analysis was used. This step was performed twice, using the same HPLC conditions and two different MS–MS analysis options for better resolution and quantification of the various analytes.

MS options. The Micromass Quattro Ultima PT was controlled *via* the MassLynx software. Argon with an inlet pressure of 0.8 bar was used. ESI (electro spray ionization source, Waters, Hungary) was flushed by nitrogen continuously produced by the nitrogen generator (Peak Scientific NM30 Nitrogen generator), including the compressor (Waters, H), with the inlet flow set at 3.6×10^{-3} mbar.

Multiple reaction monitoring settings. ESI, with a negative ESI setting, was performed with the HPLC eluent, following the ion source temperature of 85 $^{\circ}\text{C}$. The desolvation gas flow was 780 l h^{-1} , the desolvation temperature was 400 $^{\circ}\text{C}$, the cone gas flow was 10 l h^{-1} , the capillary current was 3 μA , and the cone voltage was 50 V. Aperture voltage was set at 0 V and the RF lens voltage was set at 35 V (for 1) and 0.2 V (for 2). The analyzer settings were LM1 resolution 14.5; HM1 resolution 14.5; Ion energy 1 0.7; entrance –1; collision 0 (collision parameters are set for each substance at the MS – method parameters); exit 2; LM2 resolution 14.5; HM2 resolution 14.5; ion energy 25.0 and a multiplier energy of 650 V.

Multiple reaction monitoring settings for PUFA, eicosanoids/docosanoids semi-quantification. Method A; from 0.0–9.0 min for PGF2 349.0 $\rightarrow$ 192.7, collision energy 22 eV; PD1 and PD1 isomers such as PDX 359.0 $\rightarrow$ 153.3, collision energy 17 eV; TXB2 369.0 $\rightarrow$ 195.0, collision energy 13 eV, PGE3 349.0 $\rightarrow$ 233.0, collision energy 17 eV, LXA4/LXB4 351.0 $\rightarrow$ 115.0, collision energy 12 eV, 8i-PGF3 351.0 $\rightarrow$ 193.0, collision energy 22 eV, 20-COOH-LTB4 365.0 $\rightarrow$ 195.0, collision

energy 13 eV, RvD1, RvD2 375.0 $\rightarrow$ 141.3, collision energy 13 eV; RvE1 375.1 $\rightarrow$ 141.3, collision energy 13 eV; from 9.0–12.5 min for 13-HODE 294.7 $\rightarrow$ 170.7, collision energy 16 eV; 9-HODE 294.7 $\rightarrow$ 194.7, collision energy 16 eV; 5-HEPE 317.0 $\rightarrow$ 115.0, collision energy 17 eV; 12-HEPE 317.0 $\rightarrow$ 179.0, collision energy 17 eV; 15-HEPE 317.0 $\rightarrow$ 219.0, collision energy 17 eV, LTC4 623.9 $\rightarrow$ 272.0, collision energy 14 eV from 12.5–16.0 min for LA 279.3 $\rightarrow$ 279.0, collision energy 10 eV; 8-HEPE 317.0 $\rightarrow$ 255.0, collision energy 17 eV, 18-HEPE 317.0 $\rightarrow$ 259.0, collision energy 17 eV; 5-KETE, 12-KETE and 15-KETE 317.0 $\rightarrow$ 273.0, collision energy 17 eV, 20-HETE 319.0 $\rightarrow$ 245.0, collision energy 10 eV; LTC4 623.9 $\rightarrow$ 272.0, collision energy 14 eV; from 12.5–16.0 min for LA 279.3 $\rightarrow$ 59.2, collision energy 25 eV; EPA 301.0 $\rightarrow$ 203.2, collision energy 12 eV; AA 303.0 $\rightarrow$ 259.3, collision energy 11 eV, DHA 327.1 $\rightarrow$ 29.3, collision energy 14 eV.

Method B; from 0.0–9.8 min for PGE2, d15d12PGD2, PGD2, d15d12PGJ2, PGJ2 315.0 $\rightarrow$ 271.3, collision energy 13 eV; LTB5 333.0 $\rightarrow$ 195.0, collision energy 13 eV; LTB4 335.0 $\rightarrow$ 195.0, collision energy 13 eV; RvE1 349.1 $\rightarrow$ 195.3, collision energy 13 eV; HXA3, HXB3, 20-COOH-AA 335.0 $\rightarrow$ 273.3, collision energy 13 eV; LXA5 349.0 $\rightarrow$ 115.0, collision energy 12 eV; 20-OH-LTB4 351.0 $\rightarrow$ 195.0, collision energy 13 eV; MAR 359.0 $\rightarrow$ 250.0, collision energy 13 eV; from 9.8–12.5 min for 5-HETE 318.7 $\rightarrow$ 115.0, collision energy 14 eV; 8-HETE 319.0 $\rightarrow$ 155.0, collision energy 14 eV; 11-HETE 319.0 $\rightarrow$ 167.0, collision energy 14 eV; 12-HETE 319.0 $\rightarrow$ 179.0, collision energy 14 eV; 15-HETE 319.0 $\rightarrow$ 218.9, collision energy 11 eV; 4-HDHA 343.0 $\rightarrow$ 101.0, collision energy 10 eV; 10-HDHA 343.0 $\rightarrow$ 181.0, collision energy 10 eV; 14-HDHA 343.0 $\rightarrow$ 205.0, collision energy 10 eV; 17-HDHA 343.0 $\rightarrow$ 245.0, collision energy 14 eV, 20-HDHA 343.0 $\rightarrow$ 285.0, collision energy 10 eV; 13-KODE 293.0 $\rightarrow$ 249.0, collision energy 17 eV and from 12.5–16.0 min for LA 279.3 $\rightarrow$ 59.2, collision energy 25 eV; EPA 301.0 $\rightarrow$ 203.2, collision energy 12 eV, AA 303.0 $\rightarrow$ 259.3, collision energy 11 eV, DHA 327.1 $\rightarrow$ 229.3, collision energy 14 eV.

Standard solutions. Stock solutions of the PUFAs, eicosanoids and docosanoids were prepared by dissolving the solutions obtained from Cayman-Chemicals (Tallin, EST), BioMol International (Kastel-Med KFT, Budapest, H), Sigma-Aldrich (Budapest, H), Larodan Lipids (Malmö, S) and Dr Charles Serhan (Harvard University, MS, USA) with methanol to yield a final concentration of 10 $\mu\text{g ml}^{-1}$. All stock solutions were stored in darkness at -80°C . The reference PUFAs, eicosanoids and docosanoids were used for the assay validation.

Quantification. Individual eicosanoids and docosanoids were quantified based on the determination of the “Area Under the Curve” (AUC) and compared with the AUC of standard compounds. To ensure optimal extraction, isotopically labelled standard compounds were used as described earlier.³⁹ This analytical procedure was established for liquids and tissue analysis.

