

Review

Essential fatty acids: biochemistry, physiology and pathology

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Essential fatty acids (EFAs), linoleic acid (LA), and α -linolenic acid (ALA) are essential for humans, and are freely available in the diet. Hence, EFA deficiency is extremely rare in humans. To derive the full benefits of EFAs, they need to be metabolized to their respective long-chain metabolites, *i.e.*, dihomo- γ -linolenic acid (DGLA), and arachidonic acid (AA) from LA; and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from ALA. Some of these long-chain metabolites not only form precursors to respective prostaglandins (PGs), thromboxanes (TXs), and leukotrienes (LTs), but also give rise to lipoxins (LXs) and resolvins that have potent anti-inflammatory actions. Furthermore, EFAs and their metabolites may function as endogenous angiotensin-converting enzyme and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, nitric oxide (NO) enhancers, anti-hypertensives, and anti-atherosclerotic molecules. Recent studies revealed that EFAs react with NO to yield respective nitroalkene derivatives that exert cell-signaling actions via ligation and activation of peroxisome proliferator-activated receptors. The metabolism of EFAs is altered in several diseases such as obesity, hypertension, diabetes mellitus, coronary heart disease, schizophrenia, Alzheimer's disease, atherosclerosis, and cancer. Thus, EFAs and their derivatives have varied biological actions and seem to be involved in several physiological and pathological processes.

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1 Introduction

Essential fatty acids (EFAs) are important constituents of all cell membranes. EFAs confer on membranes properties of fluidity, and, thus, determine and influence the behavior of membrane-bound enzymes and receptors. EFAs are essential for survival of humans and other mam-

mals, and they cannot be synthesized in the body; hence, they have to be obtained in our diet and, thus, are essential [1, 2]. There are two types of naturally occurring EFAs in the body, the ω -6 series derived from *cis*-linoleic acid (LA, 18:2) and the ω -3 series derived from α -linolenic acid (ALA, 18:3). There is another sequence of fatty acids derived from oleic acid (OA, 18:1 ω -9). OA is not an EFA since it can be derived from simple precursors in mammals. All the three ω -9, ω -6, and ω -3 series of unsaturated fatty acids are metabolized by the same set of enzymes to their respective long-chain metabolites. Since ω -6 and ω -3 EFAs are the EFAs, and further discussion here is centered on these two series of fatty acids and their metabolites. While some of the functions of EFAs require their conversion to eicosanoids and other products, in majority of the instances the fatty acids themselves appear to be active. The longer chain metabolites of LA and ALA are particularly important in regulating membrane function. These long-chain metabolites are of major importance in the brain, retina, liver, kidney, adrenal glands and gonads.

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Abbreviations: AA, arachidonic acid; ACE, angiotensin-converting enzyme; ALA, α -linolenic acid; CHD, coronary heart disease; CRP, C-reactive protein; DGLA, dihomo- γ -linolenic acid; DHA, docosahexaenoic acid; EFAs, essential fatty acids; EPA, eicosapentaenoic acid; GLA, γ -linolenic acid; LA, linoleic acid; LT, leukotriene; LX, lipoxin; OA, oleic acid; PA, palmitoleic acid; PG, prostaglandin; PLA₂, phospholipase A₂; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; SREBP, sterol regulatory element-binding protein; TX, thromboxane

2 Structure, nomenclature, and metabolism of EFAs

EFAs are polyunsaturated fatty acids (PUFAs) since they contain two or more double bonds. PUFAs are fatty acids some of which have at least two carbon-to-carbon double bonds in a hydrophobic hydrocarbon chain, which typically includes X-Y carbon atoms and terminates in a carboxylic acid group. PUFAs are classified in accordance with a short-hand nomenclature, which designates the number of carbon atoms present (chain length), the number of double bonds in the chain and the position of the double bonds nearest to the terminal methyl group. The notation "a:b" is used to denote the chain length and number of double bonds, and the notation "n:x" is used to describe the position of the double bond nearest to the methyl group. There are at least four independent families of PUFAs, depending on the parent fatty acid from which they are synthesized. They include: the " ω -3" series derived from ALA (18:3, ω -3); the " ω -6" series derived from *cis*-LA (18:2, ω -6); the " ω -9" series derived from OA (18:1, ω -9); and the " ω -7" series derived from palmitoleic acid (PA, 16:1, ω -7).

LA is converted to γ -linolenic acid (GLA, 18:3, n-6) by the action of the enzyme Δ^6 desaturase (d-6-d), and GLA is elongated to form dihomo-GLA (DGLA, 20:3, n-6), the precursor of the 1 series of prostaglandins (PGs). DGLA can also be converted to arachidonic acid (AA, 20:4, n-6) by the action of the enzyme Δ^5 desaturase (d-5-d). AA forms the precursor of 2 series of prostaglandins, thromboxanes and the 4 series of leukotrienes. ALA is converted to eicosapentaenoic acid (EPA, 20:5, n-3) by d-6-d and d-5-d. EPA forms the precursor of the 3 series of prostaglandins and the 5 series of leukotrienes. LA, GLA, DGLA, AA, ALA, EPA and docosahexaenoic acid (DHA, 22:6, n-3) are all PUFAs, but only LA and ALA are EFAs (see Fig. 1 for metabolism of EFAs). AA and EPA also give rise to their respective hydroxy acids, which in turn are converted to their respective leukotrienes (LTs). Both PGs and LTs are highly biologically active, have pro-inflammatory action, and are known to be involved in various pathological processes, such as atherosclerosis, bronchial asthma, inflammatory bowel disease, and several other inflammatory conditions. In the present discussion, the term "PUFAs" is used to refer to all unsaturated fatty acids: LA, GLA, DGLA, AA, ALA, EPA, and DHA; and the term EFAs refers to LA and ALA. Although the terms EFAs and PUFAs are used interchangeably for the sake of convenience, it should be understood that all EFAs are PUFAs but all PUFAs are not EFAs. It is known that many of the functions of EFAs are also brought about by PUFAs, and EFA-deficiency states can be corrected to a large extent by PUFAs. This led to the suggestion that PUFAs are "functional EFAs". Hence, in general, many authors use the terms EFAs and PUFAs interchangeably. This convention is followed in the present discussion also.

Studies revealed that EFAs/PUFAs themselves play a significant role in the pathobiology of clinical conditions such as collagen vascular diseases, hypertension, diabetes mellitus, metabolic syndrome X, psoriasis, eczema, atopic dermatitis, coronary heart disease (CHD), atherosclerosis, and cancer [3–8]. This is in addition to the role of PGs and LTs in these conditions. For instance, in inflammatory bowel disease, the inflammatory events seem to be initiated and perpetuated by PGs and LTs (such as PGE₂, PGF_{2 α '}, TXA₂ and LTB₄, LTC₄, and LTD₄) produced from AA, whereas when significant amounts of EPA and DHA are given, the inflammatory process is abrogated to a large extent. This beneficial action of EPA/DHA when supplemented from external sources has been attributed to the displacement of AA from the cell membrane phospholipid pool and to the formation of less pro-inflammatory PGs (such as PGE₃, PGF_{3 α '}, TXA₃), and LTs (such as LTB₅, LTC₅, and LTD₅) from them, and hence the favorable response. If the molecular mechanism(s) by which various stimuli are able to preferentially induce the release of AA, EPA and/or DHA and convert them to their respective products were known, then it may be possible to develop methods or strategies to treat various inflammatory conditions based on this knowledge. Armed with such knowledge, one may be able to preferentially divert the formation of anti- or less pro-inflammatory molecules from EPA/DHA, so that the disease process would be much less severe. In this context, it is interesting to note that AA, EPA and DHA could give rise to anti-inflammatory molecules such as lipoxins (LXs) and resolvins. Both LXs and resolvins suppress inflammation and help in the resolution of inflammatory events, including leukocyte infiltration and clearance of the cellular debris from the site of inflammation. This suggests that PUFAs form precursors to both pro- and anti-inflammatory molecules, and the balance between these mutually antagonistic compounds could determine the final outcome of the disease process. Yet another set of compounds, formed by the nitration of unsaturated fatty acids, called as nitrolipids, has been shown to have potent biological actions. For example, nitration of linoleate by NO-derived reactive species forms novel derivatives including nitrolinoleate, which can stimulate smooth muscle relaxation, block platelet activation, and inhibit human neutrophil function, including inhibition of superoxide generation, degranulation, and integrin expression, and, thus, suppress inflammation. These studies suggest that PUFAs have important physiological and pathological actions not only by themselves, but also by giving rise to a variety of biologically active compounds.

3 Dietary sources of EFAs

The main dietary sources of EFAs are, for LA, cereals, eggs, poultry, most vegetable oils, whole-grain breads,

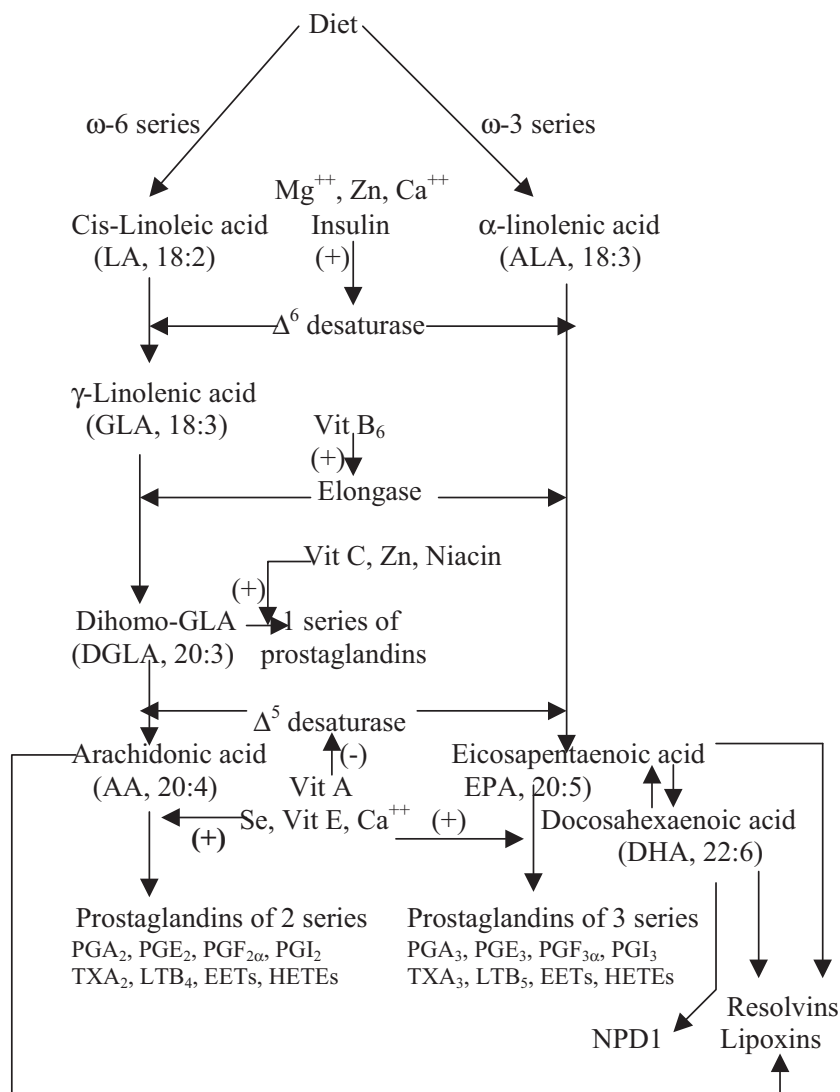


Figure 1. Scheme showing the metabolism of EFAs and co-factors that enhance the activity of Δ^6 and Δ^5 desaturases and elongases and formation of PGs. (+) Enhancement of the activity of the enzyme or increase in the formation of the product. (–) Either inhibition of the activity of the enzyme or decrease in the formation of the product.

baked goods, and margarine; sunflower, saffola, and corn oils are also rich in LA [8, 9]; and for ALA, canola oil, flaxseed oil, linseed and rapeseed oils, walnuts, and leafy green vegetables such as purslane. Human milk is particularly rich in EFAs and GLA, DGLA, AA, EPA, and DHA. Olive oil is rich in OA, whereas palm and coconut oils contain virtually none. The average daily intake of EFAs varies from country to country and again from region to region. However, in general, the intake is around 7–15 g/day in Europe and USA [8, 9].

Human milk contains 0.3–1.0% of its fat as GLA. Thus, breast fed babies get significant amounts of GLA [9, 10]. Fresh cow's milk contains small amounts of GLA (0.25% of the total fats). Evening primrose oil (EPO), borage oil, black currant oil, and hemp seed oil contain substantial amounts of GLA. GLA is present in EPO at concentrations of 7–14% of total fatty acids; in borage seed oil it is 20–27%; and in black currant seed oil at 15–20%. GLA is

also found in some fungal sources [9, 11]. Moderate amounts of DGLA are found in human milk [8–10], liver, testes, adrenals, and kidneys [9–12]. Small amounts are present in cow's milk.

AA is found in modest amounts in human milk and in small amounts in cow's milk. Meat, egg yolks, some seaweeds, and some shrimps contain substantial amounts of AA [13, 14]. The average daily intake of AA is estimated to be in the region of 100–200 mg/day [15, 16], more than enough to account for the total daily production of various PGs, which is estimated to be about 1 mg/day.

The main sources of adrenic acid (22:4 ω -6) are adrenals, kidneys, testes, and brain. The major source of EPA and DHA in the diet is from marine fish. Fresh water fish are unlikely to contain substantial amounts of EPA and DHA.

It is important to note that because of their instability, substantial loss of EFAs/PUFAs occurs during food pro-

cessing and hydrogenation. Some of these fatty acids may be denatured and converted into trans fats that are harmful to the body [17, 18]. It has been argued that the fall in the intake of biologically active ω -3 fatty acids, especially that of EPA and DHA, has been one of the major changes in Western nutrition in the last 50 years, and has contributed to the increasing incidence of atherosclerosis, CHD, hypertension, metabolic syndrome X, obesity, collagen vascular diseases and, possibly, cancer.

