

# Long Chain n-3 Polyunsaturated Fatty Acids in the Prevention of Allergic and Cardiovascular Disease

Lieke van den Elsen<sup>1</sup>, Johan Garssen<sup>1,2</sup> and Linette Willemsen<sup>1,\*</sup>

<sup>1</sup>Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584 CG, Utrecht, The Netherlands; <sup>2</sup>Danone Research – Centre for Specialised Nutrition, Bosrandweg 20, 6704 PH, Wageningen, The Netherlands

**Abstract:** The diet is considered to have a major impact on human health. Dietary lipids including long chain polyunsaturated fatty acids (LCPUFA) possess potent immunomodulatory activities. Over the last decades the incidence of inflammatory disorders including allergic and cardiovascular diseases (CVD) has been rising. This phenomenon is associated with deficiencies in N-3 LCPUFA, found in fatty fish, and increased content of N-6 LCPUFA in the Western diet. LCPUFA act via different mechanisms including membrane fluidity, raft composition, lipid mediator formation, signaling pathways and transmembrane receptors. N-3 LCPUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can reduce the development of allergic disease by affecting both the innate and adaptive immune system involved in the initiation and persistence of allergic disease. Fish oil has been shown to be effective in the primary prevention of allergic disease in infants at risk when supplemented during pregnancy and lactation. Subtle effects of N-3 LCPUFA on the outcome of the immune response may underlie these protective effects. This review describes the currently reported effects of LCPUFA on dendritic cells, T cells, B cells and mast cells. Also CVD are positively affected by N-3 LCPUFA. Populations consuming high amounts of oily fish are protected against CVD. Moreover N-3 LCPUFA are effective in the secondary prevention of cardiovascular events. Amongst other effects, EPA and DHA have been shown to suppress endothelial cell activation hereby reducing adhesion molecule expression and endothelial cell – leukocyte interactions. This review describes the mechanistic basis of the preventive role for N-3 LCPUFA in allergic disease and CVD.

**Keywords:** N-3 PUFA, prevention, allergic disease, immune cell, mediator secretion, cardiovascular disease, endothelial cell activation, cell adhesion molecule.

## SOURCE AND CONVERSION OF DIETARY POLYUNSATURATED FATTY ACIDS

The lipid composition of the cell membrane determines cell structure and function for a large part. Dietary lipids incorporate in the phospholipids of the cell membrane and can alter membrane structure and function in a large variety of cell types [1]. Lipids are hydrophobic structures, rich in nonpolar hydrocarbon regions and relatively few polar groups. Lipids play a key role in energy storage, membrane structure or have specific biological functions. One class of lipids are the fatty acids, which are generally bound to a glycerol backbone and stored as triacylglycerols (triglycerides) in animal fats and vegetable oils as well as phospholipids found in the lipid bilayer of cell membranes [2, 3]. These amphipathic phospholipids consist of two hydrophobic fatty acid chains and a polar phosphate-containing head group Fig. (1A).

Fatty acids are composed of an unbranched hydrocarbon chain with a carboxyl group at the end and contain a variable, usually even, number of carbon atoms. The most common range is from 12 to 20 carbons per chain, with 16- and 18-carbon fatty acids particularly common. The aliphatic chain is either saturated or unsaturated, depending on the absence or presence of double bonds. Polyunsaturated fatty acids (PUFA) form a subgroup within the group of fatty acids and are referred to as polyunsaturated when containing two or more double bonds [2, 3]. Double bonds can have either a *cis* or *trans* configuration. Most often unsaturated fatty acids contain *cis* double bonds which may result in bending of the structure, preventing tight packing. By contrast *trans* double bonds do not cause this change in shape and are often the result of human processing (*trans* fats) [3]. Chain length and degree of unsaturation of a fatty acid determines its melting point. Most plant triacylglycerols are liquid

at room temperature because the fatty acids of these vegetable oils are predominantly unsaturated.

PUFA are categorized into N-6 or N-3 fatty acids according to the position of the double bond nearest to the methyl end Fig. (1B). Linoleic acid, consisting of 18 carbon atoms and two double bonds as is referred to in its notation (18:2 N-6, LA), and  $\alpha$ -linolenic acid (18:3 n-3; ALA) are essential fatty acids and found in plants only, they are precursors for long chain (LC)PUFA consisting of 20 or more carbon atoms. LA is found in vegetable oils such as corn, sunflower and soybean oil. ALA can be found in green leafy vegetables, walnuts and rapeseed and flaxseed (linseed) oil [2-4]. These PUFA can be metabolized in the body into other N-6 or N-3 fatty acids by elongation of the acyl chain by elongases and insertion of extra double bonds by desaturases. Via several steps LA can be converted into arachidonic acid (20:4n-6; AA) whereas ALA can be converted into eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) [2, 3, 5, 6]. AA can be found in meat whereas oily fish is a rich source of n-3 LCPUFA EPA and DHA. Salmon, fresh tuna, mackerel, herring, pilchards and sardines are examples of fatty fish. Depending upon the type of fish the average content of EPA plus DHA for an oily fish meal varies between 1 and 3.5 g [2, 3, 7].

Besides oily fish, products rich in ALA also can add to the N-3 LCPUFA status due to conversion of ALA into N-3 LCPUFA in the body. However, the proportion of ALA converted into EPA is low and conversion to DHA is even less efficient. There is heterogeneity in the findings; highest estimated fractional conversion is 4% [8], while most other studies have reported lower estimates of conversion (0.05% or less), see Burdge *et al.* 2006 for an overview [6]. Nevertheless, an increase in the ALA intake is demonstrated to increase proportions of EPA in plasma and cell lipids, though not of DHA [9-11]. ALA has been shown to be less potent than N-3 LCPUFA in providing health benefits so ALA requires high intake. The best strategy to increase the N-3 LCPUFA content is to eat oily fish on a regular basis.

\*Address correspondence to this author at the Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands; Tel: 0031-620245763; E-mail: L.E.M.Willemsen@uu.nl

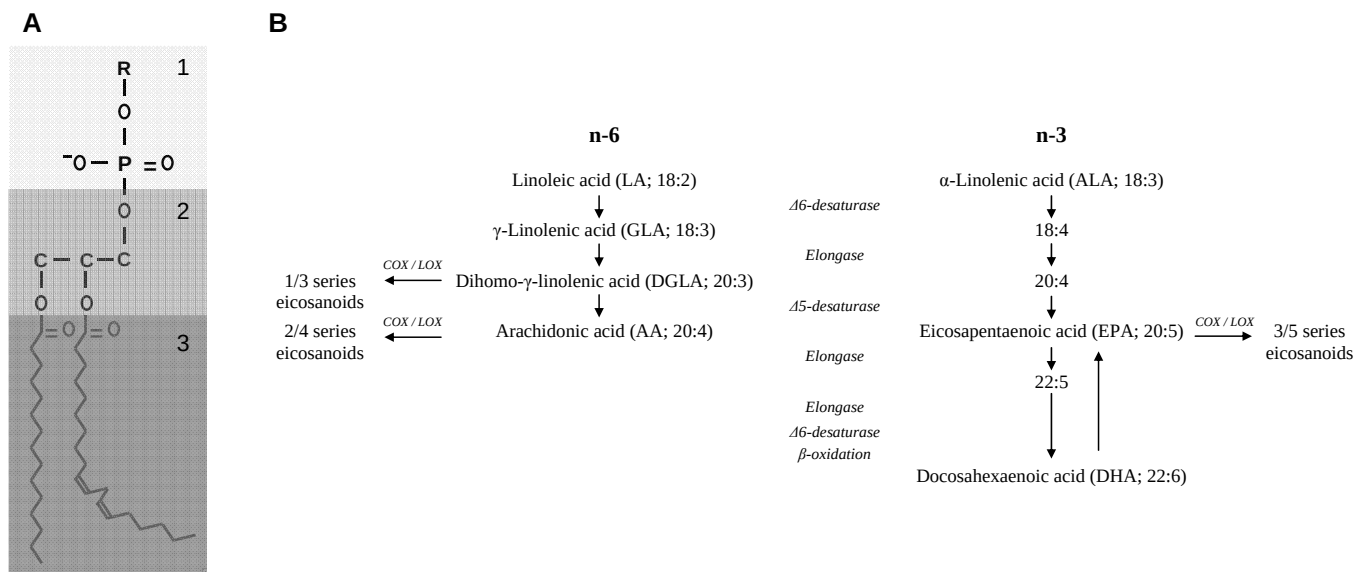


**Fig. (1).** **A.** Phospholipids are the principal components of the cell membrane. Glycerol phospholipids are most common and are composed of a polar phosphate group (1) bound to glycerol (2). R is a small polar head group such as choline. Also bound to glycerol are two fatty acids (3) that may differ from each other. **B.** The metabolic conversion pathways and eicosanoid synthesis from PUFA of the N-6 and N-3 series.

## MECHANISM OF ACTION OF LCPUFA

Among all lipids in particular LCPUFA are widely held to act on physiological functions via several cell mechanisms. Regarding the immune status, in general N-6 LCPUFA are considered as being proinflammatory and N-3 LCPUFA as being protective against inflammation. N-6 LCPUFA AA is a precursor for inflammatory type 2-series prostanoids and the 4-series leukotriens (LT) since it serves as a substrate for cyclooxygenases (COX) and lipoxygenases (LOX). By contrast N-3 LCPUFA EPA is converted by the same enzyme families into 3- and 5-series eicosanoids, known to be less biologically active or even protective against inflammation. In addition, many of the anti-inflammatory effects of N-3 LCPUFA are exerted at the level of altered gene expression through modification of transcription factor activity. For example enzymes responsible for producing inflammatory eicosanoids (e.g. COX-2 and 5-LOX), inflammatory cytokines and adhesion molecules are regulated on gene expression level by LCPUFA. This can be achieved either by direct interaction with ligand-binding transcription factors or via membrane or cytoplasmic signaling pathways finally altering transcription factor activation [1]. Peroxisome proliferator-activated receptors (PPAR), nuclear factor kappa beta (NF- $\kappa$ B) and mitogen activated protein kinase (MAPK) are examples of transcription factors being regulated by PUFA. Recently it was discovered that G protein-coupled receptor 120 (GPR120) is a transmembrane DHA receptor and moreover LCPUFA can affect the Toll-like Receptor (TLR) signaling pathway which involves NF- $\kappa$ B. Hence, LCPUFA may act on intra- and extracellular receptors which may activate the cell and result in the expression or suppression of genes encoding for proteins including cytokines.

## LCPUFA Enhance Membrane Fluidity

The fluid mosaic model is frequently used to describe the structure of cell membranes. The cell membrane consists of an asymmetric lipid bilayer with functional lipids such as cholesterol and proteins embedded; these can migrate within the membrane. The fatty acid composition of the cell is mostly cell specific but may change for example over time or due to the diet [12, 13]. Membrane composition strongly affects membrane fluidity. An increase in the content of unsaturated fatty acids like LCPUFA in membrane phos-

pholipids is accompanied by an increase in membrane fluidity so a decrease in microviscosity [13, 14]. More fluidic membranes can give rise to changes in cell function since in a more fluid membrane protein-protein and protein-lipid interaction can take place more easily.

## LCPUFA Alter Lipid Raft Composition

Lipid rafts are highly organized regions in the cell membrane that differ in composition between various cell types. Rafts are relatively rigid as compared to their surrounding and are rich in cholesterol and sphingolipids with long saturated fatty acyl chains. Many signaling proteins including receptors, enzymes and substrates are concentrated in these areas in order to facilitate efficient intracellular signaling in response to extracellular stimuli. This occurs as a result of clustering of different lipid rafts rich in signaling proteins [15, 16]. Because of the kinked structure of LCPUFA by incorporation of LCPUFA affects raft organization and interactions between lipids and proteins. This is not compatible with the highly organized lipid raft structure and will cause displacement of proteins, and by increasing membrane fluidity affect lipid raft clustering, hereby suppressing signal transduction [17].

## LCPUFA as Precursors of Lipid Mediators

### Eicosanoids

20-Carbon N-6 LCPUFA AA and dihomo- $\gamma$ -linolenic acid (DGLA) and N-3 LCPUFA EPA are precursors for eicosanoids. Eicosanoids derived from AA have the most potent biological functions. Many cell types liberate AA from membrane phospholipids upon stimulation. PUFA are liberated from membrane phosphatidylcholine or phosphatidylinositol-4, 5-bisphosphate (PIP<sub>2</sub>) by cytosolic phospholipase A<sub>2</sub> (PLA<sub>2</sub>) or by actions of phospholipase C (PLC) and a diacylglycerol (DAG) lipase respectively [18-21]. After release from the cell membrane AA can be metabolized by COX enzymes resulting in the formation of inflammatory prostanoids (PG) and thromboxanes (TX) of the 2-series (e.g. PGD<sub>2</sub>) or by 5-, 12-, and 15-LOX which yield inflammatory LT of the 4-series, lipoxins (LX), hydroperoxyeicosatetraenoic acids (HPETE) and hydroxyeicosatetraenoic acids (HETE). Major biologically active AA-metabolites are PGD<sub>2</sub>, PGE<sub>2</sub>, PGI<sub>2</sub>, PGF<sub>2a</sub> and TXA<sub>2</sub>, support-

ing the inflammatory cascade. It is cell type dependent which metabolite is formed predominantly. Half life of these mediators is very short (only seconds) and they act locally on cell surface receptors [22]. Furthermore non-esterified PUFA can be a substrate for cytochrome P450 monooxygenases (CYP). CYP-mediated oxidation of AA results in a variety of eicosanoids such as epoxides known to contribute to cell activation. N-3 LCPUFA are converted by CYP especially when little COX or LOX activity is present [23].

N-3 LCPUFA EPA and DHA competitively inhibit AA metabolism because incorporation of EPA and DHA in the cell membrane phospholipids occurs partly at the expense of AA. Subsequently, less substrate will be available for synthesis of AA-derived eicosanoids [14, 24]. Conversion of EPA is resulting in mediators of the 3-series of prostaglandins and thromboxanes via COX and the 5-series of leukotrienes via 5-LOX, which are believed to be less pro-inflammatory than the AA-derived metabolites [18, 21]. Indeed in humans fish oil supplementation has been shown to reduce production of PGE<sub>2</sub>, TXB<sub>2</sub>, LTB<sub>4</sub>, 5-HETE and LTE<sub>4</sub> by inflammatory cells while resulting in increased production of LTB<sub>5</sub>, LTE<sub>5</sub> and 5-HEPE [25, 26]. EPA and DHA are poor COX and LOX substrates compared to AA and inhibit COX activity [18, 27, 28]. Also gene expression of other proteins involved in AA-metabolism can be regulated by N-3 LCPUFA [29].

### Resolvins

N-3 LCPUFA in the cell membrane give rise to other anti-inflammatory mediators as well. These are called EPA and DHA-derived resolvins (resolution phase interaction products) [30]. Resolvins are anti-inflammatory lipid mediators derived from EPA and DHA via COX-2 activity. Resolvins of the E-series such as Resolvin E1 (RvE1) are derived from EPA, while resolvins from the D-series are products of DHA [31, 32]. RvE1 receptor activation can lead to potent anti-inflammatory effects. In a mouse model for peritonitis administration of EPA and COX inhibitor aspirin resulted in the generation of RvE1 in peritoneal exudates presumably via 5-LOX and aspirin-acetylated COX-2 as well as inhibition of leukocyte infiltration. Administration of synthetic RvE1 gave similar results. Furthermore, synthetic RvE1 was shown to protect against the development of colitis in mice via reduction of leukocyte infiltration and proinflammatory gene expression [33]. In addition, RvE1 reduced IL-12 production and migration of mouse spleen dendritic cells activated with pathogen extract [34]. Moreover, DHA conversion via COX-2 gives rise to immunoregulatory docosatrienes and neuroprotective neuroprotectins [35, 36].

### LCPUFA Alter Cell Signaling Pathways

Fatty acids may have other functions in signal transduction than discussed above. Many phospholipids are involved in signaling pathways and changes in the type of fatty acid present may alter activity and function in signal transduction [19]. It has been shown that the fatty acid composition of DAG may affect the activity of protein kinase C (PKC) [37]. Furthermore, PKC was less active in the presence of phosphatidylserine rich in LCPUFA as compared to a phosphatidylserine low in LCPUFA content [38]. However, not only the fatty acid composition of phospholipids involved in intracellular signaling is important; fatty acids can have direct effects on signal transduction pathways as well. Expression and activity of protein kinases can be altered by different fatty acids; this has been most extensively studied for PKC and was shown to be reduced by EPA and DHA. Also fish oil supplementation in mice reduced spleen lymphocyte PKC activity [39]. Furthermore, cytosolic calcium concentrations are regulated by PUFA. LA, AA, ALA, EPA and DHA have shown to induce calcium release from the endoplasmic reticulum (ER) and block the calcium influx via receptor-mediated calcium channels in T cells [40, 41]. In addition, they can enhance receptor-mediated calcium efflux [42]. Due to these kinds of downstream changes the activity of transcription factors such as NF- $\kappa$ B can be suppressed too.

