Mol. Nutr. Food Res. 2008, *52*, 885 – 897 DOI 10.1002/mnfr.200700289

Review

Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases

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With regard to inflammatory processes, the main fatty acids of interest are the n-6 PUFA arachidonic acid (AA), which is the precursor of inflammatory eicosanoids like prostaglandin E_2 and leukotriene B_4, and the n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are found in oily fish and fish oils. EPA and DHA inhibit AA metabolism to inflammatory eicosanoids. They also give rise to mediators that are less inflammatory than those produced from AA or that are anti-inflammatory. In addition to modifying the lipid mediator profile, n-3 PUFAs exert effects on other aspects of inflammation like leukocyte chemotaxis and inflammatory cytokine production. Some of these effects are likely due to changes in gene expression, as a result of altered transcription factor activity. Fish oil has been shown to decrease colonic damage and inflammation, weight loss and mortality in animal models of colitis. Fish oil supplementation in patients with inflammatory bowel diseases results in n-3 PUFA incorporation into gut mucosal tissue and modification of inflammatory mediator profiles. Clinical outcomes have been variably affected by fish oil, although some trials report improved gut histology, decreased disease activity, use of corticosteroids and relapse.

Keywords: Cytokine / Eicosanoid / Fatty acid / Fish oil / Inflammation

Received: July 25, 2007; revised: October 16, 2007; accepted: December 20, 2007

1 Fatty acid structure, nomenclature, sources, intakes and roles

Fatty acids are hydrocarbon chains with a carboxyl group at one end and a methyl group at the other. The carboxyl group is reactive and readily forms ester links with alcohol groups, for example those on glycerol or cholesterol, in turn forming acylglycerols (*e.g.* triacylglycerols, phospholipids), and cholesteryl esters. The most abundant fatty acids have straight chains of an even number of carbon atoms. Fatty acid chain lengths vary from 2 to 30 or more and the chain may contain double bonds. Fatty acids containing double bonds in the acyl chain are referred to as unsaturated fatty acids; a fatty acid containing two or more double bonds is called a PUFA. Saturated fatty acids do not contain double bonds in the acyl chain. The systematic name for a fatty acid is determined simply by the number of carbons and the number of double bonds in the acyl chain (Table 1). However, complications arise for the naming of unsaturated fatty acids. This is because there are multiple possibilities for the position of double bonds within the hydrocarbon chain and because each double bond may be in the *cis* or *trans* configuration. Therefore, when naming an unsaturated fatty acid it is important that the exact positions of double bonds and their configurations be clearly identified. Traditionally, the position of double bonds was identified by naming the carbon number (from carbon 1 (the carboxyl carbon)) on which each double bond occurs. Thus, octadecadienoic acid, an 18-carbon fatty acid with *cis* double bonds between carbons 9 and 10 and carbons 12 and 13 is correctly denoted as *cis*-9, *cis*-12-octadecadienoic acid or as *cis*, *cis*, 9,12-octadecadienoic acid. More recently, an alternative shorthand notation for fatty acids has come into frequent use. This relies upon identifying the number of carbon atoms in the chain, and the number of double bonds and their position. Thus, octadecanoic acid is notated as 18:0, indicating that it has an acyl chain of 18 carbons and

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Abbreviations: AA, arachidonic acid; **ALA,** α-linolenic acid; **DGLA,** dihomo-γ-linolenic acid; **DHA,** docosahexaenoic acid; **DSS,** dextran sulphate sodium; **EPA,** eicosapentaenoic acid; **GLA, γ-linolenic acid; IκB,** inhibitory subunit of nuclear factor κ B; **LA,** linoleic acid; **LT,** leukotriene; **NFκB,** nuclear factor κ B; **PG,** prostaglandin; **PPAR,** peroxisome proliferator activated receptor; **TNBS,** trinitrobenzene sulphonic acid; **TNF,** tumor necrosis factor; **TX,** thromboxane

Table 1. Fatty acid nomenclature

Systematic name	Trivial name	Shorthand notation
Octanoic	Caprylic	8:0
Decanoic	Capric	10:0
Dodecanoic	Lauric	12:0
Tetradecanoic	Myrsitic	14:0
Hexadecanoic	Palmitic	16:0
Octadecanoic	Stearic	18:0
cis-9-Hexadecenoic	Palmitoleic	16:1n-7
cis-9-Octadecenoic	Oleic	18:1n-9
cis-9,12-Octadecadienoic	Linoleic	18:2n-6
All *cis*-9,12,15-Octadecatrienoic	α-Linolenic	18:3n-3
All *cis*-6,9,12-Octadecatrienoic	γ-Linolenic	18:3n-6
All *cis*-8,11,14-Eicosatrienoic	Dihomo-γ-linolenic	20:3n-6
All *cis*-5,8,11,14-Eicosatetraenoic	Arachidonic	20:4n-6
All *cis*-5,8,11,14,17-Eicosapentaenoic	Eicosapentaenoic	20:5n-3
All *cis*-7,10,13,16,19-Docosapentaenoic	Docosapentaenoic	22:5n-3
All *cis*-4,7,10,13,16,19-Docosahexaenoic	Docosahexaenoic	22:6n-3