4 Factors that influence the metabolism of EFAs

Dietary LA and ALA are metabolized by the enzymes Δ^6 and Δ^5 desaturases to their respective metabolites as depicted in the Fig. 1. OA (18:1 ω -9), belonging to the ω -9 series, also forms precursor to its own series of fatty acids. However, OA is not an EFA since the body can synthesize it from simple precursors. LA, ALA, and OA are metabolized by the same set of Δ^6 and Δ^5 desaturases and elongases. The purpose of desaturases is to remove 2 hydrogens, whereas elongases is to add 2 carbons. As a result, these 3 series compete with one another for the same set of enzymes. It appears, however, that the enzymes prefer ω -3 to ω -6, and ω -6 over ω -9. Thus, in a given situation, the enzymes metabolize the fatty acids with the following sequence of preference: ω -3 > ω -6 > ω -9. Hence, under normal physiological conditions the metabolites of ω -9 are formed only in trivial amounts in the cells. This has an important biological significance since presence of significant amounts of 20:3 ω -9 suggests that there is deficiency of ω -3 and ω -6, and is used as an indicator of EFA deficiency. There are reports suggesting that this occurs in many, if not all, tumor cells. It should also be noted here that the activities of Δ^6 and Δ^5 desaturases are slow in humans ($\Delta^5 > \Delta^6$). Thus, the conversion of LA and ALA to their respective metabolites such as GLA and EPA may be inadequate under certain conditions. To bypass the block in the activities of Δ^6 and Δ^5 desaturases in some diseases and in ageing subjects, it may be necessary to directly supplement the metabolites such as GLA and DGLA (to bypass Δ^6 desaturase) and AA, EPA and DHA (to bypass Δ^6 and Δ^5 desaturases). Supplementation of AA is probably not necessary in the majority of the instances since it can be obtained from the diet. EPA and DHA can be obtained from marine fish. It is important to note that many of the PUFAs are lost during food processing and cooking, and hence direct oral supplementation of EPA and DHA may become necessary in some instances. At present, the Western diet is rich in ω -6 fatty acids compared to ω -3 fatty acids (ω -6 to ω -3 ratio is 10:1), whereas the recommended ratio is ~1:1 [8, 9].

A number of factors are known to influence the activities of desaturases and elongases involved in the metabolism of EFAs [1, 2, 11, 19, 20]. Saturated fats, cholesterol, trans-fatty acids formed by vegetable oil processing, alco-

hol, adrenaline, and glucocorticoids inhibit Δ^6 and Δ^5 desaturases. Pyridoxine, zinc, and magnesium are necessary co-factors for normal Δ^6 desaturase activity. Insulin activates Δ^6 desaturase, whereas diabetics have reduced Δ^6 desaturase activity. The activity of Δ^6 desaturase falls with age. Oncogenic viruses and radiation inhibit Δ^6 desaturase activity. Total fasting and protein deficiency reduce the activity of Δ^6 desaturase. A fat-free diet and partial caloric restriction enhances Δ^6 desaturase activity. A glucose-rich diet inhibits Δ^6 desaturase activity.

Peroxisome proliferator-activated receptor- α (PPAR- α) activator WY 14,643 significantly enhanced the transcription of hepatic Δ^6 desaturase by more than 500% [21]. When mice were fed diets containing either a 1.5% fatty acid preparation rich in conjugated linoleic acid (CLA) or a preparation rich in LA; CLA increased the mRNA expression of Δ^6 and Δ^5 desaturases, and sterol regulatory element binding protein-1 (SREBP-1). The mitochondrial and peroxisomal palmitoyl-CoA oxidation rate was about 2.5-fold higher in mice fed CLA than in those fed LA, which was associated with the up-regulation of the activity and mRNA expression of various fatty acid oxidation enzymes. On the other hand, a diet rich in palmitic acid compared to a diet rich in LA was ineffective in modulating the hepatic lipid levels or activity and mRNA levels of enzymes in fatty acid metabolism. This suggests that dietary CLA concomitantly increases the activity and mRNA levels of enzymes involved in fatty acid synthesis and oxidation, and desaturation of PUFAs in the mouse liver, which appears to be mediated by both the activation of PPAR- α and up-regulation of SREBP-1 [22]. It was reported that the hepatic expression of Δ^5 desaturase as well as Δ^6 desaturase was highly activated in transgenic mice overexpressing nuclear SREBP-1a, -1c, and -2. Disruption of the SREBP-1 gene significantly reduced the expression of both desaturases in the livers of SREBP-1-deficient mice refed after fasting. The hepatic expression of both desaturases was down-regulated by dietary PUFAs, which suppressed SREBP-1c gene expression. In contrast, sustained expression of hepatic nuclear SREBP-1c protein in the transgenic mice abolished the PUFA suppression of both desaturases. Fasting induced both the desaturases. Fibrate, a pharmacological ligand for PPAR- α , produced a significant increase in the expression of both desaturases. These data suggest that both Δ^6 and Δ^5 desaturases are regulated by SREBP-1c and PPAR- α , two reciprocal transcription factors for fatty acid metabolism, and that some of their lipogenic actions are brought about by their ability to regulate the producing PUFAs [23].

Activities of Δ^6 and Δ^5 desaturases are decreased in diabetes mellitus, hypertension, hyperlipidemia, and metabolic syndrome X. It is known that trans fats interfere with the metabolism of EFAs and promote inflammation, atherosclerosis and CHD [8, 19, 24]. The pro-inflammatory action of trans fats can be attributed to their ability to interfere with the metabolism of EFAs. Several PUFAs, es-

pecially EPA and DHA, are known to inhibit the production of pro-inflammatory cytokines: interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), IL-1, and IL-2 [4–6]. Saturated fatty acids and cholesterol also interfere with the metabolism of EFAs and, thus, promote the production of pro-inflammatory cytokines, which explains their ability to cause atherosclerosis and CHD. This suggests that trans fats, saturated fats, and cholesterol have pro-inflammatory actions, whereas PUFAs, such as GLA, DGLA, EPA and DHA, possess anti-inflammatory properties. By interfering with the metabolism of EFAs, saturated fats, cholesterol and trans fats could reduce the formation of their long-chain metabolites GLA, DGLA, AA, EPA, and DHA that are essential for the formation of biologically active and beneficial prostacyclin (PGI₂), PGI₃, LXs, and resolvins. Deficiency and/or absence of PGI₂, PGI₃, LXs and resolvins will lead to the initiation and progression of atherosclerosis, CHD, persistence of inflammation, and failure of the healing process.

5 Actions of EFAs and their metabolites

5.1 Cell membrane fluidity

Cell membrane fluidity is determined by its lipid composition. Increased incorporation of saturated fatty acids and cholesterol into the cell membrane phospholipids will render the membrane more rigid. In contrast, increased incorporation of unsaturated fatty acids into the phospholipids of the membrane will make it more fluid. Studies suggested that the number of receptors and their affinity to their respective hormones, growth factors or proteins depends on the fluidity of the cell membrane. For instance, increase in the rigidity of the cell membrane reduces the number of insulin receptors and their affinity to insulin. This, in turn, causes insulin resistance. On the other hand, increase in cell membrane fluidity due to increase in the unsaturated fatty acid content in the membrane phospholipids, increases the number of insulin receptors on the membrane and their affinity to insulin and thus, decrease in insulin resistance [25–27]. This has important therapeutic implications in diabetes mellitus.

The growth of brain during the perinatal period and adolescence depends on the availability of ω -3 and ω -6 fatty acids and various growth factors [28–30]. It is likely that decrease in the availability of ω -3 and ω -6 fatty acids during this critical period of growth may impair brain growth and the development of appropriate synaptic connections. This, in turn, may lead to the development several developmental disorders of the brain and neuropsychological conditions such as dementia, depression, schizophrenia, Alzheimer's disease, and neurodegenerative diseases: Huntington's disease, Parkinson's disease, spinocerebellar degeneration, etc.

5.2 Second messenger action

EFAs and their long-chain metabolites, and eicosanoids have second messenger like actions. Several hormones and growth factors activate phospholipase A₂ (PLA₂), which, in turn, induces the release of DGLA, AA, EPA, and DHA from the cell membrane lipid pool. These fatty acids are utilized for the formation of various eicosanoids and bring about their actions. It was noted that inhibition of PLA₂ interferes with the action of various growth factors and cytokines, and proteins. For instance, various actions of TNF- α are dependent on its ability to induce the activity of PLA₂, e.g., its tumoricidal action, and inhibitors of PLA₂ completely inhibited this action of TNF- α . Our studies showed that TNF- α -resistant tumor cells can be rendered sensitive to TNF- α by the addition of various PUFAs, especially GLA. Thus, PUFAs seem to be essential for some, if not all, actions of various growth factors and cytokines. PUFAs enhance the activity of protein kinase C (PKC), a well-known second messenger. In addition, PUFAs can activate macrophages and polymorphonuclear leukocytes (PMNs) and increase free radical generation by these cells. These important actions of PUFAs suggest that fatty acids have important second messenger actions [1, 2, 8].

5.3 Antibiotic-like actions

PUFAs show anti-biotic-like actions [31–33]. For instance, linolenic acid rapidly killed cultures of *Staphylococcus aureus*, and hydrolyzed linseed oil (which contains both LA and ALA) can inactivate methicillin-resistant *S. aureus*. ALA promotes adhesion of *Lactobacillus casei* to mucosal surfaces and, thus, augments their growth. *Lactobacilli*, in turn, suppress the growth of pathogenic bacteria like *Helicobacter pylori*, *Shigella flexneri*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Clostridium difficile*, and *Escherichia coli*. PUFAs inactivate enveloped viruses and show anti-fungal properties. The anti-inflammatory and anti-bacterial, anti-viral, and anti-fungal actions of PUFAs may explain some of their beneficial actions. In this context, it will be interesting to study whether local application or intravenous infusions of PUFAs would help patients with various bacterial, viral and fungal infections to recover faster. Since neutrophils, T cells and macrophages release PUFAs on stimulation; it is possible that this could be one of the defense mechanisms of the body to fight infections.

6 PUFAs inhibit angiotensin-converting enzyme activity and enhance endothelial nitric oxide generation

Previously, it was shown that PUFAs inhibited leukocyte angiotensin-converting enzyme (ACE) activity [31]. This

suggests that PUFAs could function as endogenous regulators of ACE activity, and thus regulate the formation of Ang-II. PUFAs enhance NO generation [34–36]. Hence, when cell/tissue concentrations of PUFAs are low, the activity of ACE will be high, leading to the formation of increased amounts of Ang-II, and the formation of endothelial NO (eNO) will be low. Plasma concentrations of PUFA and eNO are low in hypertension, diabetes mellitus, renal diseases, rheumatoid arthritis (RA), lupus, psoriasis, eczema, atopic and non-atopic dermatitis, atherosclerosis, insulin resistance, obesity; dementia, schizophrenia, bipolar disorders, Huntington's disease, Alzheimer's disease, peptic ulcer disease, and cancer [37–39]. Furthermore, a 25-nucleotide ACE deletion polymorphism increases ACE activity, and such individuals showed a higher risk of developing stroke, obesity, emphysema, bipolar affective disorders, and cancers [40, 41]. This suggests that an altered ACE activity and EFA metabolism plays a significant role in many diseases.

It has been demonstrated that transgenic rats overexpressing both human renin and angiotensinogen genes (dTGR) develop hypertension, inflammation, and renal failure, and showed renal P450-dependent AA metabolism changes that led to decreased formation epoxyeicosatrienoic acids (5,6-, 8,9-, 11,12- and 14,15-EETs) and hydroxyeicosa-tetraenoic acids (19- and 20-HETEs), which, in turn, inhibited IL-6 and TNF- α -induced activation of NF- κ B and prevented vascular inflammation [42]. These results indicate that AA and other PUFAs not only regulate ACE activity and Ang-II levels in the tissues, but also possess anti-inflammatory properties.

The interaction between various PUFAs and NO is particularly interesting in view of the pleiotropic actions of NO. PUFAs, especially EPA and AA, stimulate eNO synthesis [6, 8, 36]. NO has potent anti-atherosclerotic and anti-inflammatory actions that may explain some of the beneficial actions of PUFAs. Studies showed that the ability of aspirin to enhance the formation of eNO through the generation of epi-LXs could be linked to the anti-inflammatory action [43]. NO prevents the interaction between leukocytes and the vascular endothelium during acute inflammation by acetylation of the active site of the inducible cyclo-oxygenase (COX-2) to generate epi-LXs that have potent anti-inflammatory actions. NO stimulates the formation of PGI₂ from AA [44], and LXs are derived from AA, EPA, and DHA. Furthermore, aspirin inhibits the formation of TXA₂, a potent platelet aggregator and vasoconstrictor, and enhances PGI₂ formation, a platelet anti-aggregator and vasodilator, and thus brings about its anti-atherosclerotic actions. Thus, there is a close interaction between PUFAs, NO synthase, and COX enzymes [45].

7 PUFAs and cytokines

PUFAs (especially ALA, DGLA, EPA, and DHA), LXs and resolvins suppress IL-1, IL-2, IL-6, and TNF- α production by T cells [46–51], and thus function as endogenous anti-inflammatory molecules. Although, no studies have reported direct effect(s) of AA on the production of various cytokines, it is generally believed that PGE₂, PGF_{2 α} , TXA₂ and LTs derived from AA have modulatory role on IL-6 and TNF- α production. For instance, mast cell IL-6 production was induced by PGE₁ (derived from DGLA) and PGE₂ (derived from AA) to a similar level to that observed in anti-IgE-activated cells. In contrast, constitutive production of TNF- α was inhibited by PGE₁ and PGE₂, but not by PGD₂ [52]. It was demonstrated that PGF_{2 α} stimulates IL-6 synthesis via activation of protein kinase C in osteoblast-like MC3T3-E1 cells, and that PGE₁ induces the synthesis of IL-6 through protein kinase A activation [53]. In view of the different effects of PGE₂, PGF_{2 α} and PGE₁ on the synthesis of IL-6 and TNF- α , the local levels of IL-6 and TNF- α at the sites of inflammation and injury may depend on the balance between DGLA and AA and the respective PG products formed from them. We observed that PGE₁ and PGF_{1 α} (derived from DGLA) and TXB₂ (derived from AA) inhibit, whereas DGLA and AA *per se* do not have much influence on the growth of human lymphocytes *in vitro* at the doses tested. In contrast, PGE₁, PGE₂, PGF_{2 α} , and TXB₂ suppressed IL-2 production, and PGE₂, PGF_{2 α} , and TXB₂ enhanced IL-4 synthesis, whereas PGE₂, PGF_{2 α} , TXB₂, PGI₂, and PGF_{1 α} increased TNF- α synthesis with no action on IL-6 synthesis in human lymphocytes *in vitro* [47]. On the other hand, DGLA and AA enhanced, whereas EPA decreased, the synthesis of IL-4 in human lymphocytes *in vitro* with no action on IL-6 production, and are modulated by the doses of fatty acids used [47, 54]. DHA has been shown to suppress IL-1 β and TNF- α production by stimulated human retinal vascular endothelial cells [55]. There is evidence to suggest that some of the suppressive actions of EPA and DHA on the production of pro-inflammatory cytokines and their anti-inflammatory action seems to be mediated by their ability to increase both PPAR- γ mRNA and protein activity [56]. These results suggest that various PUFAs and their products have different, and at times diametrically opposite, actions on the synthesis of various cytokines. Hence, the local concentrations of different PUFAs and eicosanoids formed and the balance between these various modulators will ultimately determine the types and concentrations of cytokines formed and the degree of inflammation.