### N-3 LCPUFA May Suppress Generation of Reactive Oxygen Species

A variety of tissues and cells produce reactive oxygen species (ROS). Superoxide is the main relevant ROS since it is the precursor of most other ROS. Studies supplementing animals with fish oil are inconclusive about the effect of N-3 LCPUFA on ROS production in macrophages, monocytes and neutrophils (see [19] for an overview) which might be caused by differences in study setup such as cell type analyzed, diet or stimulus used. However, in most studies dietary N-3 LCPUFA reduce superoxide and/or hydrogen peroxide production [43-45]. Furthermore it has been reported that EPA (+DHA) supplementation to healthy human volunteers can lower superoxide production in blood neutrophils and monocytes [46, 47]. Many enzymes including NADPH oxidase are involved in ROS formation and different eicosanoids, cytokines and PKC control several of these enzymes [18, 48]. N-3 LCPUFA might suppress the production of ROS by affecting these molecules. This was shown by Wong *et al.* reporting that DHA inhibited NADPH oxidase and ROS generation in RAW264.7 cells while saturated lauric acid activated both.

### N-3 LCPUFA Suppress NF- $\kappa$ B and MAPK Signaling

The NF- $\kappa$ B signal transduction cascade is important in the generation of inflammatory responses. It induces the transcription of a range of inflammatory genes including COX-2, E-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL) 1 $\beta$ , IL-6 and inducible nitric oxide synthase (iNOS) [49, 50]. N-3 LCPUFA reduce NF- $\kappa$ B activity, although the exact mechanism is not clear. EPA or fish oil incubation of human monocytes was found to reduce lipopolysaccharide (LPS)-induced activation of NF- $\kappa$ B, whereas AA did not [51-54]. Zhao *et al.* showed that this was in association with a reduction in I $\kappa$ B phosphorylation, preventing dissociation of the NF- $\kappa$ B complex and consequent nuclear translocation and gene transcription. This suggests an effect of N-3 LCPUFA upstream in the NF- $\kappa$ B pathway [54]. In addition, TNF- $\alpha$  induced I $\kappa$ B degradation can be eradicated by EPA incubation in a pancreatic cell line [55]. N-3 LCPUFA can suppress activation of other signaling cascades as well, which results in the activation of various transcription factors. Lo *et al.* reported that incubation of murine macrophages with EPA reduced LPS-induced phosphorylation and activation of MAPK [56]. So far it is not exactly known how N-3 LCPUFA can diminish MAPK signaling, both PKC-dependent and independent mechanisms are involved. Furthermore they seem to act upstream and downstream of MAPK kinase (MAPKK) [57, 58].

### PUFA as Ligand for the Nuclear Receptor PPAR

Besides suppression of signaling pathways that converge on transcription factor activation, fatty acids appear to enhance the activity of the transcription factor family PPAR directly. PPAR- $\alpha$  is important in lipid metabolism and is found mostly in liver. PPAR- $\beta/\delta$  is found in many tissues, but its role is still unclear while PPAR- $\gamma$  is expressed mainly in adipose tissue and has been shown to influence adipocyte differentiation, glucose metabolism and macrophage development and function [59]. PPAR are also found in inflammatory cells and certain fatty acids and eicosanoids can act as their ligands [60, 61]. To regulate gene expression PPAR dimerize with another nuclear receptor, the retinoic-X-receptor (RXR) that among others is activated by DHA [62]. Activation of PPAR by ligand binding (e.g. fatty acids from the diet) promotes alterations in gene transcription of genes containing PPAR response elements [63]. LA, ALA, AA, EPA and DHA have been shown to be efficient activators of PPAR- $\alpha$  and - $\delta$ , and to a much lesser extent PPAR- $\gamma$  [64]. Kleiwer *et al.* reported that PPAR- $\alpha$  and - $\gamma$  were activated by LA, ALA and AA [63]. Activators of both PPAR- $\alpha$  and - $\gamma$  inhibited activation of inflammatory genes including TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, COX-2, iNOS and VCAM-1 [61, 65-69]. The anti-

inflammatory effects of PPAR might be due to stimulation of inflammatory eicosanoid breakdown via induction of peroxisomal  $\beta$ -oxidation and/or the suppression of activation of other transcription factors. For example, PPAR- $\gamma$  activation by N-3 LCPUFA may prevent NF- $\kappa$ B activation [68]. Nuclear receptors generally lack specificity for N-3 LCPUFA; therefore they are not sufficient in explaining the effects of N-3 LCPUFA on the immune system [1].

### Transmembrane Receptors for LCPUFA

#### *N-3 LCPUFA as Ligand for GPR120*

GPR120 has been discovered recently as a N-3 LCPUFA receptor. Stimulation of GPR120 by long-chain fatty acids enhanced intracellular calcium and MAPK extracellular signal-regulated kinase (ERK) (p44/42) activation in HEK 293 cells, while reducing IKK $\beta$  and c-Jun N-terminal kinase (JNK) phosphorylation, I $\kappa$ B degradation and TNF- $\alpha$  and IL-6 secretion in RAW 264.7 cells [70, 71]. GPR120 is highly expressed in macrophages, monocytic cells and adipose tissue and EPA and DHA have potent anti-inflammatory effects in these cell types. DHA showed comparable reduction of the LPS-induced inflammatory response as the synthetic GPR120 agonist GW9508. Also suppression of TNF- $\alpha$  induced inflammation was shown.  $\beta$ -Arrestin2 associates with GPR120 leading to internalization of this complex and  $\beta$ -arrestin2 then binds to TAK1 binding protein 1 (TAB1), resulting in reduced TAK1 activation. Downstream of TAK1 are the IKK $\beta$ /NF- $\kappa$ B and JNK pathway which explains the suppression of both LPS and TNF- $\alpha$  signaling after GPR120 activation. Although GPR120 is a Gq/11 G protein coupled receptor, these effects were independent of Gq/11. In addition, macrophage-mediated tissue inflammation could be reduced in obese mouse fed a N-3 LCPUFA diet via GPR120 signaling [71].

#### *PUFA Suppress TLR Activation*

In addition to GPR120, N-3 LCPUFA can signal via TLR. Saturated fatty acids are - as an integral part of the lipid A tail of LPS from Gram-negative bacteria - essential for the biological activities of this TLR4 ligand. Removal of acylated saturated fatty acids from lipid A results in complete loss of endotoxigenic activity [72, 73]. Furthermore, free fatty acids modulate TLR activation. Saturated fatty acids activate TLR4 inducing the expression of COX-2, iNOS and IL-1 $\alpha$  via NF- $\kappa$ B signaling in murine monocytic RAW 264.7 cells, while unsaturated fatty acids including LA, AA, EPA and DHA inhibit the induced expression of these inflammatory markers involving reduced degradation of I $\kappa$ B [73]. These effects involve lipid raft association. Saturated lauric acid has shown to induce receptor dimerization and recruitment of TLR4 into lipid rafts as well as downstream signaling pathways and gene expression. DHA on the other hand reduced LPS- and lauric acid-induced dimerization and recruitment of TLR4 into lipid rafts. This process occurred in a ROS-dependent manner [72]. In addition, lauric acid potentiates while DHA reduces lipopeptide-induced TLR2 activation [72, 74].

### ENHANCED N-6 OVER N-3 LCPUFA INTAKE IS ASSOCIATED WITH INCREASED INCIDENCE OF ALLERGIC AND CARDIOVASCULAR DISEASE

Epidemiological data provide strong evidence of a steady increase in incidence and prevalence of many allergic and inflammatory diseases over the last decades in the Western world. The diseases which incidences are rising since the 1950s includes allergic rhinitis, asthma, atopic dermatitis, food allergies, cardiovascular disease (CVD), inflammatory bowel disease, and autoimmune diseases like diabetes mellitus type 1 [1, 75-78]. For example between 1979 and 1991 the prevalence of asthma, hay fever and atopic dermatitis doubled in Swedish school children [79]. These changes cannot solely be explained by genetics [1]. The modern diet differs largely from more traditional diets. Amongst other changes in the diet, in the Western countries the consumption of N-3 PUFA has

gradually decreased. On the other hand the diet has excessive amounts of N-6 PUFA, mainly LA, from increased consumption of plant oils and products such as margarines. Historically the dietary ratio of N-6 to N-3 fatty acids approached 1/1 and Inuits consume a diet with a ratio of roughly 1/2.5 [76, 80, 81]. Populations consuming high levels of N-3 LCPUFA from seafood such as Inuits and Japanese have low prevalence of CVD, psoriasis, asthma, allergic sensitization towards inhalant allergens and rheumatoid arthritis [82-84]. Besides living condition, allergen exposure and genetics the diet high in N-3 LCPUFA is likely to positively affect disease prevalence. However, in current Western diets this ratio is 10/1 up to 25/1 [76, 80, 81]. This suggests an insufficient N-3 LCPUFA intake and as a consequence human inflammatory cells and structural cells like blood vessel endothelial cells contain high proportions of AA and low proportions of N-3 LCPUFA. Currently, the mean ratio for immune cells is about 20% AA, 1% EPA and 2.5% DHA [85]. The exact proportion of AA in these cells varies according to the cell type and lipid fraction used.

### Dietary Supplementation with N-3 LCPUFA Increases Membrane EPA and DHA Content

Increased exposure to N-3 LCPUFA changes the fatty acid composition of cells in a dose-dependent way. It has been shown *in vitro* that EPA and DHA fairly easily incorporate into mammalian cells [14, 24]. Also in animal studies, feeding fish oil to rodents confirms an increased EPA and DHA content in various cell types, while AA content is reduced [86, 87]. In humans consumption of N-3 LCPUFA increases the content of these fatty acids in plasma lipids and many different cell types including cord blood cells, platelets, erythrocytes, leukocytes, endothelial cells and cardiac cells [88-91]. Incorporation of EPA and DHA in human cells occurs in a dose dependent manner. Several studies show that supplementation between 1 and 6.5 g EPA plus DHA per day results in (near) linear relationships between EPA and DHA intake and content of plasma and platelet phospholipids and neutrophils [92, 93]. However, incorporation into the different cell types occurs at different rates and with different efficiencies [94]. After four weeks of supplementation with EPA and DHA incorporation of these fatty acids reaches its maximum for serum cholesteryl esters and mononuclear cells, whereas for erythrocytes this takes between 2 and 6 months in humans [90, 94]. If fish oil supplementation is abandoned EPA levels return to basal levels in mononuclear cells and erythrocytes within 8 weeks, while DHA remains elevated for a longer period of time. Erythrocyte DHA content is not even reduced to baseline levels 20 weeks after ending supplementation [89, 90, 95]. Current intake of N-3 LCPUFA in Western countries is low, for the UK < 0.25 g/day is consumed which is probably representative for other Western countries [96]. Recommendations vary in general between 0.45 – 1.2 g N-3 LCPUFA/day in adults [96, 97].

The recognition that N-3 LCPUFA exert anti-inflammatory effects has led to the insight that supplementation with N-3 LCPUFA may be of clinical benefit in the prevention or treatment of allergic or inflammatory diseases. In this review we will highlight the effects of N-3 LCPUFA in the prevention of allergic disease and cardiovascular inflammation. For allergic disease both the innate and adaptive immune response will be discussed. In view of the steadily increasing prevalence of CVD we chose to discuss this type of inflammation too and describe the effect of N-3 LCPUFA on a structural cell type, the endothelial cell (EC).

### N-3 LCPUFA IN THE PREVENTION OF ALLERGIC DISEASE

The incidence of allergic disease has been rising steadily in the Western world during the last decades and affects at least 15% of the infant population and 7% of adults is affected with asthma being only one of the major allergic diseases [98-100]. Alterations in dietary composition may have impact on the susceptibility to de-

velop allergic disease which can already be established during gestation or early infancy. Studies in both humans and animals have shown that environmental influences (including diet) during early life have profound and long-lasting effects on human health [101-103]. LCPUFA might exert positive effects on the unborn child, since they are important for the developing fetus. It requires fatty acids to build cell membranes and maintain their fluidity and conformation. Large amounts of PUFA (especially LCPUFA AA and DHA) need to be transferred to the fetus. A deficiency in LCPUFA is directly linked to poor fetal development, and consequently to defects [104]. Kabesch *et al.* have reported that in some European countries one in three children shows allergic sensitization and one in ten suffers from asthma [105]. Many factors, including dietary deficiencies in antioxidants and fibers as well as increased total fat intake have been incriminated to contribute to the increase in allergic manifestations. However, also the increased intake of N-6 over N-3 PUFA may have impact on the allergic outcome [81]. Indeed, N-6 PUFA have been shown to increase the susceptibility of allergic sensitization since these are precursors for PGE<sub>2</sub> [77, 106]. Epidemiological evidence by Hodge *et al.* showed a protective effect of N-3 LCPUFA [106]. Furthermore, in 22 children with asthma and/or allergic dermatitis aged 12-15 years and 23 non-atopic age-matched controls was observed that N-3 LCPUFA concentrations were reduced in serum of allergic adolescents in comparison to the control group. In addition, the ratio of N-6/N-3 LCPUFA was higher in the allergic group [107].

### N-3 LCPUFA Supplementation During Pregnancy and Lactation Reduce Allergic Incidence and Symptoms in Offspring

#### Pregnancy Studies

Allergic diseases are determined in early life, or even *in utero*; therefore prevention should preferably start very early in life. Supply of N-3 LCPUFA to the fetus must come from maternal sources (placenta and breast milk) which make the maternal LCPUFA status important. The maternal plasma phospholipid DHA concentration determines DHA supply to the fetus. Increased consumption of DHA during pregnancy or lactation increases the DHA content of maternal and cord plasma and breast milk [108, 109]. Regular consumption of oily fish has been shown to result in higher maternal erythrocyte DHA content [110, 111]. Possible protective effects of early N-3 LCPUFA exposure have been examined in supplementation studies with fish oil.

Recent studies report the effects of fish oil supplementation during pregnancy for the primary prevention of allergic disease. In a randomized placebo-controlled trial, women at risk of giving birth to a child at high risk of developing allergic disease were supplemented with 1.6 g EPA and 1.1 g DHA or placebo (soy oil) from week 25 of gestation to on average 3-4 months of lactation. Children (n = 117) were followed on the risk of developing allergic sensitization and disease during the first year of life. The accumulative incidence of food allergy during the first year of life was reduced by 85% in the N-3 LCPUFA group compared to the placebo group as determined by a reaction to egg or milk in presence of detectable IgE antibodies or a positive skin prick test towards these antigens. Furthermore, IgE associated eczema was reduced by more than 60% in the 12 month follow up period. This study indicated that maternal N-3 LCPUFA supplementation during late pregnancy and lactation reduced the risk of food allergy and IgE-associated eczema during the first year of life in children at risk of allergic disease [112]. Moreover, Furujehlm *et al.* have shown a reduced incidence of IgE-associated disease after N-3 LCPUFA supplementation at 2 year of age too. This was related to EPA and DHA proportions in plasma phospholipids of mother and child [113].

Dunstan *et al.* supplemented 83 pregnant woman whose offspring were at risk of developing allergic disease in a double blind, randomized, placebo-controlled trial with fish oil (3.7 g N-3 LCPUFA (1.1 g EPA + 2.2 g DHA) per day) or control capsules

(olive oil) from week 20 of pregnancy until delivery [114, 115]. Umbilical cord plasma IL-13 concentrations were significantly lower after fish oil exposure and inversely related to cord erythrocyte DHA content [114]. Neonatal mononuclear cells secreted less Th1 and Th2 cytokines in response to allergen stimulation after fish oil consumption and IL-10 secretion in response to house dust mite or cat hair extract was significantly reduced. At one year of age, infants exposed to fish oil during pregnancy were 60% less likely to be sensitized to egg allergen as determined by skin prick testing and the same tendency was shown for peanut and cow's milk protein. Although the incidence of atopic dermatitis was not different between the groups, fish oil treated children showed significantly fewer severe symptoms [115].

Instead of providing fish oil to increase N-3 LCPUFA status in pregnant woman, the Salmon in Pregnancy Study (SiPS) was the first study to increase the intake of oily fish (two portions salmon per week) from week 20 of pregnancy until delivery in pregnant women whose offspring were at high risk of developing atopic disease. The salmon, specifically farmed for the study, provided about 3.45 g EPA plus DHA per week. In the salmon group EPA and DHA percentages in plasma phosphatidylcholine were higher at weeks 34 and 38 of pregnancy as compared to 20 weeks, EPA showed the largest increase. In the control group these fatty acids decreased during pregnancy. Also in umbilical cord plasma phosphatidylcholine the EPA and DHA content was higher in the salmon group as compared to controls subjects. EPA was even 2-fold higher in the salmon group, while DHA was 1.2-fold increased. This indicates enhanced N-3 LCPUFA transfer to the fetus. Children will be followed for atopic disease status during the first years of life [116].

#### Lactation Studies

In two small studies including healthy and allergic mothers the PUFA composition of breast milk was assessed in relation to the allergic outcome of their offspring [117, 118]. In both studies it was shown that an increased N-6/N-3 PUFA ratio in breast milk was related to atopic disease in the infant. In the allergic infants the N-6/N-3 PUFA ratio was also higher in the serum cholesteryl ester fraction as a result of reduced N-3 PUFA content and in the serum lipid triglyceride fraction as a result of an increase in N-6 PUFA [118]. Fish oil supplementation during pregnancy is shown to increase breast milk N-3 PUFA levels and enhances IgA, IL-6, IL-10 and sCD14 concentrations in human milk [119]. Although the effect on the allergic outcome of N-3 LCPUFA supplementation during lactation only has not been studied yet this may be beneficial as well. Lauritzen *et al.* showed long-term immunologic effects in children at 2.5 year of age that were exposed to N-3 LCPUFA in the first 4 months of life via breastfeeding. Supplementation with 1.5 g N-3 LCPUFA per day has been associated with significantly higher production of Th1 type interferon- $\gamma$  (IFN- $\gamma$ ) in a whole blood assay compared to children with low maternal N-3 LCPUFA intake during lactation. This may reflect a faster maturation of the immune system [120].