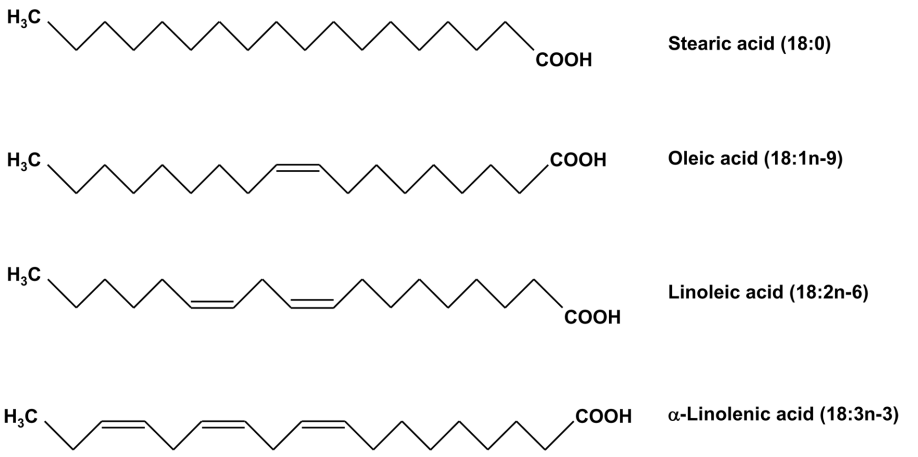

Figure 1. The structure and naming of selected 18 carbon fatty acids.

does not contain any double bonds. Unsaturated fatty acids are named simply by identifying the number of double bonds and the position of the first double bond counted from the methyl terminus (with the methyl, or ω, carbon as number 1) of the acyl chain. The way the first double bond is identified is as ω-*x*, where *x* is the carbon number on which the double bond occurs. Therefore *cis*, *cis*, 9,12-octadecadienoic acid is also known as 18:2ω-6. The ω-*x* nomenclature is sometimes referred to as omega *x* (*e.g.* 18:2 omega 6) or n-*x* (*e.g.* 18:2n-6). In addition to these nomenclatures, fatty acids are often described by their common names (Table 1). Figure 1 shows the structure of several 18-carbon fatty acids indicating the position of the double bonds in the chain and how this is reflected in their naming. Most common unsaturated fatty acids contain *cis* rather than *trans* double bonds. *Trans* double bonds do occur however as intermediates in the biosynthesis of fatty acids, in ruminant fats (*e.g.* cow's milk), in plant lipids and in some seed oils.

There are two principal families of PUFAs, the n-6 (or omega-6) and the n-3 (or omega-3) families. The simplest members of each family, linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3; ALA), cannot be synthesised by mammals. LA is found in significant quantities in many vegetable oils, including corn, sunflower and soybean oils, and in products made from such oils, such as margarines [1]. ALA is found in green plant tissues, in some common vegetable oils, including soybean and rapeseed oils, in some nuts, and in flaxseed (also known as linseed) and flaxseed oil. Between them, LA and ALA contribute over 95%, and perhaps as much as 98% of dietary PUFA intake in most Western diets [1]. The intake of LA in Western countries increased greatly in the second half of the 20th century, following the introduction and marketing of cooking oils and margarines. Typical intakes of both essential fatty acids are in excess of requirements. However, the changed pattern of consumption of LA has resulted in a marked increase in the ratio of n-6 to n-3 PUFAs in the diet. This ratio is typically between 5 and 20 in most Western populations [1, 2].

Although LA and ALA cannot be synthesised by humans they can be metabolised to other fatty acids (Fig. 2). This is achieved by the insertion of additional double bonds into