IL-1, IL-6, and TNF- α have been shown to induce insulin resistance, have cytotoxic actions and also possess neurotoxic actions. Wasting seen in patients with tuberculosis, cancer, and AIDS is due to excess production of TNF- α and other pro-inflammatory cytokines [57]. EPA and other PUFAs are known to ameliorate cachexia induced by TNF- α in animal tumor models [58, 59]. Several

retroviral agents induce lipodystrophy and insulin resistance, and this has been associated with increased levels of TNF- α and decreased concentrations of adiponectin [60, 61]. PUFAs are known to prevent/reverse insulin resistance [62–64] by decreasing TNF- α levels and, possibly, by enhancing adiponectin levels. Thus, PUFAs are likely to be useful in preventing and/or reversing some of the side effects of retroviral drugs.

8 PUFAs and 3-hydroxy-3-methylglutaryl coenzyme A reductase activity

Two SREBPs are known to control the transcription of the genes for the low-density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase. SREBP-1 and SREBP-2, each approximately 1150 amino acids in length, have a similar domain structure and operate through a similar mechanism. In the absence of sterols, soluble fragments of approximately 470 amino acids are released from both SREBP-1 and SREBP-2 by proteolytic cleavage. These soluble fragments enter the nucleus, bind to sterol regulatory elements in the promoters of genes encoding the LDL receptor and HMG-CoA synthase, thereby activating their transcription. The proteolytic processing of both SREBPs is blocked by sterol overloading, and enhanced when sterols are depleted by statins, the HMG-CoA synthesis inhibitors. Depletion of sterols by statins leads to a marked increase in the nuclear form of SREBP-2 and reciprocal decline in the nuclear form of SREBP-1, suggesting that SREBP-1 is responsible for basal transcription of the LDL receptor and HMG-CoA synthase genes in the liver, and that SREBP-2 is responsible for the increased transcription that follows sterol depletion when a statin is used [65]. Cholesterol depletion that occurs due to the use of statins leads to proteolytic activation of transcription factors of the SREBPs and also induces PPAR- γ expression. The effects of the SREBPs on PPAR- γ expression is mediated through the PPAR- γ 1 and -3 promoters, and both these promoters contain a consensus E-box motif that mediates the regulation of the PPAR- γ gene by adipocytes differentiation and determination factor 1 (ADD-1)/SREBP-1 and SREBP-2 [66]. This suggests that PPAR- γ expression is controlled by SREBPs. In addition, the 5'-flanking region of the human CASP-2 (caspase) gene contains three functional response elements for SREBPs. Exposure of human cell lines to statins induced the CASP-2 gene to an extent similar to that for known targets of SREBP proteins, adenoviral vector-mediated transfer of active SREBP-2 induced expression of the CASP-2 gene, and the caspase-2 protein, and increased the cholesterol and triacylglycerol cellular content, whereas rises in lipids were blocked following small interfering RNA-mediated silencing of the CASP-2 gene. These results suggest that human CASP-2 gene is a member of the SREBP-responsive gene battery that senses

lipid levels in cells, and raise the possibility that caspase-2 participates in the control of cholesterol and triacylglycerol levels [67]. These results are noteworthy since caspases induce apoptotic cell death, and participate in cytokine maturation, inflammation, or differentiation.

Similar to statins, EFAs and their metabolites (especially AA, EPA, and DHA) are useful in the treatment of hyperlipidemias, have anti-proliferative action on tumor cells both *in vitro* and *in vivo*, bind to DNA and regulate the expression of genes and oncogenes. More importantly, PUFAs are also potent inhibitors of the HMG-CoA reductase enzyme [68, 69]. Statins have the ability to enhance plasma AA concentrations and decrease the ratio of EPA to AA significantly [70].

SREBPs are not only membrane-bound transcription factors but also increase the synthesis of fatty acids as well as cholesterol in animal cells. All three SREBP isoforms (SREBP-1a, -1c, and -2) are subject to feedback regulation by cholesterol, which blocks their proteolytic release from membranes. SREBPs are also negatively regulated by unsaturated fatty acids by decreasing the nuclear content of SREBP-1, but not SREBP-2 in cultured human embryonic kidney (HEK)-293 cells. The potency of unsaturated fatty acids increased with increasing chain length and degree of unsaturation (docosatetraenoic acid, 22:4 ω -6, > AA > ALA > LA > palmitoleic acid, 16:1), whereas saturated fatty acids were ineffective. None of the fatty acids had a significant effect on SREBP-2 except AA, which produced a slight reduction. Unsaturated fatty acids markedly reduced the mRNAs encoding SREBP-1a and SREBP-1c, and the proteolytic processing of these SREBPs was inhibited. It is interesting to note that sterols and unsaturated fatty acids, when given together, potentiated each other in reducing nuclear SREBP-1. In the absence of fatty acids, sterols did not cause a sustained reduction of nuclear SREBP-1, but they did reduce nuclear SREBP-2. These results suggest that unsaturated fatty acids have their greatest inhibitory effects on SREBP-1a and SREBP-1c, whereas sterols have their greatest inhibitory effects on SREBP-2 [71]. Others have reported similar results [72–75]. In addition, in CaCo-2 cells, PUFAs decreased gene and protein expression of SREBP-1 and FAS mRNA by interfering with LXR activity [72], and in rats PUFAs enhanced cholesterol losses via bile acid synthesis [73]. In the intestine, dietary PUFAs suppress SREBP-1c mRNA without altering expression of its target genes, fatty acid synthase, acetyl-CoA carboxylase, or ATP citrate lyase, and decreased intestinal fatty acid synthesis by a post-transcriptional mechanism independent of the SREBP pathway [74]. Feeding mice on a tuna fish oil diet for 2 weeks decreased serum cholesterol and triacylglycerol levels by 50% and 60%, respectively; hepatic farnesyl diphosphate (FPP) synthase (a SREBP target enzyme that is subject to negative-feedback regulation by sterols in co-ordination with HMG-CoA reductase) and HMG-CoA reductase mRNAs were decreased by 70% and

40%, respectively. AA, EPA, and DHA were the most effective fatty acids in reducing FPP synthase and HMG-CoA reductase mRNA and protein levels in rat hepatoma cells compared to stearate and oleate. The suppressive effect of PUFAs on the FPP synthase was due to transcription inhibition [75], suggesting that PUFAs down-regulate hepatic cholesterol synthesis by impairing the SREBP pathway. It was reported that PUFAs reduce SREBP-mediated gene transcription by increasing intracellular cholesterol content through the hydrolysis of cellular sphingomyelin, which has a high affinity for free cholesterol, and that the lipid second messenger ceramide, a product of sphingomyelin hydrolysis, decreased SRE-mediated gene transcription of SREBP-1 and SREBP-2. These results provide evidence that sphingomyelin hydrolysis and intermediates of sphingomyelin metabolism (in addition to cholesterol and PUFAs) also regulate SRE-mediated gene transcription [76]. Thus, there is a close interaction between PUFAs, HMG-CoA reductase, SREBPs, FPP synthase, sphingolipid metabolism, and caspases that may account for the ability of both statins and PUFAs to lower cholesterol levels.

It is noteworthy that HMG-CoA reductase catalyzes the synthesis of mevalonate, which is the rate-limiting step in the mevalonate pathway. Mevalonate is the precursor of cholesterol and a variety of isoprenoid containing compounds. These isoprenoid precursors are necessary for the post-translational lipid modification (prenylation) and, hence, the function of *Ras* and other small GTPases. Therefore, inhibition of the mevalonate pathway has the potential to disrupt the function of oncogenic forms of *Ras*. This explains the ability of both statins and PUFAs to suppress *Ras* activity and anti-proliferative action, and to induce apoptosis of tumor cells. In addition, small GTPases, which are prenylated products of the mevalonate pathway, have negative control on the expression of bone morphogenetic proteins (BMPs). In view of this, inhibition of the mevalonate pathway by PUFAs will prevent the function of small GTPases and enhance the expression of several BMPs. Various BMPs are known to be essential for neuronal growth, proliferation, and differentiation. Thus, PUFAs have the ability to modulate brain growth and development, and neuronal differentiation. This action is in addition to the ability of PUFAs to form an important constituent of neuronal cell membranes and involvement in memory formation and consolidation [28, 29]. This explains why PUFAs are useful in the prevention and treatment of dementia and Alzheimer's disease [77–83]. The beneficial actions of PUFAs in Alzheimer's disease, schizophrenia and dementia have been attributed to the formation of anti-inflammatory compounds such as LXs and resolvins. Hence, a brief review of their formation is given here.

9 LXs, resolvins, and neuroprotectin D1

As already mentioned above, PUFAs not only form precursors for eicosanoids such as TXs, LTs, PGs, and PGI₂ but also gives rise to LXs and resolvins. Aspirin can convert AA, EPA and DHA to what is called as aspirin-triggered 15 epimer LXs (ATLs), which are potent inhibitors of acute inflammation [49, 84, 85]. Acetylation of COX-2 by aspirin prevents the formation of prostanoids, but the acetylated enzyme remains active *in situ* to generate 15R-hydroxyeicosatetraenoic acid (15R-HETE) from AA, which is released and converted by activated inflammatory cells such as PMNs to the 15-epimeric LXs. These LXs have potent anti-inflammatory properties [84, 85]. This interaction between endothelial cells and PMNs, leading to the formation of 15R-HETE and its subsequent conversion to 15-epimeric LXs by aspirin-acetylated COX-2, is a protective mechanism to prevent local inflammation on the vessel wall by regulating the motility of PMNs, eosinophils, and monocytes [85–89]. Endothelial cells oxidize AA (and possibly EPA and DHA) via the P450 enzyme system to form 11,12-epoxy-eicosatetraenoic acid(s), which blocks endothelial cell activation [84, 89]. These studies imply that COX-2 enzyme is essential for the formation of the beneficial LXs. In situation in which there is a deficiency or absence of LXs, interaction between PMN-endothelial cells occurs, leading to endothelial damage that may result in the development and progression of atherosclerosis, thrombus formation and coronary artery disease, and persistence of inflammation.

Compounds similar to 15R-HETE and 15-epimeric LXs are also formed from EPA and DHA. Human endothelial cells in the presence of IL-1 β (which induces COX-2) and aspirin converted EPA to 18R-HEPE, 18-HEPE, and 15R-HEPE. Activated human PMNs, in turn, converted 18R-HEPE to 5,12,18R-triHEPE and 15R-HEPE to 15-epi-LXA₅ by 5-lipoxygenase. Both 18R-HEPE and 5,12,18R-triHEPE inhibited LTB₄-stimulated PMN transendothelial migration, similar to 15-epiLXA₄. 5,12,18R-triHEPE competed with LTB₄ for its receptors and inhibited PMN infiltration. These results suggest that 5,12,18R-triHEPE suppress LT-mediated responses if present in adequate amounts at the sites of inflammation [90].

Murine brain cells expressing COX-2, when treated with aspirin, enzymatically transformed DHA to a 17R series of hydroxy DHAs (HDHAs) that, in turn, is enzymatically converted by PMNs to di- and tri-hydroxy containing docosanoids [91]. Small molecular weight compounds similar to HDHAs are generated from AA and EPA. Thus, 15R-hydroxy containing compounds are formed from AA, the 18R series from EPA, and the 17R-hydroxy series from DHA. All these compounds have potent anti-inflammatory actions and are involved in resolution of the inflammatory process, and hence have been termed as "resolvins" (see Fig. 1). Resolvins inhibited cytokine generation, leukocyte recruitment, leukocyte diapedesis, and exudate

formation. AA, EPA, and DHA-derived resolvins from acetylated COX-2 are formed via transcellular biosynthesis (e.g., due to cell-cell communication between endothelial cells and PMNs), and are potent anti-inflammatory compounds. Resolvins inhibit brain ischemia-reperfusion injury [92]. Thus, LXs and resolvins formed from AA, EPA, and DHA have cardioprotective, neuroprotective, and other cytoprotective actions.

Of the several 17-hydroxy-containing bioactive mediators derived from DHA, which were termed docosatrienes and 17S series resolvins, 10,17S-dihydroxydocosatriene, also called neuroprotectin D1 (NPD1), was found to reduce the infiltration of PMNs and possess potent anti-inflammatory and neuroprotective properties [92, 93]. NPD1 inhibited oxidative stress-induced apoptosis of human retinal pigment epithelial cells [94]. Both LXs and NPD1 enhanced wound healing [95], and promoted brain cell survival via the induction of anti-apoptotic and neuroprotective gene-expression programs that suppress A β 42-induced neurotoxicity [77, 78].

It is likely that under physiological conditions, both COX-1 and COX-2 enzymes are utilized for the formation of beneficial eicosanoids such as PGE₁, PGI₂, and LXs, resolvins, and NPD1 in various tissues such that inflammation is prevented. Failure to produce adequate amounts of LXs, resolvins, and NPD1 or interference with their action, and a simultaneous increase in the production of pro-inflammatory PGs, TXs, and LTs, and cytokines, could lead to initiation and persistence of inflammation and tissue damage.

