# Omega-3 (n-3) polyunsaturated fatty acids and inflammation: From membrane to nucleus and from bench to bedside

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Abbreviations used: COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IκB, inhibitory subunit of nuclear factor κ B; IL, interleukin; LOX, lipoxygenase; LPS, lipopolysaccharide; LT, leukotriene; MyD88, myeloid differentiation primary response gene 88; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; PPAR, peroxisome proliferator activated receptor; PUFA, polyunsaturated fatty acid; RA, rheumatoid arthritis; SPM, specialised pro-resolving mediator; TLR, toll-like receptor; TNF, tumour necrosis factor.

**Abstract**

Inflammation is a normal part of the immune response and should be self-limiting. Excessive or unresolved inflammation is linked to tissue damage, pathology and ill health. Prostaglandins and leukotrienes produced from the omega-6 fatty acid arachidonic acid are involved in inflammation. Fatty acids may also influence inflammatory processes through mechanisms not necessarily involving lipid mediators. The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) possess a range of anti-inflammatory actions. Increased content of EPA and DHA in the membranes of cells involved in inflammation has effects on the physical nature of the membranes and on the formation of signalling platforms called lipid rafts. EPA and DHA interfere with arachidonic acid metabolism which yields prostaglandins and leukotrienes involved in inflammation. EPA gives rise to weak (e.g. less inflammatory) analogues and both EPA and DHA are substrates for synthesis of specialised pro-resolving mediators. Through their effects on early signalling events in membranes and on the profile of lipid mediators produced, EPA and DHA alter both intracellular and intercellular signals. Within cells this leads to altered patterns of gene expression and of protein production. The net result is decreased production of inflammatory cytokines, chemokines, adhesion molecules, proteases and enzymes. The anti-inflammatory and inflammation resolving effects of EPA and DHA are relevant to both prevention and treatment of human diseases that have an inflammatory component. This has been widely studied in rheumatoid arthritis where there is good evidence that high doses of EPA+DHA reduce pain and other symptoms.

**Inflammation in health and disease**

Inflammation is an essential and normal component of the immune response that protects against pathogenic organisms and is involved in the response to injury [1,2]. In general, inflammation acts to create an environment that is hostile to pathogens, it initiates pathogen killing, and it causes changes in the metabolism of the host [1,2]. Many cell types play roles in the inflammatory response, which involves the production of, and responses to, a vast number of chemical mediators [1,2]. The cardinal signs of inflammation are redness, swelling, heat, pain and loss of function. These are caused by the cellular activation and chemical mediator release that occur during the initiation and perpetuation of the inflammatory response. The chemical mediators released from cells during inflammation include lipids (e.g. prostaglandins (PGs), leukotrienes (LTs), endocannabinoids, platelet activating factor), peptides (e.g. cytokines, chemokines), reactive oxygen species (e.g. superoxide anion, hydrogen peroxide), amino acid derivatives (e.g. histamine, nitric oxide) and enzymes (e.g. matrix proteases) depending upon the cell types present, the nature of the inflammatory stimulus, the anatomical site involved, and the stage during the inflammatory response [1,2]. Although the inflammatory response is designed to be damaging to pathogens, the cellular activities involved and the chemical mediators produced can cause damage to host tissues [1,2]. Fortunately therefore, inflammation is normally self-limiting and resolves, often rapidly. This is because various inhibitory mechanisms are activated as inflammation runs its course [3]. These include shedding of receptors for pro-inflammatory cytokines and increased generation of anti-inflammatory cytokines [3]. Another mechanism involved is the generation of specialised pro-resolving lipid mediators (SPMs) which act to inhibit pro-inflammatory signalling [4]. Loss of the regulatory processes involved in resolution of inflammation can result in excessive, inappropriate or on-going inflammation that can cause irreparable damage to host tissues leading to pathology and disease (Figure 1) [3-5]. Inflammation is an important component of a wide array of human conditions including classic chronic