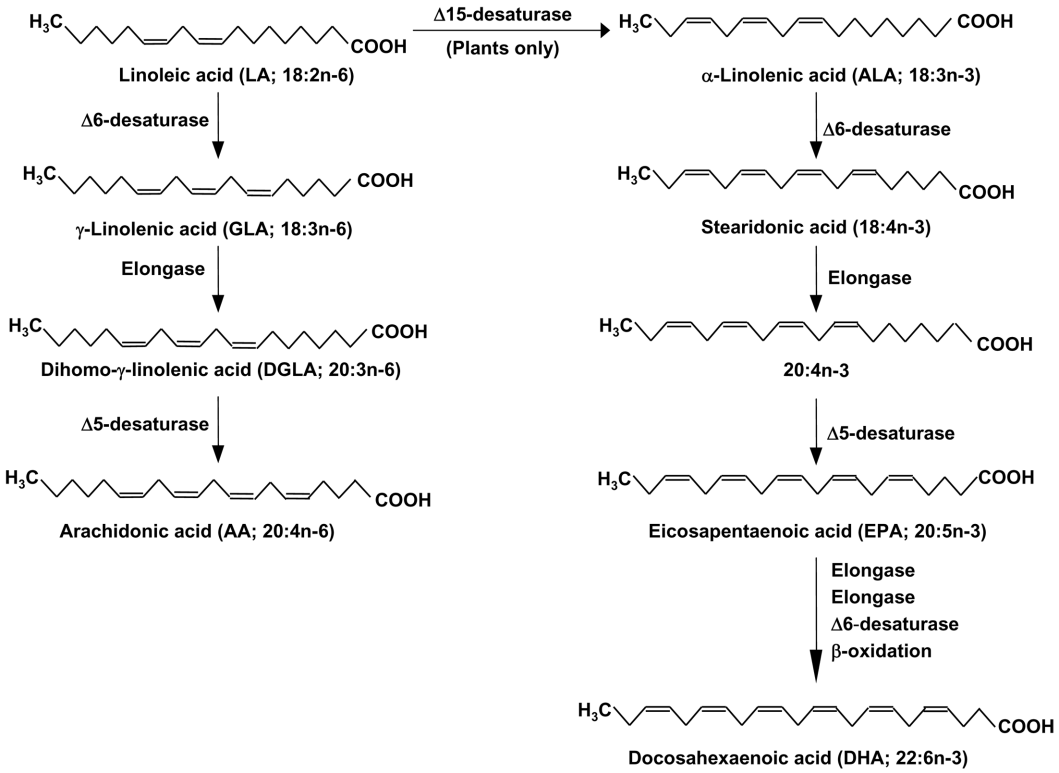

Figure 2. The biosynthesis of PUFAs. AA, arachidonic acid; ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid.

the acyl chain (*i.e.* unsaturation) and by elongation of the acyl chain. Thus, LA can be converted *via* γ-linolenic acid (18:3n-6; GLA) and dihomo-γ-linolenic acid (20:3n-6; DGLA) to arachidonic acid (20:4n-6; AA) (Fig. 2). By an analogous set of reactions catalysed by the same enzymes ALA can be converted to eicosapentaenoic acid (20:5n-3; EPA). Both AA and EPA can be further metabolised, EPA giving rise to docosapentaenoic acid (22:5n-3) and docosahexaenoic acid (22:6n-3; DHA) (Fig. 2). Dietary intakes of the longer chain, more unsaturated PUFAs are much, much lower than of LA and ALA. Some plant oils contain GLA, DGLA and stearidonic acid (18:4n-3), but typical intakes of these fatty acids from the diet are likely to be <10 mg/day. AA is found in meat and offal and intakes are estimated at 50 – 500 mg/day [1]. EPA, docosapentaenoic acid and DHA are found in fish, especially so-called ‚oily' fish (tuna, salmon, mackerel, herring, sardine). One oily fish meal can provide between 1.5 and 3.5 g of these long chain n-3 PUFAs [3]. The commercial products known as fish oils also contain these long chain n-3 PUFAs, which typically will contribute about 30% of the fatty acids present. Thus, consumption of a typical 1 g fish oil capsule *per* day can provide about 300 mg of these fatty acids. In the absence of oily fish or fish oil consumption, intake of long chain n-3 PUFAs is likely to be <100 mg/day [1, 3], although foods fortified with these fatty acids are now available in many countries.

PUFAs are important constituents of cells where they play roles assuring the correct environment for membrane protein function, maintaining membrane fluidity and regulating cell signalling, gene expression and cellular function [1]. In addition, some PUFAs, particularly AA, act as substrates for synthesis of eicosanoids, which are involved in regulation of many cell and tissue responses.

2 Eicosanoids provide a link between PUFAs and inflammatory processes

Eicosanoids are key mediators and regulators of inflammation [4, 5] and are generated from 20 carbon PUFAs. Because inflammatory cells typically contain a high proportion of the n-6 PUFA AA and low proportions of other 20-carbon PUFAs, AA is usually the major substrate for eicosanoid synthesis. Eicosanoids, which include prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs) and other oxidised derivatives, are generated from AA by the metabolic processes summarised in Fig. 3. They are involved in modulating the intensity and duration of inflammatory responses [4, 5], have cell- and stimulus-specific sources and frequently have opposing effects. Thus, the overall physiological (or pathophysiological) outcome will depend upon the cells present, the nature of the stimulus, the timing of eicosanoid generation, the concentrations of

