THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

Regulation of retinoids and n-3 PUFAs in atopy

by

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ABBREVIATIONS

AA	- arachidonic acid
AD	- atopic dermatitis
ADH	- alcohol dehydrogenase
ALA	- alpha-linolenic acid
AS	- affected skin
ATRA	- all-*trans* retinoic acid
BCMO1	- beta-carotene mono-oxygenase 1
BCMO2	- beta-carotene mono-oxygenase 2
CRBP1	- cellular retinol binding protein 1
CRABP1	- cellular retinoic acid binding protein 1
CRABP2	- cellular retinoic acid binding protein 2
CTRL	- control
cyclo	- cyclophilin A
CYP26A1	- cytochrome P450 26A1
CYP26B1	- cytochrome P450 26B1
CYP2S1	- cytochrome P450 2S1
DBD	- DNA-binding domain
DGAT	- diacylglycerol acyltransferase
DHA	- docosahexaenoic acid
DHGLA	- dihomo-gamma-linolenic acid
DPA	- docosapentaenoic acid
ELOVL	- fatty acid elongase
EPA	- eicosapentaenoic acid
FADS1	- fatty acid desaturase 1
FADS2	- fatty acid desaturase 2
GLA	- gamma-linolenic acid
HB-EGF	- heparin-binding EGF-like growth factor
HPLC-MS-MS	- high performance liquid chromatography mass spectrometry – mass spectrometry
HS	- skin from healthy volunteers
IL-13	- interleukin 13
LA	- linoleic acid

LBD	- ligand-binding domain
LRAT	- lecithin:retinol acyltransferase
LXR	- liver X receptor
MUFA	- monounsaturated fatty acid
NAS	- non-affected skin
NHR	- nuclear hormone receptor
OVA	- ovalbumin
QRT-PCR	- quantitative real time polymerase chain reaction
PBS	- phosphate buffered saline
PPAR	- peroxisome proliferator-activated receptor
PUFA	- polyunsaturated fatty acid
RA	- retinoic acid
RAL	- retinal
RALDH	- retinaldehyde dehydrogenase
RARRES	- retinoic acid receptor responder
RBP4	- retinol binding protein 4
RDH	- retinol dehydrogenase
ROL	- retinol
RAR	- retinoic acid receptor
RARE	- retinoic acid response element
RXR	- retinoid X receptor
SFA	- saturated fatty acid
TAG	- triacyl-glycerol
TGM2	- transglutaminase 2
TR	- thyroid receptor
VA	- vitamin A
VDR	- vitamin D receptor

CONTENTS

Supplement 1.

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Supplement 2.

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1. INTRODUCTION

Retinoids are known to regulate various events relevant for skin diseases like epidermal differentiation, proliferation, apoptosis, immune regulation and epidermal barrier properties. In previous studies it has been shown that retinoid supplementation evokes high IgE levels and alters Th1/Th2 balance. Over the years several retinoids, such as retinoic acid and synthetic retinoic acid analogues have been used therapeutically in skin and immune diseases and proved to be beneficial. Atopic dermatitis is a chronically relapsing inflammatory skin disease, characterized by disturbed epidermal barrier function, an increased Th2 immune response and elevated IgE levels. Polyunsaturated fatty acids (PUFAs), are essential constituents of various layers of the skin and are important precursors of several pro- and anti-inflammatory mediators. Numerous studies detected significant changes of the PUFA composition of the skin and serum in patients suffering from atopic dermatitis. So far the gene expression profiles of all known genes involved in retinoid metabolism, namely metabolizing enzymes, retinoid transport proteins, retinoid receptors and target genes have not been investigated in skin of patients with atopic dermatitis. Our interest was to find out whether retinoid concentrations change and how retinoid signaling pathways are altered in atopic dermatitis. Further we aimed to determine the impact of allergic sensitization on the PUFA composition of various serum lipid classes in mice as well as the IgE secretion in a mouse model with or without dietary docosahexaenoic acid-enriched fish oil supplementation.

2. THEORETICAL BACKGROUND

2.1 Atopy and atopic dermatitis

2.1.1. Atopy - definition and epidemiology

The term “atopy” was first coined by Coca and Cooke in 1923, the word taken from Greek meaning placelessness, out of place, unusual, special. Atopy describes the predisposition to the development of allergic diseases including bronchial asthma, allergic rhinitis and atopic dermatitis (AD) (Rocken, et al., 1998) and afflicts up to 20% of the population of the industrialized countries (Kankaanpaa, et al., 1999). Atopy can be characterized by the following phenotypical criteria: a.) increased levels of total serum IgE (where parasitic infections or other causes have to be excluded), b.) allergen specific serum IgE, c.) immediate type skin reactions. However, none of these atopic phenomena are pathognomonic for atopy and not all need to be present at the same time (Rocken, et al., 1998).

2.1.2. Atopic dermatitis - definition and epidemiology

AD is a highly pruritic, chronic and relapsing inflammatory skin disease and it can commonly occur concomitantly with asthma, food allergy, allergic rhinitis or conjunctivitis based on common patomechanism (Leung, et al., 2004; Spergel and Paller, 2003). It predominantly occurs in infancy and childhood, but it can also begin later in life and persist into adulthood having a high familial occurrence (Leung, et al., 2003; Lonne-Rahm, et al., 2004). In the prevalence of AD a great worldwide variation can be observed, ranging from 0.6 % to 20.5 % (Williams, et al., 1999) and its prevalence is higher in developed countries, although the reasons are not known (Simpson and Hanifin, 2005). Two types of AD can be distinguished: extrinsic and intrinsic types. Extrinsic or allergic AD is associated with high total serum IgE levels and the presence of specific IgE for environmental and food allergens and its incidence is 70-80 % of the patients. Intrinsic or non-allergic AD exhibits normal total IgE values and the absence of specific IgE and approximately 20 % - 30 % of the patients are involved in it, with a female predominance (Leung, et al., 2004; Tokura, 2010).

2.1.3. Pathophysiology of atopic dermatitis

Atopic dermatitis is a hereditary disease presenting a spectrum of abnormalities including permeability barrier disruption, inflammation and abnormal keratinocyte differentiation (Proksch, et al., 2008; Proksch, et al., 2006; Simpson and Hanifin, 2005).

A significant familial association has been shown for atopic dermatitis. Genetic studies indicated an association with the interleukin 13 (IL-13) gene promoter polymorphism for a cluster of cytokine genes located on chromosome 5q31-33 (Hummelshoj, et al., 2003). A genome-wide linkage research showed highly significant evidence for linkage on chromosome 3q21. Atopic dermatitis, asthma and allergic sensitization are closely associated and show strong familial clustering supporting the presence of an atopy gene in this region (Lee, et al., 2000). Various other atopy genes have been identified with lesser or no familial association.

Atopic dermatitis is characterized by the presence of epidermal barrier dysfunction. The major function of skin is to form a barrier between the external hostile environment and the internal milieu of the host (Feingold, et al., 2007). The skin must avoid the percutaneous penetration of chemical irritants and allergens, protect the host from mechanical trauma, ultraviolet light and pathogenic microorganisms, moreover it has to prevent excessive trans-epidermal water loss hindering thus the body from desiccation (Feingold, 2007). The epidermal barrier is located in the deeper part of the stratum corneum, which forms a continuous sheet of protein-enriched cells (corneocytes) connected by modified desmosomes, called corneodesmosomes and embedded in an intercellular matrix enriched in non-polar lipids and organized as lamellar lipid layers (Proksch, et al., 2008). The lipid lamellae matrix is a substance composed of ceramides, cholesterol, fatty acids and cholesterol esters. The percutaneous penetration of exogenous substances varies in the different parts of the body due to the differences in the thickness of the stratum corneum, the highest penetration is in through the thinnest parts of the stratum corneum. A larger variability can be observed between individuals (Cork, et al., 2006).

2.2. Retinoids and vitamin A

2.2.1. Definition and chemical structure

The term “retinoids” includes both naturally occurring forms of VA and other synthetic retinol analogs showing biological activities characteristic for VA (Futoryan and Gilchrest, 1994). Retinoids are a class of compounds defined as consisting of four isoprenoid units with several double bonds ($H_2C = C(CH_3)-CH = CH_2$) joined in a head-to-tail manner, as defined by the IUPAC-IUB in 1982 (Reichrath, et al., 2007; Vahlquist, 1999). The retinoid molecule can be divided into three parts: a non-aromatic ring structure, a polyprenoide side chain and a polar carbon-oxygen end group. Considering the chemical features of these molecules, their involvement in physicochemical reactions and many of their biological effects is understandable. Though natural retinoids are present mainly in the all-trans configuration, cis-trans isomerization forms of the polyprenoid side chain have also biologically relevant role (Vahlquist, 1999).

2.2.2. The importance of retinoids in skin physiology

Vitamin A and its derivatives (retinoids) play an important role in skin physiology (Roos, et al., 1998). Retinoids regulate several effects in differentiation, proliferation, apoptosis, immune regulation, epidermal barrier properties (Elias, 1987; Elias, et al., 1981) and sensorial functions (Aydogan and Karli, 2007) in numerous skin cell types. Several skin diseases like psoriasis (Saurat, 1999), ichthyosis (Mevorah, et al., 1996), skin cancers (Klaassen, et al., 2002), acne (Rollman and Vahlquist, 1985) and various other dermatoses are related to alterations in retinoid metabolism / signaling. Retinoid-based treatments have been shown to be beneficial for various therapeutic approaches of these skin diseases (Ellis, et al., 2000; Orfanos and Zouboulis, 1998). Topical and systemic treatments with retinoids like, retinoic acid and synthetic retinoic acid analogues or derivatives which modify retinoid metabolism are used already in therapy (Bissonnette, et al., 2010; Okuno, et al., 2004; Roos, et al., 1998; Vahlquist, 1999; Zouboulis, 2001).

2.2.3. Retinoid metabolism in skin

Ingested retinyl-esters via the diet are hydrolyzed to retinol (ROL) by enteral hydrolases in the intestine. ROL and carotenoids are absorbed by intestinal mucosa cells (Roos, et al., 1998). Skin expresses lecithin:retinol acyltransferase (LRAT), known to catalyze retinyl-ester synthesis (Kurlandsky, et al., 1996). After intestinal absorption, retinoids can be produced by two pathways: first, retinal (RAL) can be synthesized by oxidative cleavage of the central double bound and this step is followed by the reduction to ROL. ROL binds to cellular retinol binding proteins (CRBP1). Biosynthesis of retinoic acid (RA) from ROL can occur in two steps. ROL is oxidized to RAL by members of the alcohol dehydrogenase (ADH) and retinol dehydrogenases (RDH) through a reversible interconversion; and RAL is further oxidized to RA by means of retinaldehyde dehydrogenase enzymes (RALDH) and cytochrome P450 enzyme family members (Fisher and Voorhees, 1996; Roos, et al., 1998). The intra-cellular transportation of RA is done by means of cellular retinoic acid binding proteins 1 and 2 (CRABP1, CRABP2) (Zhelyaznik, et al., 2003) (Figure 1.).

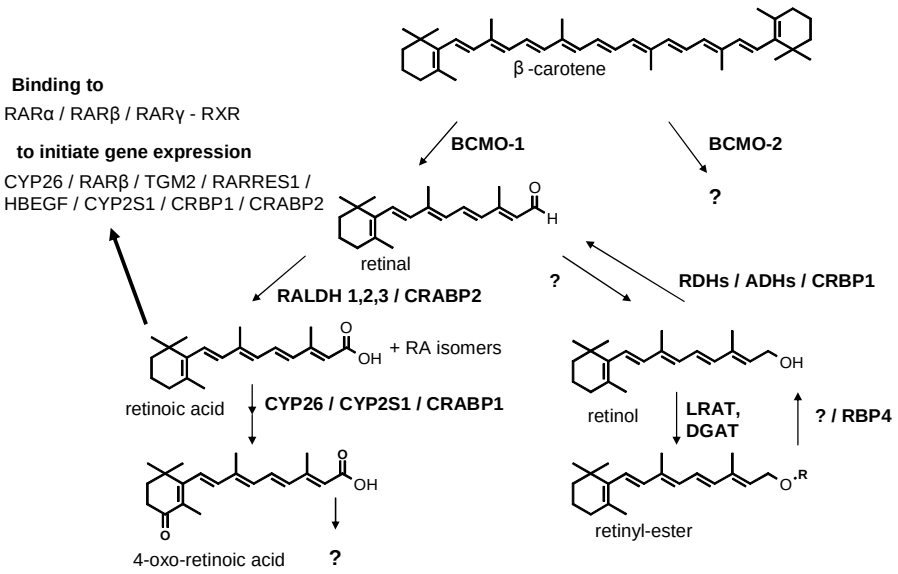

Figure 1. General scheme of retinoid signaling pathway including structures and involved binding proteins and retinoid-metabolizing enzymes: RA - retinoic acid, RDH – retinol dehydrogenase, RALDH – retinaldehyde dehydrogenase, BCMO – beta-carotene monooxygenase, CRBP - cellular retinol binding protein, CRABP – cellular retinoic acid binding protein, LRAT – lecithin:retinol acyltransferase, DGAT – diacylglycerol acyltransferase, RBP4 – retinol binding protein 4, RAR – retinoic acid receptor, RXR – retinoid X receptor, TGM2 – transglutaminase 2, RARRES1 – retinoic acid receptor responder 1, HBEGF – heparin-binding EGF-like growth factor

2.3. Polyunsaturated fatty acids

Dietary fat is an important macronutrient for the growth and development of all organisms. Besides having a nutrient value and its effect on the membrane lipid composition, dietary fat has a strong effect on gene expression as well, which results in changes of metabolism (Jump and Clarke, 1999). Furthermore, polyunsaturated fatty acids (PUFAs) modulate immune responses via their metabolites and thereby showing beneficial effects in a variety of inflammatory disorders (Stulnig, 2003).

2.3.1. Molecular structure and nomenclature of PUFAs

Fatty acids are carbon-chains with a methyl-group in one end of the molecule (termed omega=ω or *n*) and an acid group in the other end, with at least two double bonds in their structure.

Polyunsaturated fatty acids contain two or more double bonds in varying positions from the „ω" or „n" carbon. Therefore, if the first double bond is between the 3rd and 4th carbon atom from the „n" carbon, they are called omega-3 (n-3) fatty acids. When the first double bond is between the 6th and 7th carbon atom from the „n" carbon, they are called omega-6 (n-6) fatty acids (Figure 2.). PUFAs are designated by the lenght of their chain (C), the number of the double bonds in their structure (D), respectively the position of the first double bond counted from the terminal methyl group (n-). Thus, their structure can be designed by using the C:Dn-x nomenclature (Das, 2006).

