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Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?

Philip C. Calder

Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, MP887 Southampton General Hospital, Southampton, United Kingdom

Correspondence
Professor Philip C. Calder, Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, IDS Building, MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK.
Tel.: +44 23 8079 5250
Fax: +44 23 8079 5255
E-mail: pcc@soton.ac.uk

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Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are n-3 fatty acids found in oily fish and fish oil supplements. These fatty acids are able to inhibit partly a number of aspects of inflammation including leucocyte chemotaxis, adhesion molecule expression and leucocyte-endothelial adhesive interactions, production of eicosanoids like prostaglandins and leukotrienes from the n-6 fatty acid arachidonic acid, production of inflammatory cytokines and T cell reactivity. In parallel, EPA gives rise to eicosanoids that often have lower biological potency than those produced from arachidonioc acid and EPA and DHA give rise to anti-inflammatory and inflammation resolving resolvins and protectins. Mechanisms underlying the anti-inflammatory actions of n-3 fatty acids include altered cell membrane phospholipid fatty acid composition, disruption of lipid rafts, inhibition of activation of the pro-inflammatory transcription factor nuclear factor kappa B so reducing expression of inflammatory genes, activation of the anti-inflammatory transcription factor NR1C3 (i.e. peroxisome proliferator activated receptor γ) and binding to the G protein coupled receptor GPR120. These mechanisms are interlinked. In adult humans, an EPA plus DHA intake greater than 2 g day^{-1} seems to be required to elicit anti-inflammatory actions, but few dose finding studies have been performed. Animal models demonstrate benefit from n-3 fatty acids in rheumatoid arthritis (RA), inflammatory bowel disease (IBD) and asthma. Clinical trials of fish oil in patients with RA demonstrate benefit supported by meta-analyses of the data. Clinical trails of fish oil in patients with IBD and asthma are inconsistent with no overall clear evidence of efficacy.

Inflammation: a brief overview

Inflammation is a component of the normal host defence mechanism that provides protection from infection and other insults. Inflammation initiates the killing of pathogens and is involved in the processes of tissue repair so aiding restoration of homeostasis at infected or damaged sites. The inflammatory response involves interactions amongst many cell types and the production of, and responses to, a number of chemical mediators. These mediators are damaging to pathogens but may also cause damage to host tissues. It is the influx of cells into the site of inflammatory activity and the presence of the inflammatory mediators produced as a result that are responsible for the cardinal signs of inflammation: redness, swelling, heat, pain and loss of function. However, the inflammatory response is normally well regulated in order that it does not cause excessive damage to the host. It is self-limiting and resolves rapidly due to the activation of negative feedback mechanisms (e.g. secretion of anti-inflammatory cytokines or resolving lipid mediators), the inhibition of pro-inflammatory signalling cascades, the shedding of receptors for inflammatory mediators and the activation of regulatory cells. Thus, when operating properly, inflammatory responses are essential to protect the host. Nevertheless, loss of the usual regulatory processes can result in excessive, inappropriate or on-going inflammation that can cause

irreparable damage to host tissues. As a result disease can occur.

Inflammation may be classified as acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is characterized by the increased movement of plasma and leucocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events prolongs and matures the inflammatory response. These events involve the local vascular system, the immune system and various cells within the injured tissue. Typically acute inflammation will resolve within a period of hours to days. If it is prolonged, it is termed chronic inflammation and this may last for years. Chronic inflammation involves a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue by the on-going inflammatory process, although the result may be pathological indicating that the destruction predominates over the healing.

Irrespective of the cause of the inflammation, the response involves four major general events. The first is an increased supply of blood to the site of inflammation. The second is an increase in permeability of the vascular wall caused by opening of junctions between endothelial cells. This allows plasma and large molecules to cross the endothelium, so delivering soluble mediators to the site of inflammation. Thirdly, leucocytes migrate from the blood stream into the surrounding tissue, a process promoted by release of chemo-attractants from the site of inflammation and by the up-regulation of adhesion molecules on the endothelium. Finally leucocytes release chemical mediators at the site of inflammation. These mediators may include lipid-derived mediators (e.g. prostaglandins (PGs), leukotrienes (LTs), endocannabinoids, platelet activating factor), peptide mediators (e.g. cytokines, chemokines), reactive oxygen species (e.g. superoxide), amino acid derivatives (e.g. histamine) and enzymes (e.g. matrix proteases) depending upon the cell type involved, the nature of the inflammatory stimulus, the anatomical site involved and the stage during the inflammatory response. Normally these mediators play a role in host defence. However, when produced inappropriately or in an unregulated fashion they can cause damage to host tissues, leading to disease. Some of the mediators amplify the inflammatory process acting, for example, as chemo-atttactants. Some of the inflammatory mediators escape the inflammatory site into the circulation and from there they can exert systemic effects. For example, hepatic acute phase protein synthesis is regulated by cytokines like interleukin (IL)-6 released from inflammatory loci.

Inflammation is a recognized contributor to the pathology of many conditions. In some cases, such as rheumatoid arthritis (RA), inflammatory bowel diseases (IBD) and asthma, the central role of inflammation to the pathology is well recognized. Individuals with these conditions have heavy infiltration of inflammatory cells at the site of disease activity (e.g. the joints, the intestinal mucosa, the lungs) and they have elevated concentrations of inflammatory mediators at those sites and in the systemic circulation. These conditions are treated with varying levels of success by anti-inflammatory drugs. In other cases, such as atherosclerosis [1, 2] and obesity [3, 4], the role of inflammation has emerged more recently and its contribution to the pathology alongside the many other factors involved is less clear. Individuals with these conditions show infiltration of inflammatory cells at the site of disease activity (e.g. the blood vessel wall, adipose tissue), have moderately elevated concentrations of inflammatory mediators in the systemic circulation, but are not treated, primarily, with anti-inflammatory drugs.

Omega-3 (n-3) polyunsaturated fatty acids: a brief overview

The term omega-3 (ω-3 or n-3) is a structural descriptor for a family of polyunsaturated fatty acids (PUFAs). n-3 signifies the position of the double bond that is closest to the methyl terminus of the acyl chain of the fatty acid. All n-3 fatty acids have this double bond on carbon 3, counting the methyl carbon as carbon one. Like other fatty acids, n-3 fatty acids have systematic and common names, but they are frequently referred to by a shorthand nomenclature that denotes the number of carbon atoms in the chain, the number of double bonds, and the position of the first double bond relative to the methyl carbon. The simplest n-3 fatty acid is α-linolenic acid (18:3n-3). α-linolenic acid is synthesized from the n-6 fatty acid linoleic acid (18:2n-6) by desaturation, catalyzed by delta-15 desaturase (Figure 1). Animals, including humans, do not possess the delta-15 desaturase enzyme and so cannot synthesize α-linolenic acid. In contrast, plants possess delta-15 desaturase and so are able to synthesize α-linolenic acid. Although animals cannot synthesize α-linolenic acid, they can metabolize it by further desaturation and elongation. Desaturation occurs at carbon atoms below carbon number 9 (counting from the carboxyl carbon) and mainly occurs in the liver. As shown in Figure 1, α-linolenic acid can be converted to stearidonic acid (18:4n-3) by delta-6 desaturase and then stearidonic acid can be elongated to eicosatetraenoic acid (20:4n-3), which can be further desaturated by delta-5 desaturase to yield eicosapentaenoic acid (20:5n-3; known as EPA). It is important to note that the conversion of α-linolenic acid to EPA is in competition with the conversion of linoleic acid to arachidonic acid (20:4n-6) since the same enzymes are used (Figure 1). The delta-6 desaturase reaction is rate limiting in this pathway. The preferred substrate for delta-6 desaturase is α-linolenic acid. However, linoleic acid is much more prevalent in most human diets than