### EFFECTS OF LCPUFA ON IMMUNE CELLS

Although some studies have a relatively small study population, supplementation studies provide evidence that N-3 LCPUFA can exert protective effects in the development of allergic disease when supplemented early in life (during pregnancy and lactation), at least in children at high risk. The mechanisms of effect remain to be elucidated but may comprise cells of the innate as well as the adaptive immune system Fig. (2).

#### LCPUFA Suppress DC Activation *In vitro*

Dendritic cells (DC) are the link between the innate and the adaptive immune system. Both N-3 as well as N-6 LCPUFA were able to inhibit the expression of LPS-induced CD40, CD80 and/or CD86 co-stimulatory signals and suppressed T cell proliferation

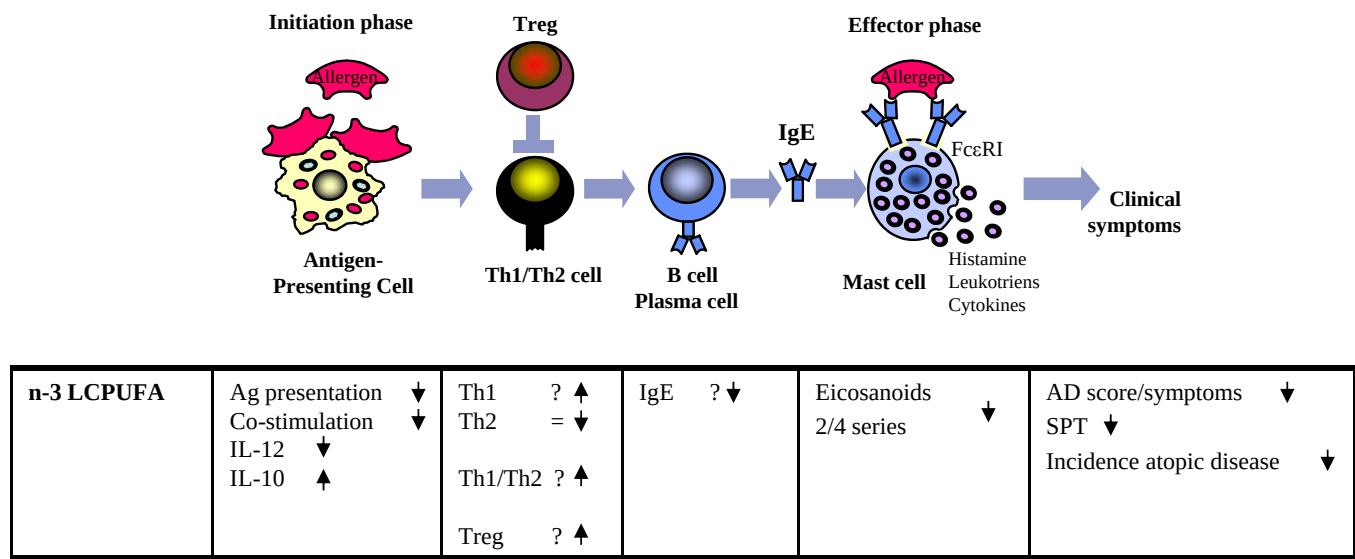


Fig. (2). Summary of the effects of moderate N-3 LCPUFA dosage on immune cell function. For details see text. AD = atopic dermatitis, SPT = skin prick test

and cytokine production when the DC were added to naive T cells, hence rendering a tolerogenic DC phenotype [121-123]. For N-3 LCPUFA these effects were obtained via PPAR- $\gamma$  activation and/or inhibition of MAPK p38 [122, 123]. Zapata-Gonzalez *et al.* and other studies have shown a suppression of LPS-induced expression of major histocompatibility complex (MHC) class II receptors on DC by N-3 LCPUFA and also a reduced capacity to present antigen to T cells [122, 123]. Also in rats fed fish oil a reduction in MHC II expression on DC and diminished antigen presentation was observed [124]. In a recent study DHA and EPA were found to suppress Th1 polarizing IL-12 secretion by LPS-stimulated bone marrow derived DC while regulatory IL-10 production was enhanced. LA did not affect cytokine secretion of the DC [125]. N-6 LCPUFA AA-derived eicosanoids such as PGE<sub>2</sub> and PGD<sub>2</sub> and other mediators such as histamine influence the activity of the DC. Both histamine and PGD<sub>2</sub> decreased IL-12 secretion by DC and promote a Th2 cell response [126-128]. Furthermore, TNF- $\alpha$  is important in migration and functional maturation of DC. Production of these inflammatory mediators also may be reduced by N-3 LCPUFA.

LCPUFA May Affect T Cell Proliferation and Polarization

Th1/Th2 polarization can be affected by the fatty acid composition of the diet. An increased dietary N-6/N-3 PUFA ratio may induce a shift in the Th1/Th2 balance towards a Th2 prone response [129]. In the study by Mizota *et al.* Balb/c mice and humans were provided with diets supplemented for four weeks with different N-6/N-3 PUFA ratios due to variations in ALA content. Secretion of IFN- $\gamma$  by splenocytes was significantly enhanced in mice fed a N-3 PUFA rich diet (N-6/N-3 PUFA ratio = 0.25) as compared to a N-6 PUFA rich diet (N-6/N-3 = 42.9). Secretion of IL-4 was reduced in both the N-6 rich and N-3 rich diet as compared to a diet with N-6/N-3 = 2.27. The ratio of IFN- $\gamma$  to IL-4 was significantly higher in mice fed the N-3 PUFA rich diet. In humans the ratio of IFN- $\gamma$ /IL-4 production by peripheral blood mononuclear cells was significantly higher during consumption of N-3 PUFA (N-6/N-3 = 3) as compared to a N-6 PUFA rich diet (N-6/N-3 = 44), as a result of reduced IL-4 secretion [129]. However, Zhang *et al.* found opposite results by fish oil in C57BL/6 mice. They reported suppression of Th1 development (reduced % IFN- $\gamma$  positive cells) by fish oil but no enhanced Th2 polarization (% IL-4 positive cells) in splenic T lymphocytes cultured under Th2 polarizing conditions [130]. Kleemann *et al.* also reported suppressed IFN- $\gamma$  but increased regulatory IL-10 mRNA expression in the Peyer's patches of fish oil-fed

BB rats [131]. However, IL-10 is often marked as a Th2 type cytokine. Unfortunately not all studies assessed regulatory cytokine secretion, which might benefit the T cell balance. Wallace *et al.* have observed that feeding a 20% fish oil diet to C57BL/6 mice had little effect on IL-4 secretion and gene expression by splenocytes but reduced IL-2 and IFN- $\gamma$  production and T cell proliferation. Also *in vitro* IL-2 secretion by murine splenocytes was inhibited with high EPA and DHA doses however; low doses tended to enhance IL-2 secretion [132]. In addition, in humans supplementation with high dose of fish oil (3-6 g/day) resulted in reduced IL-2 and IFN- $\gamma$  production and decreased T cell proliferation [133, 134]; whereas moderate fish oil supplementation (up to 2 g/day) increased T cell proliferation and IFN- $\gamma$  production *ex vivo* although no effect on IL-4 secretion was seen which enhanced the Th1/Th2 ratio [135]. Yaqoob *et al.* supplemented MF1 mice with fish oil and spleen lymphocytes were cultured for 24 hours. They have not shown an effect on IFN- $\gamma$  concentrations; however IL-4 and IL-10 concentrations in supernatants tended to be lower in the fish oil group [136]. Some studies do not report effects on these outcomes [90]. Also AA-derived PGE<sub>2</sub> has *in vitro* been shown to suppress T cell activation and proliferation including inhibition of IL-2 and IFN- $\gamma$  production, shifting the T cell response towards Th2 [137-139]. N-3 LCPUFA decreased the production of PGE<sub>2</sub> which benefits the Th1/Th2 balance in allergic individuals. On the other hand, Trebble *et al.* reported an increase in PGE<sub>2</sub> secretion by fish oil supplemented humans (2 g/day) [135]. Differences in results may be due to varying study parameters such as differences in species and cell types studied, LCPUFA type and route of cell activation. Furthermore, dose seems to be rather important. Unfortunately, many effects have been studied in Th1 type of animal models. N-3 LCPUFA do not seem to suppress either Th1 or Th2 responses. Possibly the principal immune response is targeted, this is the Th2 response in neonates and atopic subjects. So far it is unknown how N-3 LCPUFA can selectively suppress a Th response; it has been suggested that N-3 LCPUFA support regulatory T cell function [1].

T cell responses can be modulated either directly by fatty acid incorporation in the T cell membrane or indirectly e.g. via interaction with antigen-presenting B cells or DC with different fatty acid composition [140-143]. LCPUFA selectively alter protein composition of the cytoplasmic (inner) lipid leaflet by altering lipid composition since they can dislocate acetylated proteins that are anchored to the cytoplasmic lipid leaflet from the membrane. This has been shown to be a functional effect since it correlates with impaired

calcium signaling [144, 145]. Phosphorylation of linker for activation of T cells (LAT) is inhibited by N-3 LCPUFA in T cells and LAT displacement from rafts by N-3 LCPUFA leads to the suppression of T cell responses *in vitro* [17, 146]. In animal studies alteration of T cell lipid rafts has been shown to be functional and the effects of N-3 LCPUFA on early signaling events have been confirmed [147, 148]. By changing the number and activity (proliferation and cytokine secretion) of immune cells N-3 LCPUFA affect maturation and polarization of the immune system [1].

### N-3 LCPUFA May Reduce IgE Production by B Cells

As reported by dietary intervention studies during pregnancy N-3 LCPUFA show a strong tendency to reduce IgE antibody levels. Weise *et al.* found in B cells from human peripheral blood mononuclear cells that DHA inhibited IgE production [149]. However, animal feeding studies are inconclusive possibly due to different animal species used, see [19]. So far the underlying mechanism is unknown. Effects on DC, Th1/Th2 ratio as well as direct effects on the B cell may be involved. For example PGE<sub>2</sub> promotes IgE production by B cells, which will reduce after AA substitution by N-3 LCPUFA [138, 150, 151]. However, Pène *et al.* reported contrasting results, namely that PGE<sub>2</sub> inhibited IgE production by peripheral blood lymphocytes [152]. Furthermore cytokines including IL-4 and IL-13 induce IgE isotype switching in plasma cells. Possibly the production of these cytokines by T cells and mast cells is suppressed by N-3 LCPUFA [114]. Recently it has been discovered that DHA can reduce IgE production by human B cells via the IL-4 pathway or via an effect on CD40 [149].

### Are LCPUFA Able to Affect Mast Cell Activation and Signaling?

Although the anti-inflammatory effects of LCPUFA have been demonstrated in neutrophils and mononuclear cells, effects on mast cell function have not been fully addressed [153]. Modulation of mast cell degranulation and secretion of cytokines can be beneficial in the prevention of mast cell-mediated diseases including allergy.

Mast cell activation often involves the Fcε receptor I (FcεRI). Allergen-specific IgE binds to the extracellular domain of the FcεRI. As soon as IgE binds to the FcεRI receptor, the receptor is stabilized. Binding of the allergen to surface bound IgE results in FcεRI receptor aggregation and activation, followed by phosphorylation of LAT Fig. (3). Lyn phosphorylates tyrosine residues in the immunoreceptor tyrosine-based activation motifs (ITAM) of the FcεRI which causes the recruitment of Syk [154]. Activation of tyrosine kinase Syk may be affected by LCPUFA [155]. Further downstream activation of PLC is induced causing PIP<sub>2</sub> to divide into the second messengers inositol-1,4,5 triphosphate (IP<sub>3</sub>) and DAG. These mediators contain fatty acid tails and therefore might be regulated by LCPUFA. IP<sub>3</sub> binds its receptor on the ER and causes calcium to be released with as a consequence mast cell degranulation and cytokine release [156, 157]. An overview of currently reported effects of LCPUFA on mast cell degranulation and mediator secretion can be found later in this section. Together with DAG, calcium also activates PKC leading to generation of intracellular ROS such as superoxide [156, 157]. It has been described that N-3 LCPUFA may suppress both PKC and ROS generation in different cell types. However, studies assessing the impact of LCPUFA on ROS production in mast cells are rare. Nakano *et al.* found that AA and EPA enhance ROS production in mast cells [155] and canine mastocytoma cell line C2 cells incubated with LA, AA, ALA and EPA increased ROS production. The latter might involve the susceptibility of PUFA to lipid peroxidation. Antioxidant supplementation resulted in a lower increase in ROS production [158].

The pathophysiological importance of ROS generated from mast cells has not been fully elucidated. At high levels ROS are involved in the innate immune response while at low levels they are

involved in cell signaling [159]. ROS are formed in many signaling pathways in mast cells and play an important role in mast cell signaling by mediating calcium influx and the secretion of histamine, eicosanoids and cytokines including IL-8 and TNF-α via NF-κB and/or MAPK signaling [159-162]. Furthermore, tyrosine phosphorylation of PLC and LAT is ROS dependent [160].

### LCPUFA May Alter Mast Cell Degranulation

The effect of PUFA on mast cell degranulation has been studied in different cell types. Teshima *et al.* found N-6 PUFA LA, AA and γ-linolenic acid (18:2n-6) to dose-dependently increase the cytosolic free-calcium concentration and degranulation in rat basophilic leukaemia (RBL)-2H3 cells independent of IP<sub>3</sub> formation [163]. Activation of PKC by fatty acids including AA has been reported and might be involved here too [164]. Incubation with N-3 PUFA ALA and EPA did not result in activation [163]. Nakano and colleagues reported that AA significantly increased β-hexosaminidase release upon IgE-antigen stimulation and EPA showed the same tendency. This might be the result of effects early in FcεRI signaling. In AA-supplemented cells, the intracellular calcium concentration was increased and in both AA- and EPA-supplemented cells tyrosine phosphorylation of Syk and LAT was enhanced as well as intracellular ROS generation required for tyrosine phosphorylation of LAT and intracellular calcium mobilization. Fatty acid supplementation did not alter expression level of the FcεRI on the cell surface [155]. Besides this mast cell degranulation perhaps occurs more easily when the membrane is more fluid as a result of PUFA incorporation. Also in C2 cells incubation with AA and EPA increased histamine release; AA-mediated increase was significantly higher from EPA-incubated cells [165].

Others did not see an effect of LCPUFA on mast cell degranulation. Peritoneal mast cell histamine release by compound 48/80 inducing non-receptor mediated degranulation was not different for mast cells isolated from rats fed a fish oil diet as compared to a diet rich in N-6 fatty acids or saturated fatty acids [166]. Also murine mast cells (PT-18 cells) incubated with AA or EPA *in vitro* did not show an effect on histamine release [167]. Incubation of cultured human mast cells from umbilical cord mononuclear cells with EPA did not affect histamine release too [27].

Furthermore, several (LC)PUFA have been shown to reduce mast cell degranulation. Fish oil contains various N-3 LCPUFA besides EPA and DHA; 6,9,12,15,18,21-Tetracosahexaenoic acid (THA 24:6n-3) is one of these. In mouse mast cell line MC/9 cells AA increased histamine content whereas N-3 PUFA including EPA, DHA and THA reduced histamine content. Only ionophore-stimulated histamine release was affected by PUFA, while spontaneous and antigen-mediated degranulation was not. LA, AA, EPA, DHA and THA but not ALA reduced histamine release [168]. ALA incubation in RBL-2H3 cells increased both ALA and DHA content in different lipid fractions in the cell whereas LA and AA were decreased. Histamine release in this study was reduced by ALA [169]. Currently reported effects of LCPUFA on mast cell degranulation vary widely and are inconclusive.