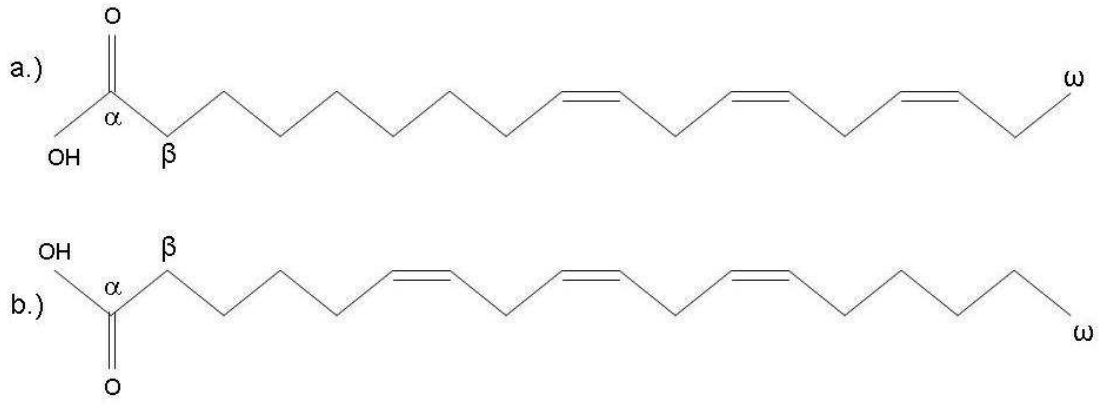

Figure 2. Chemical structure of PUFAs. a. n-3 PUFA. b. n-6 PUFA

2.3.2. Dietary sources of PUFAs

The 18-carbon long n-6 and n-3 PUFAs are synthesized by several plants, therefore they are obtained from dietary vegetable oils, such as sunflower oil. However, longer chain members of fatty acids are either obtained directly via the diet from animal or marine sources, such as fish or marine oils, or they are biosynthesized *in vivo* after the dietary ingestion of the shorter 18-carbon precursors (Ziboh, 1996). Various plant oils, such as sunflower or soybean oil, and meat products, contain mainly n-6 PUFAs, such as linoleic acid (LA) (18:2n-6) and arachidonic acid (AA) (18:3n-6), while fish oil mainly contains n-3 PUFAs: eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) (Drevon, et al., 1995; Meydani, et al., 1991).

2.3.3. Physiological functions of PUFAs

PUFAs have several physiological functions in the mammalian organism. As mentioned before they are important dietary sources, being essential macronutrients for the growth and development of all organisms (Jump and Clarke, 1999). PUFAs are structural components of the cells and they also exert dramatic effects on gene expression by regulating the activity of four families of transcription factors (Jump, 2002). Moreover PUFAs play an important role in the skin, contributing to the physical structure of the epidermal barrier, exerting profound effects on the endogenous epidermal phospholipid fatty acid composition and also take part in the generation of anti-inflammatory and antiproliferative metabolites, thus modulating immune responses (Serhan, 2008; Stulnig, 2003; Ziboh, et al., 2000).

2.3.4. PUFA metabolism

PUFA metabolism involves the alternating Δ6-desaturation, elongation and Δ5-desaturation. Thus gamma-linolenic acid (GLA) is desaturated by FADS2 (fatty acid desaturase 2) from LA which is further elongated to dihomo-gamma-linolenic acid (DHGLA) by fatty acid elongase (ELOVL) 5 and metabolized to AA acid by fatty acid desaturase 1 (FADS1). Similarly, docosapentaenoic acid (DPA) is formed by elongation from EPA and after an intermediate desaturation step, DHA is formed (Duchen and Bjorksten, 2001) (Figure 3.).

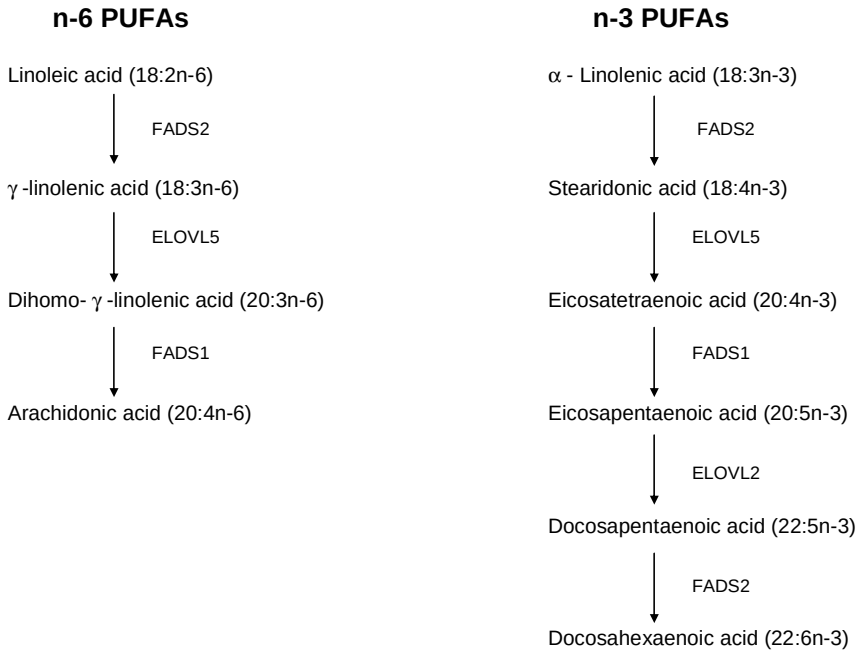

Figure 3. The biosynthesis of polyunsaturated fatty acids.

2.4. Nuclear hormone receptors in the human skin

Nuclear hormone receptors (NHRs) are transcription factors that are activated on the binding of specific ligands and that bind to specific response elements mainly located within the promoters of their target genes (Desvergne, 2007). NHRs share a common structural organization being composed of multiple functional domains. The N-terminal domain harbors a very weak to strong ligand-independent transactivation domain. The central C-domain contains the DNA binding domain (DBD) which is composed of two highly conserved zinc fingers and is the hallmark of the nuclear receptor superfamily. The DBD is linked by the hinge region to the ligand-binding domain (LBD), which is at the C-terminal half of the receptor and an area which mediates binding to other nuclear hormone receptors (Desvergne, 2007; Mangelsdorf and Evans, 1995; Mangelsdorf, et al., 1995; Nagy and Schwabe, 2004) (Figure 4.).

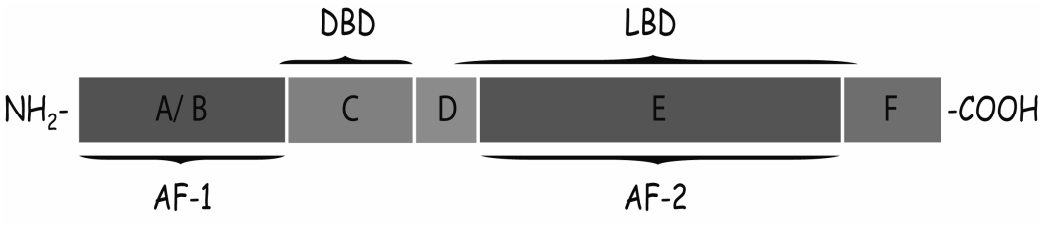

Figure 4. The structure of the nuclear hormone receptors. A/B: ligand - independent transactivation (AF-1) domain, C: DBD, DNA - binding domain, D: connects the DBD with the LBD, E: LBD, ligand binding domain, F: ligand -dependent transactivation domain

Several nuclear hormone receptors require ligand binding to be activated. A large variety of natural compounds has been identified as NHR ligands. Such ligands are steroids, vitamins A and D derivatives, thyroid hormone, PUFAs, PUFA-metabolites, oxysterols and xenobiotic lipids (Schmuth, et al., 2007). Vitamin A derivatives are ligands of RAR and RXR, while free fatty acids and especially their metabolites are nuclear receptor specific ligands of PPARs (Mangelsdorf and Evans, 1995; Schmuth, et al., 2007).

Most nuclear receptors function as dimers, either homodimers, or mainly forming heterodimers with retinoid X receptors (RXR) (Desvergne, 2007). Members of this subgroup play a critical role in cutaneous biology and include the retinoic acid receptors (RAR), the vitamin D receptor (VDR), the thyroid receptor (TR), the peroxisome proliferator-activated receptors (PPARs) and the liver X receptors (LXR) (Schmuth, et al., 2007) (Figure 5.).

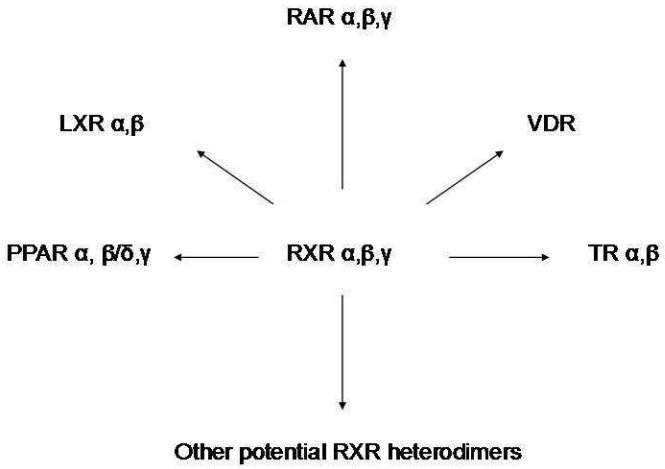

Figure 5. RXR heterodimers. RXR: retinoid X receptor, RAR: retinoic acid receptor, VDR: vitamin D receptor, TR: thyroid receptor, LXR: liver X receptor, PPAR: peroxisome proliferator-activated receptor

Retinoids mainly exert their activity via two families of nuclear hormone receptors, the retinoic acid receptors (RAR) and the retinoid X receptors (RXR) (Allenby, et al., 1993; Fisher, et al., 1994; Reichrath, et al., 1997). Both families of retinoid receptors consist of three isotypes: α, β and γ encoded by separate genes (Mangelsdorf and Evans, 1995). RARs can be mainly activated by all-*trans* retinoic acid (ATRA), while 9-*cis* retinoic acid (9CRA) is a potential candidate ligand of RXRs (Allenby, et al., 1993). Human epidermis expresses transcripts for RARα, RARγ, RXRα (Elder, et al., 1992) and RARβ in a low level (Elder, et al., 1991).

PPARs are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily. The PPAR family consists of 3 members: PPARα, PPARβ/δ and PPARγ, based on sequence homology (Michalik, et al., 2002). All 3 isotypes are expressed in both rodent and human skin, PPARα and PPARβ/δ being instrumental in skin repair after injury, both of them playing specific roles (Michalik and Wahli, 2007). PPARα has important role in the molecular mechanisms involved in skin repair and in epidermal differentiation thus contributing to the permeability barrier formation (Komuves, et al., 2000). Moreover evidence has shown the role of PPARα in controlling skin and systemic inflammatory responses in patients with AD (Staumont-Salle, et al., 2008). It has been shown that the activation of PPARβ/δ enhances keratinocyte proliferation in psoriasis by induction of heparin-binding EGF-like growth factor (HB-EGF) and that PPARβ/δ also regulates epithelial cell differentiation (al Yacoub, et al., 2008; Romanowska, et al., 2008). Recently it was demonstrated that retinoic acid is also a ligand of PPARβ/δ in cells that display a high FABP5/CRABP2 expression ratio (Berry and Noy, 2007; Schug, et al., 2007).

2.4.1. Nuclear hormone receptors and retinoids

Retinoic acid activities are mainly mediated by retinoic acid bound to its RA nuclear receptors, the RAR family and the RXR family (Allenby, et al., 1993). It has been demonstrated that RA, bound to an RAR can bind to RA response elements (RAREs) in the DNA and modulate the transcription of specific target genes. These changes in the gene expression are responsible for the biological and also for the therapeutic effects of retinoids (Futoryan and Gilchrest, 1994).

In humans three distinct RARs have been identified: RARα, RARβ and RARγ. The three RAR genes have different expression patterns in different cells and also during development.

RARα gene transcripts are found in most embryonic and adult tissues and may be therefore responsible for the effects of retinoids on growth and differentiation. RARβ is just expressed in a low level in adult human skin (Elder, et al., 1991), while most of the RAR receptors in adult skin are RARγ (Elder, et al., 1991).

The RXR family has also three receptors: RXRα, RXRβ and RXRγ. These receptors have similarities, but are quite different from the RARs both in structure and ligand specificity. RXRα appears to be the mostly expressed in human epidermis (Mangelsdorf, et al., 1992).

2.4.2. Nuclear hormone receptors and fatty acids

Although 9 - cis - retinoic acid has been described to be the natural ligand for RXR (Levin, et al., 1992), recently evidence indicates, that it can also become activated by naturally occurring polyunsaturated fatty acids, including docosahexaenoic acid (de Urquiza, et al., 2000), arachidonic acid (Lengqvist, et al., 2004) or phytanic acid (Lemotte, et al., 1996).

Certain fatty acids and their oxidation products are capable of binding and activating PPARs (Bull, et al., 2003). It has been shown that the oxidative metabolism of linoleic acid produces several bioactive metabolites that bind to PPARs (Nagy, et al., 1998). A search for natural ligands in human serum identified palmitic acid, oleic acid, linoleic acid and arachidonic acid as endogenous activators of rat PPARα (Banner, et al., 1993). Unlike the PPARα subtype, PPARγ has a clear preference for polyunsaturated fatty acids. The essential fatty acids linoleic acid, linolenic acid, arachidonic acid and eicosapentaenoic acid have been shown to bind to PPARγ at micromolar concentrations (Kliewer, et al., 1997; Xu, et al., 1999). PPARβ/δ is also a receptor for naturally occurring polyunsatureated fatty acids, such as dihomo-γ-linolenic acid, arachidonic acid and EPA with low affinities in low micromolar range (Xu, et al., 1999).

2.5. Aim of the studies

Vitamin A and its derivatives, the retinoids, are important for skin physiology. Several skin diseases were related to altered retinoid metabolism. Topical and systemic treatments with retinoids such as retinoic acid or other synthetic retinoic acid analogues or derivatives that are known to modify retinoid metabolism are already used in therapy. Retinoids have been shown to evoke high IgE levels and alter Th1/The balance in mouse supplementation studies. The aim of our study was:

- to investigate the gene expression profiles of all known genes involved in retinoid metabolism, namely metabolizing enzymes, retinoid transport proteins, retinoid receptors and target genes in the skin biopsies of atopic patients in comparison to the skin of healthy volunteers.

- to investigate the retinoid concentrations of serum and skin samples of these patients by means of high performance liquid chromatography mass spectrometry – mass spectrometry (HPLC MS-MS) methodology.

PUFA levels have been shown to be altered in the skin of atopic dermatitis patients in comparison to healthy volunteers and dietary supplementation with oils rich in n-3 PUFAs, such as EPA and DHA have been shown to induce anti-inflammatory effects in *in vitro* as well as *in vivo* experiments. Moroverer, a higher dietary intake of n-3 PUFAs was associated with lower incidences of allergic sensitization and atopic diseases in several studies. Therefore, we aimed:

- to find out how allergic sensitization, DHA-enriched fish oil supplementation and allergic sensitization with concomitantly DHA-enriched fish oil supplementation of mice influences the percentile distribution of n-3 / n-6 PUFAs bound to cholesteryl-, triacylglycerol- and phospholipid-esters of n-3 metabolism and various ratios of eicosanoid precursor PUFAs like EPA, DHA and AA in the serum as well as IgE responses.

3. MATERIALS AND METHODS

Skin biopsies and serum samples

Punch biopsy specimens were taken from involved skin of 6 patients with atopic dermatitis (2 male and 4 female patients; average age 31 years) and from 6 non-atopic healthy volunteers (2 male and 4 female individuals; average age 30 years), who were characterized by the absence of personal or family history of atopic disease. Prior informed consent was obtained according to the guidelines of the Ethical Committee and the declaration of Helsinki. In Table 1. the clinical and basic demographic data of the healthy volunteers and diseased patients are shown:

	Healthy volunteers	**AD patients**
age in years	30 ± 11	31 ± 14
gender	67 % female	67 % female
SCORAD	0 ± 0	36 ± 11
total IgE (KU/L)	32 ± 17	1879 ± 764

Table 1. Clinical and basic demographic data from healthy and diseased donors

In case of AD patients, one biopsy of affected skin and one biopsy of non-affected skin were taken from each patient. Epidermis represents ca. 20-30 % of the skin biopsy used for analysis. Specimens were immediately frozen on dry ice and stored at -70 °C until RNA isolation or HPLC analysis was performed. At the same time, serum samples were drawn from the same patients and were kept at -70 °C until analysis. Ethical approval for the study was obtained from the local ethics committee (EA1/168/06) and from each volunteer a signed informed consent was obtained.