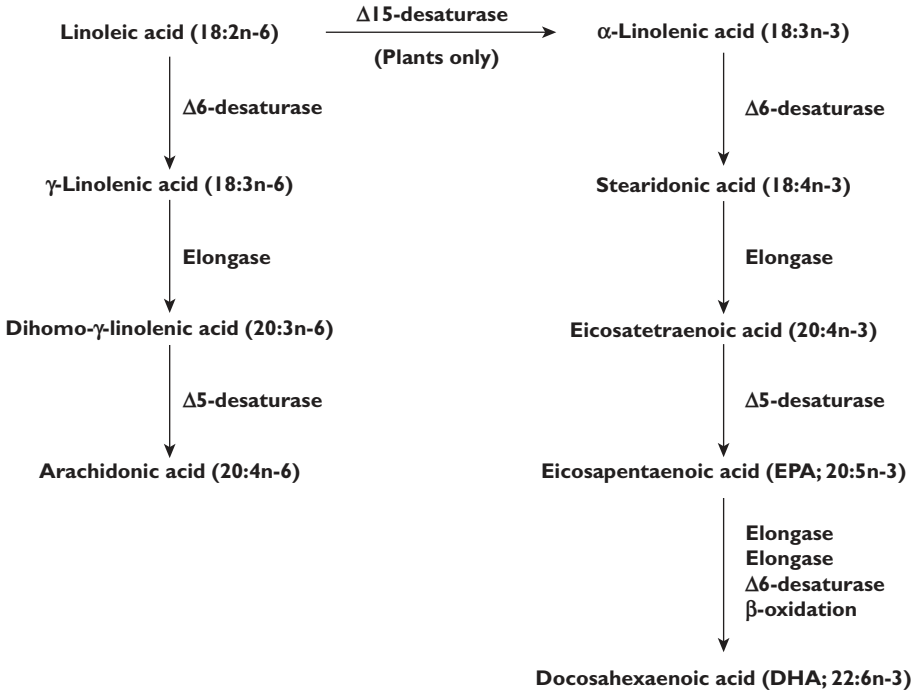

Figure 1

The conversion of plant essential n-6 and n-3 polyunsaturated fatty acids to their longer chain, more unsaturated derivatives. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid

α-linolenic acid, and so metabolism of n-6 fatty acids is quantitatively the more important. The activities of delta-6 and delta-5 desaturases are regulated by nutritional status, hormones and by feedback inhibition by end products. The pathway for conversion of EPA to docosahexaenoic acid (22:6n-3; known as DHA) involves addition of two carbons to EPA to form docosapentaenoic acid (22:5n-3; known as DPA), addition of two further carbons to produce 24:5n-3, desaturation at the delta-6 position to form 24:6n-3, translocation of 24:6n-3 from the endoplasmic reticulum to peroxisomes where two carbons are removed by limited β-oxidation to yield DHA (summarized in Figure 1). Short term studies with isotopically-labelled α-linolenic acid and long term studies using significantly increased intakes of α-linolenic acid have demonstrated that the conversion to EPA, DPA and DHA is generally poor in humans, with very limited conversion all the way to DHA being observed [5, 6]. EPA, DPA and DHA are found in significant quantities in fish (see below), and so in this article these three fatty acids are collectively referred to as marine n-3 PUFAs.

Because α-linolenic acid is produced in plants, green leaves and some plant oils, nuts and seeds contain moderate to high amounts of it and usually α-linolenic acid is the major n-3 fatty acid consumed in most human diets [7]. However, the main PUFA in most Western diets is the n-6 linoleic acid which is typically consumed in 5 to 20-fold greater amounts than α-linolenic acid [7]. Seafoods are a good source of marine n-3 PUFAs [7]. These fatty acids are found in the flesh of both lean and oily fish, with much greater amounts in the latter, and in the livers of some lean fish (e.g. cod). In people who eat little fish, intakes of marine n-3 PUFAs are low (typically <0.2 g day^{-1} [7] and probably much lower than this [8]). A single lean fish meal (e.g. one serving of cod) could provide about 0.2 to 0.3 g of marine n-3 fatty acids, while a single oily fish meal (e.g. one serving of salmon or mackerel) could provide 1.5 to 3.0 g of these fatty acids. Fish oil is prepared from the flesh of oily fish (e.g. tuna) or from the livers of lean fish (e.g. cod liver). In a typical fish oil supplement EPA and DHA together comprise about 30% of the fatty acids present, so that a 1 g fish oil capsule will provide about 0.3 g of EPA + DHA. However, the amount of n-3 fatty acids can vary between fish oils, as can the relative proportions of the individual marine n-3 PUFAs. Encapsulated oil preparations that contain n-3 fatty acids in higher amounts than found in standard fish oils are available ('fish oil concentrates'). In fish oil capsules the fatty acids are usually present in the form of triacylglycerols. However, marine n-3 fatty acids are also available in the phospholipid form (e.g. in krill oil) and as ethyl esters (e.g. in the highly concentrated pharmaceutical preparation Omacor® (Pronova Biocare, Lysaker, Norway), known as Lovaza® (GlaxoSmithKline, St Petersberg, FL, USA) in North America).

Omega-3 (n-3) polyunsaturated fatty acids and inflammatory processes

The influence of marine n-3 PUFAs on the functional responses of the various cell types involved in inflammation and on the production of the range of chemical mediators produced has been studied for many years and a number of effects have been reported. Typically the effects observed are highly suggestive that marine n-3 PUFAs act in an anti-inflammatory manner, with more recent studies suggesting that they may be involved in the resolution of inflammation. The anti-inflammatory effects of marine n-3 fatty acids are widely reviewed [9–12].

Omega-3 (n-3) polyunsaturated fatty acids and leucocyte chemotaxis

Chemotaxis is the process by which leucocytes move towards a site of inflammatory activity in response to the release of chemicals at that site. Such chemicals are termed chemo-attractants and include the arachidonic acid-derived eicosanoid LTB_4. Studies of fish oil supplementation in healthy human subjects have demonstrated a decrease in chemotaxis of neutrophils and monocytes towards various chemo-attractants including LTB_4, bacterial peptides and human serum [13–18]. These studies used between 3 and 15 g EPA + DHA day^{-1}, although a dose–response study by Schmidt *et al.* [19] suggested that near-maximum inhibition of chemotaxis occurs at an intake of 1.3 g EPA + DHA day^{-1}. The mechanism by which marine n-3 PUFAs inhibit chemotaxis is not clear but may relate to reduced expression or antagonism of receptors for chemo-attractants.