Antioxidant activity

To measure total antioxidant capacity of the airways, BAL was obtained as described above. The BAL fluid samples were cen-

trifuged (400g for 10 min at 4 °C) and then the total antioxidant potential was measured in the supernatants spectrophotometrically at 570 nm, by using a Total Antioxidant

Capacity Assay Kit (Abcam, Cambridge, UK). A standard of known 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, included in the kit) concentration was used to create a

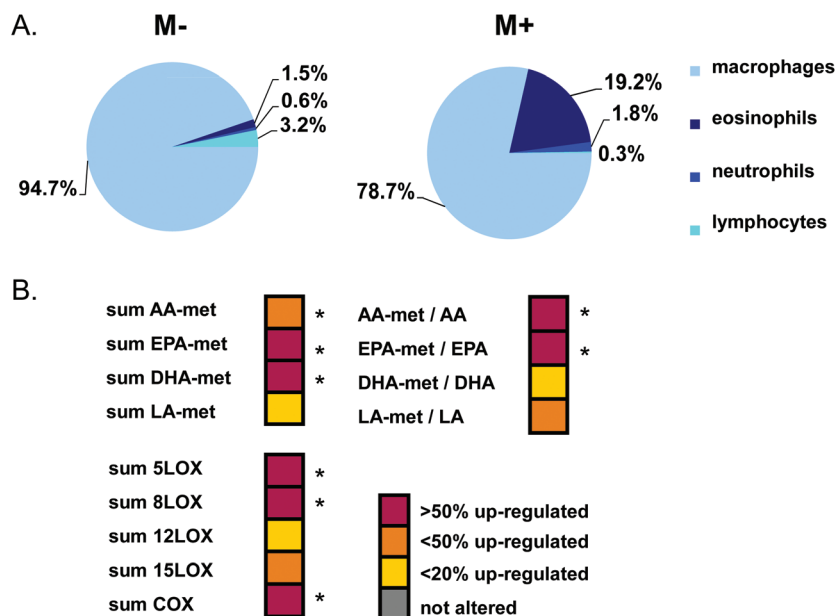


Fig. 1 (A) Percentile amounts of inflammatory cells (macrophages, eosinophils, neutrophils and lymphocytes) extracted from BAL in RWE-sensitized/PBS-challenged mice (M-, $n = 6$) and RWE-sensitized/RWE-challenged mice (M+, $n = 6$). (B) Calculated sums and ratios (M-/M+) originating from eicosanoid/docosanoid analysis displayed as heat maps. For abbreviations, please see Materials and methods section. Error bars indicate SEM and * – indicates statistical significance based on $p < 0.05$.

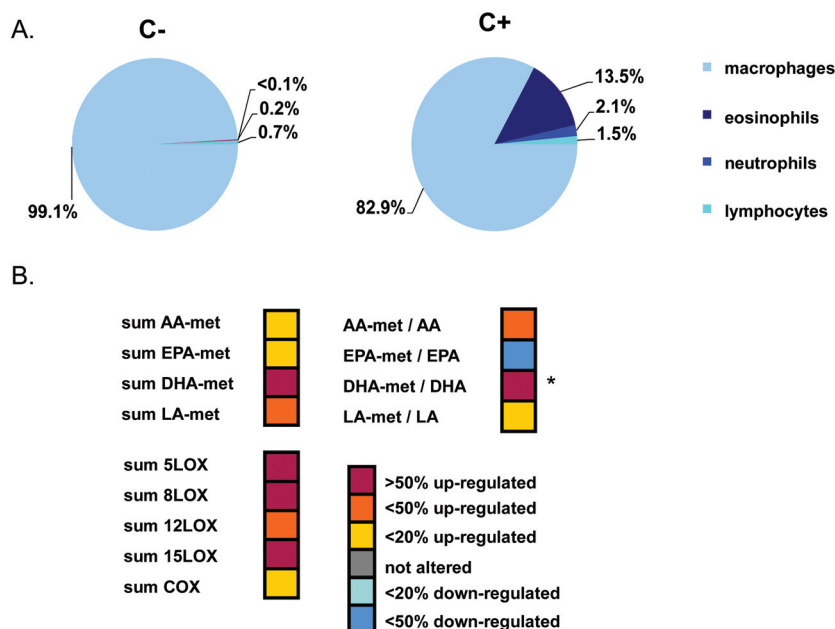


Fig. 2 (A) Percentile amounts of inflammatory cells (macrophages, eosinophils, neutrophils and lymphocytes) extracted from BAL in RWE-sensitized/PBS-challenged gerbils (C-, $n = 6$) and RWE-sensitized/RWE-challenged gerbils (C+, $n = 6$). (B) Calculated sums and ratios (M-/M+) originating from eicosanoid/docosanoid analysis displayed as heat maps. For abbreviations, please see Materials and methods section. Error bars indicate SEM and * – indicates statistical significance based on $p < 0.05$.

calibration curve ($r^2 = 0.98$). The results of the assay were expressed as $\text{nmol } \mu\text{L}^{-1}$ Trolox equivalents.

Statistics

Comparisons between two groups were performed using unpaired two-tailed Student's *t*-test for normally distributed variables with equal variance and Mann-Whitney rank sum test for data that did not pass normality or equal variance test. One-way ANOVA followed by Bonferroni's *post hoc* test was used for comparisons for more than 2 groups. The results were expressed as mean $\pm$ SEM. Analyses were performed by using Excel (Microsoft Corporation) and GraphPad Prism Version 6.0 (GraphPad Software Inc.) software. Differences were considered to be statistically significant at $P < 0.05$.

Results

Mouse allergic airway inflammation model

In this well established mouse model of allergic airway inflammation,³⁷ we observed increased percentages of eosinophils and neutrophils in BAL fluid (Fig. 1A) of sensitized mice challenged with RWE. In addition, strongly elevated sums of 5-, 8-LOX and COX pathway metabolites, as well as significantly increased sums of AA-, EPA-, and DHA-pathway metabolites (Fig. 1B), were detected in the lung of these mice. The ratios of AA-metabolites/AA and EPA-metabolites/EPA were also significantly higher (Fig. 1B). Specific lipid-mediators, such as the 5-, 8-LOX, as well as COX pathway lipid-mediators including LTB₄, LTC₄, d15d12PgD₂, PGD₂, PGJ₂, TXB₂ and PD₁, were also significantly increased in the lung of RWE-challenged mice compared to those of PBS-challenged controls (ESI Table 1†).

Gerbil allergic airway inflammation model

In the BAL fluid samples of gerbils sensitized and challenged with RWE, also increased numbers of eosinophils and neutrophils were observed (Fig. 2A). In addition, various eicosanoids and docosanoids (ESI Table 2†), as well as their calculated sums, were only increased in tendency (Fig. 2B), while the ratio of DHA-metabolites/DHA was significantly increased in the lung of these animals (Fig. 2B). Individual lipid-mediators, such as LTB₄, 8-HETE, RvD₂ and 14-HDHA as well as maresin (MAR), were significantly increased in the lung of RWE-challenged gerbils compared to those of PBS-challenged animals (ESI Table 2†).