inflammatory diseases like rheumatoid arthritis (RA), inflammatory bowel diseases, multiple sclerosis, lupus, chronic obstructive pulmonary disease, allergy and asthma which are all controlled or treated with varying degrees of success with anti-inflammatory medications [1,2]. Research in the last two decades has identified that inflammation is also a risk factor involved in atherosclerosis [6,7], cardiovascular events [6,7], neurodegenerative disorders and cognitive decline [8], and in many cancers [9]. Furthermore, an excessive inflammatory response is liked to adverse outcomes following surgery [10] and in critical illness [11]. Inflammation appears to be a driver of loss of lean mass seen in many cancers [12] and in frailty [13] and sarcopenia [14]. Finally, both obesity and ageing are associated with an elevated state of inflammation [15,16] and this might relate to the development of chronic conditions associated with both. Because of this wide-reaching adverse impact of inappropriate or excessive inflammation, strategies that control or resolve the inflammatory response could have a huge impact on human health and well-being. This review will describe mechanisms by which fatty acids can influence inflammatory processes with a focus on the effects of the omega-3 (n-3) polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). An overview of the structure, metabolism, dietary sources and intakes, and general health benefits of EPA and DHA is available elsewhere [17].

**The links between fatty acids, cell membranes and inflammation**

Many of the receptors for triggers of inflammation (e.g. several toll-like receptors (TLRs) that recognise microbial structures or products such as lipopolysaccharide (LPS)) and for inflammatory mediators such as cytokines, chemokines, prostaglandins and leukotrienes are localised in the cell membrane. Here the receptors interact with other membrane proteins to initiate intracellular signalling leading to cellular activation. In many cases, proteins need to move within the plane of cell membrane to come together and form signalling platforms. Such

movement within the membrane will obviously involve some form of interaction with the lipid components of the membrane. The term "lipid rafts" has been used to describe the signalling platforms that result, and these structures include the various proteins and their surrounding lipids, which are often specific species of phospholipids, sphingolipids and cholesterol [18]. Once rafts form, they generate signals that regulate how cells respond. For example, in monocytes and macrophages binding of LPS to TLR4 firstly initiates formation of rafts involving other proteins including myeloid differentiation primary response gene 88 (MyD88) [19]; the proteins within this raft structure in turn generate the signals that ultimately activate the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB). Activation of NFκB causes its translocation to the nucleus where it binds to the response elements in a number of genes, including those encoding classic inflammatory cytokines like tumour necrosis factor (TNF), interleukin (IL)-β, chemokines like IL-8, the PG-producing enzyme cyclooxygenase (COX) 2, inducible nitric oxide synthase, adhesion molecules and matrix metalloproteinases [20]. Ultimately these proteins are produced, establishing and perpetuating the inflammatory response. NFκB is also activated by other inflammatory stimuli including inflammatory cytokines, oxidative stress and ultraviolet irradiation. It is important to note that NFκB is just one of a number of transcription factors and signalling pathways involved in the inflammatory response.

It seems likely that the fatty acid composition of inflammatory cell membrane phospholipids will influence the ability of proteins to move within the plane of the cell membrane and will affect the ability of lipid rafts to form (this will be discussed later with regard to DHA). What this means is that modifying the fatty acid composition of the membranes of cells involved in inflammation could influence the earliest events in inflammatory signalling. There are likely to be at least two other impacts of altering cell membrane fatty acid composition in these cells. Firstly, a range of lipid second messengers are formed from cell membrane phospholipids, including lyso-phospholipids, diacylglycerols and endocannabinoids. The fatty acid composition

of these lipid second messengers affects their biological potency. For example, older data showed that the fatty acid composition of diacylglycerol affected its ability to activate protein kinase C (see Miles and Calder [21] for references), while endocannabinoids containing different fatty acids have different anti-inflammatory potency (see Calder [22] for references). It is obvious that the fatty acid composition of the lipid second messengers is determined by that of their parent phospholipid. Secondly, PUFAs released mainly from the *sn*-2 position of membrane phospholipids act as substrates for COX, lipoxygenase (LOX) and cytochrome P450 enzymes to produce lipid mediators (eicosanoids) active in inflammation including PGs and LTs (Figure 2). In this regard the omega-6 (n-6) PUFA arachidonic acid is the most common cell membrane PUFA and the most common substrate for COX, LOX and cytochrome P450 enzymes. Several mediators formed from arachidonic acid, including $PGD_2$, $PGE_2$, $LTB_4$ and the other 4-series LTs, are well described mediators and regulators of inflammation [23-25]. They act through binding to specific receptors, usually G protein-coupled receptors, and their synthesis and action are targets for a range of non-specific and specific anti-inflammatory pharmaceuticals. There is evidence from studies in both experimental animals [26] and humans [27] that the synthesis of eicosanoids from arachidonic acid is related to the amount of the fatty acid available in cell membranes.