10 EFAs in various pathological conditions

10.1 Inflammation

It is evident from the preceding discussion that PUFAs and their products participate in the pathobiology of inflammation. The amount and type of PUFA(s) released in response to inflammatory stimuli depends on the cell membrane phospholipid fatty acid content. Since the EFAs LA and ALA have to be obtained direct from diet, this suggests that dietary content EFAs could be one factor that determines the degree of inflammation. Furthermore, direct intake of various PUFAs alters the cell membrane fatty acid composition, which, in turn, modulates cell/tissue response to infection, injury and inflammatory events. Increased dietary intake of GLA, DGLA, and EPA/DHA substantially decreases inflammatory response [50, 54–56]. For instance, GLA is rapidly elongated to form DGLA, which, in turn, is converted to PGE₁ and its (DGLA) 15-lipoxygenase product 15-hydroxy-8, 11, 13-eicosatrienoic acid (15-OH-20: 3, ω 6), which have anti-inflammatory actions [96–98]. DGLA and AA inhibited IL-2 production in a dose-dependent manner without significant effects on cell viability, whereas EPA inhibited IL-2

production by peripheral blood mononuclear cells of only some human donors. It was also reported that DGLA and AA inhibited IL-2 production directly without conversion into their cyclo-oxygenase pathway products [99]. GLA showed significant inhibition of IL-1 β and TNF- α secretion by peripheral blood monocytes both *in vitro* and *in vivo* [100]. When a combination of GLA and EPA (in the form of a mixture of evening primrose oil and fish oil) and fish oil alone was given to a rat model that received an intravenous injection of *Salmonella enteritidis* endotoxin (10 mg/kg) and bronchoalveolar lavage fluid was analyzed, lung phospholipid concentrations of AA were lower and the concentrations of EPA and DHA were higher with fish oil and fish and borage oil compared with corn oil control, whereas DGLA levels were higher in the fish and borage oil group. The levels of LTB₄, LTC₄/LTD₄, 6-keto-PGF_{1 α} (a metabolite of PGL₂), and TXB₂ were significantly lower compared to the corn oil group. Lung myeloperoxidase (MPO) activity, which was significantly increased in endotoxin-treated rats, was significantly lower with either fish oil or fish and borage oil groups. However, surprisingly, there was no change in the TNF- α levels between the endotoxin-treated rats and the fish oil and fish oil plus borage oil-treated groups. In both the fish oil and fish and borage oil groups a reduction in the pulmonary neutrophil accumulation was also found [101]. These results suggest that a combination of GLA and EPA/DHA is more effective in decreasing the concentrations of AA. Similar results were obtained when a short-term enteral feeding with diets enriched with either EPA alone or in combination with GLA (using fatty acids in the place of oils) was given to adult male Sprague-Dawley rats [102]. These results have since been confirmed in human studies. It was observed that addition of EPA to GLA-supplemented diets significantly increased serum levels of EPA, but did not increase AA levels; and DGLA levels in neutrophil glycerolipids increased significantly, and neutrophils isolated from these volunteers released similar quantities of AA, but synthesized significantly lower quantities of LTs compared with their neutrophils before supplementation. This study suggested that a combination of GLA and EPA supplementation could be utilized to reduce the synthesis of pro-inflammatory AA metabolites [103]. In addition, supplementation of oils rich in ALA and EPA and DHA also reduced the plasma concentrations of soluble vascular cell adhesion molecule-1 and soluble E-selectin [104].

In summary, these studies indicate that GLA, DGLA, EPA and DHA possess anti-inflammatory actions that can be attributed to decreased formation of pro-inflammatory eicosanoids and cytokines [105], and an increase in the production of beneficial eicosanoids such as PGE₁, PGI₂, PGL₃, HPETEs, eNO, LXs, resolvins and NPD1 and early resolution of inflammation.

It is likely that when the cell membrane lipid pool is rich in GLA/DGLA/EPA/DHA and contains appropriate

amounts of AA, a specific activation of sPLA₂ and cPLA₂ (soluble and cytosolic PLA₂, respectively) could occur in response to an inflammatory stimuli that leads to the formation of increased amounts of LXs, PGD₂ and 15deoxyΔ¹²⁻¹⁴PGJ₂, eNO, GSNO, PGE₁, PGI₂, PGI₃, and HPETE_s that dampen inflammatory process and enhance resolution of inflammation. Human embryonic kidney cells, in the presence of exogenous PUFAs on exposure to IL-1, preferentially released AA due to the activation of sPLA₂-IIA, type IV cPLA₂, and type VI iPLA₂ (calcium-independent PLA₂). The degree activation of these PLA₂s was as follows: sPLA₂-IIA > type IV cPLA₂ > type VI iPLA₂. This suggests that exogenous PUFAs preferentially activate type IIA sPLA₂-mediated AA release from IL-1-stimulated cells and the order of release was AA > LA > OA [106]. This is interesting since it is known that activation of cPLA₂ and sPLA₂ would lead to the formation of anti-inflammatory LXs, PGD₂ and 15deoxyΔ¹²⁻¹⁴PGJ₂ that help in the prevention and even resolution of inflammation [107]. On the other hand, AA serves as the precursor of several pro-inflammatory compounds, and hence in the presence of excess AA, inflammation is likely to be significant.

In addition to their action on pro-inflammatory cytokines and adhesion molecules, it has also been shown that DGLA, EPA, and DHA can suppress NF-κB signaling [108–111]. In contrast, AA activates NF-κB [112], explaining its pro-inflammatory actions. In this context, it is important to note that PUFAs could serve as endogenous ligands for PPARs, which could be yet another mechanism of action by which they are able to suppress inflammatory events [113, 114]. EPA/DHA present in fish oils protect against cardiovascular disease, anti-inflammatory actions, and decrease the number of monocytic cells adherent to endothelium overlying atherosclerotic lesions by inhibiting phagocyte-endothelium interactions. In this context, it is important to note that the increased binding of a monocytic cell line (U937) to cultured endothelium due to the up-regulation of adhesion molecules by exposure to LPS, IL-1α, TNF-α, or phorbol myristate acetate (PMA) was decreased by pre-exposure of endothelial cells to oxidized EPA/DHA, whereas unoxidized PUFAs were ineffective. Oxidized PUFAs prevented LPS- or PMA-induced activation of transcription factor NF-κB and consequent induction of adhesion molecules. The active principle of oxidized PUFAs was found to be hydroperoxy fatty acids [115]. This modulation of endothelial cell adhesion molecule expression by oxidized lipids represents a natural mechanism whereby inflammation-mediated oxidation of endothelial PUFAs retard unrestrained inflammatory responses. Further studies showed that the anti-inflammatory effects of EPA/DHA present in fish oil might result from the inhibitory effects of oxidized ω-3 fatty acids on NF-κB activation via a PPAR-α-dependent pathway [116, 117].

These results are interesting since, in general, it is believed that PUFAs, being highly unsaturated, are easily

susceptible to oxidation. Hence, it is thought that increased intake of these fatty acids may enhance lipid peroxidation, and that these oxidized products are harmful to tissues. However, studies revealed that this is not true. In a double-blind, placebo-controlled trial of parallel design, 59 nonsmoking, treated-hypertensive, type 2 diabetic subjects, were randomized to 4 g daily purified EPA, DHA, or olive oil for 6 weeks, while maintaining their usual diet. It was noted that relative to the olive oil control group, post-intervention urinary F2 isoprostanes (a reliable marker of *in vivo* lipid peroxidation and oxidant stress) were decreased 19% by EPA and 20% by DHA with no significant changes in C-reactive protein (CRP), IL-6, and TNF-α following EPA or DHA supplementation [118]. This study clearly showed that either EPA or DHA reduce *in vivo* oxidant stress. These results are supported by the observation that oral supplementation of EPA/DHA does not increase *in vivo* lipid peroxidation and oxidative stress in humans [119, 120].

10.2 Atherosclerosis

Atherosclerosis is considered as a disease of low-grade systemic inflammation [121, 122]. Since PUFAs and eicosanoids modulate inflammation, it is natural that they play a significant role in atherosclerosis. The process of atherosclerosis is closely linked to the health and integrity of endothelial cells. Healthy endothelial cells synthesize and release adequate amounts of not only GLA/DGLA/EPA/DHA but also NO, PGI₂, PGI₃ and PGE₁ to prevent aggregation of platelets on their surface, and decrease the expression of adhesion molecules and production of pro-inflammatory cytokines, such as IL-1, IL-2, IL-6, and TNF-α, so that atherosclerosis would not occur. Free radicals inactivate NO and PGI₂, and thus, may initiate atherosclerosis. Pro-inflammatory cytokines such as IL-1, IL-2, IL-6, and TNF-α induce oxidant stress by enhancing the production of free radicals by monocytes, macrophages, and leukocytes. Increased production of pro-inflammatory cytokines and free radicals occurs due to shear stress, hyperglycemia, clinical or sub-clinical infections, and low-grade systemic inflammation as seen in type 2 diabetes mellitus, hypertension, hyperlipidemia, and metabolic syndrome X. EPA/ DHA and high-density lipoprotein (HDL) inhibit free radical generation and, thus, prevent oxidant stress (reviewed in [123]). Further, EPA/DHA/GLA suppress IL-6 and TNF-α synthesis and secretion, and thus behave as endogenous anti-inflammatory molecules. HDL also inhibits free radical generation, oxidant stress, adhesion molecule expression, and pro-inflammatory cytokine production and enhances eNO generation. Thus, both PUFAs and HDL have similar anti-coagulant properties on the vessel wall and anti-atherosclerotic actions. There has also been suggested that EPA/DHA enhance HDL generation. This evidence suggests that, in general, endothelial cell deficiency of

GLA/DGLA/EPA/DHA might increase the production of pro-inflammatory cytokines and free radicals, which results in the development of insulin resistance, decrease in plasma and cellular HDL concentrations, and decrease the formation of eNO, PGE₁, PGI₂, PGI₃, LXs, resolvins, and NPD1, which may ultimately initiate and perpetuate atherosclerosis. Providing adequate amounts of various PUFAs, especially EPA and DHA, can restore normalcy [123].

In addition, under normal physiological conditions there seems to be a balance maintained between pro- and anti-inflammatory cytokines and eicosanoids, and pro- and anti-platelet aggregation-inducing molecules such that atherosclerosis is prevented. For instance, both TXA₂ and PGI₂ levels are increased in subjects with atherosclerosis. TXA₂ is a potent platelet aggregator and vasoconstrictor; whereas PGI₂ has opposite actions. It was observed that *apoE*^{-/-}*TP*^{-/-} (apoE-deficient and TXA receptor-deficient) mice showed a significant delay in the development of atherosclerosis, although they had levels of serum cholesterol and triglyceride similar to those of apoE-deficient mice. On the other hand, *apoE*^{-/-}*IP*^{-/-} (apoE-deficient and PGI receptor-deficient) mice exhibited significant acceleration of the process of atherosclerosis compared to mice deficient in apoE alone. Partial endothelial disruption, increased expression of ICAM-1 and decreased expression of platelet endothelial cell adhesion molecule 1 (PECAM-1) were noted in *apoE*^{-/-}*IP*^{-/-} mice compared with those of *apoE*^{-/-}*TP*^{-/-} mice. Platelet activation with thrombin showed higher sensitivity for surface P-selectin expression in platelets of *apoE*^{-/-}*IP*^{-/-} mice and lower in *apoE*^{-/-}*TP*^{-/-} mice than in those of *apoE*^{-/-} mice. There was a significantly higher number of leukocytes rolling on the vessel wall of *apoE*^{-/-}*IP*^{-/-} mice compared to those seen in either *apoE*^{-/-}*TP*^{-/-} or *apoE*^{-/-} mice [124]. These results suggest that even though both TXA₂ and PGI₂ are derived from the same precursor AA, PGI₂ prevents, whereas TXA₂ promotes, the initiation and progression of atherosclerosis by their ability to regulate platelet activation and leukocyte-endothelial cell interaction. In a similar fashion, studies revealed that both PGE₂ and PGI₂, formed from AA, play a role in the pathogenesis of RA. PGI receptor-deficient (*IP*^{-/-}) mice exhibited significant reduction in arthritic scores, and reduction in IL-1β and IL-6 levels in the arthritic paws when were subjected to collagen-induced arthritis (CIA). Addition of an IP agonist to cultured synovial fibroblasts significantly enhanced IL-6 production. In contrast, inhibition of PGE receptor subtype (EP2 or EP4) alone did not affect inflammatory events in CIA, whereas suppression of arthritis occurred when both EP2 and EP4 receptors were inhibited. These results suggest that both PGE₂ and PGI₂ participate in RA [125]. Based on these results, it is evident that the type of products formed from the precursor PUFAs, DGLA, AA, EPA, and DHA, is important in several physiological and pathological processes. Furthermore, interactions be-

tween various PUFAs and eicosanoids also play a significant role in determining the physiological and pathological events in various tissues.

In this context, the interaction between various PUFAs is of particular significance, especially with regard to their role in atherosclerosis. For instance, in perfused vascular tissue, DGLA increased the conversion of EPA to PGI₃, a vasodilator and platelet anti-aggregator [126]. Similarly, AA enhanced the conversion of EPA to PGI₃ in the tissues [127, 128], whereas orally administered EPA enhanced AA conversion to PGI₂ [129]. On the other hand, EPA inhibits the activity of Δ⁶ and Δ⁵ desaturases (Δ⁶ > Δ⁵), as a result of which the tissue levels of DGLA will be enhanced due to decreased conversion of DGLA to AA. This can lead to increase in the formation of PGE₁, a vasodilator and platelet anti-aggregator. Thus, there is a very close interaction between DGLA, AA, and EPA [130]. Based on these lines of evidence, it is evident that optimal levels of DGLA, AA, EPA, and possibly DHA need to be present for the formation of various beneficial PGs to prevent platelet aggregation and atherosclerosis (Fig. 2). It is possible that, in a similar fashion, the conversion of DHA and EPA to resolvins and LXs may also depend on the availability of appropriate amounts of other PUFAs.

10.3 Metabolic syndrome X

Metabolic syndrome X is characterized by abdominal obesity, atherosclerosis, insulin resistance and hyperinsulinemia, hyperlipidemias, essential hypertension, type 2 diabetes mellitus, and CHD. Plasma levels of CRP, TNF-α, and IL-6, markers of inflammation, are elevated in subjects with obesity, insulin resistance, essential hypertension, type 2 diabetes, and CHD. A direct positive correlation exists between body mass index and CRP in otherwise healthy children and adults. Higher plasma CRP concentrations are associated with increased risk of CHD, ischemic stroke, peripheral arterial disease, and ischemic heart disease mortality in healthy men and women. Similarly, a strong positive and negative correlation exists between elevated CRP levels and fibrinogen, and HDL cholesterol, respectively.

IL-6, a pro-inflammatory cytokine, stimulates the production of CRP in the liver, and is absolutely required for the induced expression of CRP. In overweight and obese subjects, serum levels of TNF-α were significantly higher compared to lean subjects. Weight reduction or regular exercise decreases serum concentrations of TNF-α. A negative correlation exists between plasma TNF-α and HDL cholesterol, glycosylated hemoglobin, and serum insulin concentrations. In view of this evidence, metabolic syndrome X can be considered as a disorder of low-grade systemic inflammation [131].