Influence of lycopene-supplementation on inflammatory cell distribution in a gerbil allergic airway inflammation model

Lycopene supplementation resulted in increased numbers of lymphocytes, eosinophils and neutrophils in BAL fluid of RWE-sensitized PBS-challenged gerbils (Fig. 3A and B). It also induced some infiltration of inflammatory cells into the perivascular and peribronchial area of the airways (Fig. 5) compared to the control group. In addition, lycopene-supplementation in RWE-sensitized/RWE-challenged gerbils mainly

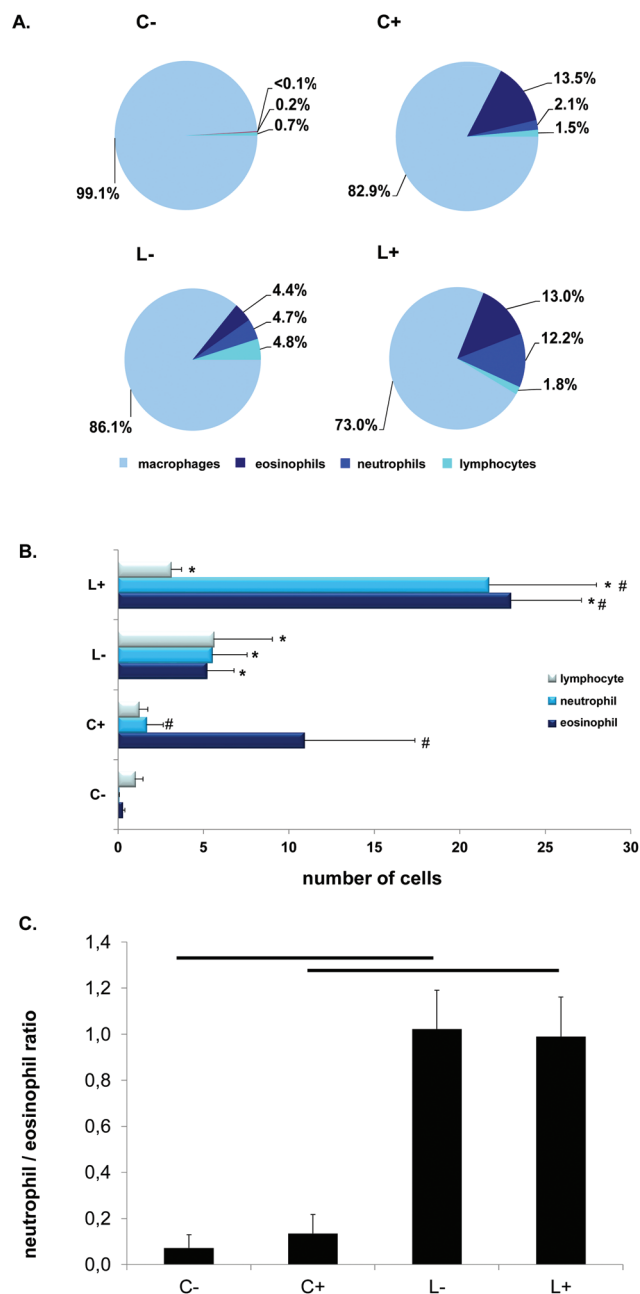


Fig. 3 (A) Percentile amounts of inflammatory cell numbers (macrophages, eosinophils, neutrophils and lymphocytes) extracted from BAL in RWE-sensitized/PBS-challenged gerbils (C-, $n = 6$), RWE-sensitized/RWE-challenged gerbils (C+, $n = 6$), lycopene-supplemented RWE-sensitized/PBS-challenged gerbils (L-, $n = 6$) and lycopene-supplemented RWE-sensitized/RWE-challenged gerbils (L+, $n = 6$). For abbreviations, please see Materials and methods section. (B) Total numbers of inflammatory cells excluding macrophages from $n = 6$ animals. For A and B; the error bars indicate SEM, * – indicates statistical significances versus base diet fed animals while # – indicates statistical significance vs. RWE-sensitized/PBS-challenged animals. (C) Calculated ratio of neutrophil/eosinophil numbers. Statistical significance ($p < 0.05$) is indicated by a bar on top of the two compared groups.

increased neutrophils, besides lymphocytes and eosinophil numbers in BAL fluid (Fig. 3A and B), as well as triggered a robust accumulation of inflammatory cells in the lungs (Fig. 5). Calculation of the ratio between neutrophils/eosinophils in the BAL fluid in the four gerbil groups indicated a strong increase of this ratio in lycopene-administered animals (Fig. 3C).

Influence of lycopene-supplementation on lung eicosanoid levels in a gerbil allergic airway inflammation model

Eicosanoid/docosanoid levels, as well as the calculated sum of COX-pathway metabolites and the sum of AA-metabolites, were significantly increased in both PBS-challenged, as well as RWE-challenged gerbils, after lycopene-supplementation