Omega-3 (n-3) polyunsaturated fatty acids, adhesion molecule expression and leucocyte adhesive interactions with the endothelium

Adhesion molecules are proteins that are expressed on the surface of endothelial cells and leucocytes. These molecules form ligand pairs that promote interaction between the different cell types. It is through these interactions that leucocytes in the bloodstream interact with the blood vessel wall and then leave the bloodstream to move to a site of inflammatory activity. Cell culture [20–23] and animal feeding studies [23–25] report decreased expression of some adhesion molecules on the surface of monocytes [22], macrophages [24], lymphocytes [25] and endothelial cells [20, 21, 23] following exposure to marine n-3 PUFAs. In some cases this was shown to result in decreased adhesion between leucocytes and endothelial cells [20, 23, 25]. EPA and DHA both reduced the adhesive interaction between monocytes and endothelial cell monolayers studied under flow conditions [26, 27]. Supplementing the diet of healthy humans with fish oil providing about 1.5 g EPA + DHA day^{-1} resulted in a lower level of expression of intercellular adhesion molecule (ICAM)-1 on the surface of blood monocytes stimulated *ex vivo* with interferon-γ [28]. Consumption of 1.8 g EPA + DHA day^{-1} by patients with peripheral vascular disease decreased the adhesive interaction of their monocytes to endothelial monolayers in culture [29]. Dietary fish oil providing 1.1 g EPA + DHA day^{-1} was found to decrease circulating levels of soluble vascular cell adhesion molecule (VCAM)-1 in elderly subjects [30], but it is not clear if this represents decreased surface expression of VCAM-1. EPA (1.8 g day^{-1}) decreased the concentrations of soluble ICAM-1 and soluble VCAM-1 in the bloodstream of patients with metabolic syndrome [23].

Omega-3 (n-3) polyunsaturated fatty acids and eicosanoid production

The eicosanoid family of lipid mediators includes oxidized derivatives of 20-carbon PUFAs, principally arachidonic acid (20:4n-6). The precursor fatty acid is released from the membrane phospholipids of inflammatory cells. PGs, thromboxanes and LTs are eicosanoids. Usually by far the most common 20-carbon PUFA in the membrane phospholipids of macrophages, neutrophils and lymphocytes is the n-6 PUFA arachidonic acid, and so arachidonic acid is the usual eicosanaoid precursor (Figure 2). Arachidonic acid is released from the phospholipids through the action of phospholipase A_2 enzymes, which are activated by inflammatory stimuli. The free arachidonic acid then acts as a substrate for cyclo-oxygenase (COX), lipoxygenase (LOX) or cytochrome P450 enzymes. COX enzymes lead to PGs and thromboxanes, LOX enzymes to LTs, and cytochrome P450 enzymes to hydroxyeicosatetraenoic and epoxyeicosatrienoic acids. Eicosanoids are long recognized as key mediators and regulators of inflammation [31–33] acting via specific receptors, usually G protein-coupled receptors, and their synthesis and action are targets for a range of non-specific and specific pharmaceuticals.

Animal studies have shown that production of arachidonic acid-derived eicosanoids like PGE_2 is decreased by EPA or DHA feeding [34–36]. Numerous studies with healthy human volunteers have described decreased production of PGE_2 and 4 series-LTs by inflammatory cells following use of fish oil supplements for a period of weeks to months [13–15, 37–40]. Similar effects of fish oil are seen in patients with chronic inflammatory diseases such as RA [41–45] and IBD [46–50]. The studies in humans demonstrating that oral marine n-3 PUFAs decrease production of arachidonic acid-derived eicosanoids have usually used fairly high intakes of EPA + DHA, most often several grams per day. A dose–response study in healthy volunteers reported that an EPA intake of 1.35 g day^{-1} for 3 months was not sufficient to influence *ex vivo* PGE_2 production by endotoxin-stimulated mononuclear cells, whereas an EPA intake of 2.7 g day^{-1} did significantly decrease PGE_2 production [51], suggesting a threshold for

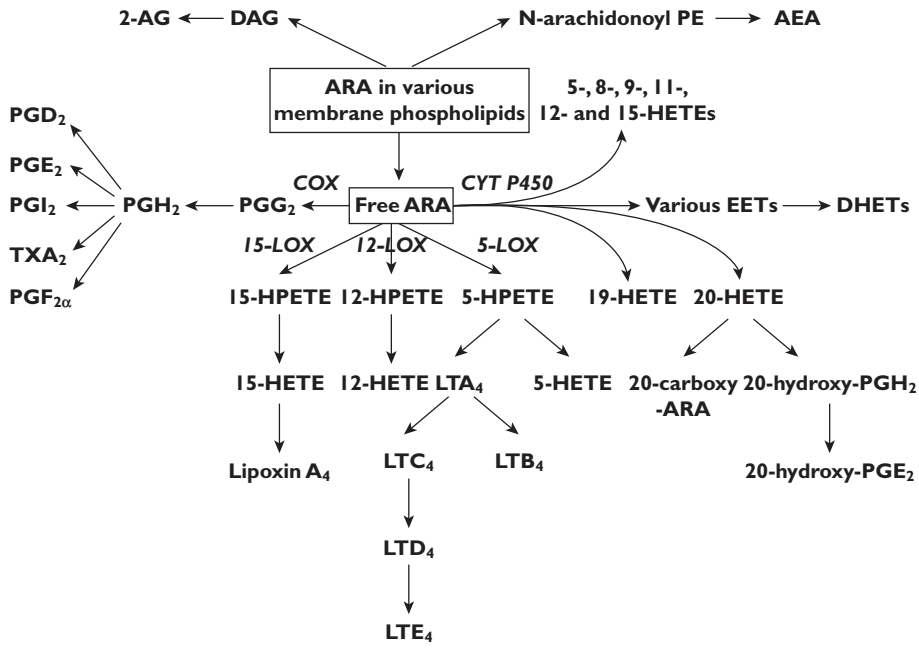

Figure 2

The pathways of eicosanoid synthesis from arachidonic acid. AEA, arachidonoyl ethanolamine (anandamide); 2-AG, 2-arachidonoyl glycerol; ARA, arachidonic acid; COX, cyclo-oxygenase; CYT p450, cytochrome P450 enzymes; DAG, diacylglycerol; DHET, dihydroxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; LOX, lipoxygenase; LT, leukotriene; PE, phosphatidyl ethanolamine; PG, prostaglandin; TX, thromboxane. Note that not all enzymes are named and that not all metabolites are shown