RNA extraction

RNA isolation was performed from human skin tissues by means of Tri® Reagent (Trizol) isolation manual technique.

1. A skin sample of ca. 100 mg is homogenised with QIAGEN Tissue Lyser in 1 ml of Trizol, using previously autoclaved QIAGEN metal beads. After 1 minute centrifugation (at 4 °C, 13000 rpm) of the samples, the supernatants are transferred into 1.5 ml Eppendorf tubes.
2. 200 µl of chloroform is added to each sample, the samples are stirred thorougly and incubated for 3 minutes on room temperature. After incubation the samples are centrifuged for 15 minutes (at 4 °C, 13000 rpm) and the upper aqueous phase is transferred into a new Eppendorf tube.
3. 500 µl of isopropanol is added to the samples, stirred and incubated for 20 minutes on room temperature. After incubation the samples are centrifuged for 10 minutes (at 4 °C, 13000 rpm). The supernatant is discarded.
4. 800 µl of 70 % ethanol is added, stirred shortly and centrifuged for 5 minutes (at 4 °C, 13000 rpm). The supernatant is discarded.
5. RNA pellets are dried for 20-25 minutes in an Eppendorf concentrator 5301.
6. 40 µl of nuclease free water (NFW) is added to each sample, stirred and incubated for 10 minutes at 65°C in a BIOSAN Dry Block Heating Thermostat. Samples are stirred briefly and cooled down on ice.
7. The concentrations and purities of the RNA samples are measured by NanoDrop ND-1000 Spectrophotometer. Samples were kept at -20 °C until further usage.

Quantitative real-time reverse transcription polymerase chain reaction (QRT-PCR)

In our study the two-step Taqman QRT-PCR was performed in order to quantify the mRNA expression level of the human genes involved in the retinoid metabolism.

Firstly, in the reverse transcription step the RNA samples are transcribed by reverse transcriptase enzyme (RT) into complementary DNA (cDNA) using the Super Script II First-

Strand Synthesis System (Invitrogen), since they are needed for the amplification. For the reaction random primer is used.

The following reaction mixture is used (Table 2.):

RT reaction	**1 reaction (μl)**
5 x SSII buffer	8
25 x dNTP	8
10 x random primer	0.2
SSII RT (200 U/ul)	0.2
DTT	4
RNA	20

Table 2. Reaction mixture used for reverse transcription

The following thermal protocol is used (Table 3.):

Time (min)	**Temperature (°C)**
10	25
120	42
5	72
∞	4

Table 3. Thermal protocol used for reverse transcription

Secondly, the previously obtained cDNA is amplified by the TaqDNA polymerase enzyme and it is measured quantitatively in order to determine the mRNA expression level. The following reaction mixture is used in case of FAM-TAMRA assays (5 µl cDNA is added to 5 µl mastermix) (Table 4.):

Compound	**Concentration**	**Volume (ul) (1 reaction)**
Nuclease Free Water		2.1
$MgCl_2$	0.1 M	1.2
Taq-buffer	10 x	1.0
dNTP	2.5 mM	0.5
Taq-polymerase	5 U	0.0625
Forward-primer	100 µM	0.0375
Reverse-primer	100 µM	0.0375
Probe (FAM-TAMRA)	20 µM	0.0625

Table 4. Reaction mixture used for quantitative real time-PCR with FAM-TAMRA probe based assays. Volumes are given for 1 reaction. Concentration of the used compounds is indicated.

In case of TaqMan ROX-MGB assays, the reaction mixture is modified according to the following table (5 µl cDNA is added to 5 µl mastermix) (Table 5.):

Compound	**Concentration**	**Volume (µl) (1 reaction)**
Nuclease Free Water		1.9
$MgCl_2$	25 mM	1.2
Taq-buffer	10 x	1.0
dNTP	2.5 mM	0.5
Taq-polymerase	5U	0.0625
50 x ROX		0.2
20 x oligo mix		0.15

Table 5. Reaction mixture used for quantitative real time-PCR with ROX-MGB assays. Volumes are given for 1 reaction. Concentration of the used compounds is indicated.

QRT-PCR was carried out in triplicate using pre-designed MGB assays or FAM-TAMRA assays ordered from Applied Biosystems, on an ABI Prism 7900. Pre-designed MGB assays

or FAM-TAMRA assays were used in our experiments. Table 6. contains a list of symbols, gene names and assays IDs of all investigated ROX-MGB assays in our experimental setup.

No.	Gene symbol	Gene name	Assay ID
1.	hBCMO1	beta-carotene mono-oxygenase 1	Hs00363176_m1
2.	hBCMO2	beta-carotene mono-oxygenase 2	Hs00230564_m1
3.	hDGAT1	diacylglycerol acyltransferase	Hs00201385_m1
4.	hLRAT	lecithin:retinol acyltransferase	Hs00428109_m1
5.	hRDH2	retinol dehydrogenase 2	Hs00331485_m1
6.	hRDH16:	retinol dehydrogenase 16	Hs00559712_m1
7.	hADH1C	alcohol dehydrogenase 1C	Hs00817827_m1
8.	hRALDH3	retinal dehydrogenase 3	Hs00167476_m1
9.	hCRABP1	cellular retinoic acid binding protein 1	Hs00171635_m1
10.	hCRBP1	cellular retinol bindig protein 1	Hs00161252_m1
11.	hRBP4	retinol bindig protein 4 4	Hs00924047_m1
12.	hCYP26A1	cytochrome P450, subfamily 26A, polypeptide 1	Hs00175627_m1
13.	hCYP26B1	cytochrome P450, subfamily 26B, polypeptide 1	Hs01595346_m1
14.	hCYP2S1	cytochrome P450, subfamily 2S, polypeptide 1	Hs00258076_m1
15.	hRARβ	retinoic acid receptor beta	Hs00233407_m1
16.	hRARRES1	retinoic acid receptor responder 1	Hs00161204_m1
17.	hHBEGF	heparin-binding EGF-like growth factor	Hs00961131_m1

Table 6. List of gene symbols, gene names and assay IDs of all the investigated ROX-MGB assays.

Table 7. includes a list symbols, gene names and forward (+) and reverse (-) primer and probe (P) sequences of all investigated FAM-TAMRA assays (when available) in our experimental setup.

No.	Gene symbol	Gene name	Forward primer, reverse primer and probe sequences	
1.	hRDH10	retinol dehydrogenase 10	Sequences not available	
2.	hRALDH1	retinal dehydrogenase 1	+	(1397+) AATTGCTATGGCGTGGTAAGT
			-	(1480) ACCGTACTCTCCCAGTTCTCTT
			p	(1422+) AGTGCCCCTTTGGTGGATTCAGGAAGATGT
3.	hRALDH2	retinal dehydrogenase 2	+	AGGCCCTCCTCGCTCAC
			-	TGCCCCAGAATGAGCTC
			p	(126+) FAM-ACCCCTCCCTCTCTTCCAAGGAGATG
4.	hCRABP2	cellular retinoic acid binding protein 2	+	(219+) ATGCTGAGGAAGATTGCTGTG
			-	(281) TCCCTCCTGTTTGATCTCCA
			p	(241+) CTGCAGCGTCCAAGCCAGCA
5.	hRARα	retinoic acid receptor alpha	+	(1206+) CCAGCACCAGCTTCCAGTTA
			-	(1302-) GGGAGGGCTGGGCAC
			p	(1284+) FAM-CTCTTCAGAACTGCTGCTCTGGGTCTCAA
6.	hRARγ	retinoic acid receptor gamma	+	(855+) TGCATCATCAAGATCGTGGAG
			-	(928-) GTGATCTGGTCAGCAATGCTG
			p	(880+) FAM-CCAAGCGGTTGCCTGGCTTTACA
7.	hRXRα	retinoid X receptor alpha	+	(1278+) GGCCTACTGCAAGCACAAGTA
			-	(1341-) CAGGCGGAGCAAGAGCTTA
			p	(1321-) FAM-CGAACCTTCCCGGCTGCTCTG
8.	hTGM2	transglutaminase 2	+	(529+) CTGGGCCACTTCATTTTGC
			-	(610-) ACTCCTGCCGCTCCTCTTC
			p	(564-) FAM-TCCAGGTACACAGCATCCGCTGGG

Table 7 List of gene symbols, gene names and primer and probe sequences of all the investigated FAM-TAMRA assays.

Relative mRNA levels were calculated using the comparative threshold cycle (C_T) method and were normalized to the level of cyclophilin A mRNA. Sequence Detector Software (version 2.1) was utilized for data analysis.

Statistics

Data are shown as mean and standard error of the mean values of three measurements per data point. Statistical analysis was performed using the program SPSS 16.0. A *p* value of less than 0.05 was considered significant.

High performance liquid chromatography mass spectrometry – mass spectrometry (HPLC MS-MS) analysis:

Concentrations of retinol and retinoic acids (RAs) were determined in human serum and skin biopsies by our previously described HPLC-MS-MS method (Rühl, 2006). The HPLC system consisted of a Waters 2695XE separation module (Waters, Budapest, Hungary), a diode-array detector (model 996, Waters, Hungary) and an MS-MS detector (Micromass Quattro Ultima Pt, Waters, Hungary). In summary, 100 mg of the skin biopsy (if samples were under 100 mg water was added up to the used standard weight: 100 mg) or 100 µl serum was diluted with a threefold volume of isopropanol, the tissues were minced by scissors, vortexed for 10 seconds, put in an ultra sonic bath for 5 minutes, shaken for 6 minutes and centrifuged at 13000 rpm in a Heraeus BIOFUGE Fresco at +4°C. After centrifugation, the supernatants were dried in an Eppendorf concentrator 5301 (Eppendorf, Germany) at 30°C. The dried extracts were resuspended with 60 µl of methanol, vortexed, shaked, diluted with 40 µl of 60 mM aqueous ammonium acetate solution and transferred into the autosampler and subsequently analysed.

Animal experiments

A set of the animal experiments were performed in the facilities of the Max Rubner Laboratory of the German Institute of Human Nutrition (DIfE) in Potsdam-Rehbrücke, Germany. The experiment was approved by the respective ethical authorities from the Land Brandenburg. A second set of experiments, for investigation of the influence of aluminium hydroxide ($Al(OH)_3$) on lipid distribution, was performed in the Laboratory Animal Core Facility of the University of Debrecen, Hungary. The experiments were performed according to the Hungarian ethical guidelines.

10-12 week old adult female C57BL/6 mice were purchased from Charles River, Sulzfeld, Germany. Mice were kept under controlled conditions at room temperature (21 ± 1°C), constant relative humidity (55 ± 5%) and 12 hour light / dark cycle with light between 6:00 AM and 6:00 PM. Mice had access to food and water *ad libitum*. The composition of the basal diet was 20% casein, 8% sucrose, 50% wheat starch, 10% sunflower oil, 5% cellulose, 5% mineral mix (Mineral-Spurenelemente-Vormischung C1000, Altromin, Lage, Germany) and 2% vitamin mix (Vitamin-Vormischung C1000 Altromin, Lage, Germany) (Schweigert, et al., 2002). In the diet supplemented with DHA-enriched fish oil, 40% of the sunflower oil was substituted by DHA-enriched fish oil (DHA 500TG SR from tuna) from Croda GmbH, Nettetal, Germany yielding a mass content of 2% DHA and 0,12 % EPA in the animals diet. The composition of the two diets is displayed in Table 8.

Fatty acid (%)	Control diet	Diet supplemented with DHA-enriched fish oil
Sum of SFA	12.4	8.3
Sum of MUFA	22.7	17.6
Sum of n-6 PUFA	64.3	42.7
Linoleic acid	64.0	40.9
Arachidonic acid	0.01	0.56
Sum of n-3 PUFA	0.63	31.4
EPA	0.12	3.6
DHA	0.26	23.4

Table 8. Percentage fatty acid composition of the administered food (data are percentages (w/w) of dry animal food.)

The basal diet and the DHA-supplemented diet groups contained each n=12 mice, for the second experiment using $Al(OH)_3$ treatment for basal and DHA-supplemented diet fed groups n=6 mice per group were used.

Before supplementation with basal- and DHA-enriched-fish oil-diet the mice were fed with standard chow diet from Sniff (Soest, Germany) and for the second set of experiments using $Al(OH)_3$ treatment the mice were fed with standard mouse diet from Altromin (Lage,

Germany). One week after the starting of the supplementation with the basal- and the DHA-enriched fish oil diet, 6 mice per group were sensitised with ovalbumin (OVA) and 6 mice were treated with phosphate buffered saline (PBS). In the second set of experiments just $Al(OH)_3$ treatment was performed in a comparable manner. Each group was treated either with three intraperitoneal injections of OVA or PBS (or $Al(OH)_3$ in PBS) after a time interval of one week. OVA injections were made according to the protocol previously described (Rühl, et al., 2003); briefly, 10 μg of OVA absorbed to 1.5 mg $Al(OH)_3$ in 100 μl PBS was given to each animal. The mice were killed by decapitation four weeks after the dietary and three weeks after the OVA/PBS treatments. At 20-30 min after the collection the blood was centrifuged at 1300 g for 3 minutes and serum was obtained. Serum was stored at – 80°C until analysis.

Fatty acid analysis

The fatty acid analysis was performed by our collaborators Tamás Décsi and Tamás Marosvölgyi. For the analysis of plasma fatty acid profiles, frozen plasma samples were thawed, and then the three internal standards (dipentadecanoylphosphatidylcholine-, cholesteryl-pentadecanoate and -tripentadecanoate) were added. Lipids were extracted by the addition of 3 ml chloroform and 1 ml methanol according to the method of Folch et al (Folch, et al., 1957). The mixture was vortexed at 2000 rpm for 10 min, and then the lower layer was aspirated into vials and evaporated under nitrogen stream. Lipid extracts were reconstituted in 70 μl chloroform and lipid classes were separated by thin layer chromatography. The solvents for thin layer chromatography of plasma lipids were as follows: hexane : diethylether : chloroform : acetic acid (21 : 6 : 3 : 1, by vol.) followed by chloroform : methanol : water (65 : 25 : 4, by vol.). The bands were stained with dichlorofluorescein, removed by scraping and transesterified in 3 N HCl-methanol solution at 84 °C for 45 min.

Fatty acids were analysed by high-resolution capillary gas chromatography using a Finnigan 9001 gas chromatograph (Finnigan/Tremetrics Inc., Austin, TX, USA) with split injection (ratio 1:25), automatic sampler (A200SE, CTC Analytic, Switzerland) and flame ionisation detector with a DB-23 cyanopropyl column of 40 m length (J & W Scientific, Folsom, CA, USA). The temperature program was the following: temperature of injector at 80 °C for 0.1 min, temperature increase by 180 °C/min up to 280 °C, temperature of detector at 280 °C, temperature of column area at 60 °C for 0.2 min, temperature increase by 40 °C/min

up to 180 °C, 5 min isothermal period, temperature increase by 1.5 °C/min up to 200 °C, 8.5-min isothermal period, temperature increase by 40 °C/min up to 240 °C and 13 min isothermal period. The constant linear velocity was 0.3 m/sec (referred to 100 °C). Peak identification was verified by comparison with authentic standards. Fatty acid results were expressed as percentages (weight by weight) of fatty acids detected with a chain length between twelve and twenty-four carbon atoms.