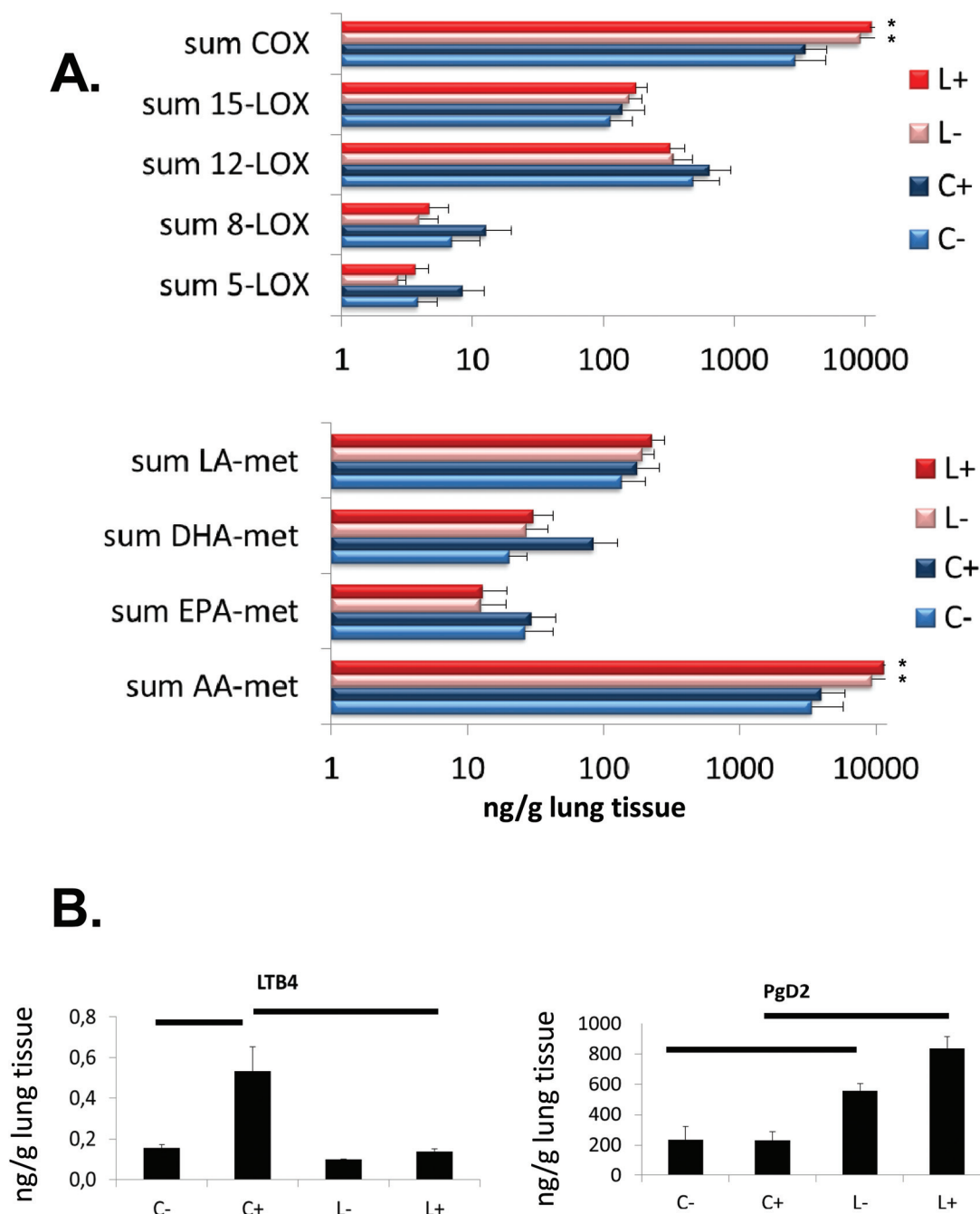


Fig. 4 (A) Calculated sums originating from eicosanoid/docosanoid analysis displayed as a bar chart. * – indicates statistical significance based on $p < 0.05$. For abbreviations, please see Materials and methods section. (B) Concentrations of LTB4 and PgD2 in lung tissue. A line on top of two bars indicates statistical significance based on $p < 0.05$. RWE-sensitized/PBS-challenged gerbils (C–), RWE-sensitized/RWE-challenged gerbils (C+), lycopene-supplemented RWE-sensitized/PBS-challenged gerbils (L–) and lycopene-supplemented RWE-sensitized/RWE-challenged gerbils (L+). For abbreviations, please see Materials and methods section. For A and B; the error bars indicate SEM.

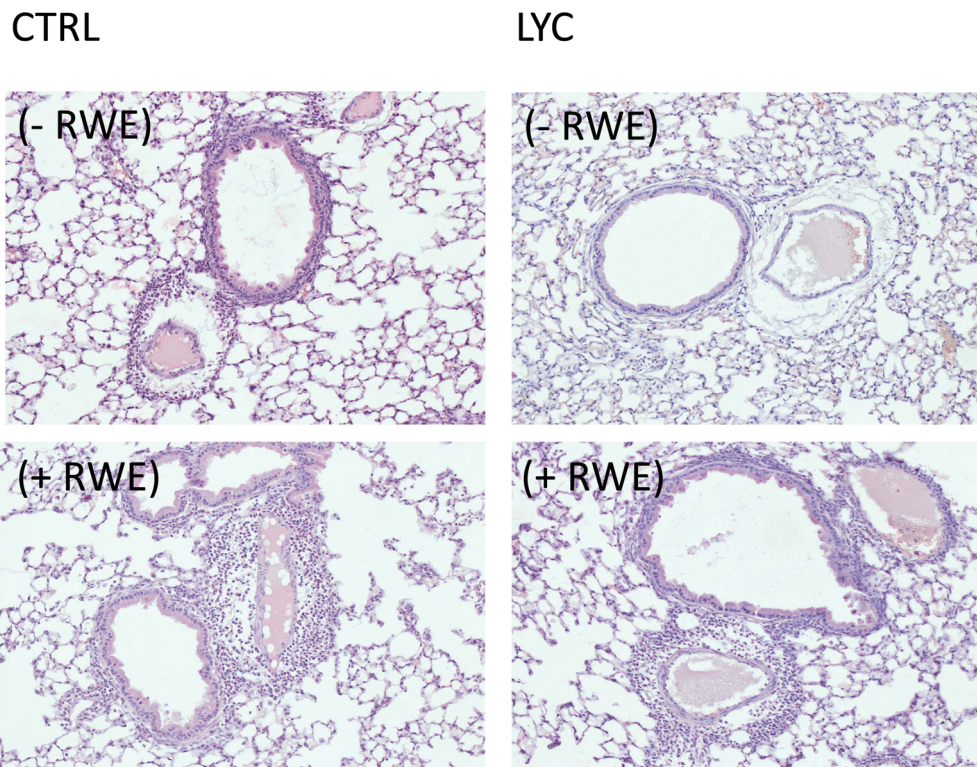


Fig. 5 Left panels (CTRL); accumulation of inflammatory cells into the lungs after intranasal PBS- or RWE-challenge of RWE-sensitized gerbils on base diet. Right panels (LYC); accumulation of inflammatory cells into the lungs after intranasal PBS- or RWE-challenge of RWE-sensitized gerbils on lycopene-supplementation diet. Lungs from gerbils obtained 72 h after intranasal challenges were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Original magnification, $\times 40$.

(Fig. 4A). The pro-inflammatory 5-LOX lipid-mediator LTB₄ was significantly increased after RWE-challenge in gerbils kept on base diet and remained low in either RWE-challenged or PBS-challenged animals on lycopene-supplementation (Fig. 4B). The COX-pathway lipid-mediators PGD₂ (shown in Fig. 4B), PGE₂, d15d12PGD₂, PGJ₂ and d15d12PGJ₂, as well as pro-resolving lipid-metabolites including LXB₄, RvD₂ and MAR, were all significantly increased in lycopene-supplemented RWE-challenged gerbils *vs.* control PBS-challenged gerbils (ESI Table 2†).

Effect of lycopene-supplementation on the total antioxidant capacity of the airways in gerbils

Intranasal RWE-challenge of the base diet and the lycopene-supplemented RWE-sensitized gerbils induced statistically significant increases in the antioxidant capacity of the BAL fluid samples when compared to the PBS challenge ($p < 0.01$ and 0.03) (Fig. 6). However, lycopene-supplementation did not alter the antioxidant capacity of the BAL fluid samples either in RWE-challenged or in PBS-challenged animals (Fig. 6). These findings suggest that effects of lycopene-supplementation on inflammatory cell distribution in the airways and lung eicosanoid levels are likely independent of antioxidant properties of lycopene.

Discussion

The effects of lycopene on asthma in animal models cannot be studied well in the commonly used mouse models, because of strong differences in carotenoid metabolism and nutri-kinetics compared to humans.³⁶ In this study, we established a gerbil asthma model; using a similar treatment schedule as in a mouse model, and we determined comparable influx of inflammatory cells into the airways. In this gerbil model, we applied lycopene to RWE-sensitized/PBS-challenged and RWE-sensitized/RWE-challenged gerbils and observed significant alterations of the influx of inflammatory cells, including lymphocytes, eosinophils and especially neutrophils, into the lungs. This increased influx of inflammatory cells was associated with an increase of COX-metabolites, with a pivotal pro-inflammatory/pro-resolving activity (ref. 41 as reviewed in ref. 9), pointing towards a predominant-neutrophil and COX-metabolite lycopene-mediated inflammatory pathway.

