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Dietary modification of inflammation with lipids

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The *n*-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found in high proportions in oily fish and fish oils. The *n*-3 PUFA are structurally and functionally distinct from the *n*-6 PUFA. Typically, human inflammatory cells contain high proportions of the *n*-6 PUFA arachidonic acid and low proportions of *n*-3 PUFA. The significance of this difference is that arachidonic acid is the precursor of 2-series prostaglandins and 4-series leukotrienes, which are highly-active mediators of inflammation. Feeding fish oil results in partial replacement of arachidonic acid in inflammatory cell membranes by EPA. This change leads to decreased production of arachidonic acid-derived mediators. This response alone is a potentially beneficial anti-inflammatory effect of *n*-3 PUFA. However, *n*-3 PUFA have a number of other effects which might occur downstream of altered eicosanoid production or might be independent of this activity. For example, animal and human studies have shown that dietary fish oil results in suppressed production of pro-inflammatory cytokines and can decrease adhesion molecule expression. These effects occur at the level of altered gene expression. This action might come about through antagonism of the effects of arachidonic acid-derived mediators or through more direct actions on the intracellular signalling pathways which lead to activation of transcription factors such as nuclear factor kappa B (NFκB). Recent studies have shown that *n*-3 PUFA can down regulate the activity of the nuclear transcription factor NFκB. Fish oil feeding has been shown to ameliorate the symptoms in some animal models of chronic inflammatory disease and to protect against the effects of endotoxin and similar inflammatory challenges. Clinical studies have reported that oral fish oil supplementation has beneficial effects in rheumatoid arthritis and among some patients with asthma, supporting the idea that the *n*-3 PUFA in fish oil are anti-inflammatory. There are indications that inclusion of *n*-3 PUFA in enteral and parenteral formulas might be beneficial to patients in intensive care or post-surgery.

Fatty acids: Fish oil: Eicosanoids: Cytokines: Inflammation

Inflammation in health and disease

Inflammation is the body's immediate response to infection or injury. It is typified by redness, swelling, heat and pain. These characteristic responses occur as a result of increased blood flow, increased permeability across blood capillaries which permits large molecules (e.g. complement, antibodies, cytokines) to leave the bloodstream and cross the endothelial wall, and increased movement of leucocytes from the bloodstream into the surrounding tissue. Inflammation functions to begin the immunological process of elimination of invading pathogens and toxins, and to repair damaged tissue. These responses must be ordered and controlled. Four interconnected proteolytic cascade systems present in the blood are activated; these systems are the coagulation, kinin, fibrinolytic and complement cascades. The role of the coagulation system is to prevent bleeding and to prevent pathogens from entering damaged vessels. The fibrinolytic system functions to remove blood clots (i.e. to antagonise the coagulation system). The kinins are inflammatory mediators such as bradykinin which is responsible for inducing pain, increasing vascular permeability and causing vasodilation. The complement system forms a link between the other three cascades and the immunological response to infection and injury. Products of complement activation can increase vascular permeability, and can trigger mast cells to release histamine resulting in vasodilation and promotion of chemotaxis of leucocytes towards the site of injury. The movement of cells into the inflammatory or infected site is induced by the up-regulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)

Abbreviations: COX, cyclo-oxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; IκB, inhibitory subunit of NFκB; IL, interleukin; LPS, lipopolysaccharide; LT, leukotriene; NFκB, nuclear factor kappa B; MCT, medium-chain triacylglycerols; PG, prostaglandin; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acids; TNF, tumour necrosis factor; TX, thromboxane; VCAM-1, vascular cell adhesion molecule-1.

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and E-selectin on the surface of endothelial cells, allowing leucocyte binding and subsequent diapedesis. The earliest cells appearing at inflamed sites are granulocytes, with monocytes and/or macrophages and lymphocytes appearing later. Granulocytes, monocytes and macrophages are involved in pathogen killing, in clearing up cellular and tissue debris, and in tissue repair. Helper T lymphocytes act to regulate monocyte and/or macrophage, natural killer cell, and B lymphocyte activity. Other lymphocytes aid in the elimination of pathogens (e.g. through the activity of cytotoxic T lymphocytes and the production of antibodies by B lymphocytes). Acute inflammation is the term applied to the early vascular and cellular responses (i.e. up to and including granulocyte infiltration), while chronic inflammation is the term applied to the later cellular events involving monocytes and/or macrophages and lymphocytes. The activity of these cells is induced by certain triggers. One important exogenous trigger is bacterial endotoxin (also known as lipopolysaccharide (LPS)), a component of the cell wall of Gram-negative bacteria. LPS can trigger complement activation (resulting in vasodilation and increased vascular permeability), coagulation, fibrinolysis and the kinin cascade. LPS can directly activate monocytes and macrophages inducing them to produce cytokines such as: tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and IL-8; eicosanoids, such as prostaglandin (PG) E_2; NO; matrix metalloproteinases; other mediators. LPS also induces adhesion molecule expression on the surface of endothelial cells and leucocytes. Histamine, peptides produced by the complement, coagulation and kinin systems, cytokines and eicosanoids are all endogenous mediators of inflammation. The cytokines produced by monocytes and macrophages also serve to regulate the whole-body response to infection and injury (Fig. 1); for example, they will act on the liver to promote acute-phase protein synthesis, on skeletal muscle and adipose tissue to promote proteolysis and lipolysis respectively (this process is believed to be the body's way of providing fuels to the immune system), and on the brain to reduce appetite and induce fever (Fig. 1). These cytokines will also interact with