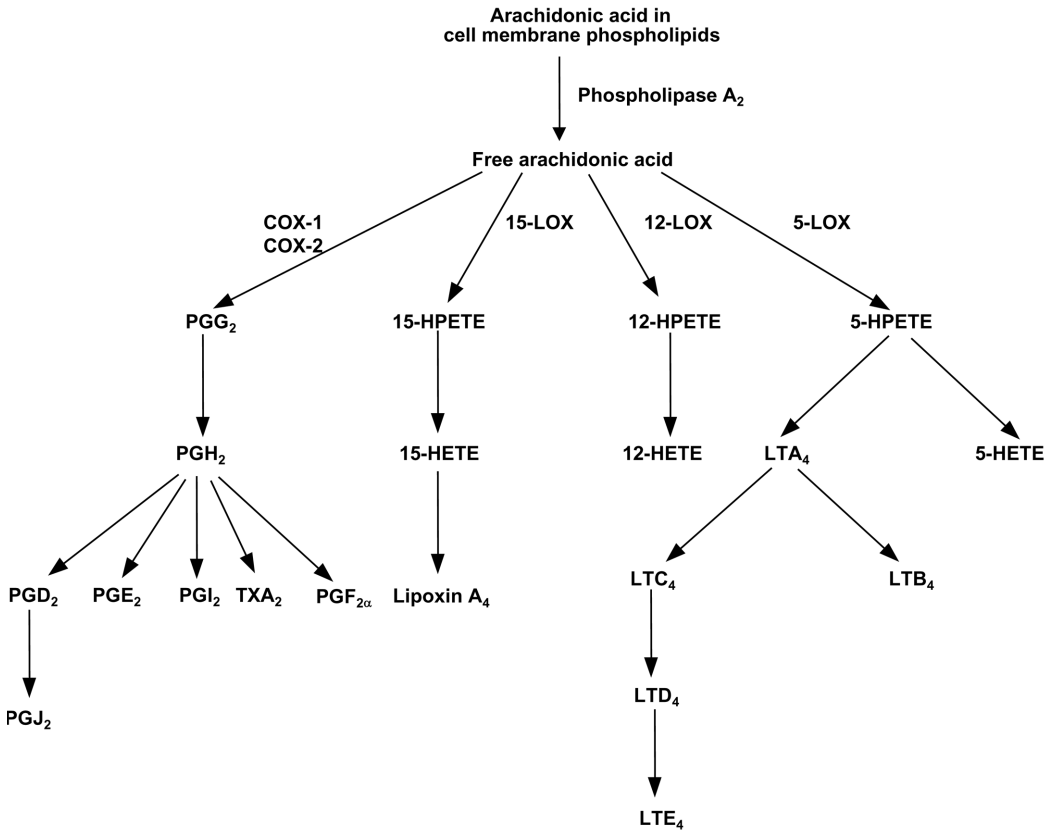

Figure 3. Outline of the pathway of eicosanoid synthesis from AA. COX, cyclooxygenase; HETE, hyroxyeicosatetraenoic acid; HPETE, hyrodoperoxyeicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane. Reproduced with permission from ref. [30].

different eicosanoids generated and the sensitivity of target cells and tissues to the eicosanoids generated.

Induction of colitis in laboratory animals results in the appearance of inflammatory eicosanoids such as PGE_2 and LTB_4 in the colonic mucosa [6, 7]. In human inflammatory bowel diseases the intestinal mucosa contains elevated levels of inflammatory eicosanoids such as LTB_4 [8]. LTB_4 has distinct pro-inflammatory actions such as inducing leukocyte infiltration and subsequent release of inflammatory mediators [4] and thus it is highly likely to be playing a pathologic role in inflammatory bowel diseases. This is supported by the reported protective effect on mucosal injury of the LT biosynthesis inhibitor MK-886 in acetic acid-induced rat colitis [9]. This effect was associated with decreased colonic LTB_4 concentrations. However, the role of PGE_2 in inflammatory bowel diseases is less certain, and there is evidence that it may be protective. For example, indomethacin, an inhibitor of PG synthesis, worsened mucosal injury in acetic acid-induced rat colitis [9]. Furthermore, the LT synthesis inhibitor not only decreased colonic LTB_4 concentrations but increased those of PGE_2 [9], suggesting that a shift of AA metabolism in favour of PGs is beneficial. Recent studies have clearly demonstrated mechanisms by which PGE_2 may act in anti-inflammatory manner. PGE_2 was shown to inhibit 5-lipoxygenase so decreasing production of the inflammatory 4-series LTs [10], and to induce 15-lipoxygenase so promoting the formation of lipoxins [10, 11] that have been found to have anti-inflammatory effects [12, 13]. Indeed, oral administration of a lipoxin A_4 analog attenuated weight loss and mortality in mice simultaneously subjected to oral treatment with dextran sodium sulphate (DSS) to induce colitis [13].

3 N-3 PUFAs modify eicosanoid profiles

Increased consumption long chain n-3 PUFAs such as EPA and DHA, results in increased proportions of those fatty acids in inflammatory cell phospholipids [14–21]. The incorporation of EPA and DHA into human inflammatory cells occurs in a dose–response fashion [20, 21] and is partly at the expense of AA. As a result of there being less substrate available for synthesis of eicosanoids from AA, n-3 PUFA supplementation of the human diet has been shown to result in decreased production of PGE_2 [15, 18, 21–24], TXB_2 [18], LTB_4 [14, 15, 24], 5-hydroxyeicosatetraenoic acid [14, 16] and LTE_4 [25] by inflammatory cells.

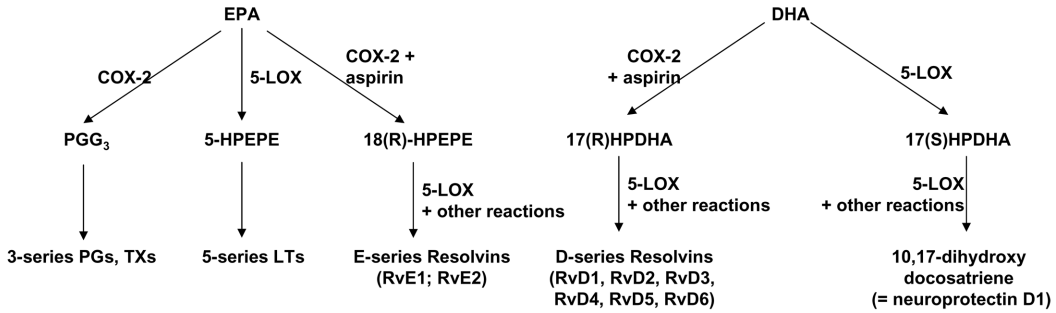

Figure 4. Outline of the pathway of synthesis of resolvins and related mediators. COX, cyclooxygenase; HPDHA, hydroperoxydocosahexaenoic acid; HPEPE, hydroperoxy-eicosapentaenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; Rv, resolvin; TX, thromboxane.