### N-3 LCPUFA Suppress PGD<sub>2</sub> and PGE<sub>2</sub> Secretion by Mast Cells

PGD<sub>2</sub> is the main prostanoid secreted by activated mast cells and associated with allergic diseases because of its functions in vasodilatation and immune cell activation [27]. In addition, PGD<sub>2</sub> is a random migration stimulating (chemokinetic) factor of neutrophils and eosinophils [170]. AA-derived mediators such as PGD<sub>2</sub> are very important for the initiation of the allergic response. C2 cells supplemented with AA and EPA showed an increase in PGE<sub>2</sub> production, although the increase in EPA was significantly lower than AA incubated cells [165]. However, in general N-3 LCPUFA have shown to be potent inhibitors of pro-inflammatory lipid mediators including PGD<sub>2</sub>. Feeding rats a diet rich in fish oil increased N-3 LCPUFA and reduced AA in peritoneal mast cell phospholipids. As a result upon mast cell stimulation PGD<sub>2</sub> and PGE<sub>2</sub> secre-

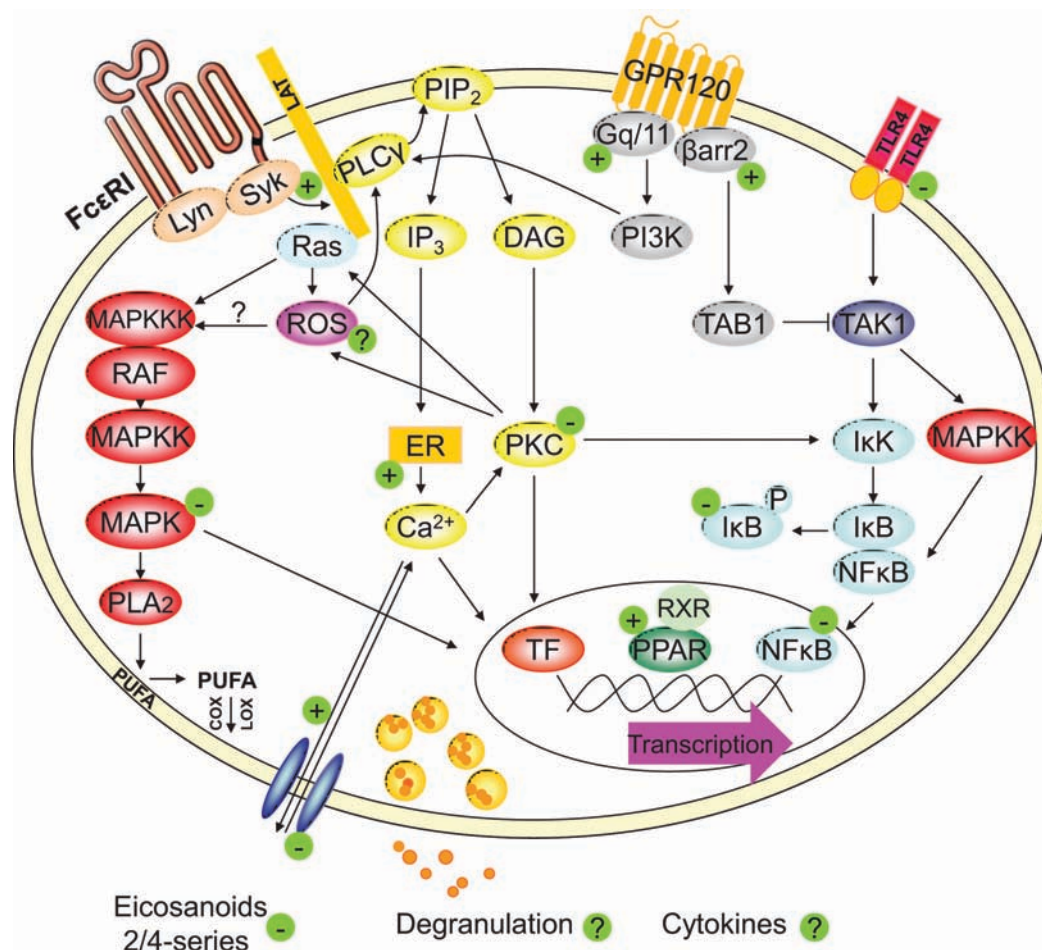


**Fig. (3).** Proposed mechanisms by which N-3 LCPUFA can affect mast cell function. Currently known effects of N-3 LCPUFA on mast cells are indicated in the figure. Furthermore effects found in other cell types, but so far not studied in mast cells are extrapolated and included in the figure. An enhancing effect of N-3 LCPUFA is indicated by (+); a reduction by (-) and (?) means inconclusive results. N-3 LCPUFA incorporate in the cell membrane and increase membrane fluidity and change lipid raft formation leading to suppressed signal transduction. FcεRI receptor activation by an allergen results in phosphorylation of Syk, which can be enhanced by EPA supplementation. Further downstream activation of PLC is induced causing PIP<sub>2</sub> to divide into the second messengers IP<sub>3</sub> and DAG. Calcium (Ca<sup>2+</sup>) is released from the ER which can be induced by N-3 LCPUFA, while calcium influx across the membrane is reduced and calcium efflux stimulated by N-3 LCPUFA. PKC is activated by DAG, this activation can be reduced by N-3 LCPUFA either directly or via altering the fatty acids composition of phospholipids involved. Together calcium and PKC contribute to mast cell degranulation; the findings for N-3 LCPUFA on mast cell degranulation are contrasting. Furthermore calcium and PKC affect gene transcription involved in cytokine release. In addition IgE receptor activation leads to activation of Ras influencing the generation of intracellular ROS. While N-3 LCPUFA have shown to be able to suppress ROS production in different cell types, the results from the few studies in mast cells show the opposite. Ras also activates the MAPK signaling route leading to proinflammatory eicosanoid formation via PLA<sub>2</sub> together with increased gene transcription involved in cytokine release. Both MAPK activation and eicosanoid formation of the 2/4 series can be reduced by N-3 LCPUFA. For mast cells the effects of N-3 LCPUFA on cytokine secretion are largely unknown. Besides FcεRI the mast cell contains TLR on their surface that signal via the NF-κB pathway thereby inducing cytokine secretion. This can be reduced by N-3 LCPUFA involving reduced receptor clustering and reduced phosphorylation of IκB. Recently the GPR120 receptor for DHA has been discovered. So far it is unknown whether this receptor is present on mast cells, but its activation by DHA can reduce NF-κB signaling or activate a G protein. Finally, PPAR can be activated by N-3 LCPUFA inhibiting the activation of inflammatory genes. For abbreviations and details see text. MAPKKK = MAPKK kinase

tion were reduced when compared to a diet mainly containing saturated fatty acids [171]. THA has shown to be effective in reducing the antigen-stimulated production of eicosanoids, like other N-3 LCPUFA do in murine MC/9 cells [168]. EPA can reduce PGD<sub>2</sub> secretion by cultured human mast cells by inhibiting COX-2 activity [27]. Furthermore, synthetic phospholipids containing DHA inhibit 5-LOX activity of rat basophilic leukemia cell line RBL-1 [169]. As a result of other lipid mediators formed in the presence of N-3 LCPUFA, the allergic response may benefit from dietary supplementation with these fatty acids.

#### **LCPUFA May be Able to Affect the Mast Cell Phenotype**

The effect of LCPUFA on mast cell mediator secretion is relevant for both the initiation and persistence of the allergic response. Although LCPUFA can regulate several transcription factors in-

involved in cytokine release by lymphocytes and macrophages [18, 19, 172], little is known about the effects of LCPUFA on cytokine secretion by mast cells. In rodents fish oil supplementation reduced the *ex vivo* production of TNF-α, IL-1β and IL-6 by macrophages [136, 173]. Dietary fish oil consumption by healthy volunteers reduces production of TNF-α, IL-1 and IL-6 by mononuclear cells. Moreover, production of these cytokines is dose dependently reduced by N-3 LCPUFA intake [135, 174]. Similar effects on cytokine secretion might occur in mast cells. Effects on cytokine production are most likely the effect of altered activity of transcription factors; reduced activation of NF-κB and activation of PPAR-γ may be involved. Furthermore, MAPK pathways are known to regulate pro-inflammatory mediator secretion. PPAR-γ is known to be expressed by mast cells and has been shown to be involved in the

release of cytokines like IL-5 and TNF- $\alpha$  but not histamine in mast cells [175]. PPAR- $\gamma$  agonists have been shown to lower the amount of intracellular ROS [176], therefore PPAR- $\gamma$  agonists including N-3 LCPUFA might have a positive effect on allergic mediator secretion. ROS can regulate tyrosine phosphorylation near the cell membrane, such as phosphorylation of LAT and PLC- $\gamma$ , finally resulting in a reduction in cytokine secretion [155, 160]. Nakano *et al.* reported that AA dose-dependently augmented TNF- $\alpha$  release in RBL-2H3 cells [155]. Incubation of murine PT-18 cells with AA or EPA but not LA *in vitro* resulted in enhanced release of platelet-activating factor independent of mast cell activation (histamine release). No antioxidants were used in these cell cultures which makes that oxidative stress may be involved in these effects [167].

There is still much unknown about the effects of LCPUFA on mast cells however several mechanisms by which N-3 LCPUFA can affect mast cell function, contributing to the prevention of allergic disease, have been discussed. Furthermore, mast cells and their products are involved in function, maturation and migration of B cells, T cells and DC and the interplay between all these cells is very important in allergic disease. T cell responses can be modulated via cytokines such as IL-4, TNF- $\alpha$  or PGD<sub>2</sub> released by mast cells [140, 141]. In addition, interaction of mast cells with B cells via CD40-CD40L can result in Th1-skewing of naïve T cells or, in the absence of T cells induce IgE production [141]. Mast cell cytokines including IL-4 and IL-13 induce IgE isotype switching of B cells [126, 140, 141, 177-179]. Histamine and PGD<sub>2</sub> can decrease IL-12 secretion by DC and promote a Th2 cell response. Further, TNF- $\alpha$  is important in migration and functional maturation of DC [128]. As a result an effect of N-3 LCPUFA on mast cell function can also affect allergic responses via other cell types. Alteration of the mast cell phenotype by N-3 LCPUFA might result in a reduced susceptibility to develop allergy or allergic inflammation.

### N-3 LCPUFA IN THE PREVENTION OF CVD

CVD have become the leading cause of morbidity and mortality in developed countries, and surprisingly in some developing countries too. Worldwide CVD result in death of approximately 16.7 million people each year. CVD include coronary heart disease (including angina pectoris, myocardial infarction and sudden death), stroke and peripheral vascular disease. Cardiovascular risk factors include hypertension and dyslipidemia (especially elevated plasma levels of low-density lipoprotein (LDL)) which are often found in metabolic disorders such as diabetic disease and obesity [180, 181].

The most important contributor to increasing CVD is atherosclerosis. Atherosclerosis is a complex, chronic inflammatory process accompanied by accumulation of lipids and fibrous elements in the large arteries [181]. Atherosclerosis is an inflammatory disease because of the interaction between modified lipoproteins, monocyte-derived macrophages, T lymphocytes and the cell elements of the vessel wall [182, 183]. Plaques formed consist of macrophage-derived foam cells, immune cells, vascular EC, smooth muscle cells, platelets, extracellular matrix, and a lipid-rich core with extensive necrosis and fibrosis of surrounding tissues [184]. The adhesion of leukocytes to endothelium is involved in the early phases of atherosclerosis [185]. Plaques may occlude the flow of oxygen-rich blood to the organs and other parts of the body and under certain conditions may rupture, leading to coronary events such as myocardial infarction.

A key event leading to the formation of plaques is the entry of LDL into the arterial intima. LDL is a major risk factor for atherosclerosis because it can be modified, mostly via oxidation, generating oxidized LDL, minimally modified LDL (mmLDL), and other biologically active forms that initiate inflammatory processes in the blood vessel wall. Modified LDL is taken up by macrophages and these cells become lipid-laden foam cells. Modified LDL also upregulates VCAM-1 and ICAM-1 expression and induces mono-

cyte adhesion to activated endothelium, an early event in the development of atherosclerotic lesions [186, 187].

Vascular inflammation, which is closely related to endothelial dysfunction is often involved in CVD and occurs at an early stage of atherosclerotic cardiovascular disorders. Vascular inflammation is caused by activation of the endothelium. This can be caused by decreased or abnormal shear stress, hypoxia, inflammatory cytokines, and leukocyte activation. EC activation is followed by an increase in leukocyte-endothelium adhesion via upregulation of endothelial cell adhesion molecules (CAM) which bind circulating leukocytes. In addition, secretion of proinflammatory factors like monocyte chemoattractant protein 1 (MCP-1), IL-6, IL-8, TNF- $\alpha$  and granulocyte-macrophage colony-stimulating factor (GM-CSF) by EC themselves enhances leukocyte adherence, and transmigration to the endothelium, and provocation of endothelial dysfunction and tissue injury [187, 188].

### N-3 LCPUFA Contribute to the Primary Prevention of CVD

Populations consuming high amounts of fish or other seafood containing high levels of N-3 LCPUFA are protected against cardiovascular morbidity and mortality. Inuit populations consuming a marine food diet (500 g/day) rich in N-3 LCPUFA (5 – 15 g/day) have a low cardiovascular mortality, despite the high fat percentage of the diet (40% of calories) [76, 82, 189-191]. The diet in the United States is equally high in fat, however is low in N-3 and high in N-6 fatty acids. Between 1974 and 1976 deaths due to ischemic heart disease counted for 5.3% of all deaths in Greenland compared to 40.4% of deaths in the US [76]. The Japanese population also has a low cardiovascular mortality [192]. Differences in genetic factors are unlikely to fully explain the difference in coronary heart disease (CHD) between Japanese and Western people. Studies of Japanese migrants to the US clearly illustrated an increase in CHD morbidity and mortality in Japanese men in the US [193]. Furthermore, the protective role of fish is supported by the difference in cardiovascular mortality between fishing and farming villages in Japan. Japanese fishers (250 g fish/day) have lower blood pressures and reduced incidence of heart disease compared to Japanese farmers (90 g fish/day). In addition, the Japanese population has a six fold lower incidence of atherosclerosis and heart disease than the US population (<20 g fish/day) [82, 194]. In both Inuit and Japanese, the EPA and DHA contents of plasma and platelet and tissue phospholipids are high whereas the relative concentration of AA is low [82].

### N-3 LCPUFA May Prevent CVD Via Epigenetic Programming

Programming of atherosclerosis and its complications might occur via an effect on vascular physiology. Epidemiological and pathological studies in animals and humans have shown that exposure to an adverse maternal environment during development increases adult vascular disease in the offspring [195, 196]. Nelson *et al.* examined that endothelial dysfunction marker ICAM-1 was elevated at birth in umbilical cord plasma in the offspring of type 1 diabetic mothers. This reflects a possible mechanism for *in utero* programming of (vascular) disease which is probably involved in an increased atherosclerotic risk later in life [196, 197]. Animal studies indicate that permanent changes either in DNA methylation and/or chromatin modification are responsible for the epigenetic programming of increased atherosclerosis susceptibility [196]. Stenvinkel *et al.* has found that inflammation is indeed linked with DNA hypermethylation in peripheral blood cell DNA. This DNA hypermethylation was associated with (cardiovascular) mortality [198]. Jiang *et al.* showed that DNA hypermethylation of specific genes is important in atherosclerosis. PPAR ligands, to which N-3 LCPUFA belong, can alter epigenetic modifications in genes participating in atherogenesis hence reducing hypermethylation [199-201]. Several PPAR activators have been shown to reduce the expression of VCAM-1 on the surface of EC during inflammatory conditions [202]. Dietary N-3 LCPUFA are associated with decreased plasma homocysteine levels, a risk factor for atherosclerosis

sis inducing DNA methylation [203, 204]. Homocysteine upregulates expression of adhesion molecules such as ICAM-1 on EC and this is reduced by PPAR agonists [205, 206]. In addition, a number of chromatin modifying enzymes, which control CAM expression, have PPAR response elements in their promoters so can be regulated via N-3 LCPUFA [207-209]. Early exposure to N-3 LCPUFA may contribute to a subtle but persistent effect on the development of CVD later in life.

### N-3 LCPUFA Contribute to the Secondary Prevention of CVD

Primary prevention avoids the development of CVD, whereas secondary prevention aims at reducing the incidence of new cardiovascular events and the increase of survival in patients with established disease. Several secondary prevention studies in post-myocardial infarction patients providing oily fish or fish oil have shown a protective effect of N-3 fatty acids on mortality, which is confirmed by meta-analysis [210-215]. The GISSI Prevenzione study investigated the effect of modest supplementation of N-3 LCPUFA (850 mg EPA and DHA daily) in approximately 11,000 post myocardial infarction patients. The risk reduction of cardiovascular death was remarkable, with an overall reduction of 30%. Furthermore, the study reported a beneficial effect of subjects also taking aspirin. Aspirin treatment enhances EPA conversion to products including RvE1, which might have caused this cardiovascular benefit [34, 210]. The DART trial (diet and reinfarction trial) was a randomized controlled trial in which it was observed that intake of two or three portions of fatty fish per week may reduce mortality in men who have recovered from myocardial infarction. They have found a 29% reduction of mortality in those advised to eat fatty fish compared to people not advised to eat fatty fish [211]. Further, the JELIS trial tested the effect of adding EPA to statin therapy in Japanese people among whom the baseline intake of N-3 LCPUFA is high compared to the Western population. Also here a 19% reduction in major coronary events was observed using EPA [215]. Hence, N-3 LCPUFA have preventive importance in humans affected with CVD. In general, 1 g/day of N-3 LCPUFA is considered effective for secondary prevention of myocardial infarction [216-218].