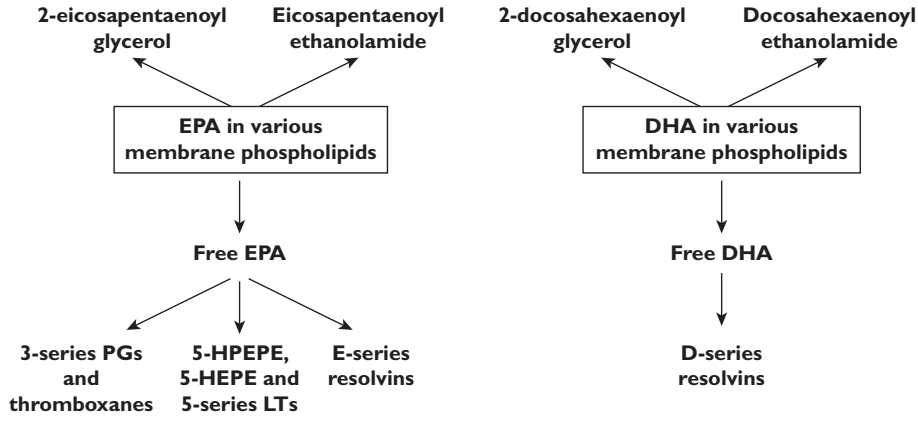

Figure 3

Overview of the pathways of lipid mediator synthesis from eicosapentaenoic and docosahexaenoic acids. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HPEPE, hydroperoxyeicosapentaenoic acid; LT, leukotriene; PG, prostaglandin. Note that the enzymes involved are not named and that not all metabolites are shown

an anti-inflammatory effect of somewhere between 1.35 and 2.7 g EPA day^{-1}.

Because EPA is a 20-carbon highly unsaturated fatty acid it is also a substrate for the COX, LOX and cytochrome P450 enzymes that produce eicosanoids (Figure 3). However, the mediators produced have a different structure from those made from arachidonic acid (e.g. PGE_3 rather than PGE_2 and LTB_5 rather than LTB_4). Increased generation of 5-series LTs has been demonstrated using macrophages from fish oil-fed mice [34] and neutrophils from humans taking fish oil supplements for several weeks [13–15]. Transgenic ('*fat*-1') mice bearing the *C. elegans* 'n-3 desaturase' gene and so able to convert n-6 to n-3 PUFAs resulting in greatly elevated n-3 PUFA content in their

tissues, were shown to generate large amounts of PGE_3 within colonic tissue after chemical induction of colonic inflammation [52]. The functional significance of generation of eicosanoids from EPA is that EPA-derived mediators are often much less biologically active than those produced from arachidonic acid [53–55]. One reason for this reduced biological potency is that eicosanoid receptors typically have a lower affinity for the EPA-derived mediator than for the arachidonic acid-derived one. This was explored in detail by Wada *et al*. [56] who identified, for example, 50 to 80% lower potency of PGE_3 compared with PGE_2 towards the EP_1, EP_2, EP_3 and EP_4 receptors. Thus, EPA results in decreased production of potent eicosanoids from arachidonic acid and increased production of weak eicosanoids. One exception to this is that the DP_1 receptor prefers PGD_3 over PDG_2 [56]. This most likely explains observations of Tull *et al*. [26] studying neutrophil binding to endothelial monolayers under flow conditions. This adhesive interaction was diminished by inhibition of COX, by EPA, by PGD_3 and by a DP_1 antagonist. The inhibitory effects of EPA were overcome by co-addition of arachidonic acid, PGD_2 or a DP_1 agonist. It was concluded that PGD_2 promotes the adhesive interaction acting via DP_1 on neutrophils and that, in the presence of EPA, PGD_3 is formed and binds to DP_1 so preventing the action of PGD_2, but not initiating an active response itself. The work of Wada *et al*. [56] demonstrating greater binding of PGD_3 than PGD_2 to DP_1 may explain how PGD_3 can prevent the action of PGD_2.

Omega-3 (n-3) polyunsaturated fatty acids and endocannabinoids

Endocannabinoids are complex eicosanoids [57]. While PGs, thromboxanes and LTs are synthesized from 20-carbon fatty acids released from membrane phospholipids by phospholipase A_2, endocannabinoids are produced by cleavage of phospholipids by other phospholipases [57]. The two major arachidonic acid containing endocannabinoids are arachidonoyl ethanolamide (AEA), also known as anandamide, and 2-arachidonoyl glycerol (2-AG). AEA is formed by a pair of reactions involving conversion of phosphatidylethanolamine to N-acyl-phosphatidylethanolamine followed by the action of phospholipase D. 2-AG is formed as a result of the sequential actions of phospholipase C and a diacylglycerol lipase. AEA and 2-AG act via the CB_1 and CB_2 receptors [57]. Both AEA and 2-AG have anti-inflammatory properties [58, 59]. Increased availability of marine n-3 PUFAs in the diets of laboratory animals results in lower concentrations of AEA and 2-AG [60–62]. Conversely, and mirroring what is seen in the classical eicosanoid system, dietary marine n-3 PUFAs enhance the concentrations of endocannabinoids with either EPA or DHA in their structure. These include docosahexaenoyl ethanolamide and eicosapentaenoyl ethanolamide [60, 62, 63]. Ethanolamides that contain marine n-3 PUFAs bind to the CB_1 and CB_2 receptors [64, 65] and have marked anti-inflammatory properties in cell culture systems [66, 67]. The first of these studies showed that both docosahexaenoyl ethanolamide and eicosapentaenoyl ethanolamide decrease endotoxin-induced IL-6 and monocyte chemotactic protein (MCP)-1 production by adipocytes [66]. The CB_2 receptor was involved in the effect of docosahexaenoyl ethanolamide on IL-6 production [66]. Meijerink *et al*. [67] showed that docosahexaenoyl ethanolamide was a potent inhibitor of nitric oxide and MCP-1 production by endotoxin-stimulated macrophages. These effects occurred at the level of expression of the MCP-1 and inducible nitric oxide synthase genes [67].

Omega-3 (n-3) polyunsaturated fatty acids and resolvins

In the last 10 years or so new families of lipid mediators produced from marine n-3 PUFAs have been discovered. These include the resolvins produced from EPA (E-series) and DHA (D-series) and protectins produced from DHA (also referred to as neuroprotectins when generated within neural tissue). The synthesis of resolvins and protectins involves the COX and LOX pathways, with different epimers being produced in the presence and absence of aspirin [68–71]. Resolvin synthesis is increased by feeding fish oil rich diets to laboratory rodents [72] and was shown to occur in *fat*-1 mice in which colitis had been induced [52]. The biological effects of resolvins and protectins have been examined extensively in cell culture and animal models of inflammation. These models have shown them to be anti-inflammatory and inflammation resolving. 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 tumour necrosis factor (TNF)-α and IL-1β production [68–71]. Resolvins reduce inflammation and protect experimental animals in models of inflammatory disease including arthritis [73], colitis [74] and asthma [75, 76]. The biological activities of resolvins are mediated via specific G-protein coupled receptors. Resolvin E1 binds to the RvE1 receptor (also known as ChemR23) [77, 78] and is a partial agonist of the LTB_4 receptor BLT_1 [78]. Through this latter action resolvin E1 can compete with LTB_4 and antagonize its action, for example as a chemo-attractant. Resolvin D1 binds to the lipoxin A_4 and annexin A1 receptor FPR2/ALX and to the RvD1 receptor (also known as GPR32) [79, 80]. Receptors for other resolvins are as yet not identified.