EPA is also able to act as a substrate for both cyclooxygenase and 5-lipoxygenase, giving rise to eicosanoids with a slightly different structure to those formed from AA. Thus, fish oil supplementation of the human diet has been shown to result in increased production of LTB_5, LTE_5 and 5-hydroxyeicosapentaenoic acid by inflammatory cells [14, 16, 25], although generation of PGE_3 has been more difficult to demonstrate. The functional significance of this is that the mediators formed from EPA are believed to be less potent than those formed from AA. For example, LTB_5 is 10 – 100-fold less potent as a neutrophil chemotactic agent than LTB_4 [26, 27], while PGE_3 is a less potent inducer of cyclooxygenase-2 gene expression in fibroblasts and of IL-6 production by macrophages than PGE_2 [28]. The reduction in generation of AA-derived mediators which accompanies fish oil consumption has lead to the idea that fish oil is anti-inflammatory and that it may be useful in the prevention and therapy of inflammatory conditions [29, 30].

4 Other anti-inflammatory actions of n-3 PUFAs

Although their action in antagonising AA metabolism is recognised as a key anti-inflammatory effect of n-3 PUFAs, these fatty acids have a number of other anti-inflammatory effects that might result from altered eicosanoid production or might be independent of this. For example, studies have shown that, when consumed in sufficient quantities, dietary fish oil results in decreased leukocyte chemotaxis, decreased adhesion molecule expression and decreased production of pro-inflammatory cytokines [28, 29]. In addition, both EPA and DHA give rise to a newly discovered family of anti-inflammatory mediators called resolvins.

4.1 Resolvins and related compounds – novel EPA and DHA derived anti-inflammatory mediators

Recent studies have identified a novel group of trihydroxyeicosapentaenoic acid mediators, termed E-series resolvins, formed from EPA by a series of reactions involving cyclooxygenase-2 (acting in the presence of aspirin) and 5-lipoxygenase (Fig. 4). These mediators appear to exert potent anti-inflammatory actions [31 – 33]. In addition, DHA-derived trihydroxydocosahexanoic acid mediators termed D-series resolvins are produced by a similar series of reactions (Fig. 4) and these too are anti-inflammatory [34, 35]. Metabolism of DHA *via* a series of steps, several involving 5-lipoxygenase, generates a dihydroxydocosatriene termed neuroprotectin D1 (Fig. 4), again a potent anti-inflammatory molecule [36]. The identification of these novel EPA and DHA derived mediators is an exciting new area of n-3 fatty acids and inflammatory mediators and the implications to a variety of conditions may be of great importance [37, 38]. With regard to inflammatory bowel diseases, Hudert *et al*. [39] showed that *fat-1* mice, which have the ability to produce n-3 PUFAs from n-6 PUFAs due to the presence of an 'n-3 PUFA desaturase' gene, are protected against DSS-induced body weight loss, colon shortening, and colonic damage and inflammation and that these mice show the presence of resolvin E1, resolvin D3 and neuroprotectin D1 in colonic tissue. Arita *et al*. [40] showed that pretreatment of mice with resolvin E1 significantly attenuated trinitrobenzene sulphonic acid (TNBS)-induced body wasting, mortality, colonic shortening and thickening, inflammatory cell infiltration, and colonic damage. These findings suggest that generation of resolvins could be an important anti-inflammatory mechanism of action of n-3 PUFAs and that such mediators may have a potent protective role in inflammatory bowel diseases. Currently there is no information on the protective role that resolvins may play in human inflammatory bowel diseases.

4.2 N-3 PUFAs and inflammatory cytokine production

Cell culture studies demonstrate that EPA and DHA can inhibit the production of IL-1β and tumor necrosis factor (TNF)-α by monocytes [41], and the production of IL-6 and IL-8 by venous endothelial cells [42, 43]. Fish oil feeding decreased *ex vivo* production of TNF-α, IL-1β and IL-6 by

rodent macrophages [44–46]. Supplementation of the diet of healthy human volunteers with fish oil providing more than 2 g EPA + DHA/day decreased production of TNF, or IL-1 or IL-6 by mononuclear cells in some studies [15, 18, 22, 47–49]. It should be noted that there are also several studies that fail to show effects of dietary long chain n-3 PUFAs on production of inflammatory cytokines in humans. Some of these studies have provided <2 g EPA + DHA/day, although others have provided higher doses (see [29, 30] for references). It is not clear what the reason for these discrepancies in the literature is, but technical factors are likely to be contributing factors [29]. The relative contributions of EPA and DHA might also be important in determining the effect of fish oil. One other factor that has recently been identified is polymorphisms in genes affecting cytokine production [50]. It was found that the effect of dietary fish oil upon cytokine production by human mononuclear cells was dependent upon the nature of the −308 TNF-α and the +252 TNF-β polymorphisms. This study raises the possibility of being able to identify those who are more likely and those who are less likely to experience specific anti-inflammatory effects of fish oil.

5 N-3 PUFAs and inflammatory gene expression

Two transcription factors that are likely to play a role in inflammation of the gastrointestinal tract are nuclear factor κ B (NFκB) and peroxisome proliferator activated receptor (PPAR)-γ. NFκB is the principal transcription factor involved in up-regulation of inflammatory cytokine, adhesion molecule and COX-2 genes [51, 52]. NFκB is activated as a result of a signalling cascade triggered by extracellular inflammatory stimuli and involving phosphorylation of an inhibitory subunit (inhibitory subunit of NFκB (IκB)) which then allows translocation of the remaining NFκB dimer to the nucleus [53]. Thus, expression of inflammatory genes is up-regulated. NFκB is a recognised target for controlling intestinal inflammation (reviewed in ref. [54–56]).