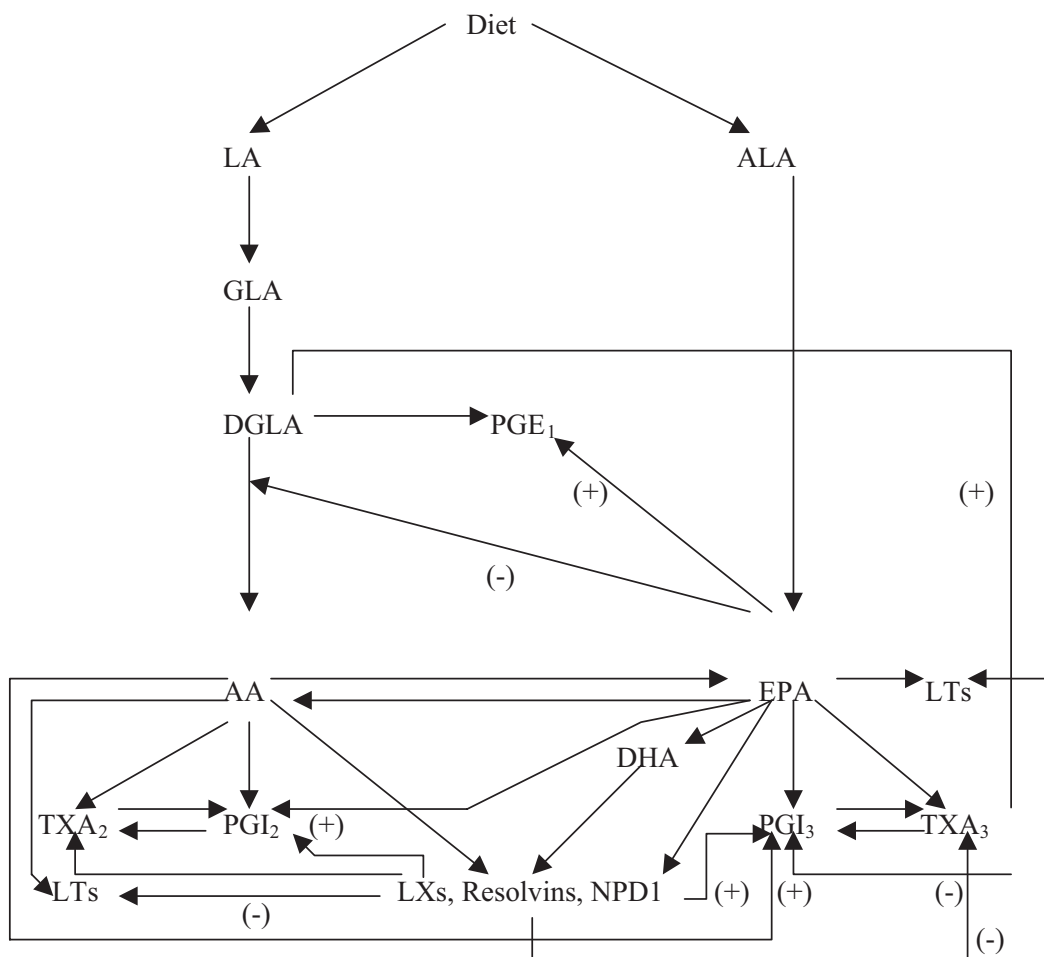


Figure 2. Scheme showing interaction(s) between ω -3 and ω -6 fatty acids and their effect on the formation of PGI₂, PGI₃, PGE₁, and LXs, resolvins and NPD1. (-) Inhibition or block in the synthesis, formation or release. (+) Enhancement in the formation or release. It is proposed that LXs, resolvins, and NPD1 enhance the formation and/or action of PGI₂ and PGI₃ and suppress that of TXA₂ and TXA₃, and thus may prevent atherosclerosis and show their cytoprotective actions. LXs, resolvins and NPD1 suppress the formation of LTs.

10.4 PUFAs and metabolic syndrome X

ω -3 and ω -6 fatty acids are essential for fetal growth and development including brain [8, 28, 29, 35, 132–135], although some studies disputed these findings [136, 137]. However, it is certain that growth and development on brain and cognitive and behavioral improvements occur in infants supplemented with AA and DHA [138, 139]. Newborn infants, especially pre-term infants, have limited capacity to form EPA, DHA and AA. EPA and DHA increase birth weight by prolonging gestation and/or by increasing the fetal growth rate, whereas AA status correlated with growth during the first year of life.

EPA, DHA, and AA, inhibit TNF- α and IL-6 production, enhance eNO generation, inhibit HMG-CoA reductase and ACE activities, function as endogenous ligands for PPARs, and suppress leptin gene expression [140, 141]. Thus, PUFAs suppress inflammation, regulate cholesterol

metabolism, enhance the production of adiponectin, and decrease insulin resistance. This may explain why PUFAs are useful to protect against CHD, prevent the progression of atherosclerosis, and decrease blood pressure. PUFAs bind to PPARs (similar to thiazolidinediones), and thus enhance adiponectin levels [142], which, in turn, improve insulin resistance. Several studies support these proposals: (i) hypertension and insulin resistance was ameliorated in experimental animals by feeding them with EPA- and DHA-rich oil; (ii) highly purified EPA reduced insulin resistance and decreased the incidence of type 2 diabetes in experimental animals; and (iii) decreased concentrations of EPA, DHA and AA in skeletal muscle phospholipids was found to be associated with decreased insulin sensitivity in humans [6, 26, 62–64, 143]. Plasma phospholipid concentrations of EPA, DHA and AA were low in subjects with hypertension, diabetes mellitus and CHD [38]. Metabolic syndrome X is uncommon in Greenland

Eskimos whose traditional diet is rich in EPA and DHA, whereas South Asian Indians, who are at high risk of developing metabolic syndrome X, have significantly lower concentrations of AA, EPA, and DHA compared to healthy Canadians and Americans [144]. It is evident from these observations that PUFAs and cytokines interact with each other and play an important role in the pathobiology of metabolic syndrome X.

10.5 Neurological conditions: schizophrenia, Huntington's disease, Alzheimer's disease

There is evidence to suggest that inflammation plays a significant role in the pathobiology of schizophrenia, Huntington's disease and Alzheimer's disease. In patients with schizophrenia, both circulating and cerebrospinal fluid (CSF) concentrations of pro-inflammatory cytokines are increased. The plasma phospholipid concentrations of EPA and DHA are decreased. Limited clinical trials showed that supplementation of EPA (especially ethyl EPA) are of significant benefit to these patients (reviewed in [39]).

Recent studies revealed that a diet high in DHA slowed the progression of Alzheimer's disease in mice. Specifically, DHA reduced the harmful brain plaques that mark the disease. Mice genetically altered to develop Alzheimer's disease, when fed with DHA-fortified chow, had 70% less buildup of amyloid protein in the brain compared with control or DHA-deficient mice [77–81]. DHA protected against damage to the "synaptic" areas and enabled mice to perform better on memory tests. These studies suggest that people who are genetically or otherwise predisposed to the disease may be able to delay it by increasing their DHA intake [79, 94].

Huntington's disease is an inherited neurodegenerative disorder due to a mutation in exon 1 of the *Huntingtin* gene that encodes a stretch of polyglutamine (polyQ) residues close to the N terminus of the Huntingtin protein. Aggregated polyQ residues are toxic to the neuronal cells. Transgenic R6/1 mice develop late-onset neurological deficits in a manner similar to the motor abnormalities of Huntington's disease seen in humans. These animals when supplemented with PUFAs, especially ethyl EPA, showed increased survival rates and decreased neurological deficits [39], suggesting that unsaturated fatty acids may prevent or arrest polyQ aggregation. Based on these results, it is tempting to suggest that PUFAs, in general, are useful in the treatment of various neurological diseases. It remains to be determined as to why and how a particular fatty acid is useful only in a particular neurological condition but not in other conditions. For instance, DHA is useful in Alzheimer's disease, whereas ethyl EPA is of benefit in Huntington's disease and schizophrenia. Understanding the molecular mechanisms of action of EPA/DHA in these conditions may throw more light on the pathobiology of these diseases.

11 Conclusions and therapeutic implications

It is evident from the preceding discussion that EFAs and their long-chain metabolites, eicosanoids, LXs, resolvins, and NPD1 have many biological actions and participate in several diseases processes (Fig. 3). In this context, it is interesting to note that NO can react with PUFAs to yield their respective nitroalkene derivatives that can be detected in plasma, and have been shown to induce vascular relaxation, inhibit neutrophil degranulation and superoxide formation, and inhibit platelet activation [145–147]. These nitroalkene derivatives of various PUFAs, which can be detected in substantial amounts both in the plasma and urine, have endogenous PPAR- γ ligand activity and decay in the blood to release NO. These reports suggest that PUFAs not only form precursors to various eicosanoids, resolvins, LXs, and NPD1, but also may react with various other molecules and form novel compounds that have biological activity.

Recent studies suggest that PUFAs may have a direct role in atherosclerosis by modulating the expression of uncoupling protein (UCP) -1 in the vascular tissue. Oxidative stress is implicated in atherosclerosis. Mitochondrial electron transport accounts for most of the reactive oxygen species (ROS) generated. UCPs (inner mitochondrial membrane anion transporters) allow protons to leak back into the mitochondrial matrix, thereby decreasing energy available for ROS generation. Superoxide anion activates UCPs [148], which limits further superoxide anion generation by dissipating the protonmotive force. This is supported by the observation that uncoupling decreases glucose-induced ROS formation, and thus prevents vascular damage in cultured endothelial cells [149]. Respiratory uncoupling is increased in the aortae of pigeons susceptible to atherosclerosis [150]. Smooth muscle cells are a major source of ROS in the vasculature [151]. In a recent study, it was reported that UCP-1 expression in aortic smooth muscle cells produced hypertension, increased high fat diet-induced atherosclerosis without affecting cholesterol levels, enhanced superoxide anion production, and decreased the availability of NO, suggesting that mild respiratory uncoupling due to mitochondrial dysfunction causes atherosclerosis [152]. In this context, it should be noted that EFA deficiency promotes respiratory uncoupling [153–155] and atherosclerosis [123, 156]. Atherosclerosis-free aortae have abundant amounts of EFA linoleate, whereas fatty streaks of early atherosclerosis are deficient in EFAs [157, 158]. Furthermore, pro-inflammatory eicosanoids generally stimulate the formation and the activity of adhesion molecules (integrins), and, thus, may promote atherosclerosis and inflammation, whereas GLA, DGLA, EPA, and DHA, and their anti-inflammatory products such as LXs, resolvins, and NPD1 are expected to suppress the expression of adhesion molecules and mitigate atherosclerosis and inflammation.

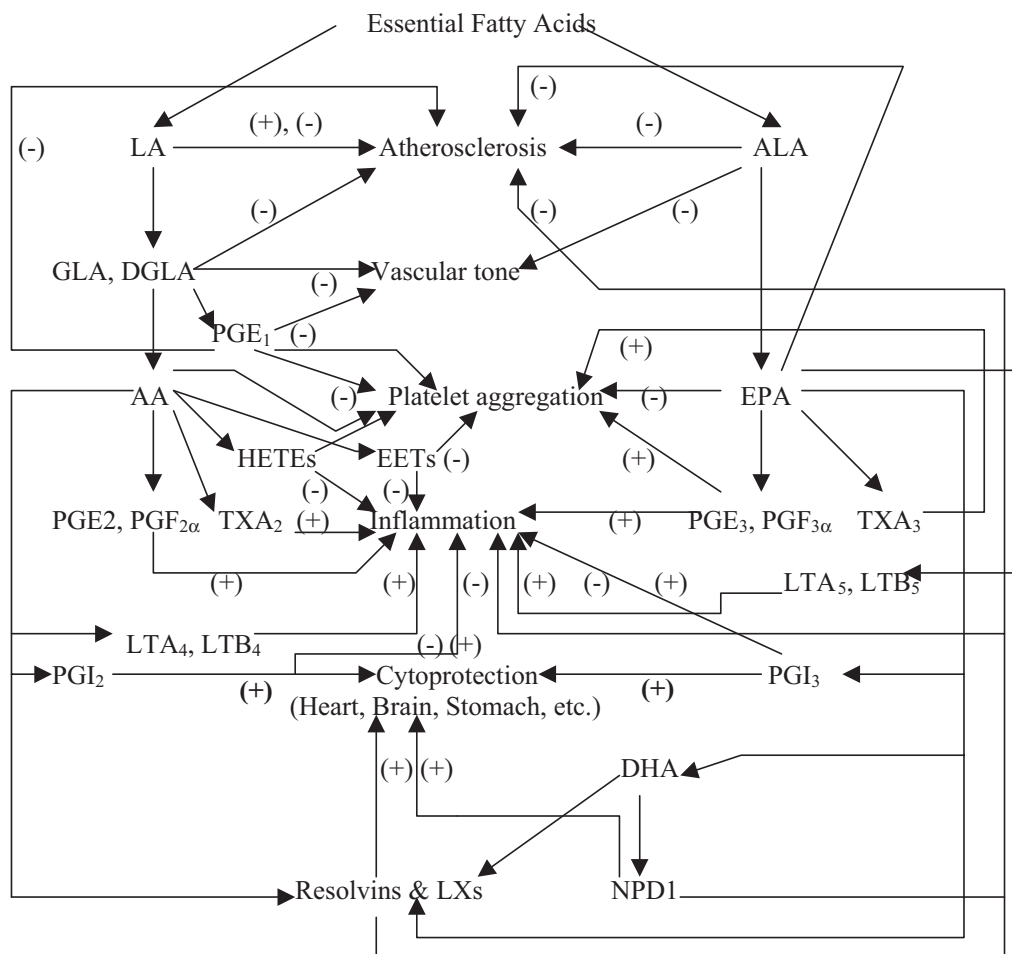


Figure 3. Scheme showing the actions of EFAs, their long-chain metabolites, and various eicosanoids formed from them. For more details see text. Note that the scheme does not depict an exhaustive list of the actions of the various molecules mentioned, with only the representative major actions depicted. (+) Positive control or enhancement of the process. (–) Negative control or inhibition or decrease of the process. Some eicosanoids may have both positive and negative actions on some processes.

LTB₄, a 5-lipoxygenase (5-LO) product derived from AA, is a potent leukocyte chemoattractant, and not only serves as a pro-inflammatory molecule but may also have a role in atherosclerosis through its receptors BLT-1 and BLT-2. Deletion of BLT-1 significantly reduced atherosclerosis in apoE^{-/-} mice only during initiating stages (4 and 8 weeks) but had no effect on the lesion size in mice fed atherogenic diet for 19 weeks. LTB₄ may also promote atherosclerosis by chemoattracting monocytes, and by converting monocytes to foam cells by enhanced expression of CD36 and fatty acid accumulation [159]. An analysis of the expression of 5-LO in human carotid plaques obtained from 60 atherosclerotic patients undergoing carotid endarterectomy, in whom the nature of the plaques was divided into asymptomatic and symptomatic according to clinical evidence of plaque instability [as determined by the occurrence of recent ischemic symptoms attributable to stenosis and by the presence of ipsilateral cerebral lesion(s) determined by computed tomography], showed

that the expression of 5-LO is elevated in symptomatic compared with asymptomatic plaques [160]. This suggests that LTB₄ may have an important role in atherosclerosis.