### N-3 LCPUFA IMPROVE ENDOTHELIAL FUNCTION

There are several mechanisms to explain cardiovascular protection of N-3 LCPUFA. Originally the beneficial effects were attributed to the decrease in production of inflammatory eicosanoids and other prothrombotic and vasoconstrictive mediators, reducing inflammatory processes within the vessel wall. In addition, N-3 LCPUFA may suppress leukocyte and smooth muscle migration into the vessel wall intima since they have been shown to dampen EC cytokine secretion and CAM expression *in vitro*, reducing the susceptibility to develop atherosclerosis [216, 219-222]. Furthermore, DHA supplementation to HUVEC altered distribution of endothelial nitric oxide synthase (eNOS) – responsible for cardiovascular homeostasis – by displacement of eNOS from caveolae [223, 224]. Hence DHA may reduce the susceptibility to develop atherosclerosis by supporting eNOS [224]. In humans N-3 LCPUFA resulted in a 43% increase in nitric oxide metabolites in urine, implicating an increase in nitric oxide which implicates an improvement of the endothelium and vasodilatory responses [225]. In animal models as well as dietary intervention trials in humans N-3 LCPUFA have been shown to reduce atherosclerosis via suppression of plaque build up. In humans N-3 LCPUFA prevented the processes that cause death such as plaque rupture, thrombosis, myocardial infarction and stroke e.g. by stabilizing atherosclerotic plaques [226, 227]. In addition, a number of risk factors involved in the development of atherosclerosis are positively affected such as reduced blood pressure and serum triacylglycerol levels. In humans, N-3 LCPUFA decrease serum triglycerides and very low-density lipoprotein cholesterol, while LDL cholesterol tends to be either elevated or unchanged [228, 229]. An impaired endothelial re-

sponse may be secondary to atherosclerotic damage or a consequence of raised blood pressure. Systolic and diastolic blood pressure are directly related to risk of CHD and stroke [230]. Only 4 mm Hg reduction in diastolic blood pressure reduces the risk of stroke by more than 34% and CHD by 14%, possibly due to the damaging effect of pressure on the vessel wall creating a spot for plaque formation [231, 232]. Supplementation of N-3 LCPUFA can act vasodilatory by reduced TXA<sub>2</sub> and increased PGI<sub>3</sub> production. In addition, PGE<sub>2</sub> and PGF<sub>2</sub> stimulate renin production resulting in increased blood pressure, whereas EPA-derived prostaglandins do not [233, 234]. A meta-analysis of 31 placebo controlled trials, with an average dose of 4.8 g EPA+DHA/day observed an overall reduction in systolic blood pressure of 3 mm Hg and a reduction of 1.5 mm Hg in diastolic blood pressure in hypertensive but not in normotensive individuals [235]. More recently an overall reduction of 2.3 mm Hg in systolic blood pressure and a 1.5 mm Hg reduction in diastolic blood pressure were observed after combining 36 individual studies with fish oil doses ranged from 0.2 – 15 g/day (3.7 g/day on average) and a treatment period of 12 weeks on average [236]. Hence, N-3 LCPUFA improve endothelial function which has an impact on the development of CVD since the endothelium is a key player in these kinds of pathologic conditions.

### N-3 LCPUFA Reduce Endothelial Cell Adhesion Molecule Expression

Adhesion of leukocytes to the endothelium is a critical event in the pathogenesis of certain vascular diseases. Increased expression of adhesion molecules on EC contributes to the early phases of inflammation, vascular injury and atherogenesis [186, 187, 222]. Conditions that trigger leukocytes to leave the circulation may occur in damaged veins in chronic venous diseases [237].

Endothelial CAM which play an important role in leukocyte adhesion and transendothelial migration include E-selectin (CD62E) mediating the initial tethering of leukocytes and ICAM-1 (CD54) and VCAM-1 (CD106) recognizing integrin ligands on the leukocyte surface and involved in the more firm adhesion of leukocytes to the endothelium [187].

### *In vitro* Studies

Incubation of primary human umbilical vein endothelial cells (HUVEC) with LCPUFA increases the content of the respective LCPUFA in cell phospholipids [221]. In *in vitro* cultures in which EC were directly exposed to pure LCPUFA, endothelial activation was attenuated. EPA and DHA can decrease the expression of ICAM-1 and VCAM-1 on EC [221, 222, 238, 239] and leukocyte adhesion to EC [221, 222, 239]. Not only N-3 LCPUFA are able to suppress CAM expression. AA and its hydroperoxy (HPETE) and hydroxy (HETE) derivatives have shown to reduce neutrophil and monocyte adhesion to HUVEC. The TNF- $\alpha$  induced E-selectin, ICAM-1 and VCAM-1 expression was suppressed by these mediators; 15-HPETE was most effective [186].

De Caterina *et al.* showed that DHA, but not EPA supplementation for 96 hours with up to 25  $\mu$ mol/L, decreased endothelial expression of E-selectin, ICAM-1 and VCAM-1 in a dose-dependent manner in human saphenous vein endothelial cells (HSVEC) after incubation with a proinflammatory stimulus (IL-1 $\beta$ ) [222, 239, 240]. Also the adhesion of monocytic cells to HSVEC was reduced by DHA incubation [239]. The inhibition of endothelial activation by LCPUFA increased with the number of double bonds. Double bond position or configuration (i.e. *cis* or *trans*) do not make a difference. DHA appears to be the most potent inhibitor of endothelial activation amongst LCPUFA [240]. Weber *et al.* demonstrated that DHA, but not EPA or AA dose-dependently decreased TNF- $\alpha$  induced surface expression of VCAM-1 on HUVEC and reduced subsequent monocytic cell adhesion. E-selectin or ICAM-1 induction by TNF- $\alpha$  or IFN- $\gamma$  and upregulation of ICAM-1 and VCAM-1 by IL-1 $\beta$  remained unaffected [221]. It has been reported by Collie-Duguid *et al.* that 65  $\mu$ mol/L EPA and DHA for 24 hours reduced

mRNA expression for E-selectin, ICAM-1 and VCAM-1 in IL-1 $\beta$  stimulated, but not in resting HUVEC [238]. Furthermore, EPA reduced IFN- $\gamma$  induced ICAM-1 expression on monocytes [241]. Yates *et al.* 2011 showed that adhesion of flowing neutrophils to HUVEC after TNF- $\alpha$  stimulation is dose-dependently inhibited by supplementation with DHA but not EPA. Cells that did adhere were activated and able to migrate across the endothelium normally; this suggests that DHA interferes with selectin-mediated tethering of adhesion. DHA reduced the expression of E-selectin on the surface of HUVEC, while mRNA levels for E-selectin were not changed by DHA supplementation [242]. E-selectin is known to be concentrated in lipid rafts [243]. DHA incorporates into these parts of the membrane, possibly affecting E-selectin expression. In addition PGD<sub>2</sub> is known to stabilize neutrophil adhesion and induction of neutrophil transmigration. In the presence of EPA PGD<sub>3</sub> is generated, which inhibited migration of neutrophils across EC [244].

#### Dietary Supplementation Studies

Increased dietary salmon intake in pregnancy dampened *ex vivo* offspring EC activation. ICAM-1 and VCAM-1 expression on resting HUVEC was not affected, but endothelial activation in response to LPS was reduced. After *ex vivo* LPS stimulation EC surface expression of ICAM-1 and VCAM-1 and EC IL-6 production were reduced. In contrast, production of G-CSF in response to LPS was higher by EC in the salmon group and secretion of some other mediators was not affected by the intervention. This implicates some selectivity or specificity to the anti-inflammatory effects of dietary N-3 LCPUFA. Reduced CAM expression on EC could result in less adherence and transmigration of leukocytes into vascular tissue. This suggests an anti-inflammatory effect of dietary N-3 LCPUFA on human EC and is a possible mechanism by which these fatty acids can lower cardiovascular risk [245]. Studies of dietary fatty acids on EC adhesion molecule expression are few. Dietary fish oil in rodents decreased expression of several adhesion molecules on lymphocytes [246], macrophages [247] and dendritic cells [248]. A fish oil supplementation study in humans decreased ICAM-1 expression on *ex vivo* IFN- $\gamma$  stimulated monocytes [249].

#### N-3 LCPUFA Reduce Endothelial Mediator Secretion

Decreased generation of inflammatory cytokines after N-3 LCPUFA incubation has been demonstrated *in vitro*.

EPA and DHA inhibit the production of IL-6 and IL-8 by venous endothelial cells, whereas N-6 LCPUFA AA was ineffective [222, 250]. Khalfoun found EPA to be more potent in inhibiting IL-6 secretion than DHA [250]. This suggests that N-3 LCPUFA may be involved in suppressing inflammation. Furthermore, incubation of EC with N-3 LCPUFA resulted in an almost completely inhibited production of platelet derived growth factor-like protein; as a result intimal smooth muscle cell proliferation may be suppressed [251].

#### N-3 LCPUFA Reduce Endothelial Cell Activation in Association with Suppression of ROS, NF- $\kappa$ B and MAPK Signaling Cascades

Endothelial CAM expression and secretion of proinflammatory factors are induced by activation of NF- $\kappa$ B and/or involve MAPK cascades in endothelium [220, 252]. Intracellular signaling mechanisms for inhibiting endothelial activation by N-3 LCPUFA are not fully elucidated, but may involve inhibition of these pathways [220, 253]. Xue *et al.* have demonstrated that both EPA and DHA diminish activation of p38 and c-Jun N-terminal kinase (JNK) kinases of TNF- $\alpha$  stimulated HUVEC. This may contribute to the inhibiting effects of N-3 LCPUFA on endothelial activation by proinflammatory stimuli [220]. Others have shown that VCAM-1 and partly E-selectin, but not ICAM-1 expression required mobilization of the transcription factor NF- $\kappa$ B in HUVEC and that DHA incorporation inhibits activation of this signal transduction pathway [221]. Wang *et al.* reported recently a reduction in surface VCAM-1 expres-

sion on EC after >40  $\mu$ mol/L DHA incubation, attributed to an inhibitory effect on the NF- $\kappa$ B pathway [254]. Critical in NF- $\kappa$ B activation is the inhibition of the production of the ROS hydrogen peroxide. N-3 LCPUFA may decrease the production of ROS, partly through the formation of active metabolites like resolvins. ROS activate NF- $\kappa$ B, probably by promoting degradation of I $\kappa$ B. N-3 LCPUFA may modulate receptor responses to atherogenic stimuli (e.g. mmLDL) by inhibiting ROS generation, leading to a decrease in endothelial activation and atherogenesis [185, 216]. Gene expression of COX-2 – involved in plaque angiogenesis and rupture – is reduced by similar mechanisms. Together this affects leukocyte adhesion to the endothelium, early atherogenesis and later stages of plaque development and plaque rupture [185].

#### PREVENTION OF CVD BY N-3 LCPUFA MAY INVOLVE MAST CELL MEDIATOR SECRETION

Recently the involvement of mast cells in atherosclerosis has been discovered. Mast cells are present in the vessel wall and in atherosclerotic lesions. They release pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6, IL-8, MCP-1 and IFN- $\gamma$  that induce plaque formation, progression and destabilization by targeting various mechanisms [255]. Also lipid mediators such as leukotrienes are involved [256]. Mast cell mediators induce adhesion molecule expression and leukocyte adhesion, leading to enhanced plaque inflammation [255, 257]. Furthermore, other mechanisms including lipid uptake and vascular permeability are promoted by mast cell mediators [255, 258, 259]. This makes the mast cell a promising novel therapeutic target in CVD. Possibly N-3 LCPUFA generate their protective effect in CVD partially by affecting the mast cell phenotype prevent CVD.

#### SUMMARY

PUFA are incorporated into the cell membranes of virtually all cells of the body. An increase in N-3 LCPUFA intake increases the n-3 LCPUFA content of the cell membranes. n-3 LCPUFA and their effects on human health are interesting in the view of the steadily increasing incidence of allergic and inflammatory diseases. N-3 PUFA, especially LCPUFA EPA and DHA, exert anti-inflammatory and immunoregulatory effects, whereas N-6 PUFA do not. However, exposure to N-3 LCPUFA is in general low in the Western world.

The beneficial effects of N-3 LCPUFA on the development of allergic disease are complex. It is the result of a range of effects on different types of cells. N-3 LCPUFA have been shown to inhibit DC activation and antigen presentation, to affect T cell polarization and reduce IgE production by B cells. Although there are several studies reporting about the effects of EPA and DHA on DC, T and B cells, the effect on mast cell degranulation and mediator release is still largely unknown. Besides the direct action on the generation of inflammatory eicosanoids, N-3 LCPUFA may act on signal transduction pathways. In general they are able to suppress ROS generation and NF- $\kappa$ B and MAPK signaling pathways while activating PPAR. However, the exact mechanism of action differs between cell type and route of stimulation. Interference with cell signaling may suppress or enhance nuclear transcription factors and the expression of genes encoding for proteins including cytokines. It is likely that these kinds of effects can alter the mast cell phenotype which may contribute to the prevention of allergic disease. All together the immune modulating effects of N-3 LCPUFA on cells of the innate and adaptive immune system may reduce the development of allergic disease.

N-3 LCPUFA not only have strong impact on the function of cells of the immune system, also structural cells benefit from these dietary fatty acids. There is an inverse relationship between the intake of N-3 LCPUFA and the incidence of CVD, at least for a part because of a reduction in atherosclerosis. The underlying mechanism for beneficial effects attributed to N-3 LCPUFA in-

cludes an endothelial mechanism of action, which lowers leukocyte recruitment to the vessel wall. A lot of information is available from *in vitro* studies in which primary HUVEC are exposed to single, pure fatty acids in high concentrations. Animal studies have also shown that dietary fish oil reduces leukocyte adhesion to EC. Cardioprotective effects have been confirmed by several studies in humans, investigating the association of fish or N-3 LCPUFA and CVD. In addition, mortality outcomes in post-myocardial infarction patients are reduced by fish and N-3 LCPUFA. Therefore, fish containing high levels of N-3 LCPUFA may be useful (in combination with other therapies) in treatment or prevention of the development of CVD. However, the precise antiatherogenic effects of N-3 LCPUFA in humans are not well established. Recently the contribution of mast cells to atherosclerotic plaque formation has been discovered. Possibly N-3 LCPUFA reduce pro-inflammatory mast cell mediator secretion leading to reduced plaque progression and rupture. This may contribute to the antiatherogenic effects.

In conclusion, N-3 LCPUFA can affect cell function and contribute to the primary prevention of allergic and cardiovascular disease. As a result they may be able to support the effectiveness of drugs currently used to treat this kind of inflammatory disorders.

## ABBREVIATIONS

|                  |   |                                                  |
|------------------|---|--------------------------------------------------|
| AA               | = | Arachidonic acid                                 |
| AD               | = | Atopic dermatitis                                |
| ALA              | = | $\alpha$ -Linolenic acid                         |
| CAM              | = | Cell adhesion molecule                           |
| CHD              | = | Coronary heart disease                           |
| COX              | = | Cyclooxygenase                                   |
| CYP              | = | Cytochrome P450                                  |
| CVD              | = | Cardiovascular disease                           |
| DAG              | = | Diacylglycerol                                   |
| DART             | = | Diet and reinfarction trial                      |
| DC               | = | Dendritic cell                                   |
| DGLA             | = | Dihomo- $\gamma$ -linolenic acid                 |
| DHA              | = | Docosahexaenoic acid                             |
| EC               | = | Endothelial cell                                 |
| eNOS             | = | Endothelial nitric oxide synthase                |
| EPA              | = | Eicosapentaenoic acid                            |
| ER               | = | Endoplasmic reticulum                            |
| ERK              | = | Extracellular signal-regulated kinase            |
| Fc $\epsilon$ RI | = | Fc $\epsilon$ receptor I                         |
| GM-CSF           | = | Granulocyte-macrophage colony-stimulating factor |
| GPR120           | = | G protein-coupled receptor 120                   |
| HETE             | = | Hydroxyeicosatetraenoic acids                    |
| HPETE            | = | Hydroperoxyeicosatetraenoic acids                |
| HSVEC            | = | Human saphenous vein endothelial cell            |
| HUVEC            | = | Human umbilical vein endothelial cell            |
| ICAM-1           | = | Intercellular adhesion molecule-1                |
| IFN- $\gamma$    | = | Interferon- $\gamma$                             |
| IgE              | = | Immunoglobulin E                                 |
| I $\kappa$ B     | = | Inhibitory subunit of NF- $\kappa$ B             |
| IKK              | = | I $\kappa$ B kinase                              |
| IL               | = | Interleukin                                      |
| iNOS             | = | Inducible nitric oxide synthase                  |
| IP <sub>3</sub>  | = | Inositol trisphosphate                           |

|                  |   |                                                 |
|------------------|---|-------------------------------------------------|
| ITAM             | = | Immunoreceptor tyrosine-based activation motifs |
| JNK              | = | c-Jun N-terminal kinase                         |
| LA               | = | Linoleic acid                                   |
| LAT              | = | Linker for activation of T cells                |
| LCPUFA           | = | Long chain polyunsaturated fatty acids          |
| LDL              | = | Low-density lipoprotein                         |
| LOX              | = | Lipoxygenase                                    |
| LPS              | = | Lipopolysaccharide                              |
| LT               | = | Leukotriene                                     |
| LX               | = | Lipoxins                                        |
| MHC              | = | Major histocompatibility complex                |
| MAPK             | = | Mitogen activated protein kinases               |
| MAPKK            | = | MAPK kinase                                     |
| MAPKKK           | = | MAPKK kinase                                    |
| MCP-1            | = | Monocyte chemoattractant protein 1              |
| MHC              | = | Major histocompatibility complex                |
| mmLDL            | = | Minimally modified LDL                          |
| NF- $\kappa$ B   | = | Nuclear factor- $\kappa$ B                      |
| PG               | = | Prostaglandin                                   |
| PIP <sub>2</sub> | = | Phosphatidylinositol-4,5-bisphosphate           |
| PKC              | = | Protein kinase C                                |
| PLA <sub>2</sub> | = | Phospholipase A <sub>2</sub>                    |
| PLC              | = | Phospholipase C                                 |
| PMA              | = | Phorbol myristate acetate                       |
| PPAR             | = | Peroxisome proliferator-activated receptors     |
| PUFA             | = | Polyunsaturated fatty acid                      |
| ROS              | = | Reactive oxygen species                         |
| RXR              | = | Retinoic-X-receptor                             |
| SPT              | = | Skin prick test                                 |
| Th               | = | T helper                                        |
| THA              | = | 6,9,12,15,18,21-Tetracosahexaenoic acid         |
| TLR              | = | Toll-like Receptor                              |
| TNF- $\alpha$    | = | Tumor necrosis factor- $\alpha$                 |
| TX               | = | Tromboxane                                      |
| VCAM-1           | = | Vascular cell adhesion molecule-1               |

## CONFLICT OF INTEREST

None declared.