Neutrophils are usually considered as major prominent cells in asthma development and maintenance, and its numbers in BAL are associated with the severity and acute exacerbation of asthma.^{42–45} We observed a strong increase of neutrophils in BAL of non-sensitized animals, as well as in sensitized animals following lycopene-administration. This may mean that lycopene is a critical risk factor in asthma

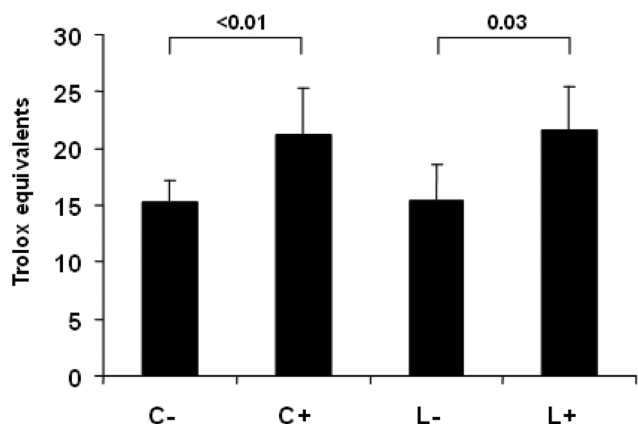


Fig. 6 Antioxidant potential was measured spectrophotometrically in the supernatant of the BAL fluid samples and expressed as Trolox equivalents. Data are presented as means $\pm$ SEM. RWE-sensitized/PBS-challenged gerbils (C-, $n = 6$), RWE-sensitized/RWE-challenged gerbils (C+; $n = 6$), lycopene-supplemented RWE-sensitized/PBS-challenged gerbils (L-, $n = 6$) and lycopene-supplemented RWE-sensitized, RWE-challenged gerbils (L+; $n = 6$).

development and chronification, which seems to be in contrast to many supplementation studies in mice and humans.^{17,26–28,46} The main difference is that (a) we used an animal model with good carotenoid absorbance and comparable metabolism to humans in contrast to the less appropriate animal model, *i.e.* mouse³⁶ and (b) we employed pure lycopene supplementation, while all other studies used tomato extracts^{26,27,46} rich in lycopene plus other carotenoids and retinoids.

Our previous studies pointed towards lycopene as a precursor of further still unidentified ligands of nuclear hormone receptors, such as peroxisome proliferator-activated receptors (PPARs), retinoid X receptor (RXR), and most importantly the retinoic acid receptor (RAR).^{31–34} RAR-ligands have been described to be involved in pro-allergic and pro-inflammatory, but also pro-resolving effects.^{25,47–53} We predict that also in the gerbil asthma model, lycopene can efficiently be converted to lycopene-derived RAR-activators and thereby stimulate immune responses. It appears that especially lycopene is a factor responsible specifically for neutrophil proliferation, confirming the theory that lycopene is a potent source for endogenous still non-identified RAR-ligands³³ and further RAR-mediated signaling.^{31,32} Another option is that RAR-mediated signaling induces and favors differentiation towards neutrophils.^{54,55} Alternative studies claim that lycopene is a potent antioxidant, while its endogenous and nutritional relevance still remains controversial.²⁹ In our studies, we could find no evidence of altered antioxidant activity upon lycopene administration (Fig. 6), confirming the previous vision of non-nutritional relevance *via* this mechanism.

This increase of mainly neutrophils in gerbils goes also “hand in hand” with strongly increased COX-metabolites, such as prostaglandins D, F, J and E, as well as thromboxane B2, indicating a general strong increase of a non-allergy specific immune response,^{42,44,45} but also a potential start of further pro-resolving activities.^{7,56–61} An allergy specific immune

response is mainly an eosinophil driven answer,^{11,27,42,62} with additional high 5-LOX pathway metabolites, such as leukotrienes and especially LTB4.^{3,63,64} After lycopene-supplementation, LTB4 levels were significantly lower or remaining low, which suggests a potential allergy beneficial activity mediated *via* increased eosinophil apoptotic effects, induced mainly by the neutrophil-originating COX-metabolite PgD2.^{7,58} Eosinophils were described as major cell types expressing 5-LOX and synthesizing leukotrienes,^{65–67} and were described to have delayed apoptosis in asthmatics.⁶⁸ Currently, it is under discussion whether a switch from the acute 5-LOX/LTB4 inflammation towards a prostaglandin-dominant answer is, in addition to a chronification of inflammation, being also the starting point for further pro-resolving processes.^{41,69} Activation of PPAR γ -mediated signaling by prostaglandins⁷⁰ for the PPAR and by the heterodimer partner RXR by the endogenous RXR-ligand 9-*cis*-13,14-dihydroretinoic acid,^{71,72} or by alternative non-identified lycopene-metabolites,^{33,73} seems to be a crucial step for further resolution and is induced by lycopene-supplementation. This potential mechanism corresponds to studies where we found that lycopene has beneficial activity for asthma prevalence and severity.

Usually, in asthmatic individuals, lycopene levels are significantly reduced,^{19,22,23} which may indicate that (a) too low lycopene is a risk factor for asthma or (b) the human body is specifically reducing its circulating lycopene levels and thereby preventing a potential acute asthma exacerbation. In addition, in human supplementation studies using lycopene-enriched food, reduced neutrophil numbers were observed in asthmatics.^{26,28} Again, in these studies not pure lycopene, but food rich in lycopene, retinoids and other carotenoids was given in nutritional relevant amounts.²⁶ In contrast and in coherence with our study, alternative studies also found pro-inflammatory effects associated with lycopene.^{74–76}

In summary, lycopene supplementation to gerbils resulted in a general increase of neutrophils and COX-metabolites in lung tissue, indicating inflammatory chronic asthma type *vs.* a pro-allergic type of asthma. The previous conclusion to recommend lycopene-supplementation and tomato-product-supplementation appears rather controversial, and dietary recommendations regarding lycopene or tomato products for asthma prevention should still be considered with care. Human supplementation or treatment studies employing pure lycopene or tomato products should be studied in further detail and under long-term supplementation in patients with chronic asthma or during asthmatic sensitization.

Conflicts of interest

There are no conflicts to declare.

References

- 1 K. S. Wan and W. F. Wu, Eicosanoids in asthma, *Acta Paediatr. Taiwan*, 2007, **48**, 299–304.