DHA constitutes about 30–50% of total fatty acids in the mammalian brain, where it is predominantly associated with membrane phospholipids [161, 162]. Thus, high levels of DHA could be released from the membrane-bound DHA in response to neurotransmitters [163] and after brain injury [164]. DHA, thus released can activate RXR in neighboring cells, since DHA serves as ligand for RXR receptor [165, 166]. In fact, it has been reported that almost all PUFAs serve as ligands for RXR receptor. DHA is essential for brain growth, development, and maturation, and DHA deficiency results in impaired spatial learning as well as other abnormalities [167–169], defects that are also seen in RXR γ -deficient mice [170]. DHA reduces blood cholesterol and enhances insulin sensitivity [171, 172], actions that are also seen with synthetic RXR lig-

ands [8, 173, 174], implying that DHA, in all probability, brings about its actions by RXR activation. Several RXR heterodimerization partners such as PPARs, the liver X receptors, and farnesoid X receptors are essential for regulating energy and nutritional homeostasis. Since DHA serves as a ligand for RXR and PPARs, it is likely that DHA modulates these and several other regulatory events by binding to these nuclear receptor heterodimers.

Since several biologically active molecules that have both pro- and anti-inflammatory actions are formed from PUFAs, it is important to know the molecular triggers that facilitate their formation. Once the mechanisms of their formation is clear, it may be possible to devise methods of selectively enhancing the synthesis of LXs, resolvins, NPD1, and nitroalkenes to suppress inappropriate inflammatory events. Synthesis of stable and more potent LXs and resolvins could be attempted, so that their usefulness in the management of several inflammatory conditions including, but not limited to, bronchial asthma, metabolic syndrome X, neurodegenerative conditions, stroke, CHD, psoriasis, collagen vascular diseases, and cancer may be attempted [8, 175, 176]. In view of their varied actions PUFAs and their products may also form the basis for the development of many nutraceuticals and drugs.



Undurti N Das graduated with a medical degree from Osmania Medical College, Hyderabad, India, where he specialized in internal medicine. Since 1976 he has turned his attentions to clinical-orientated research, with specific interests in the role of polyunsaturated fatty acids, cytokines, nitric oxide, free radicals, and anti-oxidants in various clinical conditions and especially in

cancer, inflammatory conditions, metabolic syndrome X, and schizophrenia. Undurti N Das has held several clinical and research positions including those at the Osmania University, Nizam's Institute of Medical Sciences, and L.V. Prasad Eye Institute(India) as Professor of Medicine; at the Efamol Research Institute, Kentville (Canada); at the SUNY Upstate Medical University, Syracuse, NY (USA). Undurti Das is currently the President and CEO of the privately held biotech company UND Life Sciences, Shaker Heights, OH (USA), and is a consultant to pharma companies in India and the USA. Undurti Das has more than 300 research publications and has been awarded 3 patents for novel anti-cancer molecules that are under development. Undurti N Das is the Editor-in-Chief of *Lipids in Health and Disease*.

12 References

- [1] Das, U.N., Horrobin, D.F., Begin, M.E., Huang, Y.S. *et al.*, Clinical significance of essential fatty acids. *Nutrition* 1988, 4, 337–342.
- [2] Das, U.N., Essential fatty acids: Biology and their clinical implications. *Asian Pacific J. Pharmacol.* 1991, 6, 317–330.
- [3] Das, U.N., Tumoricidal action of cis-unsaturated fatty acids and its relationship to free radicals and lipid peroxidation. *Cancer Lett.* 1991, 56, 235–243.
- [4] La Guardia, M., Giammanco, S., Di Majo, D., Tabacchi, G. *et al.*, Omega 3 fatty acids: biological activity and effects on human health. *Panminerva Med.* 2005, 47, 245–257.
- [5] Das, U.N., Mohan, I.K., Raju, T.R., Effect of corticosteroids and eicosapentaenoic acid/docosahexaenoic acid on pro-oxidant and anti-oxidant status and metabolism of essential fatty acids in patients with glomerular disorders. *Prostaglandins Leukot. Essen. Fatty Acids* 2001, 65, 197–203.
- [6] Das, U.N., Long-chain polyunsaturated fatty acids interact with nitric oxide, superoxide anion, and transforming growth factor- β to prevent human essential hypertension. *Eur. J. Clin. Nutr.* 2004, 58, 195–203.
- [7] Das, U.N., Can perinatal supplementation of long-chain polyunsaturated fatty acids prevent diabetes mellitus? *Eur. J. Clin. Nutrition* 2003, 57, 218–226.
- [8] Das, U.N., *A Perinatal Strategy for Preventing Adult Diseases: The Role of Long-Chain Polyunsaturated Fatty Acids*. Kluwer, Boston 2002.
- [9] Ollis, T.E., Meyer, B.J., Howe, P.R., Australian food sources and intakes of omega-6 and omega-3 polyunsaturated fatty acids. *Ann. Nutr. Metab.* 1999, 43, 346–355.
- [10] Harzer, G., Haug, M., Dietrich, I., Gentner, R.R., Changing patterns of human milk lipids in the course of lactation and during the day. *Am. J. Clin. Nutr.* 1983, 37, 612–621.
- [11] Horrobin, D.F., The regulation of prostaglandin biosynthesis by the manipulation of essential fatty acid metabolism. *Rev. Pure Appl. Pharmacol. Sci.* 1983, 4, 339–383.
- [12] Takayasu, K., Okuda, K., Yoshikawa, I., Fatty acid composition of human and rat adrenal lipids: occurrence of w6 docosatrienoic acid in human adrenal cholesterol esters. *Lipids* 1970, 5, 743–750.
- [13] Bezaud, J., Blond, J.P., Bernand, A., Clouet, P., The metabolism and availability of essential fatty acids in animal and human tissues. *Reprod. Nutr. Dev.* 1994, 34, 539–568.
- [14] Lilly, M.L., Bottino, N.R., Identification of arachidonic acid in Gulf of Mexico shrimp and degree of biosynthesis in *Peraeus setiferus*. *Lipids* 1975, 10, 871–875.
- [15] Doyle, W., Hare, W.R., Crawford, M.A., Comparison of fatty acid intakes in the contrasting socio-economic groups in pregnancy. *Proc. Nutr. Soc.* 1982, 42, 69A.
- [16] Meyer, B.J., Mann, N.J., Lewis, J.L., Milligan, G.C. *et al.*, Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. *Lipids* 2003, 38, 391–398.
- [17] Cantwell, M.M., Flynn, M.A., Cronin, D., O'Neill, J.P., Gibney, M.J., Contribution of foods to trans unsaturated fatty acid intake in a group of Irish adults. *J. Hum. Nutr. Diet.* 2005, 18, 377–385.
- [18] Lopez-Garcia, E., Schultze, M.B., Meigs, J.B., Manson, J.E. *et al.*, Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. *J. Nutr.* 2005, 135, 562–566.
- [19] Mozaffarian, D., Pischon, T., Hankinson, S.E., Rifai, N. *et al.*, Dietary intake of trans fatty acids and systemic inflammation in women. *Am. J. Clin. Nutr.* 2004, 79, 606–612.
- [20] Brenner, R.R., Nutritional and hormonal factors influencing desaturation of essential fatty acids. *Prog. Lipid Res.* 1982, 20, 41–48.
- [21] Takahashi, Y., Kushiro, M., Shinohara, K., Ide, T., Activity and mRNA levels of enzymes involved in hepatic fatty acid synthesis and oxidation in mice fed conjugated linoleic acid. *Biochem. Biophys. Acta* 2003, 1631, 265–273.

- [22] Tang, C., Cho, H.P., Nakamura, M.T., Clarke, S.D., Regulation of human delta-6 desaturase gene transcription: identification of a functional direct repeat-1 element. *J. Lipid Res.* 2003, *44*, 686–695.
- [23] Matsuzaka, T., Shimano, H., Yahagi, N., Amemiya-Kudo, M. *et al.*, Dual regulation of mouse Delta(5)- and Delta(6)-desaturase gene expression by SREBP-1 and PPARalpha. *J. Lipid Res.* 2002, *43*, 107–114.
- [24] Cook, H.W., The influence of trans acids on desaturation and elongation of fatty acids. *Lipids* 1981, *16*, 920–926.
- [25] Das, U.N., Insulin resistance and hyperinsulinemia: Are they secondary to an alteration in the metabolism of essential fatty acids? *Med. Sci. Res.* 1994, *22*, 243–245.
- [26] Das, U.N., A defect in the activity of Δ^6 and Δ^5 desaturases may be a factor predisposing to the development of insulin resistance syndrome. *Prostaglandins Leukot. Essent. Fatty Acids* 2005, *72*, 343–350.
- [27] Coetzer, H., Claassen, N., van Papendorp, D.H., Kruger, M.C., Calcium transport by isolated brush border and basolateral membrane vesicles: role of essential fatty acid supplementation. *Prostaglandins Leukot. Essent. Fatty Acids* 1994, *50*, 257–266.
- [28] Das, U.N., Long-chain polyunsaturated fatty acids in the growth and development of the brain and memory. *Nutrition* 2003, *19*, 62–65.
- [29] Das, U.N., Can memory be improved? A discussion on the role of *ras*, GABA, acetylcholine, NO, insulin, TNF- α , and long-chain polyunsaturated fatty acids in memory formation and consolidation. *Brain Dev.* 2003, *25*, 251–261.
- [30] Calderon, F., Kim, H.Y., Docosahexaenoic acid promotes neurite growth in hippocampal neurons. *J. Neurochem.* 2004, *90*, 979–988.
- [31] Sun, C.O., O'Connor, C.J., Robertson, A., Antibacterial actions of fatty acids and monoglycerides against *Helicobacter pylori*. *FEMS Immunol. Med. Microbiol.* 2003, *36*, 9–17.
- [32] Das, U.N., Do unsaturated fatty acids function as endogenous antibacterial and anti-viral molecules? *Am. J. Clin. Nutr.* 2006, *83*, 390–391.
- [33] Giamarellos-Bourboulis, E.J., Mouktaroudi, M., Adamis, T., Kousoulas, V. *et al.*, n-6 polyunsaturated fatty acids enhance the activities of ceftazidime and amikacin in experimental sepsis caused by multidrug-resistant *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 2004, *48*, 4713–4717.
- [34] Kumar, K.V., Das, U.N., Effect of cis-unsaturated fatty acids, prostaglandins, and free radicals on angiotensin-converting enzyme activity *in vitro*. *Proc. Soc. Exp. Biol. Med.* 1997, *214*, 374–379.
- [35] Das, U.N., Lipids that matter from infant nutrition to insulin resistance. *Prostaglandins Leukot. Essent. Fatty Acids* 2002, *67*, 1–12.
- [36] Okuda, Y., Kawashima, K., Sawada, T., Tsurumaru, K. *et al.*, Eicosapentaenoic acid enhances nitric oxide production by cultured human endothelial cells. *Biochem. Biophys. Res. Commun.* 1997, *232*, 487–491.
- [37] Das, U.N., Mohan, I.K., Raju, T.R., Effect of corticosteroids and eicosapentaenoic acid/docosahexaenoic acid on pro-oxidant and anti-oxidant status and metabolism of essential fatty acids in patients with glomerular disorders. *Prostaglandins Leukot. Essent. Fatty Acids* 2001, *65*, 197–203.
- [38] Das, U.N., Essential fatty acid metabolism in patients with essential hypertension, diabetes mellitus and coronary heart disease. *Prostaglandins Leukot. Essent. Fatty Acids* 1995, *52*, 387–391.
- [39] Das, U.N., Vaddadi, K.S., Essential fatty acids in Huntington's disease. *Nutrition* 2004, *20*, 942–947.
- [40] Bunk, S., ACEs wild. *Scientist* 2002, *16*, 22–24.
- [41] Moskowitz, D.W., Is angiotensin I-converting enzyme a 'master' disease gene? *Diabetes Tech. Therapeutics* 2002, *4*, 683–711.
- [42] Kaergel, E., Muller, D.N., Honeck, H., Theuer, J. *et al.*, P450-dependent arachidonic acid metabolism and angiotensin-II-induced renal damage. *Hypertension* 2002, *40*, 273–279.
- [43] Gilroy, D.W., New insights into the anti-inflammatory actions of aspirin – induction of nitric oxide through the generation of epi-lipoxins. *Mem. Inst. Oswaldo Cruz.* 2005, *100 Suppl 1*, 49–54.
- [44] Wang, W., Diamond, S.L., Does elevated nitric oxide production enhance the release of prostacyclin from shear stressed aortic endothelial cells? *Biochem. Biophys. Res. Commun.* 1997, *233*, 748–751.
- [45] Das, U.N., COX-2 inhibitors and metabolism of essential fatty acids. *Med. Sci. Monit.* 2005, *11*, R233–R237.
- [46] Endres, S., Ghorbani, R., Kelley, V.E., Georgilis, K. *et al.*, The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N. Engl. J. Med.* 1989, *320*, 265–271.
- [47] Kumar, G.S., Das, U.N., Effect of prostaglandins and their precursors on the proliferation of human lymphocytes and their secretion of tumor necrosis factor and various interleukins. *Prostaglandins Leukot. Essent. Fatty Acids* 1994, *50*, 331–334.
- [48] Kumar, S.G., Das, U.N., Kumar, K.V., Madhavi, N. *et al.*, Effect of n-6 and n-3 fatty acids on the proliferation and secretion of TNF and IL-2 by human lymphocytes *in vitro*. *Nutrition Res.* 1992, *12*, 815–823.
- [49] Arita, M., Bianchini, F., Aliberti, J., Sher, A. *et al.*, Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J. Exp. Med.* 2005, *201*, 713–722.
- [50] Dooper, M.M., van Riel, B., Graus, Y.M., M'Rabet, L., Dihomo-gamma-linolenic acid inhibits tumour necrosis factor-alpha production by human leucocytes independently of cyclooxygenase activity. *Immunology* 2003, *110*, 348–57.
- [51] Chavali, S.R., Zhong, W.W., Forse, R.A., Dietary alpha-linolenic acid increases TNF-alpha, and decreases IL-6, IL-10 in response to LPS: effects of sesamin on the delta-5 desaturation of omega6 and omega3 fatty acids in mice. *Prostaglandins Leukot. Essent. Fatty Acids* 1998, *58*, 185–191.
- [52] Leal-Berumen, I., O'Byrne, P., Gupta, A., Richards, C.D., Marshall, J.S., Prostanoid enhancement of interleukin-6 production by rat peritoneal mast cells. *J. Immunol.* 1995, *154*, 4759–4767.
- [53] Kozawa, O., Tokuda, H., Kaida, T., Matsuno, H., Uematsu, T., Effect of vitamin D3 on interleukin-6 synthesis induced by prostaglandins in osteoblasts. *Prostaglandins Leukot. Essent. Fatty Acids* 1998, *58*, 119–123.
- [54] Trebble, T., Arden, H.K., Stroud, M.A., Wootton, S.A. *et al.*, Inhibition of tumour necrosis factor-alpha and interleukin 6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. *Br. J. Nutr.* 2003, *90*, 405–412.
- [55] Chen, W., Esselman, W.J., Jump, D.B., Busik, J.V., Anti-inflammatory effect of docosahexaenoic acid on cytokine-induced adhesion molecule expression in human retinal vascular endothelial cells. *Invest. Ophthalmol. Vis. Sci.* 2005, *46*, 4342–4347.
- [56] Li, H., Ruan, X.Z., Powis, S.H., Fernando, R. *et al.*, EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-gamma-dependent mechanism. *Kidney Int.* 2005, *67*, 867–874.
- [57] Beutler, B., Cerami, A., Cachectin and tumor necrosis factor as two sides of the same biological coin. *Nature* 1986, *320*, 584–588.
- [58] Beck, S.A., Smith, K.L., Tisdale, M.J., Anti-cachectic and anti-tumor effect of eicosapentaenoic acid and its effect on protein turnover. *Cancer Res.* 1991, *51*, 6089–6093.
- [59] Ramos, E.J., Middleton, F.A., Laviano, A., Sato, T. *et al.*, Effects of omega-3 fatty acid supplementation on tumor-bearing rats. *J. Am. Coll. Surg.* 2004, *199*, 716–723.
- [60] Lihn, A.S., Richelsen, B., Pedersen, S.B., Hangaard, S.B. *et al.*, Increased expression of TNF-alpha, IL-6, and IL-8 in HALS; implications for reduced adiponectin expression and plasma levels. *Am. J. Physiol. Endocrinol. Metab.* 2003, *285*, E1072–E1080.