Omega-3 (n-3) polyunsaturated fatty acids and inflammatory cytokines

Early studies demonstrated that EPA and DHA inhibited endotoxin-stimulated production of IL-6 and IL-8 by cultured human endothelial cells [20, 81], while EPA or fish oil inhibited endotoxin-induced TNF-α production by cultured monocytes [82–85]. Feeding fish oil to mice

decreased production of TNF-α, IL-1β and IL-6 by endotoxin-stimulated macrophages [35, 86, 87] and decreased circulating TNF-α, IL-1β and IL-6 concentrations in mice injected with endotoxin [88]. Several studies providing fish oil supplements to healthy human volunteers have reported decreased production of TNF-α, IL-1β and IL-6 by endotoxin-stimulated monocytes or mononuclear cells [14, 37, 39, 40], although not all studies confirm this effect [9]. Some of the studies that fail to show an effect of marine n-3 PUFAs on cytokine production have provided <2 g EPA + DHA day^{-1}, which may be an insufficient dose. In patients with RA, fish oil supplements resulted in decreased IL-1 production by monocytes [89], decreased plasma IL-1β concentrations [90] and decreased serum TNF-α concentrations [91].

Omega-3 (n-3) polyunsaturated fatty acids and T-cell reactivity

In cell cultures both EPA and DHA inhibit T cell proliferation [92–95] and the production of IL-2 [93–95]. Animal feeding studies with fairly high amounts of fish oil, EPA or DHA, have also reported reduced T cell proliferative responses [96–98]. Studies in humans are less consistent, although some studies have shown that increased intake of marine n-3 PUFAs decreases human T cell proliferation [37, 99] and IL-2 production [37]. One reason for this may be an insufficient dose of n-3 PUFAs provided in some studies, but that does not explain all the inconsistency [9].

Omega-3 (n-3) fatty acids and inflammatory processes – mechanisms involved

The previous section summarized studies that show that, at a sufficiently high dose, marine n-3 PUFAs exert a range of anti-inflammatory actions including decreased adhesion molecule expression and adhesive interactions between leucocytes and endothelial cells, a decreased chemotactic response of leucocytes, decreased production of eicosanoids from arachidonic acid, increased production of eicosanoids with lower biological potency from EPA, increased production of anti-inflammatory and inflammation resolving resolvins from EPA and DHA (and protectins from DHA), decreased production of the classic inflammatory cytokines TNF, IL-1β and IL-6, and decreased T-cell reactivity (Table 1). Overall, these observations indicate a shift from a strongly pro-inflammatory environment to one of reduced inflammation, lowered cell (neutrophil, monocyte, macrophage, T-cell, endothelial cell) responsiveness and increased resolution of inflammation. A number of mechanisms by which marine n-3 PUFAs influence these separate aspects of the inflammatory response have been identified, with several new mechanistic insights gathered more recently (Table 1).

Table 1

A summary of the anti-inflammatory actions of marine n-3 PUFAs and the likely mechanisms involved

Anti-inflammatory effect	Likely mechanism involved
Reduced leucocyte chemotaxis	Decreased production of some chemo-attractants (e.g. LTB_4); Down-regulated expression of receptors for chemo-atttactants
Reduced adhesion molecule expression and decreased leucocyte-endothelium interaction	Down-regulated expression of adhesion molecule genes (via NFκB, NR1C3 (i.e. PPAR-γ) etc.)
Decreased production of eicosanoids from arachidonic acid	Lowered membrane content of arachidonic acid; Inhibition of arachidonic acid metabolism
Decreased production of arachidonic acid containing endocannabinoids	Lowered membrane content of arachidonic acid
Increased production of 'weak' eicosanoids from EPA	Increased membrane content of EPA
Increased production of anti-inflammatory EPA and DHA containing endocannabinoids	Increased membrane content of EPA and DHA
Increased production of pro-resolution resolvins and protectins	Increased membrane content of EPA and DHA; Presence of aspirin
Decreased production of inflammatory cytokines	Down-regulated expression of inflammatory cytokine genes (via NFκB, NR1C3 (i.e. PPAR-γ) etc.)
Decreased T cell reactivity	Disruption of membrane rafts (via increased content of EPA and DHA in specific membrane regions)

Functional effects of omega-3 (n-3) fatty acids involving altered cell membrane phospholipid fatty acid composition

PUFAs are key structural and functional components of the phospholipids in cell membranes. As indicated above, the phospholipids of cells involved in inflammation typically contain a rather high proportion of arachidonic acid and much less, often little, EPA or DHA [13–15, 36, 39, 93, 100–102]. However, including fish oil in the diet of experimental animals [34, 36, 95] or healthy human volunteers [13–15, 39, 51, 99, 101–104] results in increased accumulation of EPA and DHA in these cells, which is associated with a decreased content of arachidonic acid. Time course studies suggest that the net incorporation of EPA and DHA into human inflammatory cells begins within days and reaches its peak within a few weeks [51, 99, 101, 102, 104, 105], while studies that have used multiple doses of fish oil show that the incorporation of EPA and DHA (and the parallel decline in arachidonic acid) occurs in a dose–response manner [51, 102]. The resulting change in the fatty acid composition of inflammatory cell membrane phospholipids presents an altered availability of substrates for synthesis of eicosanoids, endocannabinoids, resolvins and protectins. This is most likely a major contributor to the observed effects of marine n-3 PUFAs on eicosanoid profiles, although inhibitory effects of EPA on the activities of phospholipase A_2

and COX [106] would result in decreased arachidonic acid availability and decreased arachidonic acid metabolism, respectively. Furthermore, other actions of EPA and DHA described below may lead to a decrease in COX-2 gene expression.

A second aspect of the alteration of cell membrane phospholipid fatty acids with marine n-3 PUFAs involves so called 'lipid rafts' which are now fairly well studied in T cells [107]. Rafts are structures that form by the movement of receptors, accessory proteins and enzymes within the plane of the cell membrane in order to co-localize into signalling platforms. This movement occurs in response to cell activation and is essential for the resulting intracellular signals to be properly transduced into the cytosol and beyond to the nucleus. Lipid rafts are intimately involved in T-lymphocyte responses to activation [108–110]. Cell culture and animal feeding studies have demonstrated that exposure to marine n-3 PUFAs modifies raft formation in T-cells in a way that impairs the intracellular signalling mechanisms in these cells [111–114], consistent with the observations of decreased T cell reactivity after exposure to EPA or DHA. The effect of marine n-3 PUFAs on T cells involves an altered chemical structure of the rafts which alters their functioning [115–118].