The above considerations indicate that modification of the fatty acid composition of the membranes of cells involved in inflammation could alter inflammatory responses through altered NFκB activation, altered generation or potency of lipid second messengers and altered generation of eicosanoids; in this regard there has been much interest in the effects of EPA and DHA. The phospholipids of blood cells involved in inflammatory processes (e.g. monocytes, lymphocytes (often studied together as mononuclear cells) and neutrophils) taken from humans consuming a typical Western diet typically contain 15 to 20% of fatty acids as arachidonic acid, 0.5 to 1% as EPA and 2 to 3% as DHA [27-38]. Increased intake of EPA and DHA results in increased amounts

of EPA and DHA in these phospholipids [27-38]. This enrichment in EPA and DHA occurs in a time-dependent [27,32-38] and a dose-dependent [27,35,38] manner and is largely at the expense of arachidonic acid (Figures 3 and 4). These changes in fatty acid composition seem to be important in modifying production of lipid mediators and in regulating formation of lipid rafts within membranes in response to an inflammatory stimulus (see later sections).

**N-3 PUFAs and eicosanoids**

Since increased intake of EPA and DHA decreases the amount of arachidonic acid in the membrane phospholipids of cells involved in inflammation (Figures 3 and 4), it is likely that production of arachidonic acid-derived mediators would be decreased simply because of a reduced amount of available substrate. In addition, EPA and DHA have been shown to inhibit arachidonic acid metabolism and to decrease expression of the COX-2 gene and protein [39,40]. In accordance with these arguments, a number of studies in healthy human participants have described decreased production of 2-series PGs and 4 series-LTs by inflammatory cells following use of EPA and DHA in supplements for a period of weeks to months [27-31,41-43]. Similar effects have been described in patients with chronic inflammatory diseases such as RA [44-48] and inflammatory bowel disease [49-53]. These studies have often used quite high doses of EPA and DHA. Data from a dose-response study in healthy participants with an EPA-rich supplement showed that an EPA intake of 1.35 g/day for 3 months was not sufficient to influence *ex vivo* $PGE_2$ production by LPS-stimulated mononuclear cells, whereas an EPA intake of 2.7 g/day significantly decreased $PGE_2$ production [27]. This study suggests a threshold for an anti-inflammatory effect of EPA of somewhere between 1.35 and 2.7 g EPA per day.

Like arachidonic acid, EPA is a substrate for the COX, LOX and cytochrome P450 enzymes that produce eicosanoids (Figure 2). However, because of the structural differences between EPA and arachidonic acid they generate eicosanoids with different structures, typically

containing an additional double bond. Whereas arachidonic acid gives rise to 2-series PGs and 4-series LTs, EPA gives rise to 3-series PGs and 5-series LTs. Increased generation of 5-series LTs has been demonstrated with neutrophils from humans taking EPA and DHA supplements for several weeks [28-30]. Eicosanoids produced from EPA are usually less biologically potent than those produced from arachidonic acid. For example, $LTB_5$ is 10- to 100-fold less potent than $LTB_4$ as a leukocyte chemoattractant [54-56]. One reason for this reduced biological potency is that eicosanoid receptors typically have a lower affinity for the EPA-derived mediator as described by Wada et al. [57]. For example, $PGE_3$ had 20 to 50% of the affinity of $PGE_2$ for the EP1, EP2, EP3 and EP4 receptors. Thus, in general it appears that EPA results in decreased production of potent eicosanoids from arachidonic acid and increased production of generally weak eicosanoids.