Enzyme-linked immunosorbent assay (ELISA) analysis

The ELISA analysis was performed by our collaborators Margitta Worm and Christine Weise (Koch). Plates were coated overnight with anti-mouse EM95.3 (5 µg/ml; all monoclonal antibodies used were kindly provided by Dr. Lamer, MPI, Freiburg, Germany) diluted in 0.1 M bicarbonate buffer. After blocking with 3 % milk powder/PBS, sera (diluted in 1 % milk powder/PBS) were incubated overnight and were detected with biotin conjugated anti-mouse 84 1-C (1 µg/ml). The reaction was developed with streptavidin peroxidase and tetramethylbenzidine (both from Sigma, Dreieich, Germany) and was stopped with 1 M sulphuric acid. The plates were measured at 450/490 nm and the amount of total IgE was calculated according to the standard curve. The validity was assessed by using a standard with known concentrations for total IgE and by determination of 50% saturation for OVA-specific IgE ELISA. Furthermore, all sera were measured in serial dilutions.

Statistics

Standard deviations have been calculated with SPSS 15.0 (SPSS Inc., Chicago, USA) software for Windows using a two-factor independent Mann-Whitney test; a value of $P<0.05$ was used to determine statistical significance.

4. RESULTS

4.1. Expression profiles of retinoid signalling pathways in AD skin samples

Firstly, we investigated the expression profile of genes involved in retinoid homeostasis, regulation, metabolism, retinoid receptors and retinoid target genes. These data show that a severe dysregulation of retinoid-homeostasis, -metabolism and -signalling is present in affected and non-affected AD skin. The mRNA expression levels were determined by means of QRT-PCR in non-affected and affected skin of atopic dermatitis patients in comparison to healthy volunteers. Data are shown as mean and standard error of the mean out of triplicate measurement per data point. Significant changes in the mRNA expression levels are marked with asterisks.

4.1.1. Dysregulation of retinol homeostasis / retinyl ester synthesis regulation

Retinol binding protein (RBP) 4 is the carrier protein involved in the transport of retinol and its mRNA expression was non-significantly down-regulated in atopic dermatitis non-affected and affected skin. The mRNA expression of diacylglycerol acyltransferase (DGAT) showed a slight decrease in both affected and non-affected skin compared to healthy skin, while lecithin retinol acyltransferase (LRAT) was significantly up-regulated in non-affected skin and no significant alteration was observed in affected skin of atopic dermatitis patients (Figure 6.).

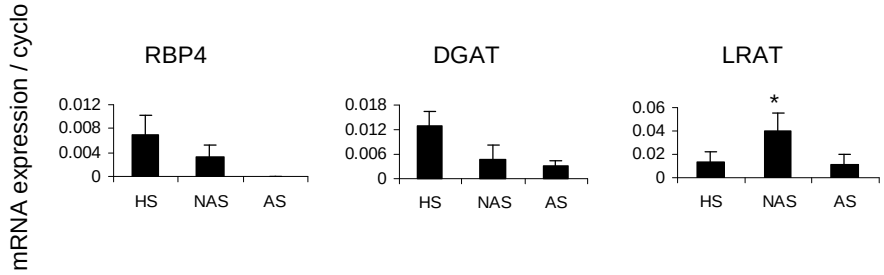

Figure 6. mRNA expression level of RBP4, DGAT and LRAT normalized to cyclophilin A (cyclo) in non-affected and affected skin of AD patients compared to healthy volunteers (n=6) ($p < 0.05$, compared to healthy skin), HS – healthy skin, NAS – non-affected skin, AS – affected skin.

4.1.2. Dysregulation of retinal synthesis

Expression levels of beta-carotene-monooxygenase (BCMO1) and beta-carotene-monooxygenase 2 (BCMO2) mRNA were not significantly altered in skin with atopic dermatitis compared to healthy skin. Retinol dehydrogenases and alcohol dehydrogenases are enzymes which are responsible for converting retinol to retinal. Retinol dehydrogenase (RDH) 2, RDH10 and RDH16 showed a very similar pattern in their mRNA expression. RDH2 was statistically significantly augmented in both affected and non-affected atopic dermatitis skin, compared to healthy skin. Induced mRNA expression of RDH10 and RDH16 was observed in atopic dermatitis affected skin vs. healthy skin. The mRNA expression of alcohol dehydrogenase (ADH) 1C was significantly reduced in non-affected and affected AD skin (Figure 7.).

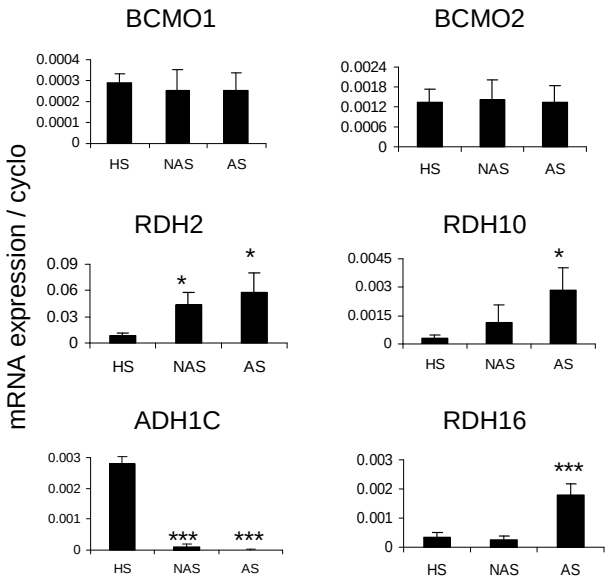

Figure 7. mRNA expression levels of BCMO1, BCMO2, RDH2, RDH10, ADH1c and RHD16 normalized to cyclo in non-affected and affected skin of AD patients compared to healthy volunteers (n=6) (* - $p < 0.05$, *** - $p < 0.001$, compared to HS). For abbreviations see Figure 6.

CRBP 1 is the intracellular carrier of retinol and its mRNA expression showed a significant down-regulation in case of skin with atopic dermatitis, both in non-affected and affected AD skin (Figure 8.).

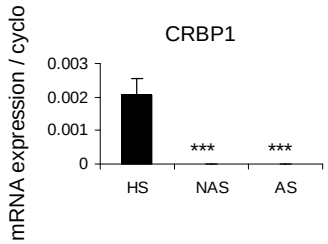

Figure 8. mRNA expression level of CRBP1 normalized to cyclo in non-affected and affected skin of AD patients compared to healthy volunteers (n=6) (*** - $p < 0.001$, compared to HS). For abbreviations see Figure 6.

4.1.3. Reduced retinoic acid synthesis in affected and non-affected skin of atopic dermatitis

Retinal dehydrogenases (RALDH) or acetaldehyde dehydrogenases convert retinaldehyde to RA. We observed a significant decrease of RALDH1 mRNA levels in skin with atopic disease, both in affected and non-affected skin, while the mRNA expression of RALDH2 and RALDH3 did not show significant alterations in our experimental setup (Figure 9.).

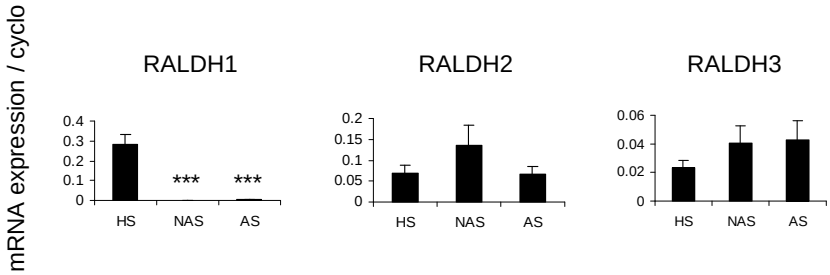

Figure 9. mRNA expression level of RALDH1, RALDH2 and RALDH3 normalized to cyclo in non-affected and affected skin of AD patients compared to healthy volunteers (n=6) (*** - $p < 0.001$, compared to HS). For abbreviations see Figure 6.

CRABP2 is an intracellular retinoic acid transporter protein and no alteration was observed in its mRNA expression in skin of atopic dermatitis patients. Also CRABP1 did not show a significant alteration in diseased skin, in comparison to healthy skin (Figure 10.).

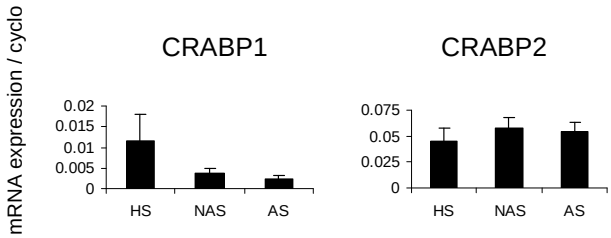

Figure 10. mRNA expression level of CRABP1 and CRABP2 normalized to cyclo in non-affected and affected skin of AD patients compared to healthy volunteers (n=6) . For abbreviations see Figure 6.

4.1.4. Reduced expression of RA degradation / metabolism enzymes in affected as well as non-affected skin of atopic dermatitis

mRNA expression level of RA-degrading enzymes was determined and revealed a significant decrease in case of CYP26A1 and CYP2S1, both in affected and non-affected AD skin. However mRNA expression of CYP26B1 could not be detected in our experimental setup (Figure 11.).

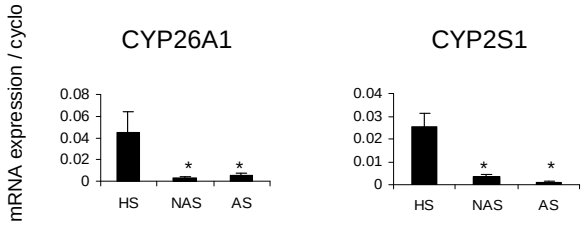

Figure 11. mRNA expression level of CYP26A1 and CYP2S1 normalized to cyclo in non-affected and affected skin of AD patients compared to healthy volunteers (n=6) (* - $p < 0.05$, compared to HS). For abbreviations see Figure 6.

4.1.5. Increased expression of RXRα in non-affected skin of atopic dermatitis

RARα mRNA levels did not show any significant alteration in diseased compared to healthy skin, while mRNA expression of RARβ was slightly but non-significantly up-regulated in non-affected AD skin. By contrast, the expression of RARγ was comparable

between atopic dermatitis and healthy skin. The expression of RXRα was significantly increased in non-affected and affected AD skin (Figure 12.).

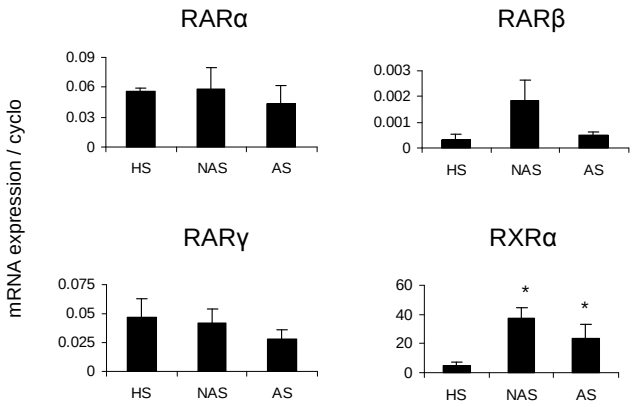

Figure 12. mRNA expression level of RARα, RARβ, RARγ and RXRα normalized to cyclo in non-affected and affected skin of AD patients compared to healthy volunteers (n=6) (* - $p < 0.05$, compared to HS). For abbreviations see Figure 6.

4.1.6. Expression of retinoid target genes in atopic dermatitis

The mRNA expression of retinoic acid receptor responder (RARRES1) was significantly decreased in case of atopic dermatitis in non-affected and affected skin, in comparison to healthy skin. Also transglutaminase 2 (TGM2) showed a significant decrease both in non-affected and in affected skin of atopic dermatitis. By contrast, the mRNA expression of heparin-binding EGF-like growth factor (HB-EGF) was comparable between AD patients and healthy volunteers (Figure 13.).

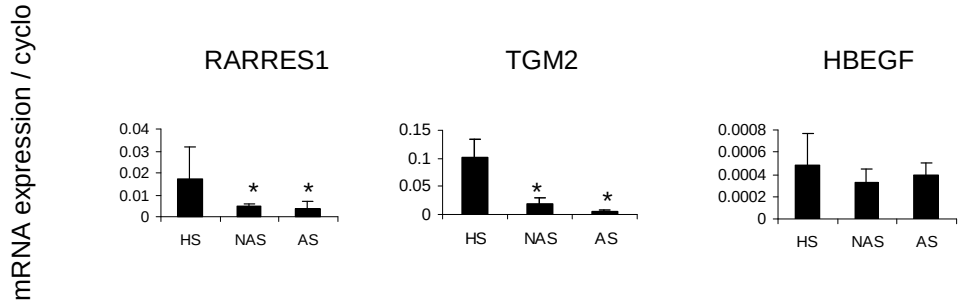

Figure 13. mRNA expression level of RARRES1, TGM2 and HBEGF normalized to cyclo in non-affected and affected skin of AD patients compared to healthy volunteers (n=6) (* - $p < 0.05$, compared to HS). For abbreviations see Figure 6.

Due to strongly reduced retinoid signalling pathways in AD skin we next analyzed ATRA and ROL concentrations in skin biopsies and serum of healthy volunteers and diseased patients:

4.2. Reduced concentrations of retinol and all-*trans* retinoic acid in AD skin, but not in serum of AD patients

Skin concentrations of ATRA and ROL were strongly reduced in affected (ATRA 0.4 / 0.5 ng/g; ROL 37 / 46 ng/g) but also in non-affected skin biopsies (ATRA 0.3 / 0.6 ng/g; ROL 32 / 54 ng/g) from an AD patient in comparison to ATRA and ROL concentrations in healthy skin (ATRA 0.7 / 1.2 ng/g; ROL 207 / 253 ng/g) (Figure 14.).

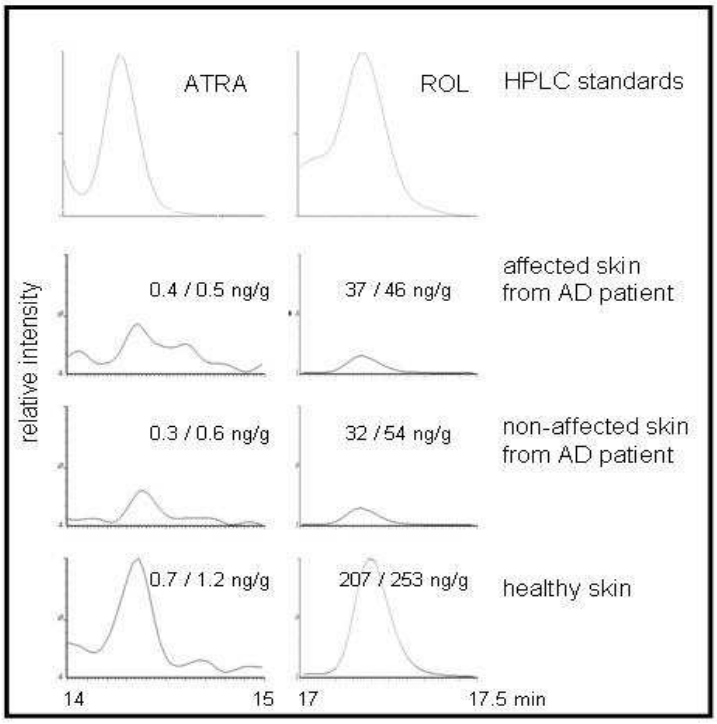

Figure 14. HPLC MS-MS chromatogram of all-*trans* retinoic acid (ATRA) and retinol (ROL) using specific MS-MS tracks for ATRA and ROL from representative human skin biopsies of one affected and one non-affected skin biopsy from one AD-patient and one healthy volunteer. The two values represent the concentrations from the two independent determinations using the skin of two different healthy as well as AD-patients. Y-Axis of HPLC MS-MS chromatograms of the measured skin samples are the same magnitude for better visualization and comparison.