The second transcription factor, PPAR-γ, is also expressed in intestinal tissue [57] where it is believed to act in an anti-inflammatory manner. Colonic biopsies of patients with ulcerative colitis show lowered PPAR-γ expression [58], PPAR-γ knock-down mice show enhanced susceptibility to TNBS-induced colitis [59] and PPAR-γ agonists reduce colitis in murine models [59, 60]. Thus, up-regulation of PPAR-γ is also a recognised target for controlling intestinal inflammation [61]. While PPAR-γ directly regulates inflammatory gene expression, it also interferes with the activation of NFκB creating an intriguing interaction between these two transcription factors [62].

Many of the effects of n-3 PUFAs on inflammatory mediator production appear to be related to altered expression of genes encoding those mediators. For example, de Caterina *et al*. [42] demonstrated that the down-regulation of vascular cell adhesion molecule (VCAM)-1 expression on endothelial cells caused by DHA was exerted at the level of VCAM-1 gene expression, and that this effect was independent of effects on eicosanoid production and on antioxidant status. Inclusion of fish oil in the diet completely abolished mRNA for TNF-α, IL-1β and IL-6 in the kidneys of autoimmune disease-prone mice [63]. Feeding mice a fish oil-rich diet significantly decreased the level of IL-1β mRNA in LPS- or phorbol ester-stimulated spleen lymphocytes [64]; the lower IL-1β mRNA level was not due to accelerated degradation but to impaired synthesis. Fish oil feeding to mice lowered basal and LPS-stimulated TNF-α mRNA levels in peritoneal macrophages [45].

The effects of n-3 PUFAs on inflammatory gene expression suggest that they might act in a way that that modifies the activity of transcription factors, most likely NFκB and/or PPAR-γ. Indeed, EPA prevented NFκB activation in response to TNF-α in cultured pancreatic cells, an effect that involved decreased degradation of IκB through decreased phosphorylation [65]. Similarly, EPA or fish oil decreased LPS-induced activation of NFκB in cultured human monocytes [66–68] and this was associated with decreased IκB phosphorylation [67, 68], perhaps due to decreased activation of mitogen-activated protein kinases [69]. These observations suggest direct effects of n-3 PUFAs on inflammatory gene expression *via* inhibition of activation of the transcription factor NFκB, although it is not entirely clear which site in the activation cascade they target.

N-3 PUFAs might also enhance PPAR-γ activity, resulting in anti-inflammatory effects perhaps involving interference in NFκB activation. PPAR-γ is known to bind, and to be activated by, various fatty acids, including n-3 PUFAs and fatty acid derivatives [70–73]. This raises the possibility that n-3 PUFAs might attenuate inflammatory processes through this novel mechanism of action.

6 N-3 PUFAs and animal models of inflammatory bowel diseases

The recognition that n-3 PUFAs possess anti-inflammatory properties has prompted a series of studies investigating their efficacy in animal models of inflammatory bowel disease [7, 39, 74–79]. These have primarily involved chemically-induced colitis. The outcomes of these studies are summarised in Table 2. Two studies report that diets rich in ALA decrease colonic damage and inflammation compared with n-6 PUFA-rich diets [76, 78]. Whether these effects are due to ALA itself or relate to conversion of ALA to its longer chain, more unsaturated derivatives, like EPA, is not clear from these studies. Four studies report that dietary fish oil decreases chemically induced colonic damage and

Table 2. Summary of studies investigating the effect of dietary n-3 PUFAs in animal models of colitis

Reference	Animal	Model	Dietary fats compared and duration	Findings
74	Rat	TNBS	Sunflower oil *vs.* cod liver oil; 4 wk prior to TNBS; up to 50 days post-TNBS	Colonic damage, ulceration and inflammation were lower in the cod liver oil group especially at 50 days; time-dependent elevation in colonic luminal thromboxane B_2 was blunted in the cod liver oil group
75	Rat	Acetic acid	Saturated fat *vs.* n-6 PUFA *vs.* fish oil; 6 wk prior to acetic acid	In the absence of misoprostol pretreatment, ileal fluid absorption was normal in the fish oil group but not in the other two groups; with misoprostol pretreatment, colonic fluid loss was reversed (to absorption) in the fish oil group but not in the other two groups and colonic injury was lower in the fish oil group than in the other two groups
76	Rat	TNBS	Safflower oil *vs.* perilla oil (rich in ALA)	Colonic ulceration was lower and colonic weight was higher in the perilla oil group; plasma LTB_4 levels were lower in the perilla oil group
77	Rat	TNBS	Cotton and sunflower oils *vs.* fish oil; 6 wk before TNBS; 14 days post-TNBS	Colonic inflammation and damage were lower in the fish oil group; colonic tissue LTB_4 and LTC_4 were lower in the fish oil group
7	Rat	TNBS	Mix of olive oil, soybean oil and coconut oil (62.5:11.1:26.4) *vs.* mix of olive oil and fish oil (70:30) *vs.* mix of olive oil and pig brain phospholipids (70:30); 1 or 2 wk post-TNBS	Colonic damage was lower in the fish oil group than in the other two groups; colonic mucosa PGE_2 was lower in the olive oil and fish oil groups than in the brain phospholipids group at both time points; colonic mucosa LTB_4 was lower in the fish oil group than in the other two groups at both time points
78	Rat	TNBS	High LA liquid diet (44 LA:1 ALA) *vs.* ALA-rich liquid diet (3 LA:1 ALA); 12 days; TNBS given intraileally after 2 days starvation; sacrifice 24 h post-TNBS	Ileal inflammation, ulceration and damage were lower in the ALA group; ileal inflammatory cell infiltrate and bleeding were lower in the ALA group; serum IL-6 concentrations were lower in the ALA group 8 h post-TNBS
39	Wild type and *fat-1* transgenic mouse	DSS	Safflower oil; 10 wk prior to DSS; 8 days postfirst DSS exposure	Body weight loss, colon shortening, and colonic damage and inflammation were lower in *fat-1* mice; colonic tissue from *fat-1* mice (but not from wild type) contained LTB_5, PGE_3, resolvin E1, resolvin D3 and neuroprotectin DI; colonic tissue tumour necrosis factor-α, IL-1β, and inducible nitric oxide synthase mRNA levels and NFκB activation were lower in *fat-1* mice; colonic tissue Toll-interacting protein and trefoil factor 3 mRNA levels were higher in *fat-1* mice
79	Mouse	IL-10 knock-out	Corn oil *vs.* fish oil; up to 10 wk	Colonic inflammation was lower in the fish oil group