**N-3 PUFAs, lipid rafts, NFκB activation and inflammatory cytokines**

As mentioned earlier, NFκB is one of the main transcription factors involved in up-regulation of the genes encoding proteins involved in inflammation including many cytokines, chemokines, adhesion molecules and COX-2 [20,58]. Inactive NFκB is a cytosolic trimer, with one of the subunits being the so-called inhibitory subunit of NFκB (IκB). NFκB is activated through signalling cascades initiated by various extracellular inflammatory stimuli, including LPS binding to TLR4. In response to such stimuli, IκB is phosphorylated and dissociates from the remaining dimer; the dissociated IκB is degraded. The active dimeric NFκB translocates to the nucleus and binds to its DNA response elements upregulating inflammatory gene expression [59]. EPA and fish oil decreased LPS-induced activation of NFκB in isolated monocytes [60-62], which was associated with decreased phosphorylation of IκB [62,63]. Similarly, DHA reduced NFκB activation in response to LPS in cultured macrophages [64] and dendritic cells [65,66]; again this involved decreased phosphorylation of IκB [64]. This suggests an effect of EPA and DHA upstream of IκB phosphorylation. In contrast to these effects of EPA and DHA, some saturated

fatty acids, but particularly lauric acid, were able to increase activation of NFκB and induce COX-2 expression in macrophages [64] and dendritic cells [65]. Lauric acid was not able to activate NFκB or induce COX-2 expression in macrophages that did not express TLR4 [64]. This suggests a direct interaction between lauric acid and TLR4. Similar to their effects in LPS-stimulated macrophages, both EPA and DHA were able to prevent the lauric acid-induced activation of NFκB and expression of COX-2 [64]. MyD88 is a cell membrane-associated adapter protein used by TLR4 in the early stages of the signalling cascade that eventually activates NFκB [19]. DHA did not inhibit COX-2 expression in macrophages not bearing constitutively active MyD88 [64], which indicates that DHA inhibits LPS and lauric acid activation of NFκB upstream of MyD88. When inflammatory cells are stimulated by LPS, TLR4, MyD88 and other signalling proteins associate into lipid rafts [19]. Lauric acid was shown to induce this same raft formation in macrophages [67]. Furthermore, DHA inhibited the ability of both LPS and lauric acid to promote recruitment of signalling proteins, including MyD88, into rafts [67]. These studies demonstrate two key points. Firstly, the differential effects of lauric acid and n-3 PUFAs on TLR4-induced inflammatory signalling that alter activation of NFκB seem to be linked to the ability of those fatty acids to promote or to disrupt raft formation within the membrane of inflammatory cells. Secondly, the well described effects of EPA and DHA on inflammatory gene (and protein) expression may actually be caused by very early events occurring in the cell membrane (Figure 5).

One of the key actions of NFκB is to upregulate expression of inflammatory genes, including those encoding a number of cytokines such as TNF and several ILs. Higher than normal levels of TNF, IL-1β, IL-6 and IL-8 are a common feature of many inflammatory conditions [1,2] and also occur in obesity [15] and with ageing [16]. Effects of EPA and DHA on NFκB activation would be expected to result in decreased production of these cytokines. Indeed, EPA and DHA decreased LPS-stimulated production of IL-6 and IL-8 by cultured human endothelial cells [68,69],

and EPA and fish oil decreased LPS-induced TNF production by cultured monocytes [60-62]. Both EPA and DHA decreased TNF-induced production of IL-6, IL-8, monocyte chemoattractant protein 1 and "regulated upon activation, normal T cell expressed and presumably secreted" by cultured human endothelial cells, with DHA being more potent [40]. Feeding fish oil to mice decreased production of TNF, IL-1β and IL-6 by LPS-stimulated macrophages [70-73] and decreased blood concentrations of TNF, IL-1β and IL-6 following intraperitoneal injection of LPS [74]. Fish oil is also reported to increase the concentration of the anti-inflammatory cytokine IL-10 [75]. Several studies providing EPA and DHA supplements to healthy human participants have reported decreased production of TNF, IL-1β and IL-6 by LPS-stimulated monocytes or mononuclear cells [29,31,41,43], although not all studies report this effect, possibly because the dose of n-3 PUFAs used was too low, although there may be other factors involved such as genotypic differences in responsiveness to n-3 fatty acids [76].