- [61] Miller, J., Carr, A., Emery, S., Law, M. *et al.*, A HIV lipodystrophy: prevalence, severity and correlates of risk in Australia. *HIV Med.* 2003, 4, 293–301.
- [62] Huang, Y.-J., Fang, V.S., Chou, Y.-C., Kwok, C.-F., Ho, L.-T., Amelioration of insulin resistance and hypertension in a fructose-fed rat model with fish oil supplementation. *Metabolism* 1997, 46, 1252–1258.
- [63] Mori, Y., Murakawa, Y., Katoh, S., Hata, S. *et al.*, Influence of highly purified eicosapentaenoic acid ethyl ester on insulin resistance in the Otsuka Long-Evans Tokushima fatty rat, a model of spontaneous non-insulin-dependent diabetes mellitus. *Metabolism* 1997, 46, 1458–1464.
- [64] Nobukata, H., Ishikawa, T., Obata, M., Shibusaki, Y., Long-term administration of highly purified eicosapentaenoic acid ethyl ester prevents diabetes and abnormalities of blood coagulation in male WBN/Kob rats. *Metabolism* 2000, 49, 912–919.
- [65] Sheng, Z., Otani, H., Brown, M.S., Goldstein, J.L., Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. *Proc. Natl. Acad. Sci. USA* 1995, 92, 935–938.
- [66] Fajas, L., Schoonjans, K., Gelman, L., Kim, J.B. *et al.*, Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. *Mol. Cell. Biol.* 1999, 19, 5495–5503.
- [67] Logette, E., Le Jossic-Corcoc, C., Masson, D., Solier, S. *et al.*, Caspase-2, a novel lipid sensor under the control of sterol regulatory element binding protein 2. *Mol. Cell. Biol.* 2005, 25, 9621–9631.
- [68] El-Sohemy, A., Archer, M.C., Regulation of mevalonate synthesis in low density lipoprotein receptor knockout mice fed n-3 or n-6 polyunsaturated fatty acids. *Lipids* 1999, 34, 1037–1043.
- [69] Das, U.N., Essential fatty acids and osteoporosis. *Nutrition* 2000, 16, 286–290.
- [70] Nakamura, N., Hamazaki, T., Jokaji, H., Minami, S., Kobayashi, M., Effect of HMG-CoA reductase inhibitors on plasma polyunsaturated fatty acid concentration in patients with hyperlipidemia. *Int. J. Clin. Lab. Res.* 1998, 28, 192–195.
- [71] Hannah, V.C., Ou, J., Luong, A., Goldstein, J.L., Brown, M.S., Unsaturated fatty acids down-regulate srebp isoforms 1a and 1c by two mechanisms in HEK-293 cells. *J. Biol. Chem.* 2001, 276, 4365–4372.
- [72] Field, F.J., Born, E., Murthy, S., Mathur, S.N., Polyunsaturated fatty acids decrease the expression of sterol regulatory element-binding protein-1 in CaCo-2 cells: effect on fatty acid synthesis and triacylglycerol transport. *Biochem. J.* 2002, 368, 855–864.
- [73] Xu, J., Cho, H., O'Malley, S., Park, J.H., Clarke, S.D., Dietary polyunsaturated fats regulate rat liver sterol regulatory element binding proteins-1 and -2 in three distinct stages and by different mechanisms. *J. Nutr.* 2002, 132, 3333–3339.
- [74] Field, F.J., Born, E., Mathur, S.N., Fatty acid flux suppresses fatty acid synthesis in hamster intestine independently of SREBP-1 expression. *J. Lipid Res.* 2003, 44, 1199–1208.
- [75] Le Jossic-Corcoc, C., Gonther, C., Zaghini, I., Logette, E. *et al.*, Hepatic farnesyl diphosphate synthase expression is suppressed by polyunsaturated fatty acids. *Biochem. J.* 2005, 385, 787–794.
- [76] Worgall, T.S., Johnson, R.A., Seo, T., Gierens, H., Deckelbaum, R.J., Unsaturated fatty acid-mediated decreases in sterol regulatory element-mediated gene transcription are linked to cellular sphingolipid metabolism. *J. Biol. Chem.* 2002, 277, 3878–3885.
- [77] Calon, F., Lim, G.P., Yang, F., Morihara, T. *et al.*, Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron* 2004, 43, 633–645.
- [78] Lukiw, W.J., Cui, J.-G., Marcheselli, V.L., Bodker, M. *et al.*, A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. *J. Clin. Invest.* 2005, 115, 2774–2783.
- [79] Calon, F., Lim, G.P., Morihara, T., Yang, F. *et al.*, Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's. *Eur. J. Neurosci.* 2005, 22, 617–626.
- [80] Akbar, M., Calderon, F., Wen, Z., Kim, H.Y., Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. *Proc. Natl. Acad. Sci. USA* 2005, 102, 10858–10863.
- [81] Hashimoto, M., Tanabe, Y., Fujii, Y., Kikuta, T. *et al.*, Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid beta-infused rats. *J. Nutr.* 2005, 135, 549–555.
- [82] Aravindakshan, M., Ghate, M., Ranjekar, P.K., Evans, D.R., Mahadik, S.P., Supplementation with a combination of omega-3 fatty acids and antioxidants (vitamins E and C) improves the outcome of schizophrenia. *Schizophr. Res.* 2003, 62, 195–204.
- [83] Das, U.N., Can perinatal supplementation of long-chain polyunsaturated fatty acids prevent schizophrenia in adult life? *Med. Sci. Monit.* 2004, 10, HY33–HY37.
- [84] Claria, J., Serhan, C.N., Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proc. Natl. Acad. Sci. USA* 1995, 92, 9475–9479.
- [85] Chiang, N., Gronert, K., Clish, C.B., O'Brien, J.A. *et al.*, Leukotriene B₄ receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. *J. Clin. Invest.* 1999, 104, 309–316.
- [86] Xiao, G., Tasi, A.-L., Palmer, G., Boyar, W.C. *et al.*, Analysis of hydroperoxide-induced tyrosyl radicals and lipoxygenase activity in aspirin-treated human prostaglandin synthase-2. *Biochemistry* 1997, 36, 1836–1845.
- [87] Levy, B.D., Clish, C.B., Schmidt, B., Gronert, K., Serhan, C.N., Lipid mediator class switching during acute inflammation signals in resolution. *Nat. Immunol.* 2001, 2, 612–619.
- [88] Bendeira-Melo, C., Serra, M.F., Diaz, B.I., Cordeiro, R.S.B. *et al.*, Cyclooxygenase-2-derived prostaglandin E₂ and lipoxin A₄ accelerate resolution of allergic edema in *Angiostrongylus costaricensis*-infected rats: relationship with concurrent eosinophilia. *J. Immunol.* 2000, 164, 1029–1036.
- [89] Serhan, C.N., Maddox, J.F., Petasis, N.A., Akritopoulou-Zanze, I. *et al.*, Design of lipoxin A₄ stable analogs that block transmigration and adhesion of human neutrophils. *Biochemistry* 1995, 34, 14609–14615.
- [90] Serhan, C.N., Clish, C.B., Brannon, J., Colgan, S.P. *et al.*, Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nons teroidal antiinflammatory drugs and transcellular processing. *J. Exp. Med.* 2000, 192, 1197–1204.
- [91] Serhan, C.N., Hong, S., Gronert, K., Colgan, S.P. *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, 1025–1037.
- [92] Marcheselli, V.L., Hong, S., Lukiw, W.J., Tian, X.H. *et al.*, Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. *J. Biol. Chem.* 2003, 278, 43807–43817.
- [93] Hong, S., Gronert, K., Devchand, P.R., Moussignac, R.L., Serhan, C.N., 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, 14677–14687.
- [94] Mukherjee, P.K., Marcheselli, V.L., Serhan, C.N., Bazan, N.G., Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. *Proc. Natl. Acad. Sci. USA* 2004, 101, 8491–8496.
- [95] Gronert, K., Maheshwari, N., Khan, N., Hasan, I.R. *et al.*, A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense. *J. Biol. Chem.* 2005, 280, 15267–15278.

- [96] Miller, C.C., McCreedy, C.A., Jones, A.D., Ziboh, V.A., Oxidative metabolism of dihomo-gammalinolenic acid by guinea pig epidermis: evidence of generation of anti-inflammatory products. *Prostaglandins* 1988, *35*, 917–938.
- [97] Tate, G., Mandell, B.F., Laposata, M., Ohliger, D. *et al.*, Suppression of acute and chronic inflammation by dietary gamma linolenic acid. *J. Rheumatol.* 1989, *16*, 729–734.
- [98] Heitmann, J., Iversen, L., Kragballe, K., Ziboh, V.A., Incorporation of 15-hydroxyeicosatrienoic acid in specific phospholipids of cultured human keratinocytes and psoriatic plaques. *Exp. Dermatol.* 1995, *4*, 74–78.
- [99] Santoli, D., Zurier, R.B., Prostaglandin E precursor fatty acids inhibit human IL-2 production by a prostaglandin E-independent mechanism. *J. Immunol.* 1989, *143*, 1303–1309.
- [100] DeLuca, P., Rossetti, R.G., Alavian, C., Karim, P., Zurier, R.B., Effects of gammalinolenic acid on interleukin-1 beta and tumor necrosis factor-alpha secretion by stimulated human peripheral blood monocytes: studies *in vitro* and *in vivo*. *J. Invest. Med.* 1999, *47*, 246–250.
- [101] Mancuso, P., Whelan, J., DeMichele, S.J., Snider, C.C. *et al.*, Dietary fish oil and fish and borage oil suppress intrapulmonary proinflammatory eicosanoid biosynthesis and attenuate pulmonary neutrophil accumulation in endotoxic rats. *Crit. Care Med.* 1997, *25*, 1198–1206.
- [102] Palombo, J.D., DeMichele, S.J., Boyce, P.J., Lydon, E.E. *et al.*, Effect of short-term enteral feeding with eicosapentaenoic and gammalinolenic acids on alveolar macrophage eicosanoid synthesis and bactericidal function in rats. *Crit. Care Med.* 1999, *27*, 1908–1915.
- [103] Barham, J.B., Edens, M.B., Fonteh, A.N., Johnson, M.M. *et al.*, Addition of eicosapentaenoic acid to gamma-linolenic acid-supplemented diets prevents serum arachidonic acid accumulation in humans. *J. Nutr.* 2000, *130*, 1925–1931.
- [104] Thies, F., Miles, E.A., Nebe-von-Caron, G., Powell, J.R. *et al.*, Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. *Lipids* 2001, *36*, 1183–1193.
- [105] Lo, C.J., Chiu, K.C., Fu, M., Lo, R., Helton, S., Fish oil decreases macrophage tumor necrosis factor gene transcription by altering the NF kappa B activity. *J. Surg.* 1999, *82*, 216–221.
- [106] Kambe, T., Murakami, M., Kudo, I., Polyunsaturated fatty acids potentiate interleukin-1-stimulated arachidonic acid release by cells overexpressing type IIA secretory phospholipase A2. *FEBS Lett.* 1999, *453*, 81–84.
- [107] Gilroy, D.W., Newson, J., Sawmynaden, P., Willoughby, D.A., Croxtall, J.D., A novel role for phospholipase A₂ isoforms in the checkpoint control of acute inflammation. *FASEB J.* 2004, *18*, 489–498.
- [108] Borghaei, H., Borghaei, R.C., Ni, X., Pease, E. *et al.*, Evidence that suppression of IL-1 induced collagenase mRNA expression by dihomo-gamma-linolenic acid (DGLA) involves inhibition of NF kappa B binding. *Inflamm. Res.* 1997, *46 Suppl. 2*, S177–178.
- [109] Zhao, Y., Joshi-Barve, S., Barve, S., Chen, L.H., Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. *J. Am. Coll. Nutr.* 2004, *23*, 71–78.
- [110] Schley, P.D., Jijon, H.B., Robinson, L.E., Field, C.J., Mechanisms of omega-3 fatty acid-induced growth inhibition in MDA-MB-231 human breast cancer cells. *Breast Cancer Res. Treat.* 2005, *92*, 187–195.
- [111] Weber, C., Erl, W., Pietsch, A., Danesch, U., Weber, P.C., 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*, 622–628.
- [112] Camandola, S., Leonarduzzi, G., Musso, T., Varesio, L. *et al.*, Nuclear factor kB is activated by arachidonic acid but not by eicosapentaenoic acid. *Biochem. Biophys. Res. Commun.* 1996, *229*, 643–647.
- [113] Li, H., Ruan, X.Z., Powis, S.H., Fernando, R. *et al.*, EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-gamma-dependent mechanism. *Kidney Int.* 2005, *67*, 867–874.
- [114] Kliewer, S.A., Sundseth, S.S., Jones, S.A., Brown, P.J. *et al.*, Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc. Natl. Acad. Sci. USA* 1997, *94*, 4318–4323.
- [115] Sethi, S., Eastman, A.Y., Eaton, J.W., Inhibition of phagocyte-endothelium interactions by oxidized fatty acids: a natural anti-inflammatory mechanism? *J. Lab. Clin. Med.* 1996, *128*, 27–38.
- [116] Mishra, A., Chaudhary, A., Sethi, S., Oxidized omega-3 fatty acids inhibit NF-kappaB activation via a PPARalpha-dependent pathway. *Arterioscler. Thromb. Vasc. Biol.* 2004, *24*, 1621–1627.
- [117] Chaudhary, A., Mishra, A., Sethi, S., Oxidized omega-3 fatty acids inhibit pro-inflammatory responses in glomerular endothelial cells. *Nephron. Exp. Nephrol.* 2004, *97*, e136–145.
- [118] Mori, T.A., Woodman, R.J., Burke, V., Puddey, I.B. *et al.*, Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects. *Free Radic. Biol. Med.* 2003, *35*, 772–781.
- [119] Mori, T.A., Puddey, I.B., Burke, V., Croft, K.D. *et al.*, Effect of omega 3 fatty acids on oxidative stress in humans: GC-MS measurement of urinary F2-isoprostane excretion. *Redox. Rep.* 2000, *5*, 45–46.
- [120] Barden, A.E., Mori, T.A., Dunstan, J.A., Taylor, A.L. *et al.*, Fish oil supplementation in pregnancy lowers F2-isoprostanes in neonates at high risk of atopy. *Free Radic. Res.* 2004, *38*, 233–239.
- [121] Mullenix, P.S., Andersen, C.A., Starnes, B.W., Atherosclerosis as inflammation. *Ann. Vasc. Surg.* 2005, *19*, 130–138.
- [122] Stokes, K.Y., Granger, D.N., The microcirculation: a motor for the systemic inflammatory response and large vessel disease induced by hypercholesterolaemia? *J. Physiol.* 2005, *562*, 647–653.
- [123] Das, U.N., Polyunsaturated fatty acids, endothelial lipase and atherosclerosis. *Prostaglandins Leukot. Essen. Fatty Acids* 2005, *72*, 173–179.
- [124] Kobayashi, T., Tahara, Y., Matsumoto, M., Iguchi, M. *et al.*, Roles of thromboxane A₂ and prostacyclin in the development of atherosclerosis in apoE-deficient mice. *J. Clin. Invest.* 2004, *114*, 784–794.
- [125] Honda, T., Segi-Nishida, E., Miyachi, Y., Narumiya, S., Prostacyclin-IP signaling and prostaglandin E₂-EP2/EP4 signaling both mediate joint inflammation in mouse collagen-induced arthritis. *J. Exp. Med.* 2006, *203*, 325–335.
- [126] Juan, H., Sametz, W., Dihomo-gamma-linolenic acid increases the metabolism of eicosapentaenoic acid in perfused vascular tissue. *Prostaglandins Leukot. Med.* 1985, *19*, 79–86.
- [127] Bordet, J.C., Guichardant, M., Lagarde, M., Hydroperoxides produced by n-6 lipoxygenation of arachidonic and linoleic acids potentiate synthesis of prostacyclin related compounds. *Biochim. Biophys. Acta* 1988, *958*, 460–468.
- [128] Bordet, J.C., Guichardant, M., Lagarde, M., Arachidonic acid strongly stimulates prostaglandin I₃ (PGI₃) production from eicosapentaenoic acid in human endothelial cells. *Biochem. Biophys. Res. Commun.* 1986, *135*, 403–410.
- [129] Hamazaki, T., Hirai, A., Terano, T., Sajiki, J. *et al.*, Effects of orally administered ethyl ester of eicosapentaenoic acid (EPA; C20:5, omega-3) on PGI₂-like substance production by rat aorta. *Prostaglandins* 1982, *23*, 557–567.
- [130] Das, U.N., Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: but, why and how? *Prostaglandins Leukot. Essen. Fatty Acids* 2000, *63*, 351–362.