## ACKNOWLEDGMENT

None declared.

## REFERENCES

- [1] Calder PC, Krauss-Etschmann S, de Jong EC, *et al.* Early nutrition and immunity - progress and perspectives. *Br J Nutr* 2006; 96: 774-90.
- [2] Calder PC, Yaqoob P. Omega-3 polyunsaturated fatty acids and human health outcomes. *Biofactors* 2009; 35: 266-72.
- [3] Sala-Vila A, Miles EA, Calder PC. Fatty acid composition abnormalities in atopic disease: evidence explored and role in the disease process examined. *Clin Exp Allergy* 2008; 38: 1432-50.
- [4] Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* 2005; 45: 581-97.
- [5] Sprecher H. Metabolism of highly unsaturated N-3 and N-6 fatty acids. *Biochim Biophys Acta* 2000; 1486: 219-31.

- [6] Burdge GC, Calder PC. Dietary alpha-linolenic acid and health-related outcomes: a metabolic perspective. *Nutr Res Rev* 2006; 19: 26-52.
- [7] British Nutrition Foundation. Briefing paper: N-3 fatty acids and health. London; 1999.
- [8] Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. *Biochim Biophys Acta* 1994; 1213: 277-88.
- [9] Finnegan YE, Minihane AM, Leigh-Firbank EC, *et al.* Plant- and marine-derived N-3 polyunsaturated fatty acids have differential effects on fasting and postprandial blood lipid concentrations and on the susceptibility of LDL to oxidative modification in moderately hyperlipidemic subjects. *Am J Clin Nutr* 2003; 77: 783-95.
- [10] Sanders TA, Younger KM. The effect of dietary supplements of omega 3 polyunsaturated fatty acids on the fatty acid composition of platelets and plasma choline phosphoglycerides. *Br J Nutr* 1981; 45: 613-6.
- [11] Cunnane SC, Hamadeh MJ, Liede AC, Thompson LU, Wolever TM, Jenkins DJ. Nutritional attributes of traditional flaxseed in healthy young adults. *Am J Clin Nutr* 1995; 61: 62-8.
- [12] Gibney MJ, Hunter B. The effects of short- and long-term supplementation with fish oil on the incorporation of N-3 polyunsaturated fatty acids into cells of the immune system in healthy volunteers. *Eur J Clin Nutr* 1993; 47: 255-9.
- [13] Stubbs CD, Smith AD. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim Biophys Acta* 1984; 779: 89-137.
- [14] Calder PC, Yaqoob P, Harvey DJ, Watts A, Newsholme EA. Incorporation of fatty acids by concanavalin A-stimulated lymphocytes and the effect on fatty acid composition and membrane fluidity. *Biochem J* 1994; 300 ( Pt 2): 509-18.
- [15] Pike LJ. Lipid rafts: bringing order to chaos. *J Lipid Res* 2003; 44: 655-67.
- [16] Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol* 2000; 1: 31-9.
- [17] Zeyda M, Staffler G, Horejsi V, Waldhausl W, Stulnig TM. LAT displacement from lipid rafts as a molecular mechanism for the inhibition of T cell signaling by polyunsaturated fatty acids. *J Biol Chem* 2002; 277: 28418-23.
- [18] Stulnig TM. Immunomodulation by polyunsaturated fatty acids: mechanisms and effects. *Int Arch Allergy Immunol* 2003; 132: 310-21.
- [19] Calder PC. N-3 polyunsaturated fatty acids and immune cell function. *Adv Enzyme Regul* 1997; 37: 197-237.
- [20] Calder PC. Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids* 2006; 75: 197-202.
- [21] Calder PC, Grimble RF. Polyunsaturated fatty acids, inflammation and immunity. *Eur J Clin Nutr* 2002; 56 Suppl 3: S14-9.
- [22] Jump DB, Clarke SD. Regulation of gene expression by dietary fat. *Annu Rev Nutr* 1999; 19: 63-90.
- [23] Jump DB. The biochemistry of N-3 polyunsaturated fatty acids. *J Biol Chem* 2002; 277: 8755-8.
- [24] Calder PC, Bond JA, Harvey DJ, Gordon S, Newsholme EA. Uptake and incorporation of saturated and unsaturated fatty acids into macrophage lipids and their effect upon macrophage adhesion and phagocytosis. *Biochem J* 1990; 269: 807-14.
- [25] Lee TH, Hoover RL, Williams JD, *et al.* Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on *in vitro* neutrophil and monocyte leukotriene generation and neutrophil function. *N Engl J Med* 1985; 312: 1217-24.
- [26] von Schacky C, Kiehl R, Jendraschak E, Kaminski WE. N-3 fatty acids and cysteinyl-leukotriene formation in humans *in vitro*, *ex vivo*, and *in vivo*. *J Lab Clin Med* 1993; 121: 302-9.
- [27] Obata T, Nagakura T, Masaki T, Maekawa K, Yamashita K. Eicosapentaenoic acid inhibits prostaglandin D2 generation by inhibiting cyclo-oxygenase-2 in cultured human mast cells. *Clin Exp Allergy* 1999; 29: 1129-35.
- [28] Calder PC. Dietary modification of inflammation with lipids. *Proc Nutr Soc* 2002; 61: 345-58.
- [29] Bagga D, Wang L, Farias-Eisner R, Glaspy JA, Reddy ST. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. *Proc Natl Acad Sci USA* 2003; 100: 1751-6.
- [30] Calder PC. N-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr* 2006; 83: 1505S-19S.
- [31] Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J Exp Med* 2000; 192: 1197-204.
- [32] Serhan CN, Hong S, Gronert K, *et al.* Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med* 2002; 196: 1025-37.
- [33] Arita M, Yoshida M, Hong S, *et al.* Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. *Proc Natl Acad Sci U S A* 2005; 102: 7671-6.
- [34] Arita M, Bianchini F, Aliberti J, *et al.* Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J Exp Med* 2005; 201: 713-22.
- [35] Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. *J Biol Chem* 2003; 278: 14677-87.
- [36] Marcheselli VL, Hong S, Lukiw WJ, *et al.* Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. *J Biol Chem* 2003; 278: 43807-17.
- [37] Kishimoto A, Takai Y, Mori T, Kikkawa U, Nishizuka Y. Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. *J Biol Chem* 1980; 255: 2273-6.
- [38] Bell MV, Sargent JR. Effects of the fatty acid composition of phosphatidylserine and diacylglycerol on the *in vitro* activity of protein kinase C from rat spleen: influences of (N-3) and (N-6) polyunsaturated fatty acids. *Comp Biochem Physiol B* 1987; 86: 227-32.
- [39] VanMeter AR, Ehringer WD, Stillwell W, Blumenthal EJ, Jeniski LJ. Aged lymphocyte proliferation following incorporation and retention of dietary omega-3 fatty acids. *Mech Ageing Dev* 1994; 75: 95-114.
- [40] Chow SC, Jondal M. Polyunsaturated free fatty acids stimulate an increase in cytosolic Ca<sup>2+</sup> by mobilizing the inositol 1,4,5-trisphosphate-sensitive Ca<sup>2+</sup> pool in T cells through a mechanism independent of phosphoinositide turnover. *J Biol Chem* 1990; 265: 902-7.
- [41] Chow SC, Ansotegui IJ, Jondal M. Inhibition of receptor-mediated calcium influx in T cells by unsaturated non-esterified fatty acids. *Biochem J* 1990; 267: 727-32.
- [42] Breittmayer JP, Pelassy C, Cousin JL, Bernard A, Aussel C. The inhibition by fatty acids of receptor-mediated calcium movements in Jurkat T-cells is due to increased calcium extrusion. *J Biol Chem* 1993; 268: 20812-7.
- [43] Joe B, Lokesh BR. Role of capsaicin, curcumin and dietary N-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. *Biochim Biophys Acta* 1994; 1224: 255-63.
- [44] Fletcher MP, Ziboh VA. Effects of dietary supplementation with eicosapentaenoic acid or gamma-linolenic acid on neutrophil phospholipid fatty acid composition and activation responses. *Inflammation* 1990; 14: 585-97.
- [45] Hubbard NE, Somers SD, Erickson KL. Effect of dietary fish oil on development and selected functions of murine inflammatory macrophages. *J Leukoc Biol* 1991; 49: 592-8.
- [46] Fisher M, Levine PH, Weiner BH, *et al.* Dietary N-3 fatty acid supplementation reduces superoxide production and chemiluminescence in a monocyte-enriched preparation of leukocytes. *Am J Clin Nutr* 1990; 51: 804-8.
- [47] Thompson PJ, Misso NL, Passarelli M, Phillips MJ. The effect of eicosapentaenoic acid consumption on human neutrophil chemiluminescence. *Lipids* 1991; 26: 1223-6.
- [48] James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr* 2000; 71: 343S-8S.
- [49] Christman JW, Lancaster LH, Blackwell TS. Nuclear factor kappa B: a pivotal role in the systemic inflammatory response syndrome and new target for therapy. *Intensive Care Med* 1998; 24: 1131-8.
- [50] Chen F, Castranova V, Shi X, Demers LM. New insights into the role of nuclear factor-kappaB, a ubiquitous transcription factor in the initiation of diseases. *Clin Chem* 1999; 45: 7-17.

- [51] Camandola S, Leonarduzzi G, Musso T, *et al.* Nuclear factor  $\kappa$ B is activated by arachidonic acid but not by eicosapentaenoic acid. *Biochem Biophys Res Commun* 1996; 229: 643-7.
- [52] Lo CJ, Chiu KC, Fu M, Lo R, Helton S. Fish oil decreases macrophage tumor necrosis factor gene transcription by altering the NF  $\kappa$ B activity. *J Surg Res* 1999; 82: 216-21.
- [53] Novak TE, Babcock TA, Jho DH, Helton WS, Espat NJ. NF- $\kappa$ B inhibition by omega-3 fatty acids modulates LPS-stimulated macrophage TNF- $\alpha$  transcription. *Am J Physiol Lung Cell Mol Physiol* 2003; 284: L84-9.
- [54] Zhao Y, Joshi-Barve S, Barve S, Chen LH. Eicosapentaenoic acid prevents LPS-induced TNF- $\alpha$  expression by preventing NF- $\kappa$ B activation. *J Am Coll Nutr* 2004; 23: 71-8.
- [55] Ross JA, Moses AG, Fearon KC. The anti-catabolic effects of N-3 fatty acids. *Curr Opin Clin Nutr Metab Care* 1999; 2: 219-26.
- [56] Lo CJ, Chiu KC, Fu M, Chu A, Helton S. Fish oil modulates macrophage P44/P42 mitogen-activated protein kinase activity induced by lipopolysaccharide. *JPEN J Parenter Enteral Nutr* 2000; 24: 159-63.
- [57] Denys A, Hichami A, Khan NA. Eicosapentaenoic acid and docosahexaenoic acid modulate MAP kinase (ERK1/ERK2) signaling in human T cells. *J Lipid Res* 2001; 42: 2015-20.
- [58] Denys A, Hichami A, Khan NA. Eicosapentaenoic acid and docosahexaenoic acid modulate MAP kinase enzyme activity in human T-cells. *Mol Cell Biochem* 2002; 232: 143-8.
- [59] Schoonjans K, Staels B, Auwerx J. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J Lipid Res* 1996; 37: 907-25.
- [60] Chinetti G, Griglio S, Antonucci M, *et al.* Activation of proliferator-activated receptors  $\alpha$  and  $\gamma$  induces apoptosis of human monocyte-derived macrophages. *J Biol Chem* 1998; 273: 25573-80.
- [61] Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor- $\gamma$  is a negative regulator of macrophage activation. *Nature* 1998; 391: 79-82.
- [62] de Urquiza AM, Liu S, Sjöberg M, *et al.* Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. *Science* 2000; 290: 2140-4.
- [63] Kliewer SA, Sundseth SS, Jones SA, *et al.* Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors  $\alpha$  and  $\gamma$ . *Proc Natl Acad Sci U S A* 1997; 94: 4318-23.
- [64] Forman BM, Chen J, Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors  $\alpha$  and  $\delta$ . *Proc Natl Acad Sci U S A* 1997; 94: 4312-7.
- [65] Jiang C, Ting AT, Seed B. PPAR- $\gamma$  agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998; 391: 82-6.
- [66] Poynter ME, Daynes RA. Peroxisome proliferator-activated receptor  $\alpha$  activation modulates cellular redox status, represses nuclear factor- $\kappa$ B signaling, and reduces inflammatory cytokine production in aging. *J Biol Chem* 1998; 273: 32833-41.
- [67] Takano H, Nagai T, Asakawa M, *et al.* Peroxisome proliferator-activated receptor activators inhibit lipopolysaccharide-induced tumor necrosis factor- $\alpha$  expression in neonatal rat cardiac myocytes. *Circ Res* 2000; 87: 596-602.
- [68] Wang P, Anderson PO, Chen S, Paulsson KM, Sjögren HO, Li S. Inhibition of the transcription factors AP-1 and NF- $\kappa$ B in CD4 T cells by peroxisome proliferator-activated receptor  $\gamma$  ligands. *Int Immunopharmacol* 2001; 1: 803-12.
- [69] Xu X, Otsuki M, Saito H, *et al.* PPAR $\alpha$  and GR differentially down-regulate the expression of nuclear factor- $\kappa$ B-responsive genes in vascular endothelial cells. *Endocrinology* 2001; 142: 3332-9.
- [70] Hirasawa A, Tsumaya K, Awaji T, *et al.* Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat Med* 2005; 11: 90-4.
- [71] Oh DY, Talukdar S, Bae EJ, *et al.* GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 2004; 142: 687-98.
- [72] Wong SW, Kwon MJ, Choi AM, Kim HP, Nakahira K, Hwang DH. Fatty acids modulate Toll-like receptor 4 activation through regulation of receptor dimerization and recruitment into lipid rafts in a reactive oxygen species-dependent manner. *J Biol Chem* 2009; 284: 27384-92.
- [73] Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. *J Biol Chem* 2001; 276: 16683-9.
- [74] Lee JY, Zhao L, Youn HS, *et al.* Saturated fatty acid activates but polyunsaturated fatty acid inhibits Toll-like receptor 2 dimerized with Toll-like receptor 6 or 1. *J Biol Chem* 2004; 279: 16971-9.
- [75] Prescott SL, Calder PC. N-3 polyunsaturated fatty acids and allergic disease. *Curr Opin Clin Nutr Metab Care* 2004; 7: 123-9.
- [76] Leaf A, Weber PC. A new era for science in nutrition. *Am J Clin Nutr* 1987; 45: 1048-53.
- [77] Black PN, Sharpe S. Dietary fat and asthma: is there a connection? *Eur Respir J* 1997; 10: 6-12.
- [78] Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002; 347: 911-20.
- [79] Aberg N, Hesselmar B, Aberg B, Eriksson B. Increase of asthma, allergic rhinitis and eczema in Swedish schoolchildren between 1979 and 1991. *Clin Exp Allergy* 1995; 25: 815-9.
- [80] Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr* 1991; 54: 438-63.
- [81] Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood)* 2008; 233: 674-88.
- [82] Kinsella JE, Lokesh B, Stone RA. Dietary N-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: possible mechanisms. *Am J Clin Nutr* 1990; 52: 1-28.
- [83] Krause TG, Koch A, Poulsen LK, Kristensen B, Olsen OR, Melbye M. Atopic sensitization among children in an arctic environment. *Clin Exp Allergy* 2002; 32: 367-72.
- [84] Horrobin DF. Low prevalences of coronary heart disease (CHD), psoriasis, asthma and rheumatoid arthritis in Eskimos: are they caused by high dietary intake of eicosapentaenoic acid (EPA), a genetic variation of essential fatty acid (EFA) metabolism or a combination of both? *Med Hypotheses* 1987; 22: 421-8.
- [85] Calder PC. Immunomodulation by omega-3 fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 2007; 77: 327-35.
- [86] Yaqoob P, Sherrington EJ, Jeffery NM, *et al.* Comparison of the effects of a range of dietary lipids upon serum and tissue lipid composition in the rat. *Int J Biochem Cell Biol* 1995; 27: 297-310.
- [87] Yaqoob P, Newsholme EA, Calder PC. Influence of cell culture conditions on diet-induced changes in lymphocyte fatty acid composition. *Biochim Biophys Acta* 1995; 1255: 333-40.
- [88] Faber J, Berkhout M, Vos AP, *et al.* Supplementation with a fish oil-enriched, high-protein medical food leads to rapid incorporation of EPA into white blood cells and modulates immune responses within one week in healthy men and women. *J Nutr* 2011; 141: 964-70.
- [89] von Schacky C, Fischer S, Weber PC. Long-term effects of dietary marine omega-3 fatty acids upon plasma and cellular lipids, platelet function, and eicosanoid formation in humans. *J Clin Invest* 1985; 76: 1626-31.
- [90] Yaqoob P, Pala HS, Cortina-Borja M, Newsholme EA, Calder PC. Encapsulated fish oil enriched in alpha-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. *Eur J Clin Invest* 2000; 30: 260-74.
- [91] Harris WS, Sands SA, Windsor SL, *et al.* Omega-3 fatty acids in cardiac biopsies from heart transplantation patients: correlation with erythrocytes and response to supplementation. *Circulation* 2004; 110: 1645-9.
- [92] Harris WS, Windsor SL, Dujovne CA. Effects of four doses of N-3 fatty acids given to hyperlipidemic patients for six months. *J Am Coll Nutr* 1991; 10: 220-7.
- [93] Marsen TA, Pollok M, Oette K, Baldamus CA. Pharmacokinetics of omega-3-fatty acids during ingestion of fish oil preparations. *Prostaglandins Leukot Essent Fatty Acids* 1992; 46: 191-6.
- [94] Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J Lipid Res* 1997; 38: 2012-22.
- [95] Popp-Snijders C, Schouten JA, van Blitterswijk WJ, van der Veen EA. Changes in membrane lipid composition of human erythrocytes after dietary supplementation of (N-3) polyunsaturated fatty acids. Maintenance of membrane fluidity. *Biochim Biophys Acta* 1986; 854: 31-7.