**N-3 PUFAs and peroxisome proliferator activated receptor (PPAR)-γ**

Peroxisome proliferator activated receptor (PPAR)-γ is a transcription factor which has anti-inflammatory effects [77]. Agonists of PPAR-γ reduce murine colitis (78-80) and mice with PPAR-γ knock-down show enhanced susceptibility to chemically-induced colitis [78 PPAR-γ is able to physically interfere with the translocation of NFκB to the nucleus [81]. EPA and DHA can activate PPAR-γ [82-85]. Furthermore, DHA induced PPAR-γ in dendritic cells, which was associated with inhibition of NFκB activation and reduced production of TNF and IL-6 following LPS stimulation [66]. DHA also induced a number of PPAR-γ target genes in dendritic cells [86]. The EPA derivatives $PGD_3$ and 15-deoxy-$PGD_3$ activated PPAR-γ in adipocytes, which is linked to the induction of the anti-inflammatory adipokine adiponectin [87]. These observations suggest that one mechanism of the anti-inflammatory action of EPA and DHA is activation of PPAR-

γ. This may be another means through which these fatty acids inhibit NFκB activation (Figure 5).

**N-3 PUFAs and specialised pro-resolving mediators**

Both EPA and DHA are substrates for the synthesis of specialised pro-resolving lipid mediators (SPMs) (Figure 6). SPMs include the E- and D-series resolvins produced from EPA and DHA, respectively, and protectins (aka neuroprotectins) and maresins produced from DHA. Generation of classic eicosanoids involves either the COX or LOX pathways operating separately from one another (Figure 2). However, SPMs are synthesised using COX and LOX enzymes in the same pathway (Figure 6). Furthermore, synthesis of many SPMs is promoted by aspirin and different epimers are produced in the presence and absence of aspirin [88-90]. Both aspirin-triggered and non-aspirin-triggered SPMs have biological activity [88-90]. A variety of SPMs have been reported in human blood [91-93], including umbilical cord blood [94,95], adipose tissue [96], breast milk [97] and synovial fluid [98]. Supplemental EPA and DHA has been shown to result in higher concentrations of some SPMs in human blood [91-93,99]. Ostermann et al. [100] demonstrated a dose-dependent increase in the plasma concentrations of multiple precursors of SPMs in healthy participants supplemented with different doses of EPA+DHA for one year (Figure 7), although the SPMs themselves were not detected. Maternal supplementation with high dose fish oil during pregnancy resulted in higher concentrations of the SPM precursors 17-hydroxy DHA and 18-hydroxy EPA, but not of the SPMs themselves, in umbilical cord blood [94]. Patients with peripheral artery disease showed increased plasma resolvin E3 after 3 months supplementation with high dose EPA+DHA [101]. In healthy participants, single dosing with an SPM-enriched fish oil resulted in dose-dependent elevation in plasma SPM concentrations over the following hours [102].

The biological effects of SPMs have been examined extensively in cell culture and animal models of inflammation [88-90] and are now beginning to be explored in humans. The cell and animal models have shown that all SPMs tested to date have anti-inflammatory and inflammation resolving actions. For example, resolvin E1, resolvin D1 and protectin D1 all inhibited transendothelial migration of neutrophils, so preventing the infiltration of neutrophils into sites of inflammation; resolvin D1 inhibited IL-1β production; and protectin D1 inhibited TNF and IL-1β production [88-90]. Resolvins reduce inflammation and protect experimental animals in models of inflammatory disease including arthritis [103,104], colitis [105], asthma [106-109] and other states of inflammation including sepsis [110,111] and acute lung injury [112-114]. The potent activity of SPMs may explain many of the documented actions of EPA and DHA in inflammation.

**N-3 PUFAs and rheumatoid arthritis (RA)**

RA is a chronic inflammatory disease that affects the joints, with infiltration of inflammatory cells [115], increased expression of both COX-1 and COX-2 in the synovium along with high concentrations of pro-inflammatory eicosanoid products of arachidonic acid metabolism (e.g. $PGE_2$) in the synovial fluid [116] and high concentrations of pro-inflammatory cytokines including TNF, IL-1β, IL-6, IL-8 and granulocyte/macrophage colony stimulating factor in the synovial fluid and circulation [117]. These observations indicate that RA may be a target for EPA+DHA. Mice fed fish oil had delayed onset and reduced incidence and severity of collagen-induced arthritis compared to mice fed vegetable oil [118]. EPA and DHA both suppressed streptococcal cell wall-induced arthritis in rats, with EPA being more effective [119]. Both fish oil and krill oil slowed the onset of collagen-induced arthritis in mice, decreasing its severity, paw swelling, and knee joint pathology compared with the control group [120]. EPA and DHA supplements lowered blood concentrations of inflammatory cytokines [121-124] and eicosanoids [46,125,126] in patients with RA. In a recent trial, DHA at 2.1 g/day for 10 weeks increased plasma concentrations of SPM