- [131] Das, U.N., Is metabolic syndrome X an inflammatory condition? *Exp. Biol. Med.* 2002, *227*, 989–997.
- [132] Lauritzen, L., Hoppe, C., Straarup, E.M., Michaelsen, K.F., Maternal fish oil supplementation in lactation and growth during the first 2.5 years of life. *Pediatr. Res.* 2005, *58*, 235–242.
- [133] Clandinin, M.T., Van Aerde, J.E., Merkel, K.L., Marris, C.L. *et al.*, Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. *J. Pediatr.* 2005, *146*, 461–468.
- [134] Agostoni, C., Gali, C., Riva, E., Colombo, C. *et al.*, Reduced docosahexaenoic acid synthesis may contribute to growth restriction in infants born to mothers who smoke. *J. Pediatr.* 2005, *147*, 854–856.
- [135] Llanos, A., Li, Y., Mena, P., Salem, N.J., Uauy, R., Infants with intrauterine growth restriction have impaired formation of docosahexaenoic acid in early neonatal life: a stable isotope study. *Pediatr. Res.* 2005, *58*, 735–740.
- [136] Makrides, M., Gibson, R.A., Udell, T., Ried, K., International LCPUFA Investigators. Supplementation of infant formula with long-chain polyunsaturated fatty acids does not influence the growth of term infants. *Am. J. Clin. Nutr.* 2005, *81*, 1094–1101.
- [137] Udell, T., Gibson, R.A., Makrides, M., PUFA Study Group. The effect of alpha-linolenic acid and linoleic acid on the growth and development of formula-fed infants: a systematic review and meta-analysis of randomized controlled trials. *Lipids* 2005, *40*, 1–11.
- [138] McCann, J.C., Ames, B.N., Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *Am. J. Clin. Nutr.* 2005, *82*, 281–295.
- [139] Auestad, N., Scott, D.T., Janowsky, J.S., Jacobsen, C. *et al.*, Visual, cognitive, and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. *Pediatrics* 2003, *112*, e177–83.
- [140] Peyron-Caso, E., Taverna, M., Guerre-Millo, M., Veronese, A. *et al.*, Dietary (n-3) polyunsaturated fatty acids up-regulate plasma leptin in insulin-resistant rats. *J. Nutr.* 2002, *132*, 2235–2240.
- [141] Reseland, J.E., Haugen, F., Hollung, K., Solvoll, K. *et al.*, Reduction of leptin gene expression by dietary polyunsaturated fatty acids. *J. Lipid Res.* 2001, *42*, 743–750.
- [142] Flachs, P., Mohamed-Ali, V., Horakova, O., Rossmel, M. *et al.*, Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Diabetologia* 2006, *49*, 394–397.
- [143] Borkman, M., Storlien, L.H., Pan, D.A., Jenkins, A.B. *et al.*, The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *N. Engl. J. Med.* 1993, *328*, 238–244.
- [144] Das, U.N., Kumar, K.V., Ramesh, G., Essential fatty acid metabolism in South Indians. *Prostaglandins Leukot. Essen. Fatty Acids* 1994, *50*, 253–255.
- [145] Baker, P.R.S., Lin, Y., Schopfer, F.J., Woodcock, S.R. *et al.*, Fatty acid transduction of nitric oxide signaling: Multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands. *J. Biol. Chem.* 2005, *280*, 42464–42475.
- [146] Coles, B., Bloodworth, A., Clark, S.R., Lewis, M.J. *et al.*, Nitrooleate inhibits superoxide generation, degranulation, and integrin expression by human neutrophils. *Circ. Res.* 2002, *91*, 375–381.
- [147] Lima, E.S., Bonim, M.G., Augusto, O., Barbeiro, H.V. *et al.*, Nitrated lipids decompose to nitric oxide and lipid radicals and cause vasorelaxation. *Free Radic. Biol. Med.* 2005, *39*, 532–539.
- [148] Eghtay, K.S., Roussel, D., St-Pierre, J., Jekabsons, M.B. *et al.*, Superoxide activates mitochondrial uncoupling proteins. *Nature* 2002, *415*, 96–99.
- [149] Nishikawa, T., Edelstein, D., Du, X.L., Tamagishi, S. *et al.*, Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000, *404*, 787–790.
- [150] Santerre, R.F., Nicolosi, R.J., Smith, S.C., Respiratory control in preatherosclerotic susceptible and resistant pigeon aortas. *Exp. Mol. Pathol.* 1974, *20*, 397–406.
- [151] Droge, W., Free radicals in the physiological control of cell function. *Physiol. Rev.* 2002, *82*, 47–95.
- [152] Bernal-Mizrachi, C., Gates, A.C., Weng, S., Imamura, T. *et al.*, Vascular respiratory uncoupling increases blood pressure and atherosclerosis. *Nature* 2005, *435*, 502–506.
- [153] Klein, P.D., Johnson, R.M., Phosphorus metabolism in unsaturated fatty acid-deficient rats. *J. Biol. Chem.* 1954, *211*, 103–110.
- [154] Hayashida, T., Portman, O.W., Swelling of liver mitochondria from rats fed diets deficient in essential fatty acids. *Proc. Soc. Exp. Biol. Med.* 1960, *103*, 656–659.
- [155] Cha, S.H., Fukushima, A., Sakuma, K., Kagawa, Y., Chronic docosahexaenoic acid intake enhances expression of the gene for uncoupling protein 3 and affects pleiotropic mRNA levels in skeletal muscle aged C57BL/6Njcl mice. *J. Nutr.* 2001, *131*, 2636–2642.
- [156] Cornwell, D.G., Panganamala, R.V., Atherosclerosis: an intracellular deficiency in essential fatty acids. *Prog. Lipid Res.* 1981, *20*, 365–376.
- [157] Smith, E.B., The effects of age and of early atheroma on the intimal lipids in men. *Biochem. J.* 1962, *84*, 49p.
- [158] Smith, E.B., Lipids carried by S₁ O-12 lipoprotein in normal and hypercholesterolaemic serum. *Lancet* 1962, *2*, 530–534.
- [159] Subbarao, K., Jala, V.R., Mathias, S., Suttles, J. *et al.*, Role of leukotriene B₄ receptors in the development of atherosclerosis: potential mechanisms. *Arterioscler. Thromb. Vasc. Biol.* 2004, *24*, 369–3675.
- [160] Cipollone, F., Mezzetti, A., Fazia, M.L., Cucurullo, C. *et al.*, Association between 5-lipoxygenase expression and plaque instability in humans. *Arterioscler. Thromb. Vasc. Biol.* 2005, *25*, 1665–1670.
- [161] Connor, W.E., Neuringer, M., Reisbick, S., Essential fatty acids: the importance of n-3 fatty acids in the retina and brain. *Nutr. Rev.* 1992, *50*, 21–29.
- [162] Connor, W.E., Neuringer, M., Reisbick, S., Essentiality of omega 3 fatty acids: evidence from the primate model and implications for human nutrition. *World Rev. Nutr. Diet.* 1991, *66*, 118–132.
- [163] Garcia, M.C., Kim, H.-Y., Mobilization of arachidonate and docosahexaenoate by stimulation of the 5-HT_{2A} receptor in rat C6 glioma cells. *Brain Res.* 1997, *768*, 43–48.
- [164] Homayoun, P., Parkins, N.E., Soblosky, J., Carey, M.E. *et al.*, Cortical impact injury in rats promotes a rapid and sustained increase in polyunsaturated free fatty acids and diacylglycerols. *Neurochem. Res.* 2000, *25*, 269–276.
- [165] Mata de Urquiza, A., Liu, S., Sjöberg, M., Zetterstrom, R.H. *et al.*, Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. *Science* 2000, *290*, 2140–2144.
- [166] Lengqvist, J., Mata de Urquiza, A., Bergman, A.C., Willson, T.M. *et al.*, Polyunsaturated fatty acids including docosahexaenoic and arachidonic acid bind to the retinoid X receptor a ligand-binding domain. *Mol. Cell. Proteomics* 2004, *3*, 692–703.
- [167] Lim, S.Y., Hoshiba, J., Moriguchi, T., Salem, N. Jr., N-3 fatty acid deficiency induced by a modified artificial rearing method leads to poorer performance in spatial learning tasks. *Pediatr. Res.* 2005, *58*, 741–748.
- [168] Lim, S.Y., Hoshiba, J., Salem, N. Jr., An extraordinary degree of structural specificity is required in neural phospholipids for optimal brain function: n-6 docosapentaenoic acid substitution for docosahexaenoic acid leads to a loss in spatial task performance. *J. Neurochem.* 2005, *95*, 848–857.

- [169] Garcia-Calatayud, S., Redondo, C., Martin, E., Ruiz, J.I. *et al.*, Brain docosahexaenoic acid status and learning in young rats submitted to dietary long-chain polyunsaturated fatty acid deficiency and supplementation limited to lactation. *Pediatr. Res.* 2005, *57*, 719–723.
- [170] Chiang, M.Y., Misner, D., Kempermann, G., Schikorski, T. *et al.*, An essential role for retinoid receptors RARbeta and RXRgamma in long-term potentiation and depression. *Neuron* 1998, *21*, 1353–1361.
- [171] Grimsgaard, S., Bonna, K.H., Hansen, J.B., Nordoy, A., Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. *Am. J. Clin. Nutr.* 1997, *66*, 649–659.
- [172] Flachs, P., Mohamed-Ali, V., Horakova, O., Rossmeisl, M. *et al.*, Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Diabetologia* 2006, *49*, 394–397.
- [173] Argmann, C.A., Sawyez, C.G., McNeil, C.J., Hegele, R.A., Huff, M.W., Activation of peroxisome proliferator-activated receptor gamma and retinoid X receptor results in net depletion of cellular cholesteryl esters in macrophages exposed to oxidized lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* 2003, *23*, 475–482.
- [174] Kim, H.I., Cha, J.Y., Kim, S.Y., Kim, J.W. *et al.*, Peroxisomal proliferator-activated receptor-gamma upregulates glucokinase gene expression in beta-cells. *Diabetes* 2002, *51*, 676–685.
- [175] Das, U.N., From bench to the clinic: γ -linolenic acid therapy of human gliomas. *Prostaglandins Leukot. Essen. Fatty Acids* 2004, *70*, 419–426.
- [176] Emsley, R., Myburgh, C., Oosthuizen, P., van Rensburg, S.J., Randomized, placebo controlled study of ethyl-eicosapentaenoic acid as supplemental treatment in schizophrenia. *Am. J. Psychiatry* 2002, *159*, 1596–1598.