Serum concentrations of ATRA 2,8 ± 0,8 ng/ml and ROL 510 ± 217 ng/ml were comparable in healthy volunteers and AD patients ATRA 2,9 ± 1,0 ng/ml and ROL 573 ± 191 ng/ml. No significant differences could be observed between healthy volunteers and AD patients (Table 9.):

	ATRA	**ROL**
HEALTHY	2.8 ± 0.8	510 ± 217
AD	2.9 ± 1.0	573 ± 191

Table 9. Concentrations of ATRA and ROL in ng/ml in serum of healthy volunteers (HEALTHY, n=6) and atopic dermatitis patients (AD, n=6).

4.3. Fatty acid composition of serum lipid classes in mice following allergic sensitization with or without dietary docosahexaenoic acid-enriched fish oil substitution

Concentrations of DHA and EPA have been shown to be increased after DHA supplementation in serum lipids but so far to the best of our knowledge the effect of allergic sensitization on the composition of serum lipids has not been investigated. We aimed to find out how does the fatty acid composition of serum lipid classes in mice change following allergic sensitization with or without dietary docosahexaenoic acid-enriched fish oil substitution.

The effect of the different diets and treatments are discussed separately: the effect of diet (basal diet (control diet; CTRL) and PBS injections (CTRL-PBS) vs. DHA-enriched fish oil-supplementation and PBS injections (DHA-PBS); basal diet and OVA injections (CTRL-OVA) vs. DHA-enriched fish oil supplementation and OVA injections (DHA-OVA)); respectively the effect of allergic sensitization without dietary supplementation (basal diet with PBS injections (CTRL-PBS) vs. basal diet with OVA injections (CTRL-OVA)) and with dietary DHA supplementation (DHA-enriched fish oil supplementation with PBS injections (DHA-PBS) vs. DHA-enriched fish oil supplementation with OVA injections (DHA-OVA)).

In a second set of experiments we treated both the animals fed the basal diet and the animals fed the DHA-enriched fish oil supplementation with $Al(OH)_3$. Fatty acid composition of plasma cholesteryl esters, phospholipids and triacylglycerols are shown in consequent tables, as it follows.

4.3.1. The effect of dietary docosahexaenoic acid (DHA)-enriched fish oil-supplementation without allergic sensitization - control diet and phosphate-buffered saline treatment (CTRL-PBS) vs. docosahexaenoic acid (DHA)-enriched diet and phosphate-buffered saline treatment (DHA-PBS)

The sum of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) were comparable between both groups, except for higher values of MUFAs in triacylglycerols (TAG) in the control animals. *n*-3 PUFA levels were strongly increased after DHA-enriched fish oil supplementation in all the three lipid classes. Inconsistent results were seen in the individual *n*-6 PUFAs. Values of linoleic acid (LA) increased significantly in cholesteryl esters, but decreased in TAG. Similarly, γ-linolenic acid (GLA) values were higher in phospholipids, but significantly lower in TAG and cholesteryl esters in the animals treated with *n*-3 PUFAs in comparison to the controls. Values of DHGLA were significantly higher in both cholesteryl esters and phospholipids in animals treated with n-3 PUFAs than in control mice. In contrast, percentages (w/w) of arachidonic acid (AA) were significantly lower in all three serum lipid classes of animals receiving *n*-3 PUFA supplementation compared to the controls. The sum of *n*-6 PUFAs was always lower in animals receiving *n*-3 PUFA supplementation. Table 10. shows the fatty acid composition of plasma cholesteryl ester lipids in mice fed either with diet supplemented with docosahexaenoic acid (DHA) enriched fish oil or control diet (CTRL) and subsequently treated with PBS.

Fatty acid composition of plasma cholesterol esters in CTRL-PBS vs. DHA-PBS

Fatty acid	CTRL-PBS		DHA-PBS	
	Mean	SD	Mean	SD
Sum of SFA	8.96	*0.34*	11.47	*0.63*
Sum of MUFA	12.00	*0.51*	11.36	*0.44*
18-2n6 (LA)	31.53	*15.44*	43.07 *	*4.10*
18-3n6 (GLA)	0.51	*0.32*	0.29 *	*0.06*
20-2n6	0.30	*0.17*	0.16 *	*0.19*
20-3n6 (DHGLA)	0.58	*0.17*	0.95 *	*0.10*
20-4n6 (AA)	42.51	*12.14*	20.16 *	*5.00*
22-4n6	0.63	*0.25*	0.15 *	*0.06*
22-5n6	0.91	*0.53*	0.18	*0.12*
Sum of n-6 PUFA	76.98	*0.77*	64.96 *	*1.02*
18-3n3 (ALA)	0.05	*0.01*	0.24	*0.39*
20-3n3	0.29	*0.26*	1.04 *	*1.35*
20-5n3 (EPA)	0.05	*0.05*	4.21 *	*1.53*
22-5n3 (DPA)	0.03	*0.01*	0.15 *	*0.02*
22-6n3 (DHA)	1.64	*0.75*	6.58 *	*2.84*
Sum of n-3 PUFA	2.07	*0.15*	12.20 *	*0.63*

Table 10. Fatty acid composition of plasma cholesteryl ester lipids in mice fed either with diet supplemented with docosahexaenoic acid (DHA)-enriched fish oil or control diet (CTRL) and subsequently treated with phosphate-buffered saline (PBS).

LA - linoleic acid, GLA - γ-linolenic acid, DHGLA – dihomo-γ-linolenic acid, AA – arachidonic acid, ALA – α-linolenic acid, DPA- docosapentaenoic acid.

* Indicates significant differences ($p < 0.05$)

Table 11. shows the fatty acid composition of plasma phospholipids in mice fed either with diet supplemented with docosahexaenoic acid (DHA) enriched fish oil or control diet (CTRL) and subsequently treated with phosphate-buffered saline (PBS).

Fatty acid composition of plasma phospholipids in CTRL-PBS vs. DHA-PBS

Fatty acid	CTRL-PBS		DHA-PBS	
	Mean	SD	Mean	SD
Sum of SFA	49.01	*0.72*	45.97	*0.51*
Sum of MUFA	9.13	*0.32*	10.01	*0.33*
18-2n6 (LA)	22.75	*1.58*	24.72	*2.85*
18-3n6 (GLA)	0.10	*0.04*	0.67*	*0.59*
20-2n6	0.31	*0.04*	0.27	*0.04*
20-3n6 (DHGLA)	1.00	*0.19*	1.63 *	*0.30*
20-4n6 (AA)	14.01	*3.63*	6.10 *	*1.78*
22-4n6	1.50	*0.70*	0.44 *	*0.25*
22-5n6	0.03	*0.01*	0.49 *	*0.81*
Sum of n-6 PUFA	39.69	*0.70*	34.32	*0.82*
18-3n3 (ALA)	0.02	*0.01*	0.24	*0.22*
20-3n3	0.12	*0.08*	0.40	*0.22*
20-5n3 (EPA)	0.007	*0.005*	0.94 *	*0.32*
22-5n3 (DPA)	0.02	*0.01*	0.27 *	*0.08*
22-6n3 (DHA)	2.00	*1.02*	7.86 *	*3.78*
Sum of n-3 PUFA	2.17	*0.21*	9.70 *	*0.79*

Table 11. Fatty acid composition of plasma phospholipids in mice fed either with diet supplemented with DHA-enriched fish oil or control diet (CTRL) and subsequently treated with phosphate-buffered saline (PBS). For abbreviations see Table 10.

Table 12. shows the fatty acid composition of plasma triacylglycerol lipids in mice fed either with diet supplemented with DHA-enriched fish oil or control diet (CTRL) and subsequently treated with phosphate-buffered saline (PBS).

Fatty acid composition of plasma triacylglycerols in CTRL-PBS vs. DHA-PBS

Fatty acid	CTRL-PBS		DHA-PBS	
	Mean	SD	Mean	SD
Sum of SFA	25.21	*0.30*	26.91	*0.70*
Sum of MUFA	36.24	*0.46*	30.39 *	*0.51*
18-2n6 (LA)	33.20	*2.58*	27.19*	*4.47*
18-3n6 (GLA)	0.27	*0.05*	0.16 *	*0.04*
20-2n6	0.53	*0.20*	0.26 *	*0.04*
20-3n6 (DHGLA)	0.64	*0.29*	0.40	*0.17*
20-4n6 (AA)	2.53	*0.91*	1.02 *	*0.26*
22-4n6	0.54	*0.21*	0.49	*0.20*
22-5n6	0.41	*0.26*	0.39	*0.30*
Sum of n-6 PUFA	38.11	*0.49*	29.91 *	*1.14*
18-3n3 (ALA)	0.04	*0.03*	0.19 *	*0.02*
20-3n3	0.26	*0.19*	0.32	*0.17*
20-5n3 (EPA)	0.02	*0.01*	1.47 *	*0.42*
22-5n3 (DPA)	0.09	*0.03*	0.87 *	*0.45*
22-6n3 (DHA)	0.03	*0.01*	9.94 *	*2.64*
Sum of n-3 PUFA	0.43	*0.05*	12.80 *	*0.62*

Table 12. Fatty acid composition of plasma triacylglycerol lipids in mice fed either with diet supplemented with DHA-enriched fish oil or control diet (CTRL) and subsequently treated either with phosphate-buffered saline (PBS). For abbreviations see Table 10.

4.3.2. Effect of dietary DHA-enriched fish oil-supplementation with allergic sensitization (CTRL-OVA vs. DHA-OVA)

Values of the relevant *n*-3 PUFAs were all significantly higher in the animals supplemented with DHA and EPA. The sums of SFAs were significantly higher in all serum lipid classes in the animals receiving *n*-3 PUFA supplementation. The sums of MUFAs were higher in phospholipids and cholesteryl esters, but were significantly lower in TAG in the animals treated with *n*-3 PUFAs than in the controls. Values of LA were significantly higher in cholesteryl esters, but significantly lower in TAG in the animals receiving DHA and EPA

supplementation. AA values as well as the sum of *n*-6 PUFAs were significantly lower in all serum lipid classes in the animals treated with *n*-3 PUFAs than in the controls. In general, all *n*-3 PUFA levels were higher after DHA-supplementation. Table 13. shows the fatty acid composition of plasma cholesteryl ester lipids in mice fed either with diet supplemented with docosahexaenoic acid (DHA) enriched fish oil or control diet (CTRL) and subsequently treated with ovalbumin (OVA).

Fatty acid composition of plasma cholesterol esters in CTRL-OVA vs. DHA-OVA

Fatty acid	CTRL-OVA		DHA-OVA	
	Mean	SD	Mean	SD
Sum of SFA	6.09	*0.18*	12.78 *	*0.68*
Sum of MUFA	10.97	*0.31*	13.24	*0.27*
18-2n6 (LA)	32.73	*9.22*	43.02 *	*2.07*
18-3n6 (GLA)	0.42	*0.04*	0.55	*0.73*
20-2n6	0.13	*0.02*	0.19	*0.17*
20-3n6 (DHGLA)	0.48	*0.06*	1.13 *	*0.25*
20-4n6 (AA)	46.61	*7.86*	18.84 *	*5.49*
22-4n6	0.54	*0.15*	0.14 *	*0.04*
22-5n6	0.08	*0.03*	0.58	*0.69*
Sum of n-6 PUFA	80.99	*0.42*	64.44 *	*0.65*
18-3n3 (ALA)	0.03	*0.03*	0.19 *	*0.27*
20-3n3	0.12	*0.06*	0.53 *	*0.17*
20-5n3 (EPA)	0.02	*0.01*	3.12 *	*1.13*
22-5n3 (DPA)	0.03	*0.01*	0.15	*0.08*
22-6n3 (DHA)	1.75	*0.34*	5.55 *	*2.61*
Sum of n-3 PUFA	1.95	*0.08*	9.54 *	*0.67*

Table 13. Fatty acid composition of plasma cholesteryl ester lipids in mice fed either with diet supplemented with DHA-enriched fish oil or control diet (CTRL) and subsequently treated with ovalbumin (OVA). For abbreviations see Table 10.

Table 14. shows the fatty acid composition of plasma phospholipids in mice fed either with diet supplemented with DHA-enriched fish oil or control diet (CTRL) and subsequently treated with ovalbumin (OVA).

Fatty acid composition of plasma phospholipids CTRL-OVA vs. DHA-OVA

Fatty acid	CTRL-OVA		DHA-OVA	
	Mean	SD	Mean	SD
Sum of SFA	46.06	*0.29*	52.99 *	*0.44*
Sum of MUFA	10.08	*0.27*	11.70	*0.32*
18-2n6 (LA)	22.11	*1.98*	20.82	*1.23*
18-3n6 (GLA)	0.14	*0.12*	0.05 *	*0.02*
20-2n6	0.33	*0.04*	0.30	*0.03*
20-3n6 (DHGLA)	1.03	*0.12*	1.64 *	*0.32*
20-4n6 (AA)	15.88	*1.98*	5.48 *	*1.46*
22-4n6	1.69	*0.56*	0.25 *	*0.07*
22-5n6	0.09	*0.10*	0.13	*0.15*
Sum of n-6 PUFA	41.26	*0.51*	28.68 *	*0.34*
18-3n3 (ALA)	0.04	*0.03*	0.05	*0.03*
20-3n3	0.13	*0.08*	0.18 *	*0.12*
20-5n3 (EPA)	0.003	*0.001*	0.64 *	*0.17*
22-5n3 (DPA)	0.03	*0.00*	0.23 *	*0.04*
22-6n3 (DHA)	2.40	*0.40*	5.54 *	*2.74*
Sum of n-3 PUFA	2.60	*0.07*	6.64 *	*0.57*

Table 14. Fatty acid composition of plasma phospholipids in mice fed either with diet supplemented with DHA-enriched fish oil or control diet (CTRL) and subsequently treated with ovalbumin (OVA). For abbreviations see Table 10.

Table 15. shows the fatty acid composition of plasma triacylglycerol lipids in mice fed either with diet supplemented with DHA-enriched fish oil or control diet (CTRL) and subsequently treated with ovalbumin (OVA).