precursors (14- and 17-hydroxy DHA) in patients with RA [127]. These effects should reduce pain and cartilage destruction; if pain is reduced then patients may decrease their use of pain-controlling drugs like non-steroidal anti-inflammatory drugs (NSAIDs). In agreement with this, Cleland et al. [47] reported that patients with RA who used fish oil supplements were more likely to reduce use of NSAIDs and to be in remission than those patients who did not use fish oil. A number of randomised controlled trials of fish oil in RA report improvements in several clinical outcomes including reduced duration of morning stiffness, reduced number of tender or swollen joints, reduced joint pain, reduced time to fatigue, increased grip strength and decreased use of NSAIDs (see [128,129] for references). The dose of EPA+DHA used in these trials has usually been quite high, between about 1 and 7 g per day, averaging about 3.5 g per day. In keeping with the difference in prevalence of RA between males and females, most of these studies recruited many more females than males; no trials have investigated or compared the effect of n-3 fatty acids in females and males with RA. Several systematic reviews and meta-analyses of trials of fish oil in patients with RA have been conducted [130-133]. One meta-analysis included data from nine trials published between 1985 and 1992 inclusive and from one unpublished trial and concluded that dietary fish oil supplementation for 3 months significantly reduced tender joint count and morning stiffness [130]. A second meta-analysis of n-3 fatty acids and pain included data from 17 trials [131]; this analysis indicated that fish oil reduces patient assessed joint pain, duration of morning stiffness, number of painful and/or tender joints, and use of NSAIDs. In 2017 a systematic review [132] and a systematic review and meta-analysis (133) of n-3 PUFA supplements and arthritic pan were published. The first of these included 18 randomised controlled trials that used between 2.1 ad 9.1 g EPA+DHA/day for durations of 12 to 52 weeks. Ten of these studies supported the hypothesis that n-3 PUFAs reduce patient or physician assessment of pain in patients with RA [132]. The meta-analysis of 22 trials identified a significant reduction in pain with n-3 PUFAs in patients with RA [133]. Thus, based upon the findings of

individual trials and meta-analyses of those findings, there is fairly robust evidence of the efficacy of n-3 fatty acids in RA, although high doses seem to be needed. A recent trial using foods enriched with algal oil providing 2.1 g DHA per day for 10 weeks in RA patients maintaining their medication regimen found a significant reduction in tender and swollen joints, disease activity score and ultrasound score [127].

One study has examined the relationship between EPA status and responsiveness of patients with RA to treatment with antibodies against TNF [134]. It was identified that the greatest reduction in disease activity score at 12 weeks of treatment was seen in those in the highest tertile of plasma phospholipid EPA. Furthermore, plasma phospholipid EPA was significantly positively associated with European League Against Rheumatism response. These observations indicate that response to anti-TNF antibodies is better in those with higher EPA status. An increase in Th17 cells has been associated with non-response to anti-TNF antibodies. *In vitro* incubation of blood mononuclear cells with the antibodies increased the frequency of Th17 cells, but co-incubation with EPA prevented this. It was suggested that increasing EPA status might improve response of patients with RA to anti-TNF antibodies by preventing generation of pro-inflammatory Th17 cells.