- 2 A. P. Sampson, R. U. Thomas, J. F. Costello and P. J. Piper, Enhanced leukotriene synthesis in leukocytes of atopic and asthmatic subjects, *Br. J. Clin. Pharmacol.*, 1992, **33**, 423–430.
- 3 M. K. Oyoshi, R. He, Y. Li, S. Mondal, J. Yoon, R. Afshar, M. Chen, D. M. Lee, H. R. Luo, A. D. Luster, J. S. Cho, L. S. Miller, A. Larson, G. F. Murphy and R. S. Geha, Leukotriene B₄-driven neutrophil recruitment to the skin is essential for allergic skin inflammation, *Immunity*, 2012, **37**, 747–758.
- 4 V. Capra, M. D. Thompson, A. Sala, D. E. Cole, G. Folco and G. E. Rovati, Cysteinyl-leukotrienes and their receptors in asthma and other inflammatory diseases: critical update and emerging trends, *Med. Res. Rev.*, 2007, **27**, 469–527.
- 5 A. J. Jame, P. M. Lackie, A. M. Cazaly, I. Sayers, J. F. Penrose, S. T. Holgate and A. P. Sampson, Human bronchial epithelial cells express an active and inducible biosynthetic pathway for leukotrienes B₄ and C₄, *Clin. Exp. Allergy*, 2007, **37**, 880–892.
- 6 S. H. Wu, P. L. Yin, Y. M. Zhang and H. X. Tao, Reversed changes of lipoxin A₄ and leukotrienes in children with asthma in different severity degree, *Pediatr. Pulmonol.*, 2010, **45**, 333–340.
- 7 T. Murata, K. Aritake, Y. Tsubosaka, T. Maruyama, T. Nakagawa, M. Hori, H. Hirai, M. Nakamura, S. Narumiya, Y. Urade and H. Ozaki, Anti-inflammatory role of PGD₂ in acute lung inflammation and therapeutic application of its signal enhancement, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 5205–5210.
- 8 L. Swedin, R. Ellis, T. Neimert-Andersson, A. Ryrfeldt, G. Nilsson, M. Inman, S. E. Dahlen and M. Adner, Prostaglandin modulation of airway inflammation and hyperresponsiveness in mice sensitized without adjuvant, *Prostaglandins Other Lipid Mediators*, 2010, **92**, 44–53.
- 9 T. Oguma, K. Asano and A. Ishizaka, Role of prostaglandin D₂ and its receptors in the pathophysiology of asthma, *Allergol. Int.*, 2008, **57**, 307–312.
- 10 L. S. Harrington, R. Lucas, S. K. McMaster, L. Moreno, G. Scadding, T. D. Warner and J. A. Mitchell, COX-1, and not COX-2 activity, regulates airway function: relevance to aspirin-sensitive asthma, *FASEB J.*, 2008, **22**, 4005–4010.
- 11 S. Aggarwal, Y. P. Moodley, P. J. Thompson and N. L. Misso, Prostaglandin E₂ and cysteinyl leukotriene concentrations in sputum: association with asthma severity and eosinophilic inflammation, *Clin. Exp. Allergy*, 2010, **40**, 85–93.
- 12 K. Kontogianni, P. Bakakos, K. Kostikas, G. Hillas, A. Papaportfyriou, S. Papiris, N. G. Koulouris and S. Loukides, Levels of prostaglandin E₂ and Cysteinyl-leukotrienes in sputum supernatant of patients with asthma: the effect of smoking, *Clin. Exp. Allergy*, 2012, **43**, 616–624.
- 13 O. Quehenberger, A. M. Armando, A. H. Brown, S. B. Milne, D. S. Myers, A. H. Merrill, S. Bandyopadhyay, K. N. Jones, S. Kelly, R. L. Shaner, C. M. Sullards, E. Wang, R. C. Murphy, R. M. Barkley, T. J. Leiker, C. R. Raetz, Z. Guan, G. M. Laird, D. A. Six, D. W. Russell, J. G. McDonald, S. Subramaniam, E. Fahy and E. A. Dennis, Lipidomics reveals a remarkable diversity of lipids in human plasma, *J. Lipid Res.*, 2010, **51**, 3299–3305.
- 14 M. W. Buczynski, D. S. Dumlao and E. A. Dennis, Thematic Review Series: Proteomics. An integrated omics analysis of eicosanoid biology, *J. Lipid Res.*, 2009, **50**, 1015–1038.
- 15 N. L. Misso, J. Brooks-Wildhaber, S. Ray, H. Vally and P. J. Thompson, Plasma concentrations of dietary and non-dietary antioxidants are low in severe asthma, *Eur. Respir. J.*, 2005, **26**, 257–264.
- 16 G. Devereux and A. Seaton, Diet as a risk factor for atopy and asthma, *J. Allergy Clin. Immunol.*, 2005, **115**, 1109–1117, quiz 1118.
- 17 G. Riccioni, M. Barbara, T. Bucciarelli, C. di Ilio and N. D'Orazio, Antioxidant vitamin supplementation in asthma, *Ann. Clin. Lab. Sci.*, 2007, **37**, 96–101.
- 18 R. Rühl, Non-pro-vitamin A and pro-vitamin A carotenoids in atopy development, *Int. Arch. Allergy Appl. Immunol.*, 2013, **161**, 99–115.
- 19 G. Riccioni, T. Bucciarelli, B. Mancini, C. Di Ilio, R. Della Vecchia and N. D'Orazio, Plasma lycopene and antioxidant vitamins in asthma: the PLAVA study, *J. Asthma*, 2007, **44**, 429–432.
- 20 B. C. Schock, I. S. Young, V. Brown, P. S. Fitch, M. D. Shields and M. Ennis, Antioxidants and oxidative stress in BAL fluid of atopic asthmatic children, *Pediatr. Res.*, 2003, **53**, 375–381.
- 21 I. Kompauer, J. Heinrich, G. Wolfram and J. Linseisen, Association of carotenoids, tocopherols and vitamin C in plasma with allergic rhinitis and allergic sensitisation in adults, *Public Health Nutr.*, 2006, **9**, 472–479.
- 22 L. G. Wood, M. L. Garg, R. J. Blake, S. Garcia-Caraballo and P. G. Gibson, Airway and circulating levels of carotenoids in asthma and healthy controls, *J. Am. Coll. Nutr.*, 2005, **24**, 448–455.
- 23 G. Riccioni, T. Bucciarelli, B. Mancini, C. Di Ilio and N. D'Orazio, Plasma lycopene concentration is low in subjects with stable asthma, *Allergy*, 2006, **61**, 1371–1372.
- 24 A. M. Al Senaidy, Serum vitamin A and beta-carotene levels in children with asthma, *J. Asthma*, 2009, **46**, 699–702.
- 25 R. Rühl, Effects of dietary retinoids and carotenoids on immune development, *Proc. Nutr. Soc.*, 2007, **66**, 458–469.
- 26 L. G. Wood, M. L. Garg, H. Powell and P. G. Gibson, Lycopene-rich treatments modify noneosinophilic airway inflammation in asthma: proof of concept, *Free Radical Res.*, 2008, **42**, 94–102.
- 27 L. C. Hazlewood, L. G. Wood, P. M. Hansbro and P. S. Foster, Dietary lycopene supplementation suppresses Th₂ responses and lung eosinophilia in a mouse model of allergic asthma, *J. Nutr. Biochem.*, 2010, **22**, 95–100.
- 28 C. M. Lee, J. H. Chang, D. O. Moon, Y. H. Choi, I. W. Choi, Y. M. Park and G. Y. Kim, Lycopene suppresses ovalbumin-induced airway inflammation in a murine model of asthma, *Biochem. Biophys. Res. Commun.*, 2008, **374**, 248–252.
- 29 J. W. Erdman, Jr., N. A. Ford and B. L. Lindshield, Are the health attributes of lycopene related to its antioxidant function?, *Arch. Biochem. Biophys.*, 2009, **483**, 229–235.