Fatty acid composition of plasma triacylglycerols CTRL-OVA vs. DHA-OVA

Fatty acid	CTRL-OVA		DHA-OVA	
	Mean	SD	Mean	SD
Sum of SFA	23.18	*0.34*	34.12 *	*0.83*
Sum of MUFA	40.39	*0.52*	29.70 *	*0.56*
18-2n6 (LA)	31.51	*4.42*	22.06 *	*2.34*
18-3n6 (GLA)	0.27	*0.03*	0.27	*0.20*
20-2n6	0.47	*0.12*	0.25 *	*0.03*
20-3n6 (DHGLA)	0.49	*0.11*	0.36	*0.09*
20-4n6 (AA)	2.44	*0.31*	0.99 *	*0.64*
22-4n6	0.47	*0.13*	0.43	*0.12*
22-5n6	0.19	*0.19*	2.19 *	*1.84*
Sum of n-6 PUFA	35.84	*1.11*	26.54 *	*0.59*
18-3n3 (ALA)	0.11	*0.05*	0.24 *	*0.04*
20-3n3	0.26	*0.27*	0.57	*0.34*
20-5n3 (EPA)	0.01	*0.01*	1.20 *	*0.55*
22-5n3 (DPA)	0.03	*0.01*	0.72 *	*0.25*
22-6n3 (DHA)	0.18	*0.02*	6.92 *	*3.64*
Sum of n-3 PUFA	0.59	*0.07*	9.64 *	*0.83*

Table 15. Fatty acid composition of plasma triacylglycerol lipids in mice fed either with diet supplemented with DHA-enriched fish oil or control diet (CTRL) and subsequently treated with ovalbumin (OVA). For abbreviations see Table 10.

4.3.3. Effect of allergic sensitization without dietary DHA-enriched fish oil-supplementation: control diet and phosphate-buffered saline treatment vs. control diet and ovalbumin treatment (CTRL-PBS vs. CTRL-OVA)

The sums of SFAs were comparable in phospholipids and triacylglycerols in sensitized and non-sensitized animals, while in cholesteryl esters they were significantly lower in the sensitized animals. The sums of MUFAs were similar in phospholipids, triacylglycerols and cholesteryl esters. Allergic sensitization did not cause significant changes in *n*-6 PUFA values. *n*-3 PUFA values exhibited significant changes upon sensitization mainly in triacylglycerols: values of ALA, and DHA significantly increased, whereas those of EPA and docosapentaenoic acid (22:5*n*-3) significantly decreased. EPA was significantly lower in triacylglycerols and phospholipids and borderline significantly lower in cholesteryl esters. In tables 16., 17. and 18., the fatty acid composition of plasma cholesteryl ester lipids, phospholipids and triacilglycerol lipids are shown subsequently for control diet and phosphate-buffered treatment vs. control diet and ovalbumin treatment (CTRL-PBS vs. CTRL-OVA).

Fatty acid composition of plasma cholesterol esters in CTRL-PBS vs. CTRL-OVA

Fatty acid	CTRL-PBS		CTRL-OVA	
	Mean	SD	Mean	SD
Sum of SFA	8.96	*0.34*	6.09 *	*0.18*
Sum of MUFA	12.00	*0.51*	10.97	*0.31*
18-2n6 (LA)	31.53	*15.44*	32.73	*9.22*
18-3n6 (GLA)	0.51	*0.32*	0.42	*0.04*
20-2n6	0.30	*0.17*	0.13	*0.02*
20-3n6 (DHGLA)	0.58	*0.17*	0.48	*0.06*
20-4n6 (AA)	42.51	*12.14*	46.61	*7.86*
22-4n6	0.63	*0.25*	0.54	*0.15*
22-5n6	0.91	*0.53*	0.08	*0.03*
Sum of n-6 PUFA	76.98	*0.77*	80.99	*0.42*
18-3n3 (ALA)	0.05	*0.01*	0.03	*0.03*
20-3n3	0.29	*0.26*	0.12 *	*0.06*
20-5n3 (EPA)	0.05	*0.05*	0.02	*0.01*
22-5n3 (DPA)	0.03	*0.01*	0.03	*0.01*
22-6n3 (DHA)	1.64	*0.75*	1.75	*0.34*
Sum of n-3 PUFA	2.07	*0.15*	1.95	*0.08*

Table 16. Fatty acid composition of plasma cholesteryl ester lipids in mice fed with a control diet and phosphate-buffered treatment vs. control diet and ovalbumin treatment (CTRL-PBS vs. CTRL-OVA). For abbreviations see Table 10.

Fatty acid composition of plasma phospholipids CTRL-PBS vs. CTRL-OVA

Fatty acid	CTRL-PBS		CTRL-OVA	
	Mean	SD	Mean	SD
Sum of SFA	49.01	*0.72*	46.06	*0.29*
Sum of MUFA	9.13	*0.32*	10.08	*0.27*
18-2n6 (LA)	22.75	*1.58*	22.11	*1.98*
18-3n6 (GLA)	0.10	*0.04*	0.14	*0.12*
20-2n6	0.31	*0.04*	0.33	*0.04*
20-3n6 (DHGLA)	1.00	*0.19*	1.03	*0.12*
20-4n6 (AA)	14.01	*3.63*	15.88	*1.98*
22-4n6	1.50	*0.70*	1.69	*0.56*
22-5n6	0.03	*0.01*	0.09	*0.10*
Sum of n-6 PUFA	39.69	*0.70*	41.26	*0.51*
18-3n3 (ALA)	0.02	*0.01*	0.04	*0.03*
20-3n3	0.12	*0.08*	0.13	*0.08*
20-5n3 (EPA)	0.007	*0.005*	0.003*	*0.001*
22-5n3 (DPA)	0.02	*0.01*	0.03	*0.00*
22-6n3 (DHA)	2.00	*1.02*	2.40	*0.40*
Sum of n-3 PUFA	2.17	*0.21*	2.60	*0.07*

Table 17. Fatty acid composition of plasma phospholipids in mice fed with a control diet and phosphate-buffered treatment vs. control diet and ovalbumin treatment (CTRL-PBS vs. CTRL-OVA). For abbreviations see Table 10.

Fatty acid composition of plasma triacylglycerols CTRL-PBS vs. CTRL-OVA

Fatty acid	CTRL-PBS		CTRL-OVA	
	Mean	SD	Mean	SD
Sum of SFA	25.21	*0.30*	23.18	*0.34*
Sum of MUFA	36.24	*0.46*	40.39	*0.52*
18-2n6 (LA)	33.20	*2.58*	31.51	*4.42*
18-3n6 (GLA)	0.27	*0.05*	0.27	*0.03*
20-2n6	0.53	*0.20*	0.47	*0.12*
20-3n6 (DHGLA)	0.64	*0.29*	0.49	*0.11*
20-4n6 (AA)	2.53	*0.91*	2.44	*0.31*
22-4n6	0.54	*0.21*	0.47	*0.13*
22-5n6	0.41	*0.26*	0.19	*0.19*
Sum of n-6 PUFA	38.11	*0.49*	35.84	*1.11*
18-3n3 (ALA)	0.04	*0.03*	0.11 *	*0.05*
20-3n3	0.26	*0.19*	0.26	*0.27*
20-5n3 (EPA)	0.02	*0.01*	0.01 *	*0.01*
22-5n3 (DPA)	0.09	*0.03*	0.03 *	*0.01*
22-6n3 (DHA)	0.03	*0.01*	0.18 *	*0.02*
Sum of n-3 PUFA	0.43	*0.05*	0.59	*0.07*

Table 18. Fatty acid composition of plasma triacylglycerol lipids in mice fed with a control diet and phosphate-buffered treatment vs. control diet and ovalbumin treatment (CTRL-PBS vs. CTRL-OVA). For abbreviations see Table 10.

4.3.4. Effect of allergic-sensitisation with dietary DHA-enriched fish oil-supplementation: docosahexaenoic acid-enriched diet and phosphate buffered saline treatment vs. docosahexaenoic acid-enriched diet and ovalbumin treatment (DHA-PBS vs. DHA-OVA).

Sensitization with ovalbumin with dietary interventions resulted in similar SFA levels in cholesteryl esters, but significantly increased levels in triacylglycerols and phospholipids. The sums of MUFAs were similar in all lipid classes. *n*-6 PUFA values were similar in cholesteryl esters and triacylglycerols, while in phospholipids the sum of *n*-6 PUFAs and LA levels were significantly lower in the sensitized animals. In tables 19., 20. and 21., the fatty acid composition of plasma cholesteryl ester lipids, phospholipids and triacilglycerol lipids are shown subsequently for docosahexaenoic acid-enriched diet and phosphate-buffered treatment vs. docosahexaenoic acid-enriched diet and ovalbumin treatment (DHA-PBS vs. DHA-OVA).

Fatty acid composition of plasma cholesterol esters in DHA-PBS vs. DHA-OVA

Fatty acid	DHA-PBS		DHA-OVA	
	Mean	SD	Mean	SD
Sum of SFA	11.47	*0.63*	12.78	*0.68*
Sum of MUFA	11.36	*0.44*	13.24	*0.27*
18-2n6 (LA)	43.07	*4.10*	43.02	*2.07*
18-3n6 (GLA)	0.29	*0.06*	0.55	*0.73*
20-2n6	0.16	*0.19*	0.19	*0.17*
20-3n6 (DHGLA)	0.95	*0.10*	1.13	*0.25*
20-4n6 (AA)	20.16	*5.00*	18.84	*5.49*
22-4n6	0.15	*0.06*	0.14	*0.04*
22-5n6	0.18	*0.12*	0.58	*0.69*
Sum of n-6 PUFA	64.96	*1.02*	64.44	*0.65*
18-3n3 (ALA)	0.24	*0.39*	0.19	*0.27*
20-3n3	1.04	*1.35*	0.53	*0.17*
20-5n3 (EPA)	4.21	*1.53*	3.12	*1.13*
22-5n3 (DPA)	0.15	*0.02*	0.15	*0.08*
22-6n3 (DHA)	6.58	*2.84*	5.55	*2.61*
Sum of n-3 PUFA	12.20	*0.63*	9.54	*0.67*

Table 19. Fatty acid composition of plasma cholesteryl ester lipids in mice fed with a DHA-enriched fish oil diet and phosphate-buffered treatment vs. DHA-enriched fish oil diet and ovalbumin treatment (DHA-PBS vs. DHA-OVA). For abbreviations see Table 10.

Fatty acid composition of plasma phospholipids in DHA-PBS vs. DHA-OVA

Fatty acid	DHA-PBS		DHA-OVA	
	Mean	SD	Mean	SD
Sum of SFA	45.97	*0.51*	52.99 *	*0.44*
Sum of MUFA	10.01	*0.33*	11.70	*0.32*
18-2n6 (LA)	24.72	*2.85*	20.82 *	*1.23*
18-3n6 (GLA)	0.67	*0.59*	0.05 *	*0.02*
20-2n6	0.27	*0.04*	0.30	*0.03*
20-3n6 (DHGLA)	1.63	*0.30*	1.64	*0.32*
20-4n6 (AA)	6.10	*1.78*	5.48	*1.46*
22-4n6	0.44	*0.25*	0.25	*0.07*
22-5n6	0.49	*0.81*	0.13	*0.15*
Sum of n-6 PUFA	34.32	*0.82*	28.68 *	*0.34*
18-3n3 (ALA)	0.24	*0.22*	0.05	*0.03*
20-3n3	0.40	*0.22*	0.18 *	*0.12*
20-5n3 (EPA)	0.94	*0.32*	0.64 *	*0.17*
22-5n3 (DPA)	0.27	*0.08*	0.23 *	*0.04*
22-6n3 (DHA)	7.86	*3.78*	5.54 *	*2.74*
Sum of n-3 PUFA	9.70	*0.79*	6.64 *	*0.57*

Table 20. Fatty acid composition of plasma phospholipids in mice fed with a DHA-enriched fish oil diet and phosphate-buffered treatment vs. DHA-enriched fish oil diet and ovalbumin treatment (DHA-PBS vs. DHA-OVA). For abbreviations see Table 10.

Fatty acid composition of plasma triacylglycerols in DHA-PBS vs. DHA-OVA

Fatty acid	DHA-PBS		DHA-OVA	
	Mean	SD	Mean	SD
Sum of SFA	26.91	*0.70*	34.12 *	*0.83*
Sum of MUFA	30.39	*0.51*	29.70	*0.56*
18-2n6 (LA)	27.19	*4.47*	22.06 *	*2.34*
18-3n6 (GLA)	0.16	*0.04*	0.27	*0.20*
20-2n6	0.26	*0.04*	0.25	*0.03*
20-3n6 (DHGLA)	0.40	*0.17*	0.36	*0.09*
20-4n6 (AA)	1.02	*0.26*	0.99	*0.64*
22-4n6	0.49	*0.20*	0.43	*0.12*
22-5n6	0.39	*0.30*	2.19 *	*1.84*
Sum of n-6 PUFA	29.91	*1.14*	26.54	*0.59*
18-3n3 (ALA)	0.19	*0.02*	0.24	*0.04*
20-3n3	0.32	*0.17*	0.57	*0.34*
20-5n3 (EPA)	1.47	*0.42*	1.20	*0.55*
22-5n3 (DPA)	0.87	*0.45*	0.72	*0.25*
22-6n3 (DHA)	9.94	*2.64*	6.92	*3.64*
Sum of n-3 PUFA	12.80	*0.62*	9.64	*0.83*

Table 21. Fatty acid composition of plasma triacylglycerol lipids in mice fed with a DHA-enriched fish oil diet and phosphate-buffered treatment vs. DHA-enriched fish oil diet and ovalbumin treatment (DHA-PBS vs. DHA-OVA). For abbreviations see Table 10.

Table 22. is a comprehensive summary of the most important changes in the fatty acid composition of plasma cholesteryl esters, phospholipids and triacylglycerols after the administration of the different diets: CTRL-PBS vs. DHA-PBS, CTRL-OVA vs. DHA-OVA, CTRL-PBS vs. CTRL-OVA and DHA-PBS vs. DHA-OVA.