Effects of omega-3 (n-3) fatty acids on NFκB-mediated inflammatory signalling

Nuclear factor kappa B (NFκB) is one of the most important transcription factors involved in inflammatory responses. It is the main transcription factor involved in up-regulation of the genes encoding inflammatory cytokines, adhesion molecules and COX-2 [119, 120]. NFκB is activated in a signalling cascade triggered by extracellular inflammatory stimuli, including endotoxin, and involving phosphorylation of an inhibitory subunit [inhibitory subunit of NFκB (IκB)] which then allows translocation of the remaining NFκB dimer to the nucleus [121]. As described earlier, marine n-3 PUFAs decrease expression of adhesion molecules and production of inflammatory cytokines and COX-2 metabolites. One common mechanism to explain these effects would be an impact on the NFκB system. Indeed, EPA or fish oil decreased endotoxin-induced activation of NFκB in human monocytes [82, 84, 85] and this was associated with decreased IκB phosphorylation [84, 85]. Likewise, DHA reduced NFκB activation in response to endotoxin in cultured macrophages [122] and dendritic dells [123, 124], an effect that involved decreased IκB phosphorylation [122]. In contrast, saturated fatty acids, especially lauric acid (12:0), enhanced NFκB activation in macrophages [122] and dendritic cells [123]. Lee *et al*. [122] reported that EPA and DHA were able to prevent this effect of lauric acid in macrophages. These observations suggest a general effect of marine n-3 PUFAs on inflammatory gene expression via inhibition of activation of the transcription factor NFκB in response to exogenous inflammatory stimuli.

Effects of omega-3 (n-3) fatty acids on the anti-inflammatory transcription factor PPAR-γ

NR1C3 or peroxisome proliferator activated receptor (PPAR)-γ is a transcription factor which is thought to act in an anti-inflammatory manner [125]. PPAR-γ knock-down mice show enhanced susceptibility to chemically-induced colitis [126] and PPAR-γ agonists reduce colitis in murine models [126, 127]. Thus, up-regulation of PPAR-γ is a likely target for controlling inflammation [128]. While PPAR-γ directly regulates inflammatory gene expression, it also interferes with the translocation of NFκB to the nucleus [129]. PPAR-γ may be activated by n-3 PUFAs [130–133] and in inflammatory cells DHA induced PPAR-γ [124] and a number of known PPAR-γ target genes [134]. These effects were linked to decreased production of the inflammatory cytokines TNF-α and IL-6 upon endotoxin stimulation [124]. Thus, activation of PPAR-γ may be one of the anti-inflammatory mechanisms of action of marine n-3 PUFAs and this may link to the inhibition of NFκB activation described above.

Effects of omega-3 (n-3) fatty acids on the G-protein coupled receptor GPR120

Several G-protein coupled cell membrane receptors can bind fatty acids. There is some specificity to this with two of these receptors, FFA_1 (also known as GPR40) and GPR120, being able to bind long chain fatty acids. Inflammatory macrophages express GPR120 abundantly but do not express FFA_1 [135]. A synthetic agonist of GPR120 inhibited the macrophage response to endotoxin, an effect which involved maintenance of cytosolic IκB and a decrease in production of TNF-α and IL-6 [135]. These effects are similar to those of EPA and DHA and indicate that GPR120 is involved in anti-inflammatory signalling. Oh *et al*. [135] studied the effect of EPA and DHA on GPR120-mediated gene activation in macrophages. They found that both EPA and DHA enhanced this and went on to study the effects of DHA in further detail. The ability of DHA to inhibit responsiveness of macrophages to endotoxin, already well demonstrated, was abolished in GPR120 knockdown cells. These findings indicate that the inhibitory effect of DHA (and probably also of EPA) on NFκB occurs via GPR120. Thus, there appear to be at least two mechanisms by which marine n-3 PUFAs inhibit NFκB activation, one involving GPR120 and the other involving PPAR-γ, although these may be linked.

Omega-3 (n-3) fatty acids and inflammatory processes – evidence of efficacy

General comments

The ability of marine n-3 PUFAs to down-regulate several aspects of inflammation (summarized in Table 1) suggest

that these fatty acids might be important in determining the development and severity of inflammatory diseases and further that they may be useful as a component of therapy. The evolving identification of candidate mechanisms of action to explain the functional effects observed (see Table 1) adds significant biological plausibility to this approach. Consequently fish oil supplements have been evaluated to differing extents and with varying success in a range of inflammatory conditions [9, 136, 137].

Rheumatoid arthritis

Amongst the classic inflammatory conditions, fish oil has been most thoroughly examined in RA and the studies have been reviewed in detail elsewhere [136]. Animal models have demonstrated that marine n-3 PUFAs can delay the onset of arthritis, reduce its severity and improve joint pathology [138–140]. Cleland *et al*. [44] found that patients with RA who use fish oil supplements were more likely to reduce use of non-steroidal anti-inflammatory drugs (NSAIDs) and to be in remission than those patients who did not use fish oil. Randomized 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 [136]. The dose of n-3 PUFAs used in these trials has typically been high, between about 1 and 7 g day^{-1} and averaging about 3.5 g day^{-1} [136]. This dose would equate to 50 mg kg^{-1} body weight day^{-1} and would be difficult to achieve through the diet, but can be achieved through use of supplements or liquid oil. Meta-analyses that include trials of fish oil in RA have been conducted [141, 142]. 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 [141]. A more recent meta-analysis of n-3 PUFAs and pain included data from 17 trials, including one trial in RA with flaxseed oil and two trials of fish oil not in RA patients, but which reported joint pain [142]. This analysis indicated that fish oil reduces patient assessed joint pain, duration of morning stiffness, number of painful and/or tender joints, and consumption of NSAIDs. Thus there is fairly robust evidence of the efficacy of marine n-3 PUFAs in RA.

Inflammatory bowel diseases

Animal models have demonstrated that marine n-3 PUFAs decrease chemically-induced colonic damage and inflammation [137]. The effects on disease severity were, in all cases, associated with a reduction in production of arachidonic acid-derived eicosanoids [137]. A more recent study investigated chemically-induced colitis in *fat-1* mice [52]. The mice showed much less colonic damage and inflammation than wild type mice and this was associated with a marked change in the pattern of inflammatory mediators present in colonic tissue. A study in IL-10 knock-out mice that spontaneously develop colitis demonstrated significantly reduced colonic inflammation if the mice were fed fish oil [143]. EPA and DHA are incorporated into gut mucosal tissue of patients with IBD who supplement their diet with fish oil [47, 144, 145] and this is associated with reduced inflammation [46–50]. Some randomized controlled trials of fish oil in IBD have reported clinical benefits including improved clinical score, improved gut mucosal histology, improved sigmoidoscopic score, lower rate of relapse and decreased use of corticosteroids [137]. The dose of marine n-3 PUFAs used in these trials has typically been high, between 2.5 and 6 g day^{-1} and averaging about 4 g day^{-1}, equivalent to about 55 mg kg^{-1} body weight day^{-1}. However, a number of trials do not report benefits [137]. One study with an enterically coated fish oil showed a significantly lower rate of relapse over 12 months in patients with Crohn's disease [146] but two recent trials with a similar design and fish oil preparation and using a similar dose of n-3 PUFAs could not replicate this finding [147]. A meta-analysis identified 13 studies of fish oil supplementation in IBD reporting outcomes related to clinical score, sigmoidoscope score, gut mucosal histology score, induced remission and relapse, but concluded that that there were sufficient data to perform meta-analysis only for relapse and only for ulcerative colitis. There was no benefit seen [148]. More recent meta-analyses considering maintenance of remission in patients with Crohn's disease or with ulcerative colitis have identified marginal effects if any [149–151]. Thus, despite some favourable studies, there is at best only weak evidence that marine n-3 PUFAs have clinical benefits in human IBD.