ALA, α-linolenic acid; DDS, dextran sulphate sodium; IL, interleukin; LT, leukotriene; PG, prostaglandin; TNBS, trinitrobenzene sulphonic acid.

inflammation compared with an n-6 PUFA-rich diet [7, 74, 75, 77]. These effects on disease severity were, in all cases, associated with a reduction in production of AA-derived eicosanoids (see Table 2 for details). A recent study investigated DSS-induced colitis in *fat-1* mice [39]. These mice are able to produce n-3 PUFAs from n-6 PUFAs due to the presence of an 'n-3 PUFA desaturase' gene. They show much less colonic damage and inflammation than wild-type mice and this is associated with a marked change in the pattern of inflammatory mediators present in colonic tissue. Another recent study in IL-10 knock-out mice, that spontaneously develop colitis, demonstrated significantly reduced colonic inflammation of the mice that were feed fish oil compared with n-6 PUFA-rich corn oil [79].

7 Trials of n-3 PUFAs in inflammatory bowel diseases

Long chain n-3 PUFAs are incorporated into gut mucosal tissue of patients with inflammatory bowel disease who supplement their diet with fish oil [80–82] and there are reports that this results in anti-inflammatory effects, such as decreased LTB_4 production by neutrophils [82–84] and colonic mucosa [84, 85], decreased PGE_2 and TXB_2 production by colonic mucosa [81] and decreased production of PGE_2 and INF-γ by blood mononuclear cells [86]. Small open-label or pilot studies reported clinical benefit of fish oil supplementation in ulcerative colitis [83, 87]. A number of randomised, placebo-controlled, double-blind studies of

Table 3. Summary of the results of placebo-controlled studies using dietary long chain n-3 PUFAs (in the form of fish oil) in patients with inflammatory bowel diseases

Reference	Disease	Dose of EPA + DHA (g/day)	Duration (wk)	Placebo	Effect of long chain n-3 PUFAs on clinical outcomes
80	UC and CD	1.8 + 1.3	12	Olive oil	Decreased sigmoidoscope score; decreased disease activity in UC (n.s.); no effect on disease activity in CD
88	UC	2.7 + 1.8	12	Mixed oils	Decreased disease activity; decreased use of corticosteroids; no effect on sigmoidoscope score; no effect on gut mucosal histology score
82	UC	4.5 + 1.1	52	Olive oil	Decreased use of corticosteroids in relapsing patients; induction of remission in relapsing patients; no effect on relapse for patients in remission
85	UC	3.2 + 2.2	16	Linoleic acid-rich vegetable oil	Decreased gut mucosal histology score; decreased use of corticosteroids; increased weight gain
89	UC	2.2 + 1.5 for 4 wk; then 1.1 + 0.75 for 20 wk	24	Olive oil	No effect on sigmoidoscope score; no effect on gut mucosal histology score; no effect on relapse; no effect on rectal bleeding
90	CD	1.8 + 0.9	52	SCFAs	Increased maintenance in remission; decreased relapse
91	UC	Total 5.1	104	Corn oil	No effect on gut mucosal histology score; no effect on disease activity; no effect on relapse
92	CD	2.8 + 1.5	52	Corn oil	No effect on relapse
93	UC	3.2 + 2.1	52	Olive oil	No effect on relapse
94, 95	UC	3.2 + 2.4	24	Sunflower oil	Decreased sigmoidoscope score; decreased gut mucosal histology score; decreased disease activity
96	UC	Total 5.6	24	Sunflower oil	Decreased disease activity; decreased sigmoidoscope score
97	CD	1.6 + 1.1	24	Olive oil	No effect on disease activity; no effect on body composition

CD, Crohn's disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; UC, ulcerative colitis. Modified with permission from ref. [30].