**Decreased inflammation with n-3 PUFAs: any concern about infection?**

Because inflammation is the earliest stage of the host response to infection, whether the anti-inflammatory effects of n-3 PUFAs increase susceptibility to infection requires consideration. This is discussed in some detail elsewhere [135, 136, 137], although the focus has been mainly on studies in mice, rats and guinea pigs. The conclusion of the most recent discussion [137], based on these animal studies, is that low dose EPA+DHA "is beneficial against experimental infections caused by extracellular pathogens [which induce a strong inflammatory response] including *Staphylococcus pneumoniae, Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus*

*aureus* by reducing inflammation". A concern was raised about higher doses of EPA+DHA and infections by intracellular pathogens such as *Mycobacterium tuberculosis*, *Salmonella*, influenza virus and Herpes simplex virus [137]. Since these conclusions are based on animal studies, where the extrapolation of dietary intakes to humans is uncertain, and where there are also other dietary differences as well as differences in gut microbiota, lipid metabolism and the immune system, they must be treated cautiously. Follow-up over 10 years of 38,378 male US health professionals aged 44 to 79 years at study outset identified no significant relationship between EPA or DHA intake and risk of community acquired pneumonia [138]. In a cohort of 63,257 Chinese men and women aged 45 to 74 years recruited between 1993 and 1998, intake of EPA+DHA was associated with a reduced risk of developing active tuberculosis in the follow-up period to 2013 in a dose-dependent manner [139]. Two intervention studies in children indicate that EPA+DHA do not increase, and may even decrease, the risk of infections. Thai schoolchildren aged 9 to 12 years consuming milk fortified with fish oil providing 200 mg EPA + 1 g DHA daily on 5 days per week for 6 months had significantly fewer episodes and shorter duration of illness (mainly upper respiratory tract) than the placebo group [140]. Iron-deficient South African schoolchildren aged 6 to 11 years received iron, EPA+DHA (80 mg EPA + 420 mg DHA) or iron+EPA+DHA four times per week for 8.5 months [141]. Iron supplementation increased the number of days with illness and illness caused by respiratory symptoms, whereas EPA+DHA reduced the number of days with illness at school. Furthermore, the combination of EPA+DHA with iron prevented the adverse effect of iron alone. Studies of n-3 PUFAs and infection in adults have mainly been studied in hospitalised patients. Intravenous administration of lipid emulsions containing fish oil to surgical patients has been reported to reduce infections in a number of trials; as discussed elsewhere [142] six meta-analyses published between 2010 and 2018 all report significantly reduced risk of infection with odds/risk ratios of between 0.36 and 0.56 compared with placebo. In critically ill patients the evidence for intravenous fish oil is less clear [142], but the most recent

meta-analysis [143] identified a risk ratio of 0.64 for infections in patients receiving intravenous fish oil compared to placebo. Enteral feeds containing fish oil in combination with other bioactive nutrients have been demonstrated through meta-analysis to reduce infections in surgical [144] and critically ill [145] patients.

**Summary and conclusions**

Inflammation is a normal part of the immune response and should be self-limiting. It involves a multitude of cell types, chemical mediators, and interactions. Excessive or unresolved inflammation is linked to tissue damage, pathology and many conditions of ill health. Chemical mediators produced from PUFAs are known to play a role in the initiation, perpetuation and termination of inflammatory responses and changes in fatty acid composition can modify lipid raft formation and cell signalling leading to altered gene expression and an altered pattern of lipid mediator production. Cells involved in the inflammatory response are typically rich in the n-6 PUFA arachidonic acid, but the contents of arachidonic acid and of EPA and DHA can be altered through oral administration of EPA and DHA. Eicosanoids produced from arachidonic acid, like $PGE_2$ and 4-series LTs, have roles in inflammation. EPA also gives rise to eicosanoids but these are usually less potent than those produced from arachidonic acid. EPA and DHA give rise to resolvins, and DHA to protectins and maresins which are anti-inflammatory and inflammation resolving. The effects of EPA and DHA on inflammatory gene expression are due at least in part to reduced activation of NFκB which seems to relate to membrane-mediated events including inhibition of lipid raft formation in response to inflammatory triggers. Dose-dependent actions of n-3 PUFAs on inflammatory responses have not been well described, but it appears that a dose of at least 2 g per day is necessary to achieve an anti-inflammatory effect in humans. As a result of their anti-inflammatory actions, EPA and DHA may have therapeutic efficacy in inflammatory diseases. Work with animal models of RA has demonstrated efficacy of fish oil and of mediators