Fatty acids	CHOLESTERYL ESTERS		PHOSPHOLIPIDS		TRIACYLGLYCEROLS	
	CTRL-*PBS*	DHA-*PBS*	CTRL-*PBS*	DHA-*PBS*	CTRL-*PBS*	DHA-*PBS*
20:4n-6 (AA)	42.51 ± 12.14	**20.16 ± 5.00 (*)**	14.01 ± 3.63	**6.10 ± 1.78 (*)**	2.53 ± 0.91	**1.02 ± 0.26 (*)**
Σ n-6 PUFA	76.98 ± 0.01	**64.96 ± 1.02 (*)**	39.69 ± 0.70	34.52 ± 0.82	38.11 ± 0.49	**29.91 ± 1.14 (*)**
20:5n-3 (EPA)	0.05 ± 0.05	**4.21 ± 1.53 (**)**	0.007 ± 0.005	**0.94 ± 0.32 (**)**	0.02 ± 0.01	**1.47 ± 0.42 (**)**
22:6n-3 (DHA)	1.64 ± 0.75	**6.58 ± 2.84 (**)**	2.00 ± 1.02	**7.86 ± 3.78 (**)**	0.03 ± 0.01	**9.94 ± 2.64 (**)**
Σ n-3 PUFA	2.07 ± 0.15	**12.20 ± 0.63 (**)**	2.17 ± 0.21	**9.70 ± 0.79 (**)**	0.43 ± 0.05	**12.80 ± 0.62 (**)**

	CTRL-*OVA*	DHA-*OVA*	CTRL-*OVA*	DHA-*OVA*	CTRL-*OVA*	DHA-*OVA*
20:4n-6 (AA)	46.61 ± 7.86	**18.84 ± 5.49 (*)**	15.88 ± 1.98	**5.48 ± 1.46 (*)**	2.44 ± 0.31	**0.99 ± 0.64 (*)**
Σ n-6 PUFA	80.99 ± 0.42	**64.44 ± 0.65 (*)**	41.26 ± 0.51	**28.68 ± 0.34 (*)**	35.84 ± 1.11	**26.54 ± 0.59 (*)**
20:5n-3 (EPA)	0.02 ± 0.01	**3.12 ± 1.13 (**)**	0.003 ± 0.001	**0.64 ± 0.17 (**)**	0.01 ± 0.01	**1.20 ± 0.55 (**)**
22:6n-3 (DHA)	1.75 ± 0.34	**5.55 ± 2.61 (**)**	2.40 ± 0.40	**5.54 ± 2.74 (**)**	0.18 ± 0.02	**6.92 ± 3.64 (**)**
Σ n-3 PUFA	1.95 ± 0.08	**9.54 ± 0.67 (**)**	2.60 ± 0.70	**6.64 ± 0.57 (**)**	0.59 ± 0.07	**9.64 ± 0.83 (**)**

	CTRL-*PBS*	CTRL-*OVA*	CTRL-*PBS*	CTRL-*OVA*	CTRL-*PBS*	CTRL-*OVA*
20:4n-6 (AA)	42.51 ± 12.14	46.61 ± 7.86	14.01 ± 3.63	15.88 ± 1.98	2.53 ± 0.91	2.44 ± 0.31
Σ n-6 PUFA	76.98 ± 0.01	80.99 ± 0.42	39.69 ± 0.70	41.26 ± 0.51	38.11 ± 0.49	35.84 ± 1.11
20:5n-3 (EPA)	0.05 ± 0.05	0.02 ± 0.01	0.007 ± 0.005	**0.003 ± 0.001 (*)**	0.02 ± 0.01	**0.01 ± 0.01 (*)**
22:6n-3 (DHA)	1.64 ± 0.75	1.75 ± 0.34	2.00 ± 1.02	2.40 ± 0.40	0.03 ± 0.01	**0.18 ± 0.02 (**)**
Σ n-3 PUFA	2.07 ± 0.15	1.95 ± 0.08	2.17 ± 0.21	2.60 ± 0.70	0.43 ± 0.05	0.59 ± 0.07

	DHA-*PBS*	DHA-*OVA*	DHA-*PBS*	DHA-*OVA*	DHA-*PBS*	DHA-*OVA*
20:4n-6 (AA)	20.16 ± 5.00	18.84 ± 5.49	6.10 ± 1.78	5.48 ± 1.46	1.02 ± 0.26	0.99 ± 0.64
Σ n-6 PUFA	64.96 ± 1.02	64.44 ± 0.65	34.52 ± 0.82	**28.68 ± 0.34 (*)**	29.91 ± 1.14	26.54 ± 0.59
20:5n-3 (EPA)	4.21 ± 1.53	3.12 ±1.13	0.94 ± 0.32	**0.64 ± 0.17 (*)**	1.47 ± 0.42	1.20 ± 0.55
22:6n-3 (DHA)	6.58 ± 2.84	5.55 ±2.61	7.86 ± 3.78	**5.54 ± 2.74 (*)**	9.94 ± 2.64	6.92 ± 3.64
Σ n-3 PUFA	12.20 ± 0.63	9.54 ±0.67	9.70 ± 0.79	**6.64 ± 0.57 (*)**	12.80 ± 0.62	9.64 ± 0.83

Table 22. Comprehensive summary of the most important changes in the fatty acid composition of plasma cholesteryl esters, phospholipids and triacylglycerols after the administration of the different diets: CTRL-PBS vs. DHA-PBS, CTRL-OVA vs. DHA-OVA, CTRL-PBS vs. CTRL-OVA and DHA-PBS vs. DHA-OVA.

* - significantly lower difference ($p < 0.05$)

** - significantly higher difference ($p < 0.05$)

4.3.5. Inter-comparison of lipid classes

DHA-supplementation affected the fatty acid compositions of all three lipid classes vs. control. The increase in the sum of n-3 PUFAs expressed as DHA-PBS/CTRL-PBS was the most pronounced in serum triacylglycerols (30-times), in comparison to phospholipids (4-times) and cholesteryl esters (6-times).

4.3.6. Ratios of EPA/AA, EPA/DHGLA and EPA/DHA

The ratios in the PBS-treated animals were always set as 100% for better comparison of the OVA sensitization values after basal- or DHA-enriched fish oil supplementation diet. The EPA/AA ratio for triacylglycerols decreased significantly and just non-significantly in cholesteryl esters and phospholipids in non-supplemented animals to 23 ± 26%, 29 ± 6%, 29 ± 15% after allergic sensitization, whereas the decrease was only to 70 ± 31%, 80 ± 18%, 76 ± 17% after accompanying DHA-enriched fish oil-supplementation.

The EPA/DHGLA ratio also decreased in non-supplemented animals to 20 ± 18% for triacylglycerols (significantly), 42 ± 15% for cholesteryl esters and 36 ± 22% for phospholipids after allergic sensitization, whereas the decrease was much smaller (to 92 ± 51% for triacylglycerols, 66 ± 26% for cholesteryl esters and 68 ± 13% for phospholipids) after DHA-enriched fish oil-supplementation.

The EPA vs. DHA ratio decreased after sensitization without DHA-enriched fish oil-supplementation to 3 ± 4% significantly for triacylglycerols, 26 ± 6% for cholesteryl esters and 19 ± 9% for phospholipids of the original value, while after DHA-enriched fish oil-supplementation it was significantly higher after sensitisation with 119 ± 11% for triacylglycerols, 88 ± 17% for cholesteryl esters and 93 ± 26% for phospholipids.

Triacylglycerols	EPA/AA	EPA/DHGLA	EPA/DHA
	Mean ± SD	Mean ± SD	Mean ± SD
CTRL PBS (%)	100 ± 41	100 ± 73	100 ± 42
CTRL OVA (%)	23 ± 26 *	20 ± 19 *	3 ± 4 *
DHA PBS (%)	100 ± 26	100 ± 37	100 ± 19
DHA OVA (%)	70 ± 31	92 ± 51 **	119 ± 11 **

Cholesteryl esters	EPA/AA	EPA/DHGLA	EPA/DHA
	Mean ± SD	Mean ± SD	Mean ± SD
CTRL PBS (%)	100 ± 106	100 ± 91	100 ± 105
CTRL OVA (%)	29 ± 6	42 ± 15	26 ± 6
DHA PBS (%)	100 ± 21	100 ± 34	100 ± 16
DHA OVA (%)	80 ± 18 **	66 ± 26	88 ± 17 **

Phospholipids	EPA/AA	EPA/DHGLA	EPA/DHA
	Mean ± SD	Mean ± SD	Mean ± SD
CTRL PBS (%)	100 ± 92	100 ± 79	100 ± 122
CTRL OVA (%)	29 ± 15	36 ± 22	19 ± 9
DHA PBS (%)	100 ± 21	100 ± 23	100 ± 46
DHA OVA (%)	76 ± 17 **	68 ± 13 **,*	93 ± 26 **

Table 23. Ratios of selected n-3 and n-6 PUFA (Mean values and standard deviations). Ratios detected in PBS-treated groups were set as 100% (grey shadows). Data are calculated on the basis of six independent samples.

* - Mean value is significantly different from that of the PBS-treated animals ($p<0.05$)

** - Mean value is significantly different from that of the CTRL animals ($p<0.05$).

4.3.7. Influence of $Al(OH)_3$-treatment on the fatty acid composition of plasma phospholipids

Concentrations of AA, DHGLA, DHA and EPA were not significantly altered after $Al(OH)_3$ or $Al(OH)_3$-OVA treatments in basal diet as well in *n*-3-PUFA supplemented animals. Concentrations of EPA were always lower (non-significantly) in OVA - $Al(OH)_3$-treated mice in comparison to PBS-treated mice.

Ratios of EPA/AA, EPA/DHGLA and EPA/DHA were in the same range after $Al(OH)_3$-treatment like for animals with PBS-treatment in basal diet fed animals, while the ratios were much, but non-significantly, lower in OVA-$Al(OH)_3$-treated animals. Ratios of EPA/AA, EPA/DHGLA and EPA/DHA were just slightly lower after $Al(OH)_3$-treatment, ratios of EPA/AA and EPA/DHA were lower and just EPA/DHGLA ratios were significantly lower in the *n*-3 PUFA diet supplemented mice.

n-3 PUFA supplementation diet significantly ameliorated the decrease of the EPA/AA, EPA/DHGLA and EPA/DHA ratios in plasma phospholipids after OVA-$Al(OH)_3$-treatment but not after $Al(OH)_3$-treatment alone in comparison to basal diet fed animals.

Fatty acid	AA	DHGLA	DHA	EPA	EPA/AA (%)	EPA/DHGLA(%)	EPA/DHA(%)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
CTRL-PBS	14.0 ± 3.6	1.00 ± 0.19	2.00 ± 1.02	0.007 ± 0.005	100 ± 92	100 ± 79	100 ± 122
CTRL-$Al(OH)_3$	11.2 ± 0.8	0.88 ± 0.08	2.08 ± 0.30	0.010 ± 0.006	128 ± 59	155 ± 83	82 ± 44
CTRL-OVA-$Al(OH)_3$	15.9 ± 2.0	1.03 ± 0.12	2.40 ± 0.40	0.003 ± 0.001	29 ± 15	36 ± 22	19 ± 9
DHA-PBS	6.10 ± 1.78 *	1.63 ± 0.30 *	7.86 ± 3.78 *	0.94 ± 0.32 *	100 ± 21	100 ± 23	100 ± 46
DHA-$Al(OH)_3$	4.69 ± 0.36 *	1.61 ± 0.23 *	7.29 ± 1.17 *	0.68 ± 0.11 *	94 ± 10	75 ± 9	68 ± 7
DHA-OVA-$Al(OH)_3$	5.48 ± 1.46 *	1.64 ± 0.32 *	5.54 ± 2.74 *	0.64 ± 0.17 *	76 ± 17 *	68 ± 13 **,*	93 ± 26 *

Table 24 Fatty acid composition of plasma phospholipids in mice fed either with diet supplemented with docosahexaenoic acid enriched fish oil or control diet and subsequently treated either with ovalbumin or phosphate-buffered saline and ratios of selected n-3 and n-6 PUFA. Ratios detected in PBS-treated groups were set as 100% (grey shadows). Data are shown as percentages (w/w) calculated on the basis of six independent samples.

* - Mean value is significantly different from that of the PBS-treated animals ($p<0.05$),

** - Mean value is significantly different from that of the CTRL animals ($p<0.05$).

4.3.8. Allergic sensitisation

Serum IgE levels increased significantly after OVA sensitization in the control diet fed group from 0.4 ± 0.2 μg/ml to 3.2 ± 2.0 μg/ml and in the DHA-enriched fish oil supplemented group from 0.7 ± 0.1 μg/ml to 2.8 ± 1.3 μg/ml. There was no significant difference between the IgE levels of the control diet fed group and the DHA-enriched fish oil supplemented group. Additionally, the specificity of the sensitization was supported by measuring OVA-specific IgE titers in sensitized mice whereas in non-sensitized mice no OVA-specific IgE was detected (data not shown).

5. DISCUSSION

Retinoids are known to regulate several events relevant for skin diseases such as epidermal differentiation, proliferation, apoptosis, immune regulation and epidermal barrier properties. In previous studies it has been shown that retinoid supplementation evokes high IgE levels and alters Th1/Th2 balance (Cantorna, et al., 1994; Rühl, et al., 2004; Tokuyama and Tokuyama, 1996). Several retinoids, such as retinoic acid and synthetic retinoic acid analogues have been used therapeutically in skin and immune diseases and proved to be beneficial. Atopic dermatitis is a chronically relapsing inflammatory skin disorder, characterized by disturbed epidermal barrier function, an increased Th2 immune response and elevated IgE levels. So far the expression profiles of the genes involved in retinoid homeostasis, regulation and metabolism, retinoid receptors and target genes have not been investigated in skin of patients with atopic dermatitis in comparison to healthy volunteers.

5.1. Decreased retinoid concentration and retinoid signaling pathways in human atopic dermatitis

In this study we demonstrated that in affected and in non-affected human tissue biopsies the retinoid transport, synthesis, concentrations, signaling and homeostasis are severely dysregulated in comparison to skin from healthy volunteers. Surprisingly, even the skin of non-affected areas of atopic dermatitis patients displayed dysfunction of retinoid signaling, suggesting an intrinsic disease specific dysfunction for the regulation of retinoid binding proteins, metabolizing enzymes, retinoid response target gene expression as well as retinoid concentrations. Interestingly, the mRNA expression of the majority of retinoid response target genes like CRBP1, CYP26A1, CYP2S1, TGM2 and RARRES1 was significantly down-regulated, which is in accordance with the decreased level of retinoic acid determined in AD skin.

ATRA is the major RAR ligand and its concentration in the mammalian skin is tightly regulated in a specific spatiotemporal manner (Chapellier, et al., 2002; Everts, et al., 2004; Everts, et al., 2007). Various cell types in the skin and especially in the inflamed skin have been shown to be able to synthesize the bioactive retinoic acid (Jurukovski, et al., 1999; Spiegl, et al., 2008). We found that retinoid response target genes like RARRES1, CRBP1,

CYP26A1, CYP2S1 and TGM2 are significantly decreased in affected as well as non-affected human skin of AD patients, while the expression of other retinoid target genes like RARβ, CRABP2 and HB-EGF was not altered. HPLC MS-MS data additionally confirmed that the concentration of ATRA is much lower in affected and in non-affected AD skin in comparison to skin from healthy volunteers; no difference was detected between affected and non-affected AD skin. This might also be a cause or result of lower delivery of the retinoic acid precursor, retinol (ROL) via RBP4 to the skin, while both ROL levels as well as RBP4 expression are lower in affected AD skin samples in comparison to healthy volunteers.

Additionally the analysis of serum concentrations from the same patients and volunteers displayed comparable ATRA levels between AD patients and healthy volunteers. These data suggest a systemic non-RA based origin for this skin specific dysfunction of retinoid-mediated signaling in AD. We suggest and partly know already that besides ATRA (the main signaling molecule for retinoid target gene expression), which is much lower in skin of atopic patients, also other relevant and still non-identified bioactive retinoids or / and other retinoid mediated response pathways involving retinoid-activated nuclear receptors must be present. Alternative activators of RAR and RXR may be responsible for stable and non-altered expression of the retinoid target genes RARβ, CRABP2 and HB-EGF in atopic skin even when ATRA levels are present in much lower concentrations. Identification of novel endogenous RAR as well as RXR ligands is currently under investigation in our laboratories.

The expression of the major retinoic acid synthesizing enzyme in the skin the RALDH1 is significantly decreased in AD skin vs. healthy skin (Park, et al., 2007). This strong down-regulation in affected and non-affected AD skin is suggested to be mainly responsible for lower ATRA concentrations and thereby for the significantly lower retinoid mediated signaling in the skin of AD patients.

Deficiency of retinoids / retinoid signaling in the skin or general vitamin A deficiency has been associated to various symptoms also seen in the atopic dermatitis skin phenotype. Th1 / Th2 shift (Cantorna, et al., 1994), altered apoptosis (Trautmann, et al., 2000), altered skin differentiation and proliferation (Gibbs, et al., 1996) and increased bacterial skin colonization (Wiedermann, et al., 1996) were associated with vitamin A deficiency or deletion of retinoid receptor mediated signaling in transgenic skin specific mouse models (Chapellier, et al., 2002; Li, et al., 2005). Whether lower retinoid signaling and lower retinoic acid concentration in AD

skin is based on an intrinsic abnormality is still under examination in our various *in vivo* studies.