Asthma

Arachidonic acid-derived eicosanoids, such as PGD_2, LTC_4, LTD_4 and LTE_4, are produced by the cells that mediate pulmonary inflammation in asthma (e.g. mast cells) and are believed to be major mediators of asthmatic bronchoconstriction. The 4-series LTs have been detected in the blood, bronchoalveolar lavage fluid and urine of asthmatics [152]. In addition to the role of arachidonic acid-derived eicosanoids as mediators of asthma, PGE_2 is also involved in regulating the development of the T helper type 2 phenotype of T lymphocytes that predisposes to allergic inflammation [153] and promotes the formation of immunoglobulin E by B lymphocytes [154]. Thus, a hypothesis has evolved that an increased intake of n-6 PUFAs coupled to an insufficient intake of n-3 PUFAs has played a causal role in increased asthma incidence [155]. DHA reduced lung inflammation and improved lung function in a murine model of asthma [156]. Epidemiologic data link high n-6 PUFA or low n-3 PUFA consumption with childhood asthma [157]. Studies have reported anti-inflammatory effects of fish oil in patients with asthma, such as decreased 4-series LT production [158–160] and leucocyte chemotaxis [159, 160]. Randomized controlled trials of fish oil in adult asthma

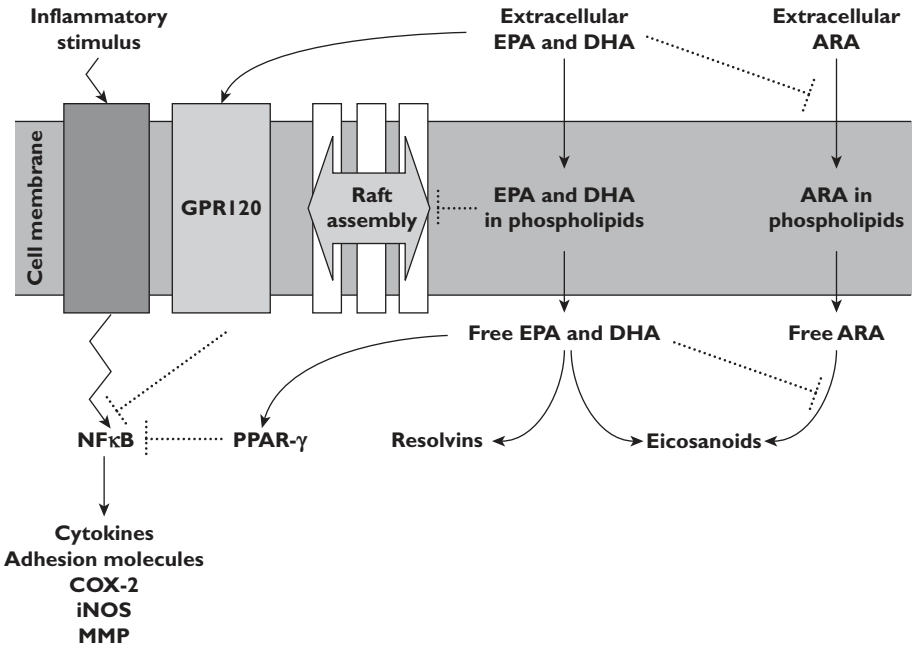

Figure 4

Summary of the anti-inflammatory actions of marine n-3 polyunsaturated fatty acids. ARA, arachidonic acid; COX, cyclo-oxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GPR, G-protein coupled receptor; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase; NFκB, nuclear factor kappa B, PPAR, peroxisome proliferator activated receptor. Dotted lines indicate inhibition

have reported no benefit [9]. One small trial in school children with asthma found a trend towards improved lung function after low dose marine n-3 PUFAs [161]. A second trial in school children reported significant improvement in lung function and a significant decline in disease score [162]. Thien *et al*. [163] included eight studies published between 1988 and 2000 in a systematic review. They identified that there was no consistent effect of fish oil on lung function, asthma symptoms or asthma medication use, although they stated one study in children showed improved lung function and reduced asthma medication use. Clearly, more needs to be done in this area.

Omega-3 (n-3) polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology?

Marine n-3 PUFAs are undoubtedly biologically active with an array of anti-inflammatory effects reported in cell culture studies, animal models, trials in healthy human volunteers and clinical trials in various patient groups. Effects on a variety of inflammatory responses (chemotaxis, adhesion molecule expression, adhesive interactions, eicosanoid and cytokine production) and on a variety of cell types (neutrophils, monocytes, macrophages, dendritic cells, T lymphocytes, endothelial cells) are reported. Long explored and more recently discovered mechanisms explain the anti-inflammatory effects of marine n-3 PUFAs. Many of these mechanisms are interlinked (Figure 4) and a key aspect appears to be incorporation of the fatty acids into cell membrane phospholipids. Marine n-3 PUFAs exert actions within the membrane itself or following controlled release from the membrane by phospholipase enzymes. The anti-inflammatory actions of the 'free' marine n-3 fatty acids are now recognized to be at least partly mediated by cell surface and intracellular receptors, GPR120 and NR1C3 (i.e. PPAR-γ), respectively. Both these receptors appear to be involved in inhibiting activation of NFκB, the prototypical pro-inflammatory transcription factor. PPAR-γ acts by a physical interaction [129], while GPR120 inhibits signalling upstream of phosphorylation of IκB [135]. This suggests two separate mechanisms by which EPA and DHA can suppress activation of NFκB. Both GPR120 and PPAR-γ are pharmaceutical targets. EPA and DHA inhibit arachidonic acid metabolism, a long established target for pharmaceutical agents, and EPA gives rise to eicosanoid mediators that may block the action of those derived from arachidonic acid [26], perhaps explaining some of the effects of the fatty acids on chemotaxis and on the leucocyte-endothelium adhesive interaction. EPA- and DHA-derived mediators like eicosanoids, resolvins and protectins obviously act via receptors. Those for the eicosanoids are

shared with arachidonic acid derived eicosanoids [56] and those for resolvins and protectins are only just becoming identified. The clear role of EPA- and DHA-derived mediators in resolution of inflammation [68–72] and their apparent potency is an exciting development in both the biology of n-3 fatty acids and the biology of inflammation. In this regard, although the resolvins are under development as pharmaceutical agents, EPA and DHA could be regarded as pro-drugs. Marine n-3 PUFA actions in the membrane, in cell signalling, in regulating gene expression, in acting via receptors and as pro-drugs giving rise to potent lipid mediators that in turn act via receptors, suggest a place for EPA and DHA in pharmacology. However, EPA and DHA are naturally occurring substances, commonly consumed in the diet, although in rather low amounts in non-fish eaters who are not using fish oil supplements. Nevertheless, marine n-3 PUFAs are nutrients. Furthermore, they can be produced endogenously (Figure 1), although the extent to which humans carry out this process is said to be limited [5, 6]. Having established that EPA and DHA can impact on inflammation and that their mechanisms of action align them with pharmaceuticals, perhaps the primary criterion in establishing whether these effects are nutrition or pharmacology is a consideration of dose required to elicit an anti-inflammatory effect.