- 30 P. C. Calder, P. Yaqoob, F. Thies, F. A. Wallace and E. A. Miles, Fatty acids and lymphocyte functions, *Br. J. Nutr.*, 2002, **87**(Suppl. 1), S31–S48.
- 31 G. Aydemir, H. Carlsen, R. Blomhoff and R. Rühl, Lycopene induces Retinoic Acid Receptor transcriptional activation in mice, *Mol. Nutr. Food Res.*, 2012, **56**, 702–712.
- 32 G. Aydemir, Y. Kasiri, E. M. Bartok, E. Birta, K. Frohlich, V. Bohm, J. Mihaly and R. Rühl, Lycopene supplementation restores vitamin A deficiency in mice and possesses thereby partial pro-vitamin A activity transmitted via RAR-signaling, *Mol. Nutr. Food Res.*, 2016, **60**, 203–211.
- 33 G. Aydemir, Y. Kasiri, E. Birta, G. Beke, A. L. Garcia, E. M. Bartok and R. Rühl, Lycopene-derived bioactive retinoic acid receptors/retinoid-X receptors-activating metabolites may be relevant for lycopene's anti-cancer potential, *Mol. Nutr. Food Res.*, 2013, **57**, 739–747.
- 34 C. Caris-Veyrat, A. L. Garcia, E. Reynaud, R. Lucas, G. Aydemir and R. Rühl, A review about lycopene-induced nuclear hormone receptor signalling in inflammation and lipid metabolism via still unknown endogenous Apo-10'-lycopenoids, *Int. J. Vitam. Nutr. Res.*, 2016, **86**, 62–70.
- 35 C. M. Lee, J. D. Lederman, N. E. Hofmann and J. W. Erdman, The mongolian gerbil (*Meriones unguiculatus*) is an appropriate animal model for evaluation of the conversion of beta-carotene to vitamin A, *J. Nutr.*, 1998, **128**, 280–286.
- 36 C. M. Lee, A. C. Boileau, T. W. Boileau, A. W. Williams, K. S. Swanson, K. A. Heintz and J. W. Erdman, Jr., Review of animal models in carotenoid research, *J. Nutr.*, 1999, **129**, 2271–2277.
- 37 I. Boldogh, A. Bacsı, B. K. Choudhury, N. Dharajiya, R. Alam, T. K. Hazra, S. Mitra, R. M. Goldblum and S. Sur, ROS generated by pollen NADPH oxidase provide a signal that augments antigen-induced allergic airway inflammation, *J. Clin. Invest.*, 2005, **115**, 2169–2179.
- 38 A. Bacsı, B. K. Choudhury, N. Dharajiya, S. Sur and I. Boldogh, Subpollen particles: carriers of allergenic proteins and oxidases, *J. Allergy Clin. Immunol.*, 2006, **118**, 844–850.
- 39 M. Szklenar, J. Kalkowski, V. Stangl, M. Lorenz and R. Rühl, Eicosanoids and docosanoids in plasma and aorta of healthy and atherosclerotic rabbits, *J. Vasc. Res.*, 2013, **50**, 372–382.
- 40 E. Csanky, R. Rühl, B. Scholtz, A. Vasko, L. Takacs and W. M. Hempel, Lipid metabolite levels of prostaglandin D2 and eicosapentaenoic acid recovered from bronchoalveolar lavage fluid correlate with lung function of chronic obstructive pulmonary disease patients and controls, *Electrophoresis*, 2009, **30**, 1228–1234.
- 41 D. W. Gilroy, P. R. Colville-Nash, D. Willis, J. Chivers, M. J. Paul-Clark and D. A. Willoughby, Inducible cyclooxygenase may have anti-inflammatory properties, *Nat. Med.*, 1999, **5**, 698–701.
- 42 P. Bogaert, T. Naessens, S. De Koker, B. Hennuy, J. Hacha, M. Smet, D. Cataldo, E. Di Valentin, J. Piette, K. G. Tournoy and J. Grooten, Inflammatory signatures for eosinophilic vs. neutrophilic allergic pulmonary inflammation reveal critical regulatory checkpoints, *Am. J. Physiol.: Lung Cell. Mol. Physiol.*, 2011, **300**, L679–L690.
- 43 A. C. Drews, M. M. Pizzichini, E. Pizzichini, M. U. Pereira, P. M. Pitrez, M. H. Jones, P. D. Sly and R. T. Stein, Neutrophilic airway inflammation is a main feature of induced sputum in nonatopic asthmatic children, *Allergy*, 2009, **64**, 1597–1601.
- 44 W. C. Moore, A. T. Hastie, X. Li, H. Li, W. W. Busse, N. N. Jarjour, S. E. Wenzel, S. P. Peters, D. A. Meyers and E. R. Bleeker, Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis, *J. Allergy Clin. Immunol.*, 2013, **133**, 1557–1563.e1555.
- 45 A. V. Kamath, I. D. Pavord, P. R. Ruparelia and E. R. Chilvers, Is the neutrophil the key effector cell in severe asthma?, *Thorax*, 2005, **60**, 529–530.
- 46 I. Neuman, H. Nahum and A. Ben-Amotz, Reduction of exercise-induced asthma oxidative stress by lycopene, a natural antioxidant, *Allergy*, 2000, **55**, 1184–1189.
- 47 R. Rühl, A. Garcia, F. J. Schweigert and M. Worm, Modulation of cytokine production by low and high retinoid diets in ovalbumin-sensitized mice, *Int. J. Vitam. Nutr. Res.*, 2004, **74**, 279–284.
- 48 R. Rühl, A. Hanel, A. L. Garcia, A. Dahten, U. Herz, F. J. Schweigert and M. Worm, Role of vitamin A elimination or supplementation diets during postnatal development on the allergic sensitisation in mice, *Mol. Nutr. Food Res.*, 2007, **51**, 1173–1181.
- 49 J. Gericke, J. Ittensohn, J. Mihaly, S. Dubrac and R. Rühl, Allergen-induced dermatitis causes alterations in cutaneous retinoid-mediated signaling in mice, *PLoS One*, 2013, **8**, e71244.
- 50 J. Gericke, J. Ittensohn, J. Mihaly, S. Alvarez, R. Alvarez, D. Töröcsik, A. R. de Lera and R. Rühl, Regulation of retinoid-mediated signaling involved in skin homeostasis by RAR and RXR agonists/antagonists in mouse skin, *PLoS One*, 2013, **8**, e62643.
- 51 A. L. Garcia, R. Rühl, U. Herz, C. Koebnick, F. J. Schweigert and M. Worm, Retinoid- and carotenoid-enriched diets influence the ontogenesis of the immune system in mice, *Immunology*, 2003, **110**, 180–187.
- 52 C. B. Stephensen, R. Rasooly, X. Jiang, M. A. Ceddia, C. T. Weaver, R. A. Chandraratna and R. P. Bucy, Vitamin A enhances in vitro Th2 development via retinoid X receptor pathway, *J. Immunol.*, 2002, **168**, 4495–4503.
- 53 V. Julia, L. Macia and D. Dombrowicz, The impact of diet on asthma and allergic diseases, *Nat. Rev. Immunol.*, 2015, **15**, 308–322.
- 54 M. Shibakura, T. Koyama, M. Ohsawa, R. Kamiyama and S. Hirosawa, A retinoic acid receptor-alpha (RAR alpha) selective agonist modulates procoagulant activity of acute promyelocytic cells and induces their differentiation into neutrophils, *Blood*, 1998, **91**, 724–725.
- 55 S. Tsai, S. Bartelmez, R. Heyman, K. Damm, R. Evans and S. J. Collins, A mutated retinoic acid receptor-alpha exhibiting dominant-negative activity alters the lineage develop-