- [96] Scientific Advisory Committee on Nutrition/Committee on Toxicity; Advice on fish consumption; benefits and risks. TSO, London; 2004.
- [97] Simopoulos AP, Leaf A, Salem N, Jr. Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Ann Nutr Metab* 1999; 43: 127-30.
- [98] Devereux G. The increase in the prevalence of asthma and allergy: food for thought. *Nat Rev Immunol* 2006; 6: 869-74.
- [99] Floistrup H, Swartz J, Bergstrom A, *et al.* Allergic disease and sensitization in Steiner school children. *J Allergy Clin Immunol* 2006; 117: 59-66.
- [100] Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006; 6: 761-71.
- [101] Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocr Rev* 2006; 27: 141-69.
- [102] Waterland RA, Garza C. Potential mechanisms of metabolic imprinting that lead to chronic disease. *Am J Clin Nutr* 1999; 69: 179-97.
- [103] Lucas A. Programming by early nutrition: an experimental approach. *J Nutr* 1998; 128: 401S-6S.
- [104] Enke U, Seyfarth L, Schleussner E, Markert UR. Impact of PUFA on early immune and fetal development. *Br J Nutr* 2008; 1-11.
- [105] Kabesch M, Schaal W, Nicolai T, von Mutius E. Lower prevalence of asthma and atopy in Turkish children living in Germany. *Eur Respir J* 1999; 13: 577-82.
- [106] Hodge L, Salome CM, Peat JK, Haby MM, Xuan W, Woolcock AJ. Consumption of oily fish and childhood asthma risk. *Med J Aust* 1996; 164: 137-40.
- [107] Yu G, Bjorksten B. Polyunsaturated fatty acids in school children in relation to allergy and serum IgE levels. *Pediatr Allergy Immunol* 1998; 9: 133-8.
- [108] Connor WE, Lowensohn R, Hatcher L. Increased docosahexaenoic acid levels in human newborn infants by administration of sardines and fish oil during pregnancy. *Lipids* 1996; 31 Suppl: S183-7.
- [109] Harris WS, Connor WE, Lindsey S. Will dietary omega-3 fatty acids change the composition of human milk? *Am J Clin Nutr* 1984; 40: 780-5.
- [110] Olsen SF, Hansen HS, Sommer S, *et al.* Gestational age in relation to marine N-3 fatty acids in maternal erythrocytes: a study of women in the Faroe Islands and Denmark. *Am J Obstet Gynecol* 1991; 164: 1203-9.
- [111] Sanjurjo P, Matorras R, Perteagudo L. Influence of fatty fish intake during pregnancy in the polyunsaturated fatty acids of erythrocyte phospholipids in the mother at labor and newborn infant. *Acta Obstet Gynecol Scand* 1995; 74: 594-8.
- [112] Furuhielm C, Warstedt K, Larsson J, *et al.* Fish oil supplementation in pregnancy and lactation may decrease the risk of infant allergy. *Acta Paediatr* 2009; 98: 1461-7.
- [113] Furuhielm C, Warstedt K, Fageras M, *et al.* Allergic disease in infants up to 2 years of age in relation to plasma omega-3 fatty acids and maternal fish oil supplementation in pregnancy and lactation. *Pediatr Allergy Immunol* 2011; 22: 505-14.
- [114] Dunstan JA, Mori TA, Barden A, *et al.* Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy. *Clin Exp Allergy* 2003; 33: 442-8.
- [115] Dunstan JA, Mori TA, Barden A, *et al.* Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized, controlled trial. *J Allergy Clin Immunol* 2003; 112: 1178-84.
- [116] Miles E, Noakes P, Kremmyda L-S, *et al.* The salmon in pregnancy study- study design, subject characteristics, maternal fish and marine N-3 fatty acid intake, and marine N-3 fatty acid status in maternal and umbilical cord blood. *Am J Clin Nutr* 2011; 94(6): 1986S-92S.
- [117] Duchon K, Yu G, Bjorksten B. Atopic sensitization during the first year of life in relation to long chain polyunsaturated fatty acid levels in human milk. *Pediatr Res* 1998; 44: 478-84.
- [118] Kankaanpaa P, Nurmela K, Erkkila A, *et al.* Polyunsaturated fatty acids in maternal diet, breast milk, and serum lipid fatty acids of infants in relation to atopy. *Allergy* 2001; 56: 633-8.
- [119] Dunstan JA, Roper J, Mitoulas L, Hartmann PE, Simmer K, Prescott SL. The effect of supplementation with fish oil during pregnancy on breast milk immunoglobulin A, soluble CD14, cytokine levels and fatty acid composition. *Clin Exp Allergy* 2004; 34: 1237-42.
- [120] Lauritzen L, Kjaer TM, Fruekilde MB, Michaelsen KF, Frokiaer H. Fish oil supplementation of lactating mothers affects cytokine production in 2 1/2-year-old children. *Lipids* 2005; 40: 669-76.
- [121] Zeyda M, Saemann MD, Stuhlmeier KM, *et al.* Polyunsaturated fatty acids block dendritic cell activation and function independently of NF-kappaB activation. *J Biol Chem* 2005; 280: 14293-301.
- [122] Zapata-Gonzalez F, Rueda F, Petriz J, *et al.* Human dendritic cell activities are modulated by the omega-3 fatty acid, docosahexaenoic acid, mainly through PPAR(gamma):RXR heterodimers: comparison with other polyunsaturated fatty acids. *J Leukoc Biol* 2008; 84: 1172-82.
- [123] Wang H, Hao Q, Li QR, *et al.* Omega-3 polyunsaturated fatty acids affect lipopolysaccharide-induced maturation of dendritic cells through mitogen-activated protein kinases p38. *Nutrition* 2007; 23: 474-82.
- [124] Sanderson P, MacPherson GG, Jenkins CH, Calder PC. Dietary fish oil diminishes the antigen presentation activity of rat dendritic cells. *Biochem Soc Trans* 1997; 25: 351S.
- [125] Draper E, Reynolds CM, Canavan M, Mills KH, Loscher CE, Roche HM. Omega-3 fatty acids attenuate dendritic cell function via NF-kappaB independent of PPARgamma. *J Nutr Biochem* 2002; 22: 784-90.
- [126] Caron G, Delneste Y, Roelands E, *et al.* Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. *J Immunol* 2001; 167: 3682-6.
- [127] Gosset P, Pichavant M, Faveeuw C, Bureau F, Tonnel AB, Trottein F. Prostaglandin D2 affects the differentiation and functions of human dendritic cells: impact on the T cell response. *Eur J Immunol* 2005; 35: 1491-500.
- [128] Theiner G, Gessner A, Lutz MB. The mast cell mediator PGD2 suppresses IL-12 release by dendritic cells leading to Th2 polarized immune responses *in vivo*. *Immunobiology* 2006; 211: 463-72.
- [129] Mizota T, Fujita-Kambara C, Matsuya N, *et al.* Effect of dietary fatty acid composition on Th1/Th2 polarization in lymphocytes. *JPN J Parenter Enteral Nutr* 2009; 33: 390-6.
- [130] Zhang P, Smith R, Chapkin RS, McMurray DN. Dietary (N-3) polyunsaturated fatty acids modulate murine Th1/Th2 balance toward the Th2 pole by suppression of Th1 development. *J Nutr* 2005; 135: 1745-51.
- [131] Kleemann R, Scott FW, Worz-Pagenstert U, Nimal Ratnayake WM, Kolb H. Impact of dietary fat on Th1/Th2 cytokine gene expression in the pancreas and gut of diabetes-prone BB rats. *J Autoimmun* 1998; 11: 97-103.
- [132] Wallace FA, Miles EA, Evans C, Stock TE, Yaqoob P, Calder PC. Dietary fatty acids influence the production of Th1- but not Th2-type cytokines. *J Leukoc Biol* 2001; 69: 449-57.
- [133] Gallai V, Sarchielli P, Trequattrini A, *et al.* Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with N-3 polyunsaturated fatty acids. *J Neuroimmunol* 1995; 56: 143-53.
- [134] Meydani SN, Endres S, Woods MM, *et al.* Oral (N-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *J Nutr* 1991; 121: 547-55.
- [135] Trebble TM, Wootton SA, Miles EA, *et al.* Prostaglandin E2 production and T cell function after fish-oil supplementation: response to antioxidant cosupplementation. *Am J Clin Nutr* 2003; 78: 376-82.
- [136] Yaqoob P, Calder P. Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. *Cell Immunol* 1995; 163: 120-8.
- [137] Miles EA, Aston L, Calder PC. *In vitro* effects of eicosanoids derived from different 20-carbon fatty acids on T helper type 1 and T helper type 2 cytokine production in human whole-blood cultures. *Clin Exp Allergy* 2003; 33: 624-32.
- [138] Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest* 2001; 108: 15-23.
- [139] Chouaib S, Welte K, Mertelsmann R, Dupont B. Prostaglandin E2 acts at two distinct pathways of T lymphocyte activation: inhibition of interleukin 2 production and down-regulation of transferrin receptor expression. *J Immunol* 1985; 135: 1172-9.

- [140] Stelekati E, Orinska Z, Bulfone-Paus S. Mast cells in allergy: innate instructors of adaptive responses. *Immunobiology* 2007; 212: 505-19.
- [141] Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. *Nat Immunol* 2005; 6: 135-42.
- [142] Calder PC, Yaqoob P, Thies F, Wallace FA, Miles EA. Fatty acids and lymphocyte functions. *Br J Nutr* 2002; 87 Suppl 1: S31-48.
- [143] Stulnig TM, Zeyda M. Immunomodulation by polyunsaturated fatty acids: impact on T-cell signaling. *Lipids* 2004; 39: 1171-5.
- [144] Stulnig TM, Berger M, Sigmund T, Raederstorff D, Stockinger H, Waldhausl W. Polyunsaturated fatty acids inhibit T cell signal transduction by modification of detergent-insoluble membrane domains. *J Cell Biol* 1998; 143: 637-44.
- [145] Stulnig TM, Huber J, Leitinger N, et al. Polyunsaturated eicosapentaenoic acid displaces proteins from membrane rafts by altering raft lipid composition. *J Biol Chem* 2001; 276: 37335-40.
- [146] Zeyda M, Szekeres AB, Saemann MD, et al. Suppression of T cell signaling by polyunsaturated fatty acids: selectivity in inhibition of mitogen-activated protein kinase and nuclear factor activation. *J Immunol* 2003; 170: 6033-9.
- [147] Sanderson P, Calder PC. Dietary fish oil appears to prevent the activation of phospholipase C-gamma in lymphocytes. *Biochim Biophys Acta* 1998; 1392: 300-8.
- [148] Fan YY, Ly LH, Barhoumi R, McMurray DN, Chapkin RS. Dietary docosahexaenoic acid suppresses T cell protein kinase C theta lipid raft recruitment and IL-2 production. *J Immunol* 2004; 173: 6151-60.
- [149] Weise C, Hilt K, Milovanovic M, Ernst D, Ruhl R, Worm M. Inhibition of IgE production by docosahexaenoic acid is mediated by direct interference with STAT6 and NFkappaB pathway in human B cells. *J Nutr Biochem* 2011; 22: 269-75.
- [150] Roper RL, Phipps RP. Prostaglandin E2 regulation of the immune response. *Adv Prostaglandin Thromboxane Leukot Res* 1994; 22: 101-11.
- [151] Kaur K, Harris SG, Padilla J, Graf BA, Phipps RP. Prostaglandin E2 as a modulator of lymphocyte mediated inflammatory and humoral responses. *Adv Exp Med Biol* 1999; 469: 409-12.
- [152] Pene J, Rousset F, Briere F, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. *Proc Natl Acad Sci U S A* 1988; 85: 6880-4.
- [153] Nilsson G, Blom T, Kusche-Gullberg M, et al. Phenotypic characterization of the human mast-cell line HMC-1. *Scand J Immunol* 1994; 39: 489-98.
- [154] Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 125: S73-80.
- [155] Nakano N, Nakao A, Uchida T, et al. Effects of arachidonic acid analogs on FcepsilonRI-mediated activation of mast cells. *Biochim Biophys Acta* 2005; 1738: 19-28.
- [156] Inoue T, Suzuki Y, Yoshimaru T, Ra C. Reactive oxygen species produced up- or downstream of calcium influx regulate proinflammatory mediator release from mast cells: role of NADPH oxidase and mitochondria. *Biochim Biophys Acta* 2008; 1783: 789-802.
- [157] Gilfillan AM, Tkaczky C. Integrated signalling pathways for mast-cell activation. *Nat Rev Immunol* 2006; 6: 218-30.
- [158] Schmutzler S, Bachmann L, Fuhrmann H, Schumann J. PUFA-dependent alteration of oxidative parameters of a canine mastocytoma cell line. *Acta Vet Hung* 2010; 58: 453-64.
- [159] Swindle EJ, Metcalfe DD. The role of reactive oxygen species and nitric oxide in mast cell-dependent inflammatory processes. *Immunol Rev* 2007; 217: 186-205.
- [160] Suzuki Y, Yoshimaru T, Matsui T, et al. Fc epsilon RI signaling of mast cells activates intracellular production of hydrogen peroxide: role in the regulation of calcium signals. *J Immunol* 2003; 171: 6119-27.
- [161] Kim JY, Ro JY. Signal pathway of cytokines produced by reactive oxygen species generated from phorbol myristate acetate-stimulated HMC-1 cells. *Scand J Immunol* 2005; 62: 25-35.
- [162] Suzuki Y, Yoshimaru T, Inoue T, Niide O, Ra C. Role of oxidants in mast cell activation. *Chem Immunol Allergy* 2005; 87: 32-42.
- [163] Teshima R, Amano F, Nakamura R, Tanaka Y, Sawada J. Effects of polyunsaturated fatty acids on calcium response and degranulation from RBL-2H3 cells. *Int Immunopharmacol* 2007; 7: 205-10.
- [164] Khan WA, Blobe GC, Hannun YA. Arachidonic acid and free fatty acids as second messengers and the role of protein kinase C. *Cell Signal* 1995; 7: 171-84.
- [165] Gueck T, Seidel A, Fuhrmann H. Consequences of eicosapentaenoic acid (N-3) and arachidonic acid (N-6) supplementation on mast cell mediators. *J Anim Physiol Anim Nutr (Berl)* 2004; 88: 259-65.
- [166] Whelan J, Broughton KS, Lokesh B, Kinsella JE. *In vivo* formation of leukotriene E5 by murine peritoneal cells. *Prostaglandins* 1991; 41: 29-42.
- [167] Triggiani M, Connell TR, Chilton FH. Evidence that increasing the cellular content of eicosapentaenoic acid does not reduce the biosynthesis of platelet-activating factor. *J Immunol* 1990; 145: 2241-8.
- [168] Ishihara K, Murata M, Kaneniwa M, et al. Effect of tetracosahexaenoic acid on the content and release of histamine, and eicosanoid production in MC/9 mouse mast cell. *Lipids* 1998; 33: 1107-14.
- [169] Matsumoto K, Morita I, Hibino H, Murota S. Inhibitory effect of docosahexaenoic acid-containing phospholipids on 5-lipoxygenase in rat basophilic leukemia cells. *Prostaglandins Leukot Essent Fatty Acids* 1993; 49: 861-6.
- [170] Lewis RA. Leukotrienes and other lipid mediators of asthma. *Chest* 1985; 87: 5S-10S.
- [171] van Haaster CM, Engels W, Lemmens PJ, Hornstra G, van der Vusse GJ. Formation of prostanoids and hydroxy fatty acids by stimulated peritoneal mast cells: role of the dietary fat type in rat. *Biochim Biophys Acta* 1993; 1167: 147-54.
- [172] Calder PC. Sir David Cuthbertson Medal Lecture. Immunomodulatory and anti-inflammatory effects of N-3 polyunsaturated fatty acids. *Proc Nutr Soc* 1996; 55: 737-74.
- [173] Renier G, Skamene E, DeSanctis J, Radzioch D. Dietary N-3 polyunsaturated fatty acids prevent the development of atherosclerotic lesions in mice. Modulation of macrophage secretory activities. *Arterioscler Thromb* 1993; 13: 1515-24.
- [174] Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ. The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in N-3 fatty acids from vegetable oil or fish oil. *Am J Clin Nutr* 1996; 63: 116-22.
- [175] Sugiyama H, Nonaka T, Kishimoto T, Komoriya K, Tsuji K, Nakahata T. Peroxisome proliferator-activated receptors are expressed in human cultured mast cells: a possible role of these receptors in negative regulation of mast cell activation. *Eur J Immunol* 2000; 30: 3363-70.
- [176] Lee KS, Kim SR, Park SJ, et al. Peroxisome proliferator activated receptor-gamma modulates reactive oxygen species generation and activation of nuclear factor-kappaB and hypoxia-inducible factor 1alpha in allergic airway disease of mice. *J Allergy Clin Immunol* 2006; 118: 120-7.
- [177] Gosset P, Bureau F, Angeli V, et al. Prostaglandin D2 affects the maturation of human monocyte-derived dendritic cells: consequence on the polarization of naive Th cells. *J Immunol* 2003; 170: 4943-52.
- [178] Faveeuw C, Gosset P, Bureau F, et al. Prostaglandin D2 inhibits the production of interleukin-12 in murine dendritic cells through multiple signaling pathways. *Eur J Immunol* 2003; 33: 889-98.
- [179] Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. *J Clin Invest* 2001; 108: 1865-73.
- [180] World Health Organisation, The world health report 2003 - Shaping the future; 2003.
- [181] Libby P. Inflammation in atherosclerosis. *Nature* 2002; 420: 868-74.
- [182] Glass CK, Witztum JL. Atherosclerosis. the road ahead. *Cell* 2001; 104: 503-16.
- [183] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993; 362: 801-9.
- [184] Kim YS, Ahn Y, Hong MH, et al. Rosuvastatin suppresses the inflammatory responses through inhibition of c-Jun N-terminal kinase and Nuclear Factor-kappaB in endothelial cells. *J Cardiovasc Pharmacol* 2007; 49: 376-83.
- [185] De Caterina R, Massaro M. Omega-3 fatty acids and the regulation of expression of endothelial pro-atherogenic and pro-inflammatory genes. *J Membr Biol* 2005; 206: 103-16.
- [186] Huang ZH, Bates EJ, Ferrante JV, et al. Inhibition of stimulus-induced endothelial cell intercellular adhesion molecule-1, E-selectin, and vascular cellular adhesion molecule-1 expression by arachidonic acid and its hydroxy and hydroperoxy derivatives. *Circ Res* 1997; 80: 149-58.