Typical intakes of marine n-3 PUFAs are in the tens to low hundreds of mg per day [7, 8, 164], even in people consuming lean fish or taking standard fish oil capsules. The UK Government recommendation for health of the general adult population is a minimum intake of 0.45 g EPA + DHA day^{-1}, this being based upon one lean and one oily fish meal per week [164]. The FAO/WHO recently made a recommendation for adults of at least 0.25 g EPA + DHA day^{-1} [165], a recommended intake mirrored by the European Food Safety Authority [166]. Such intakes can be achieved by regular consumption of oily fish or by use of fish oil supplements. However, it seems unlikely that 'nutritional' intakes of the order of a few hundreds of mg per day will affect inflammation particularly in those in which it is chronic. Studies that report effects of marine n-3 PUFAS on inflammatory cell functions or inflammatory mediator production or concentrations have typically used intakes of EPA + DHA >2 g day^{-1}, equivalent to about 30 mg kg^{-1} body weight day^{-1}, although not all studies using high doses report effects [9]. Conversely, studies using doses <2 g day^{-1} rarely report effects of marine n-3 PUFAs on inflammation, although again there are exceptions to this. Data from Rees *et al*. [51] suggest a threshold intake of between 1.35 and 2.7 g EPA day^{-1} is required to impact on PGE_2 production by mononuclear cells stimulated with endotoxin, while studies in RA, the inflammatory condition where evidence of clinical benefit is most robust, have used an average dose of about 3.5 g EPA + DHA day^{-1} [136]. To achieve such a high intake through the diet is only possible by consuming oily fish at least once per day. Depending upon its source and the serving size, salmon can provide 1.5 to 3 g EPA + DHA per serving. The need to consume these high amounts of EPA + DHA on a daily basis to elicit anti-inflammatory effects suggests the action is more pharmacologic than nutritional.

Conclusions

EPA and DHA are the major n-3 PUFAs found in oily fish and fish oil supplements. There is substantial evidence that these fatty acids are able to inhibit partly a number of aspects of inflammation including leukocyte chemotaxis, adhesion molecule expression and leucocyte-endothelial adhesive interactions, production of eicosanoids like PGs and LTs from the n-6 fatty acid arachidonic acid, production of inflammatory cytokines and T cell reactivity. EPA and DHA act through a variety of mechanisms, including acting via cell surface (GPR120) and intracellular [NR1C3 (i.e. PPAR-γ)] receptors that control inflammatory cell signalling and gene expression patterns. Some effects of marine n-3 fatty acids on inflammatory cells appear to be mediated by, or at least are associated with, changes in fatty acid composition of cell membranes. Changes in fatty acid composition can modify lipid raft formation, cell signalling leading to altered gene expression and the pattern of lipid and peptide mediator production. Cells involved in the inflammatory response are typically rich in the n-6 fatty acid 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, 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 which are anti-inflammatory and inflammation resolving. Increased membrane content of EPA and DHA (and decreased arachidonic acid content) results in a changed pattern of production of eicosanoids, including endocannabinoids, and resolvins. Thus, fatty acid exposure and the fatty acid composition of human inflammatory cells influence the function of those cells and the contents of arachidonic acid, EPA and DHA appear to be especially important. Dose-dependent actions of marine n-3 PUFAs on inflammatory responses have not been well described, but it appears that a dose of at least 2 g day^{-1} is necessary to achieve an anti-inflammatory effect. This would equate to about 30 mg kg^{-1} body weight day^{-1}. Although it is possible to obtain such a dose from the diet (e.g. one meal of salmon or mackerel every day), this seems an unlikely strategy for most people. It would also be difficult to achieve this dose with standard fish oil capsules, about seven 1 g capsules day^{-1} would be needed, but 2 g day^{-1} is more achievable with fish oil concentrates and with pharmaceutical preparations like Omacor® (aka Lovaza®). As a result of their anti-inflammatory actions marine n-3 PUFAs may have therapeutic efficacy in inflammatory diseases. Work

with animal models of RA, IBD and asthma has demonstrated efficacy of fish oil and of mediators derived from EPA and DHA. There have been a number of clinical trials of fish oil in patients with RA, IBD or asthma. These trials have typically used high doses of EPA + DHA, often above the anti-inflammatory threshold of 2 g day^{-1}. Most 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 [141, 142]. Thus, evidence for clinical efficacy of marine n-3 PUFAs in RA is fairly robust. Some trials of fish oil in IBD indicate benefits (e.g. improved clinical score, improved gut mucosal histology, improved sigmoidoscopic score, lower rate of relapse and decreased use of corticosteroids) but the findings are inconsistent and meta-analyses conclude that there is currently no clear evidence of efficacy of marine n-3 fatty acids in human IBD [148–151]. Trials of fish oil in adult asthma do not show benefit. In childhood asthma one trial showed a reduction of disease severity and an improvement in lung function [162], but another did not [161]. Meta-analyses combining findings from studies in adults and in children conclude that there is currently no clear evidence of efficacy of marine n-3 fatty acids in asthma [163]. It is not clear why anti-inflammatory effects observed with marine n-3 PUFAs in IBD and asthma do not translate into more consistent clinical improvements. Thus, there is a need to know more about the actions of marine n-3 PUFAs in patients with inflammatory disorders in order optimize the strategy for their therapeutic use. In particular, a better understanding of dose–response relationships in different patient groups, of the relative importance of EPA and DHA and of those factors that limit the effectiveness of EPA and DHA is needed.

Competing Interests

The author serves on Scientific Advisory Boards of the Danone Research Centre in Specialized Nutrition and Aker Biomarine. He acts as a consultant to Mead Johnson Nutritionals, Vifor Pharma and Amarin Corporation. He has received speaking honoraria from Solvay Healthcare, Solvay Pharmaceuticals, Pronova Biocare, Fresenius Kabi, B. Braun, Abbott Nutrition, Baxter Healthcare, Nestle, Unilever and DSM. He currently receives research funding from Vifor Pharma. He is Past-President of the International Society for the Study of Fatty Acids and Lipids, an organization that is partly supported by corporate membership fees, mainly the food and supplements industries. He is a member of the Board of Directors of ILSI Europe, the Board of Directors of the European Nutraceutical Association, and the Council of the British Nutrition Foundation. These organizations are each supported in part by the food and supplements industries.

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