- ment of a multipotent hematopoietic cell line, *Genes Dev.*, 1992, **6**, 2258–2269.
- 56 T. Liu, T. M. Laidlaw, C. Feng, W. Xing, S. Shen, G. L. Milne and J. A. Boyce, Prostaglandin E2 deficiency uncovers a dominant role for thromboxane A2 in house dust mite-induced allergic pulmonary inflammation, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 12692–12697.
 - 57 L. Vong, J. G. Ferraz, R. Panaccione, P. L. Beck and J. L. Wallace, A pro-resolution mediator, prostaglandin D(2), is specifically up-regulated in individuals in long-term remission from ulcerative colitis, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 12023–12027.
 - 58 C. Ward, I. Dransfield, J. Murray, S. N. Farrow, C. Haslett and A. G. Rossi, Prostaglandin D2 and its metabolites induce caspase-dependent granulocyte apoptosis that is mediated via inhibition of I kappa B alpha degradation using a peroxisome proliferator-activated receptor-gamma-independent mechanism, *J. Immunol.*, 2002, **168**, 6232–6243.
 - 59 G. M. Gauvreau, R. M. Watson and P. M. O'Byrne, Protective effects of inhaled PGE2 on allergen-induced airway responses and airway inflammation, *Am. J. Respir. Crit. Care Med.*, 1999, **159**, 31–36.
 - 60 Y. Honma, I. Arai, Y. Hashimoto, N. Futaki, M. Sugimoto, M. Tanaka and S. Nakaike, Prostaglandin D2 and prostaglandin E2 accelerate the recovery of cutaneous barrier disruption induced by mechanical scratching in mice, *Eur. J. Pharmacol.*, 2005, **518**, 56–62.
 - 61 C. D. Buckley, D. W. Gilroy and C. N. Serhan, Proresolving lipid mediators and mechanisms in the resolution of acute inflammation, *Immunity*, 2004, **40**, 315–327.
 - 62 C. E. Rose, Jr., J. A. Lannigan, P. Kim, J. J. Lee, S. M. Fu and S. S. Sung, Murine lung eosinophil activation and chemokine production in allergic airway inflammation, *Cell. Mol. Immunol.*, 2010, **7**, 361–374.
 - 63 H. Ohnishi, N. Miyahara and E. W. Gelfand, The role of leukotriene B(4) in allergic diseases, *Allergol. Int.*, 2008, **57**, 291–298.
 - 64 R. A. Luz, P. Xavier-Elsas, B. de Luca, D. Masid-de-Brito, P. S. Cauduro, L. C. Arcanjo, A. C. dos Santos, I. C. de Oliveira and M. I. Gaspar-Elsas, 5-lipoxygenase-dependent recruitment of neutrophils and macrophages by eotaxin-stimulated murine eosinophils, *Mediators Inflammation*, 2014, **2014**, 102160.
 - 65 A. S. Cowburn, S. T. Holgate and A. P. Sampson, IL-5 increases expression of 5-lipoxygenase-activating protein and translocates 5-lipoxygenase to the nucleus in human blood eosinophils, *J. Immunol.*, 1999, **163**, 456–465.
 - 66 T. Nabe, M. Miura, T. Kamiki and S. Kohno, Arachidonate 5-lipoxygenase and cyclooxygenase metabolites from guinea pig eosinophils and macrophages, *Jpn. J. Pharmacol.*, 2000, **83**, 261–264.
 - 67 O. Kalayci, E. Birben, C. Sackesen, O. Keskin, F. Tahan, M. E. Wechsler, E. Civelek, O. U. Soyer, G. Adalioglu, A. Tuncer, E. Israel and C. Lilly, ALOX5 promoter genotype, asthma severity and LTC production by eosinophils, *Allergy*, 2006, **61**, 97–103.
 - 68 H. Kankaanranta, M. A. Lindsay, M. A. Gienbycz, X. Zhang, E. Moilanen and P. J. Barnes, Delayed eosinophil apoptosis in asthma, *J. Allergy Clin. Immunol.*, 2000, **106**, 77–83.
 - 69 O. Haworth and C. D. Buckley, Resolving the problem of persistence in the switch from acute to chronic inflammation, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 20647–20648.
 - 70 B. M. Forman, P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman and R. M. Evans, 15-Deoxy-delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma, *Cell*, 1995, **83**, 803–812.
 - 71 A. R. de Lera, W. Krezel and R. Rühl, An Endogenous Mammalian Retinoid X Receptor Ligand, At Last!, *ChemMedChem*, 2016, **11**, 1027–1037.
 - 72 R. Rühl, A. Krzyzosiak, A. Niewiadomska-Cimicka, N. Rochel, L. Szeles, B. Vaz, M. Wietrzyk-Schindler, S. Alvarez, M. Szklenar, L. Nagy, A. R. de Lera and W. Krezel, 9-cis-13,14-dihydroretinoic acid is an endogenous retinoid acting as RXR ligand in mice, *PLoS Genet.*, 2015, **11**, e1005213.
 - 73 C. Caris-Veyrat, A. Schmid, M. Carail and V. Böhm, Cleavage products of lycopene produced by in vitro oxidations: characterization and mechanisms of formation, *J. Agric. Food Chem.*, 2003, **51**, 7318–7325.
 - 74 S. Veeramachaneni, L. M. Ausman, S. W. Choi, R. M. Russell and X. D. Wang, High dose lycopene supplementation increases hepatic cytochrome P450E1 protein and inflammation in alcohol-fed rats, *J. Nutr.*, 2008, **138**, 1329–1335.
 - 75 B. Watzl, A. Bub, B. R. Brandstetter and G. Rechkemmer, Modulation of human T-lymphocyte functions by the consumption of carotenoid-rich vegetables, *Br. J. Nutr.*, 1999, **82**, 383–389.
 - 76 H. Bessler, H. Salman, M. Bergman, Y. Alcalay and M. Djaldetti, In vitro effect of lycopene on cytokine production by human peripheral blood mononuclear cells, *Immunol. Invest.*, 2008, **37**, 183–190.