- [187] Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007; 27: 2292-301.
- [188] Rao RM, Yang L, Garcia-Cardena G, Luscinskas FW. Endothelial-dependent mechanisms of leukocyte recruitment to the vascular wall. *Circ Res* 2007; 101: 234-47.
- [189] Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* 1978; 2: 117-9.
- [190] Kromann N, Green A. Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic diseases 1950-1974. *Acta Med Scand* 1980; 208: 401-6.
- [191] Newman WP, Middaugh JP, Probst MT, Rogers DR. Atherosclerosis in Alaska Natives and non-natives. *Lancet* 1993; 341: 1056-7.
- [192] Yano K, MacLean CJ, Reed DM, *et al.* A comparison of the 12-year mortality and predictive factors of coronary heart disease among Japanese men in Japan and Hawaii. *Am J Epidemiol* 1988; 127: 476-87.
- [193] Worth RM, Kato H, Rhoads GG, Kagan K, Syme SL. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: mortality. *Am J Epidemiol* 1975; 102: 481-90.
- [194] Toshima H, Koga Y, Menotti A, *et al.* The seven countries study in Japan. Twenty-five-year experience in cardiovascular and all-causes deaths. *Jpn Heart J* 1995; 36: 179-89.
- [195] Barker DJ. Fetal origins of coronary heart disease. *Bmj* 1995; 311: 171-4.
- [196] DeRuiter MC, Alkemade FE, Gittenberger-de Groot AC, Poelmann RE, Havekes LM, van Dijk KW. Maternal transmission of risk for atherosclerosis. *Curr Opin Lipidol* 2008; 19: 333-7.
- [197] Nelson SM, Sattar N, Freeman DJ, Walker JD, Lindsay RS. Inflammation and endothelial activation is evident at birth in offspring of mothers with type 1 diabetes. *Diabetes* 2007; 56: 2697-704.
- [198] Stenvinkel P, Karimi M, Johansson S, *et al.* Impact of inflammation on epigenetic DNA methylation - a novel risk factor for cardiovascular disease? *J Intern Med* 2007; 261: 488-99.
- [199] Jiang Y, Zhang J, Xiong J, Cao J, Li G, Wang S. Ligands of peroxisome proliferator-activated receptor inhibit homocysteine-induced DNA methylation of inducible nitric oxide synthase gene. *Acta Biochim Biophys Sin (Shanghai)* 2007; 39: 366-76.
- [200] Lund G, Zaina S. Atherosclerosis, lipids, inflammation and epigenetics. *Curr Opin Lipidol* 2007; 18: 699-701.
- [201] Benatti P, Peluso G, Nicolai R, Calvani M. Polyunsaturated fatty acids: biochemical, nutritional and epigenetic properties. *J Am Coll Nutr* 2004; 23: 281-302.
- [202] Jackson SM, Parhami F, Xi XP, *et al.* Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction. *Arterioscler Thromb Vasc Biol* 1999; 19: 2094-104.
- [203] Huang T, Tucker K, Lee Y, *et al.* MAT1A variants modulate the effect of dietary fatty acids on plasma homocysteine concentrations. *Nutr Metab Cardiovasc Dis* 2011; 22(4): 362-8.
- [204] Huang T, Wahlqvist ML, Li D. Docosahexaenoic acid decreases plasma homocysteine via regulating enzyme activity and mRNA expression involved in methionine metabolism. *Nutrition* 2010; 26: 112-9.
- [205] Alkhoury K, Parkin SM, Homer-Vanniasinkam S, Graham AM. Chronic Homocysteine Exposure Upregulates Endothelial Adhesion Molecules and Mediates Leukocyte: Endothelial Cell Interactions under Flow Conditions. *Eur J Vasc Endovasc Surg* 2011; 41(3): 429-35.
- [206] Bai YP, Liu YH, Chen J, *et al.* Rosiglitazone attenuates NF-kappaB-dependent ICAM-1 and TNF-alpha production caused by homocysteine via inhibiting ERK1/2/p38MAPK activation. *Biochem Biophys Res Commun* 2007; 360: 20-6.
- [207] Joss-Moore LA, Wang Y, Baack ML, *et al.* IUGR decreases PPARgamma and SETD8 Expression in neonatal rat lung and these effects are ameliorated by maternal DHA supplementation. *Early Hum Dev* 2010; 86: 785-91.
- [208] Hellebrekers DM, Castermans K, Vire E, *et al.* Epigenetic regulation of tumor endothelial cell anergy: silencing of intercellular adhesion molecule-1 by histone modifications. *Cancer Res* 2006; 66: 10770-7.
- [209] Rose JL, Huang H, Wray SF, Hoyt DG. Integrin engagement increases histone H3 acetylation and reduces histone H1 association with DNA in murine lung endothelial cells. *Mol Pharmacol* 2005; 68: 439-46.
- [210] Dietary supplementation with N-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999; 354: 447-55.
- [211] Burr ML, Fehily AM, Gilbert JF, *et al.* Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* 1989; 2: 757-61.
- [212] Calder PC. N-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin Sci (Lond)* 2004; 107: 1-11.
- [213] Bucher HC, Griffith LE, Guyatt GH. Systematic review on the risk and benefit of different cholesterol-lowering interventions. *Arterioscler Thromb Vasc Biol* 1999; 19: 187-95.
- [214] Studer M, Briel M, Leimenstoll B, Glass TR, Bucher HC. Effect of different antilipidemic agents and diets on mortality: a systematic review. *Arch Intern Med* 2005; 165: 725-30.
- [215] Yokoyama M, Origasa H, Matsuzaki M, *et al.* Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 2007; 369: 1090-8.
- [216] De Caterina R, Zampolli A. Omega-3 fatty acids, atherogenesis, and endothelial activation. *J Cardiovasc Med (Hagerstown)* 2007; 8 Suppl 1: S11-4.
- [217] Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 2002; 106: 2747-57.
- [218] Van de Werf F, Ardissino D, Betriu A, *et al.* Management of acute myocardial infarction in patients presenting with ST-segment elevation. The Task Force on the Management of Acute Myocardial Infarction of the European Society of Cardiology. *Eur Heart J* 2003; 24: 28-66.
- [219] Mo SJ, Son EW, Lee SR, Lee SM, Shin DH, Pyo S. CML-1 inhibits TNF-alpha-induced NF-kappaB activation and adhesion molecule expression in endothelial cells through inhibition of Ikbalpha kinase. *J Ethnopharmacol* 2007; 109: 78-86.
- [220] Xue H, Wan M, Song D, Li Y, Li J. Eicosapentaenoic acid and docosahexaenoic acid modulate mitogen-activated protein kinase activity in endothelium. *Vascul Pharmacol* 2006; 44: 434-9.
- [221] Weber C, Erl W, Pietsch A, Danesch U, Weber PC. Docosahexaenoic acid selectively attenuates induction of vascular cell adhesion molecule-1 and subsequent monocytic cell adhesion to human endothelial cells stimulated by tumor necrosis factor-alpha. *Arterioscler Thromb Vasc Biol* 1995; 15: 622-8.
- [222] De Caterina R, Cybulsky MI, Clinton SK, Gimbrone MA, Jr., Libby P. The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arterioscler Thromb* 1994; 14: 1829-36.
- [223] Ma DW, Seo J, Switzer KC, *et al.* N-3 PUFA and membrane microdomains: a new frontier in bioactive lipid research. *J Nutr Biochem* 2004; 15: 700-6.
- [224] Li Q, Zhang Q, Wang M, *et al.* Docosahexaenoic acid affects endothelial nitric oxide synthase in caveolae. *Arch Biochem Biophys* 2007; 466: 250-9.
- [225] Harris WS, Rambjor GS, Windsor SL, Diederich D. N-3 fatty acids and urinary excretion of nitric oxide metabolites in humans. *Am J Clin Nutr* 1997; 65: 459-64.
- [226] Felton CV, Crook D, Davies MJ, Oliver MF. Relation of plaque lipid composition and morphology to the stability of human aortic plaques. *Arterioscler Thromb Vasc Biol* 1997; 17: 1337-45.
- [227] Thies F, Garry JM, Yaquob P, *et al.* Association of N-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* 2003; 361: 477-85.
- [228] Leaf A, Weber PC. Cardiovascular effects of N-3 fatty acids. *N Engl J Med* 1988; 318: 549-57.
- [229] Harris WS. N-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* 1997; 65: 1645S-54S.
- [230] Stamler J, Neaton JD, Wentworth DN. Blood pressure (systolic and diastolic) and risk of fatal coronary heart disease. *Hypertension* 1989; 13: 12-12.
- [231] Collins R, Peto R, MacMahon S, *et al.* Blood pressure, stroke, and coronary heart disease. Part 2, Short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *Lancet* 1990; 335: 827-38.

- [232] MacMahon S, Peto R, Cutler J, *et al.* Blood pressure, stroke, and coronary heart disease. Part 1, Prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet* 1990; 335: 765-74.
- [233] Webber PC, Larsson C, Anggard E, *et al.* Stimulation of renin release from rabbit renal cortex by arachidonic acid and prostaglandin endoperoxides. *Circ Res* 1976; 39: 868-74.
- [234] Gerkens JF, Williams A, Branch RA. Effect of precursors of the 1, 2, and 3 series prostaglandins on renin release and renal blood flow in the dog. *Prostaglandins* 1981; 22: 513-20.
- [235] Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* 1993; 88: 523-33.
- [236] Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR, Kok FJ. Blood pressure response to fish oil supplementation: meta-regression analysis of randomized trials. *J Hypertens* 2002; 20: 1493-9.
- [237] Boisseau MR. Leukocyte involvement in the signs and symptoms of chronic venous disease. Perspectives for therapy. *Clin Hemorheol Microcirc* 2007; 37: 277-90.
- [238] Collie-Duguid ES, Wahle KW. Inhibitory effect of fish oil N-3 polyunsaturated fatty acids on the expression of endothelial cell adhesion molecules. *Biochem Biophys Res Commun* 1996; 220: 969-74.
- [239] De Caterina R, Cybulsky MA, Clinton SK, Gimbrone MA, Jr., Libby P. Omega-3 fatty acids and endothelial leukocyte adhesion molecules. *Prostaglandins Leukot Essent Fatty Acids* 1995; 52: 191-5.
- [240] De Caterina R, Bernini W, Carluccio MA, Liao JK, Libby P. Structural requirements for inhibition of cytokine-induced endothelial activation by unsaturated fatty acids. *J Lipid Res* 1998; 39: 1062-70.
- [241] Hughes DA, Southon S, Pinder AC. (N-3) Polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes *in vitro*. *J Nutr* 1996; 126: 603-10.
- [242] Yates CM, Tull SP, Madden J, *et al.* The (N-3) PUFA, DHA, Inhibits the Adhesion of Flowing Neutrophils to Cytokine Stimulated Human Umbilical Vein Endothelial Cells. *J Nutr* 2011; 141: 1331-4.
- [243] Setiadi H, McEver RP. Clustering endothelial E-selectin in clathrin-coated pits and lipid rafts enhances leukocyte adhesion under flow. *Blood* 2008; 111: 1989-98.
- [244] Tull SP, Yates CM, Maskrey BH, *et al.* Omega-3 Fatty acids and inflammation: novel interactions reveal a new step in neutrophil recruitment. *PLoS Biol* 2009; 7: e1000177.
- [245] van den Elsen LW, Noakes PS, van der Maarel MA, *et al.* Salmon consumption by pregnant women reduces ex vivo umbilical cord endothelial cell activation. *Am J Clin Nutr* 2011; 94: 1418-25.
- [246] Sanderson P, Calder PC. Dietary fish oil diminishes lymphocyte adhesion to macrophage and endothelial cell monolayers. *Immunology* 1998; 94: 79-87.
- [247] Miles EA, Wallace FA, Calder PC. Dietary fish oil reduces intercellular adhesion molecule 1 and scavenger receptor expression on murine macrophages. *Atherosclerosis* 2000; 152: 43-50.
- [248] Sanderson P, MacPherson GG, Jenkins CH, Calder PC. Dietary fish oil diminishes the antigen presentation activity of rat dendritic cells. *J Leukoc Biol* 1997; 62: 771-7.
- [249] Hughes DA, Pinder AC, Piper Z, Johnson IT, Lund EK. Fish oil supplementation inhibits the expression of major histocompatibility complex class II molecules and adhesion molecules on human monocytes. *Am J Clin Nutr* 1996; 63: 267-72.
- [250] Khalfoun B, Thibault F, Watier H, Bardos P, Lebranchu Y. Docosahexaenoic and eicosapentaenoic acids inhibit *in vitro* human endothelial cell production of interleukin-6. *Adv Exp Med Biol* 1997; 400B: 589-97.
- [251] Fox PL, DiCorleto PE. Fish oils inhibit endothelial cell production of platelet-derived growth factor-like protein. *Science* 1988; 241: 453-6.
- [252] Kang DG, Moon MK, Lee AS, Kwon TO, Kim JS, Lee HS. Cornuside suppresses cytokine-induced proinflammatory and adhesion molecules in the human umbilical vein endothelial cells. *Biol Pharm Bull* 2007; 30: 1796-9.
- [253] Carluccio MA, Massaro M, Bonfrate C, *et al.* Oleic acid inhibits endothelial activation : A direct vascular antiatherogenic mechanism of a nutritional component in the mediterranean diet. *Arterioscler Thromb Vasc Biol* 1999; 19: 220-8.
- [254] Wang TM, Chen CJ, Lee TS, *et al.* Docosahexaenoic acid attenuates VCAM-1 expression and NF-kappaB activation in TNF-alpha-treated human aortic endothelial cells. *J Nutr Biochem* 2011; 22: 187-94.
- [255] Bot I, Biessen EA. Mast cells in atherosclerosis. *Thromb Haemost* 2011; 106: 820-6.
- [256] Soehnlein O, Weber C. Myeloid cells in atherosclerosis: initiators and decision shapers. *Semin Immunopathol* 2009; 31: 35-47.
- [257] Zhang J, Alcaide P, Liu L, *et al.* Regulation of endothelial cell adhesion molecule expression by mast cells, macrophages, and neutrophils. *PLoS One* 2011; 6: e14525.
- [258] Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis (\*). *Annu Rev Immunol* 2009; 27: 165-97.
- [259] Heikkila HM, Trosien J, Metso J, *et al.* Mast cells promote atherosclerosis by inducing both an atherogenic lipid profile and vascular inflammation. *J Cell Biochem* 2010; 109: 615-23.