derived from EPA and DHA, like some of the resolvins. There have been a number of clinical trials of fish oil in patients with RA and these trials have typically used high doses of EPA+DHA, often above the anti-inflammatory threshold of 2 g per day. Many trials in RA report clinical improvements (e.g. improved patient assessed pain, decreased morning stiffness, fewer painful or tender joints, decreased use of NSAIDs), and when the trials have been pooled in meta-analyses statistically significant clinical benefit has emerged [130-133]. Thus, based upon the findings of individual trials and meta-analyses of those findings, there is fairly robust evidence of the efficacy of n-3 fatty acids in RA, although high doses seem to be needed. Decreasing inflammation with n-3 PUFAs appears not to be associated with an impairment of host defence where that has been tested in humans, and may even enhance protection. Trials of EPA+DHA and infection in adults in the community (i.e. non-hospitalised) are warranted.

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**Figure captions**

Figure 1. Schematic representation of self-limiting and chronic inflammation. Modified from Prostaglandins Leukotrienes and Essential Fatty Acids, Vol 131, J.K. Innes and P.C. Calder, Omega-6 fatty acids and inflammation, pp 41-48, Copyright 2018, with permission from Elsevier. [5]

Figure 2. Summary of eicosanoid synthesis from arachidonic and eicosapentaenoic acids. COX, cyclooxygenase; Cyt P450, cytochrome P450; HETE, hydroxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane.

Figure 3. Time-dependent changes in eicosapentaenoic acid (EPA) and arachidonic acid content in human mononuclear cells. Healthy human participants consumed fish oil providing 2.1 g EPA and 1.1 g DHA per day for 1 week [37] or for 12 weeks [32]. Participants in Faber et al. [37] were 6 females and 6 males while participants in Yaqoob et al. [32] were 1 female and 7 males. Blood was sampled at several time points in each study and mononuclear cells prepared. Fatty acid composition of the cells was determined by gas chromatography. Mean values are shown. Squares represent EPA and triangles represent arachidonic acid. Black symbols represent data from Faber et al. [37] and grey symbols represent data from Yaqoob et al. [32]. Reprinted from Biochimica et Biophysica Molecular and Cell Biology of Lipids, Vol 1851, P.C. Calder, Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance, pp 469-484, Copyright 2015, with permission ofromElsevier. [22]

Figure 4. Dose-dependent changes in eicosapentaenoic acid (EPA) and arachidonic acid content in human mononuclear cells. Healthy males consumed a supplement providing 0, 1.35,

2.7 or 4.05 g EPA per day for 12 weeks (n = 15 or 16 per group). Blood was sampled at 0 and 12 weeks and mononuclear cells prepared. Fatty acid composition of the cells was determined by gas chromatography. Mean values for change from week 0 are shown; data for arachidonic acid have been normalised so that the change from week 0 in the group receiving no supplemental EPA is zero. Squares represent EPA and triangles represent arachidonic. Data are for the older males reported in Rees et al. [27] Reprinted from Biochimica et Biophysica Molecular and Cell Biology of Lipids, Vol 1851, P.C. Calder, Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance, pp 469-484, Copyright 2015, with permission from Elsevier. [22]

Figure 5. Overview of the key anti-inflammatory actions of EPA and DHA. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PPAR, peroxisome proliferator activated receptor; TLR, toll-like receptor.

Figure 6. Overview of the synthesis of specialised pro-resolving lipid mediators from eicosapentenoic acid (EPA) and docosahexaenoic acid (DHA). COX, cyclooxygenase; Cyt P450, cytochrome P450 enzymes; LOX, lipoxygenase; MaR, maresin; P, protectin; Rv, resolvin.

Figure 7. Plasma concentrations of three precursors of specialised pro-resolving mediators in humans. Healthy human participants consumed fish oil providing different amounts eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) per week for one yr. The different groups comprised 14 males and 14 females (placebo), 17 males and 18 females (3.27 g EPA+DHA/week), 14 males and 15 females (6.54 g EPA+DHA per week) and 15 males

and 14 females (13.08 g EPA+DHA/week). Lipid mediator concentrations were determined by liquid chromatography-mass spectrometry. Circles represent 8-hydroxy-EPA; squares represent 14-hydroxy-DHA; triangles represent 17-hydroxy-DHA. Data are taken from Ostermann et al. [100].