fish oil in inflammatory bowel disease have been reported [80, 82, 85, 88–97]. The characteristics and findings of these trials are summarised in Table 3. The dose of long chain n-3 PUFAs used in these trials was between 2.7 and 5.6 g/day and averaged about 4.5 g/day (see Table 3). Some of these trials indicate benefits of fish oil which include improved clinical score, improved gut mucosal histology, improved sigmoidoscopic score, lower rate of relapse, and decreased use of corticosteroids (Table 3). One study of special note is that of Belluzzi *et al*. [90] in which patients with Crohn's disease in remission were randomised to receive placebo or 2.7 g long chain n-3 PUFAs/day from an enterically coated fish oil preparation for 1 year. The primary outcome was relapse. There was a significant difference in the proportion of patients who relapsed over 12 months: 11/39 (28%) in the fish oil group *versus* 27/39 (69%) in the placebo group ($p < 0.001$). Likewise there was a significant difference in the proportion of patients who remained in remission at 12 months: 59% in the fish oil group *versus* 26% in the placebo group ($p = 0.003$). Two studies not listed in Table 3 also merit discussion [98, 99]. In a randomised, placebo-controlled trial, Middleton *et al*.

[98] gave patients with ulcerative colitis a preparation that included the n-6 PUFA GLA (1.62 g/day) in addition to EPA (0.27 g/day) and DHA (0.045 g/day) for 12 months. The rationale for providing GLA is that its longer chain, more unsaturated derivative DGLA (Fig. 2) is converted to PGE_1 *via* cyclooxygenase and to 15-hydroxy-DGLA by 15-lipoxygenase. PGE_1 is considered anti-inflammatory [100] while 15-hydroxy-DGLA inhibits 5-lipoxygenase, so decreasing formation of inflammatory 4-series LTs. The therapeutic potential of enriching inflammatory cells in DGLA is shown by studies using the PGE_1 analog misoprostol which is protective in animal models of chemically induced colitis [101 – 104]. Middleton *et al*. [98] found no differences in relapse rate or sigmoidoscope score between the placebo and PUFA groups. Dichi *et al*. [99] randomised patients with ulcerative colitis to sulphasalazine (2 g/day) or fish oil (3.2 g EPA + 2.16 g DHA/day) for 2 months in a crossover design. Neither treatment changed colonic histology score from study entry; however, fish oil, but not sulphasalazine, significantly decreased the sigmoidoscope score [99].

Reviews of trials of fish oil in inflammatory bowel diseases have been published [105 – 107] and these conclude that there is some benefit from fish oil in inflammatory bowel diseases. A meta-analysis identified 13 studies of fish oil supplementation in inflammatory bowel diseases reporting outcomes related to clinical score, sigmoidoscope score, gut mucosal histology score, induced remission and relapse [108]. However, it was concluded that that there were sufficient data to perform meta-analysis only for relapse and only for ulcerative colitis. Relapse was reported in five studies in ulcerative colitis (see Table 3), and three of these were used for meta-analysis [82, 91, 93]. Two of these studies reported a higher rate of relapse with fish oil compared with placebo [82, 91], although this was not significant in either study, while one reported no effect [93]. The pooled risk of relapse with long chain n-3 PUFAs relative to placebo was 1.13 (95% CI: 0.91, 1.57). This meta-analysis concluded that ‚n-3 fatty acids have no effect on relative risk of relapse in ulcerative colitis' but that ‚there was a statistically nonsignificant reduction in requirement for corticosteroids for n-3 fatty acids relative to placebo in two studies' [108]. A recent study, not considered in the meta-analysis, reported no effect of 2.7 g EPA + DHA/day for 24 wk on disease activity in patients with Crohn's disease [97]. Thus, despite several favourable studies, the overall view at the moment must be that there is only weak evidence that long chain-3 PUFAs have clinical benefits in inflammatory bowel diseases. However, the apparent ability of long chain n-3 PUFAs to retain Crohn's disease patients in remission [90] is a striking finding and merits further investigation. One reason why fish oil might be more effective in animal models than in patients with disease is that the dose of long chain n-3 PUFAs provided to patients, although rather high in terms of habitual consumption of these fatty acids, is low compared with that used in animal studies.

8 Overall conclusions

Eicosanoids derived from the n-6 PUFA AA, particularly LTB_4, play a role in inflammatory bowel diseases. The role of PGE_2 is not clear and it may be protective; recent studies indicate mechanisms to support a protective role. Long chain n-3 PUFAs decrease the production of inflammatory eicosanoids from AA and promote the production of less inflammatory eicosanoids from EPA and of anti-inflammatory resolvins and similar mediators from EPA and DHA. Long chain n-3 PUFAs have other anti-inflammatory actions including decreasing leukocyte chemotaxis, adhesion molecule expression and inflammatory cytokine production. Some of these effects are exerted through decreased activation of the pro-inflammatory transcription factor NFκB and perhaps through increased activation of the anti-inflammatory transcription factor PPAR-γ. Thus, n-3 PUFAs act *via* both lipid mediator-related and nonlipid mediator-related mechanisms. Whatever their mechanism of action, n-3 PUFAs are potentially useful anti-inflammatory agents. Work with chemically induced colitis in animal models has consistently demonstrated efficacy of fish oil. There have been a number of clinical trials of fish oil in patients with inflammatory bowel diseases. Although some trials report clinical improvement (*e.g.* improved gut histology, decreased disease activity, decreased use of corticosteroids, decreased relapse), when the trials have been pooled no consistently strong clinical effect has emerged. Perhaps more, better designed and larger trials are required to assess the therapeutic potential of long chain n-3 PUFAs in inflammatory bowel diseases.

The author has declared no conflict of interest.

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