Remarkable is the reduced gene expression of the retinoid-target genes CRBP1, CYP26A1, CYP2S1, TGM2, RALDH1, RARRES1 and the ADH1C in non-affected AD skin comparably to affected AD skin. We suggest that a general and intrinsic abnormality is responsible for this dysregulation and may be a result of systemic chronic inflammation. A different expression profile was observed for LRAT and RXRα which are exclusively increased in non-affected AD skin, confirming also a general intrinsic abnormality responsible for this dysregulation of retinoid-signaling (LRAT, RXRα) and maybe of other RXRα-heterodimer mediated pathways in non-affected AD skin. This increased expression of LRAT and RXRα may be a response of the non-affected skin on intrinsic chronic inflammation to further enable and balance reduced retinoid signaling. Additionally the increased expression of RDH2 and RDH10 may be a skin based response to enable and balance retinoid signaling in the skin.

An altered nutrition with high vitamin A as well as pro-vitamin A carotenoids resulting in significantly higher serum levels of all-*trans* retinoic acid (Rühl, et al., 2008) or increased ingestion of dietary fats which lead to increased expression of various factors / enzymes important for retinoid signaling (Jeyakumar, et al., 2008) might contribute also to this altered retinoid signaling in affected as well as non-affected skin of AD patients.

Several approaches using nutritional supplementations with carotenoids and various retinoids as well as systemic inflammation / allergic sensitization are in progress to elucidate why both in affected as well as in non-affected skin of atopic dermatitis patients retinoid transport, synthesis, concentrations and signaling are strongly decreased. We suggest that the answer to this question may help to understand the pathogenesis of atopic dermatitis and may lead to strategies for atopy prevention. Based on our observations we suggest that topical retinoid applications using single retinoids or combinations of some selective retinoids would be highly beneficial therapeutic tools in atopic dermatitis.

In summary, more studies are needed in order to identify how retinoid transport, metabolism, concentrations and signaling are regulated in the skin and the regulation of key players like RALDH1, which is the major enzyme important for retinoic acid synthesis in

human skin, in AD patients in comparison to healthy volunteers. Animal studies using topical as well as systemic application of various retinoids and KO animal models of retinoid synthesizing enzymes and retinoid receptors are in progress. We conclude that the retinoid signaling pathway is dysregulated in AD patients based on an abnormal retinoid transport, synthesis and concentrations in skin which might contribute to the pathogenesis of AD, but also offer novel therapeutic approaches.

In our study we have shown that retinoid signaling pathways and retinoic acid levels are lower in skin of AD patients in comparison to healthy volunteers, additionally, other studies have shown significant changes of the PUFA composition in the skin and serum of atopic dermatitis patients. On the other hand, retinoid supplementation studies have shown to evoke high IgE levels and alter Th1/Th2 balance. We aimed to investigate the impact of allergic sensitization on the PUFA composition of various serum lipid classes as well as IgE secretion in a mouse model with or without dietary DHA-enriched fish oil supplementation.

5.2. Fatty acid composition of serum lipid classes in mice following allergic sensitization with or without dietary docosahexaenoic acid-enriched fish oil substitution

In this study, we demonstrate that both DHA-enriched fish oil-supplementation and allergic sensitization significantly influence the fatty acid composition of different serum lipid classes. Allergic sensitization after three intra-peritoneal injections of OVA associated to $Al(OH)_3$ has been shown in various publications of our group (Rühl, et al., 2003; Rühl, et al., 2004; Worm, et al., 2001) and additionally in this study we determined an increased total IgE level after OVA sensitization. The levels of IgE were just slightly lower in the DHA-enriched fish oil-supplemented group in comparison to the control diet fed group. The fact that the alteration of ratios of individual fatty acids, being precursors of pro-inflammatory or anti-inflammatory PUFA-metabolites, changed after allergic sensitization in a manner depending on DHA-enriched fish oil-supplementation is firstly described in literature.

Supplementation of DHA has been shown, in several studies carried out in various organisms, to yield in increased concentrations of DHA as well as EPA in serum lipids (Kew, et al., 2004; Mueller, et al., 2005; Mueller, et al., 2004). Our data support this aspect of previous reports in a mouse model investigating the fatty acid composition of serum triacylglycerols, phospholipids and cholesteryl esters. The effect of DHA-enriched fish oil-

supplementation on EPA levels could be mediated via three pathways: a) Increased concentrations of EPA in the DHA-enriched fish oil, b) *retro*-conversion of DHA to EPA (Hansen, et al., 1998) and c) decreased conversion of EPA to DHA (Hansen, et al., 1998). In addition to the increased levels of the main n-3 long-chain PUFAs, DHA and EPA, the levels of the n-6 long chain PUFA, AA markedly decreased. This finding is in accordance with previous observations (Kew, et al., 2004; Mueller, et al., 2005), and may reflect the inhibitory effects of abundance of DHA on FADS2, the rate limiting step also in AA biosynthesis (Horrobin, 1993), based on higher concentrations of AA precursors in sunflower oil in comparison to DHA-enriched fish oil.

The exact interrelationship of fatty acids in circulating serum lipids to tissue fatty acid metabolism remains to be clarified (Di Stasi, et al., 2004; Nikolaidis, et al., 2005). However, fatty acid composition of serum lipid classes is still considered an important indicator of fatty acid status (Aro, 2003). In the present study, effects of dietary intervention were detectable in all the three serum lipid classes analyzed, whereas marked effect of allergic sensitization on n-3 long-chain PUFAs could mainly be observed in serum triacylglycerol esters.

The major outcome of our study was the influence of the allergic sensitization on the fatty acid composition of various serum lipid classes. To the best of our knowledge, no study has previously focused on the effect of allergic sensitization on serum fatty acid patterns, whereas several studies compared fatty acid status in humans with and without allergic disease (Hoff, et al., 2005; Korotkova, et al., 2004). Possibly these alterations are partly due to an altered lipoprotein distribution after acute phase response (Schweigert, 2001).

In the present study, we investigated the effects of allergic sensitization induced by triple intra-peritoneal OVA-injections in adult mice, and observed several significant alterations of plasma phospholipids fatty acid levels. Our experiments showed that $Al(OH)_3$ has no or just marginal effects on lipid composition. This biochemical inactive $Al(OH)_3$ adjuvant is used to potentiate the immune responses to vaccines by adsorbing the antigen (Jefferson, et al., 2004; Verdier, et al., 2005). $Al(OH)_3$ does not alter serum lipid concentrations, because if given intra-peritoneally and absorbed to allergen, while aluminium or aluminium ions could be quite active in alteration of lipid metabolism (Sarin, et al., 1997). No relevant amounts of aluminium and aluminium ions will reach in blood circulation in case if biochemical stable

$Al(OH)_3$ is used under physiological pH conditions after intra-peritoneal injections (Berthon and Dayde, 1992).

After allergic sensitization but without accompanying DHA-supplementation, the levels of mainly the n-3 PUFAs, ALA, EPA, DPA and DHA were significantly altered in triacylglycerols. EPA and DPA were significantly down-regulated, whereas DHA and ALA were significantly up-regulated. For better visualization of the results obtained, the ratios of precursors of pro- and anti-inflammatory bioactive lipids EPA, DHGLA and AA were calculated in table 24.

Ratios of EPA/AA and EPA/DHGLA were significantly reduced upon allergic sensitization and without accompanying DHA-enriched fish oil-supplementation. After accompanying DHA-enriched fish oil-supplementation, however, the reduction of the ratios was much lower. Consequently, the ratios were significantly lower in the non-supplemented than in supplemented animals.

Metabolism via cycloxygenase (COX) and lipoxygenase (LOX) pathways has been shown to be highly dependent on the availability of lipid-precursors for further metabolism (Caughey, et al., 1996). It has been convincingly demonstrated that n-3 PUFAs were better substrates for the conversion by lipoxygenases (Jakschik, et al., 1980), but much weaker for the COX-2 mediated pathways to PgE derivatives (Malkowski, et al., 2001). In addition, leukotriens originating from EPA have been shown to be much less active in comparison to analogues from the AA cascade (Leitch, et al., 1984). EPA and other n-3 PUFAs were also found to inhibit COX and LOX activity (Corey, et al., 1983; Obata, et al., 1999; Saku, et al., 1999). Our recent results indicate that the LOX and COX pathways were mainly significantly down-regulated in the peripheral blood mononuclear cells and serum of atopic dermatitis patients in comparison to healthy volunteers (data not shown, Mihály et al, in preparation).

Our data strongly support the hypothesis that DHA-enriched fish oil-supplementation significantly alters the levels and ratios of n-3/n-6 precursor fatty acids for further bio-activation to pro-inflammatory prostaglandins, which are mainly Th2 skewing, and leukotriens (Arcoleo, et al., 1995; Miles, et al., 2003). Various immune-competent cells like lymphocytes, macrophages, dendritic cells etc. could alter lipid metabolism via various enzymes like lipoxygenases, cycloxygenases, cytochromes, etc. as well as various immune

reactions could be influenced by dietary lipids and their active metabolites (Calder, 2001; Rustan and Drevon, 2001). Novel studies support that EPA and DHA via COX- and LOX-pathways serve as precursors for anti-inflammatory bioactive lipids like lipoxins, neuroprotectins and resolvins (Serhan, 2005; Serhan and Savill, 2005). Higher levels of DHA and EPA may lead to increased production of these novel-described derivatives in the mammalian organism after DHA-enriched fish oil-supplementation and accompanying allergic sensitization, possibly resulting in reduced severity of the allergic phenotype.

In summary, the data obtained in the present study indicate that fatty acid levels and especially the ratios of fatty acid representing precursors of bioactive lipids after allergic sensitization highly depend on accompanying DHA-enriched fish oil-supplementation. This DHA-enriched fish oil-supplementation mediated alteration of lipid ratios of bioactive precursor lipids may explain the allergy-ameliorating effects of DHA in particular and/or n-3 PUFAs in general (Abba, et al., 2005; Mueller, et al., 2005; Mueller, et al., 2004; Nagakura, et al., 2000; Watanabe and Kuroda, 1999; Yokoyama, et al., 2000; Zhang, et al., 2005). Unfortunately, in our experimental setup we could not observe any allergy ameliorating effect. Further studies of our group will focus on lipid metabolism and molecular mechanisms of possible allergy-preventive effects of DHA in animal studies, human supplementation trials and human cohort studies.

6. SUMMARY

Polyunsaturated fatty acids (PUFAs) are present in high concentrations in the human organism and are essential components of various layers of the skin, furthermore they are important precursors of several pro- and anti-inflammatory mediators. Several studies investigated the PUFA composition in atopic patients and healthy volunteers and PUFA levels have been shown to be decreased in atopic dermatitis skin in comparison to healthy skin. Alterations of retinoid metabolism and signaling have been related to several skin diseases including atopic dermatitis (AD), but so far the expression profiles of the genes involved in retinoid homeostasis regulation and metabolism, retinoid receptors and target genes as well as retinoid concentrations have not been investigated in the human skin. In previous studies, in mice, retinoids have been shown to enhance Th2-mediated responses, respectively elevate IgE levels.

Our results show that the target gene expression of retinoid receptor regulated pathways is significantly decreased in AD patients. The main retinoic acid synthesizing enzyme, retinal-dehydrogenase 1, was significantly lower expressed in AD patients. Analysis of retinoid concentrations in serum and skin showed comparable all-*trans* retinoic acid (ATRA) and retinol (ROL) concentrations in AD and healthy serum, but strongly reduced ATRA and ROL concentrations in AD skin in comparison to healthy skin. Our data indicate that retinoid transport, synthesis, concentrations, signaling and homeostasis are severely dysregulated in affected and non-affected human skin biopsies of AD patients in comparison to the skin of healthy volunteers.

In the case of PUFAs the missing point was to find out how supplementation of non-sensitized and sensitized mice with DHA-enriched fish oil diet influences the percentile contribution of n-3 / n-6 PUFAs. Significantly increased percentile contributions of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in case of triacylglycerols, cholesteryl esters and phospholipids, while significantly lower arachidonic acid (AA) levels were observed in the serum of both non-sensitized and sensitized mice fed the DHA-enriched diet. Allergic sensitization was reflected by the decreased EPA/AA ratio in all lipid classes in non-supplemented animals and to a lesser extent DHA-enriched fish oil supplemented mice. Serum IgE levels significantly increased after allergic sensitization both in the group fed the basal diet and the group fed the DHA-enriched fish oil diet, but no significant changes could be observed in between the two diet groups.

ÖSSZEFOGLALÁS

A többszörösen telítetlen zsírsavak (PUFA) magas koncentrációban vannak jelen az emberi szervezetben és alapvető összetevői a bőr különböző rétegeinek, továbbá számos pro- és anti-inflammatorikus mediátorok prekurzorai. Több tanulmány vizsgálta a PUFA összetételt atópiás dermatitiszes betegek és egészséges önkéntesek esetén, és kimutatták, hogy a PUFA mennyisége csökkent az atópiás dermatitiszes bőrben az egészséges bőrhöz képest. A retinoid metabolizmus és jelátvitel változásait több bőrbetegséggel hozták összefüggésbe, beleértve az atópiás dermatitiszt (AD) is. A retinoid homeosztázisban, szabályozásban és anyagcserében résztvevő gének, a retinoid receptorok és célgének génexpresszióját, valamint a retinoid koncentrációt még nem vizsgálták humán AD bőrben. Egereken végzett korábbi tanulmányok kimutatták, hogy a retinoidok fokozzák a Th2-mediált válaszokat, illetve IgE-szint emelkedést váltanak ki.

Eredményeink azt mutatják, hogy a retinoid receptor által szabályozott útvonalak célgénjeinek génexpressziója jelentősen csökkent az AD betegekben. A fő retinsav szintetizáló enzim, a retinal-dehidrogenáz 1, szignifikánsan alacsonyabban fejeződött ki AD betegek esetében. A retinoid koncentráció elemzése hasonló csupa-transz retinsav (ATRA) és retinol (ROL) koncentrációt mutatott az AD és egészséges szérumban, de szignifikánsan csökkent az ATRA és ROL koncentrációja az AD bőrben az egészséges bőrhöz képest. Adataink azt mutatják, hogy a retinoid transzport, szintézis, koncentráció, jelátvitel és homeosztázis jelentősen diszregulálódott mind az érintett, mind a nem érintett AD bőrbiopsziákban az egészséges önkéntesekéhez képest.

A PUFA esetében a hiányzó pont az volt, hogy meghatározzuk, hogyan változik az n-3 ill. n-6 PUFA százalékos összetétele a nem szenzitizált és szenzitizált DHA-dúsított halolaj tápon élő egerek esetében. A dokozahexaénsav (DHA) és eikozapentaénsav (EPA) szignifikánsan emelkedett százalékos kontribúciója volt megfigyelhető a trigliceridek, koleszteril észterek és foszfolipidek esetén, míg jelentősen alacsonyabb arachidonsav (AA) szintet figyeltünk meg a nem szenzitizált és szenzitizált DHA-dúsított halolaj tápon élő egerek szérumában. Az allergiás szenzitizációt a csökkent EPA/AA arány mutatta minden vizsgált lipid osztályban az alaptápon élő egerek esetében, kisebb mértékben a DHA-dúsított halolaj tápon élő egerek esetében. Allergiás szenzitizáció után a szérum IgE szint jelentősen emelkedett mind az alaptápon, mind a DHA-dúsított halolaj tápon tartott egerek esetében, de jelentős változás nem volt megfigyelhető a két csoport között.

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retinoidok, retinol, génexpresszió, hormon magreceptorok, atópiás dermatitiss, allergiás szenzitizáció, n-3 többszörösen telítetlen zsírsavak, eikozapentaénsav, dokozahexaénsav

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