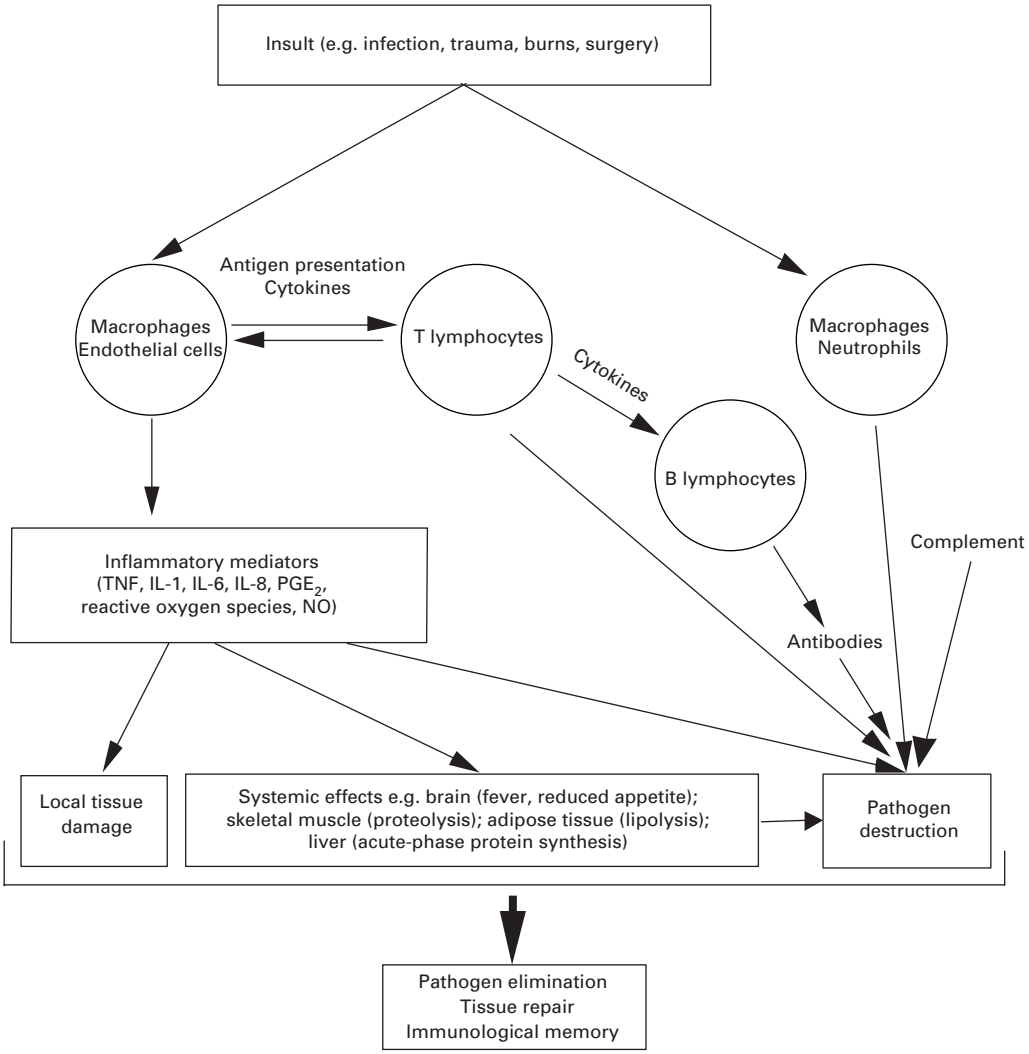

Fig. 1. The interrelationship between components of the innate and acquired immune responses. IL, interleukin; PG, prostaglandin; TNF, tumour necrosis factor.

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T lymphocytes. Antigen-presenting cells, which include activated monocytes and macrophages, will present antigen to T lymphocytes, and so the acquired immune response will be triggered (Fig. 1). Now there will be a cell-mediated response to the antigen. T lymphocytes will produce cytokines (e.g. interferon (IFN)-γ) which will regulate the activity of the cells involved in the innate response (monocytes, macrophages, natural killer cells), promote the proliferation of B and T lymphocytes and promote antibody production by B lymphocytes. By virtue of the integrated innate and acquired responses the source of the antigen should be eliminated and a component of immunological memory will remain (Fig. 1).

Thus, inflammation and the inflammatory response are part of the normal innate immune response. However, when inflammation occurs in an uncontrolled manner disease ensues. High levels of TNF-α, IL-1β and IL-6 are particularly destructive. Chronic overproduction of TNF-α and IL-1 may cause muscle wasting and loss of bone mass. TNF-α, IL-1 and IL-6 are implicated in causing some of the pathological responses which occur in endotoxic shock, and in adult respiratory distress syndrome and chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. It is this highly destructive nature of these and other inflammatory mediators which prompted Thomas (1972) to say 'Our arsenals for fighting off bacteria are so powerful, and involve so many different defence mechanisms, that we are more in danger from them than from the invaders. We live in the midst of explosive devices; we are mined'.

Fatty acids and eicosanoids

The fatty acid composition of inflammatory cells

Inflammatory and immune cells from laboratory animals consuming a typical laboratory chow and from human subjects consuming a typical Western diet contain a high proportion of the *n*-6 polyunsaturated fatty acid (PUFA) arachidonic acid (20:4*n*-6) and low proportions of *n*-3 PUFA, especially eicosapentaenoic acid (20:5*n*-3; EPA; see Calder, 1998, 2001*a*). The exact proportion of arachidonic acid in human inflammatory cells varies according to cell type and the lipid fraction examined (Gibney & Hunter, 1993; Sperling *et al*. 1993). The phospholipids of human mononuclear cells (an approximately 70:20:10 mixture of T lymphocytes, B lymphocytes and monocytes purified from human blood) contain (% total fatty acids) 6–10 linoleic acid (18:2*n*-6), 1–2 dihomo-γ-linolenic acid (20:3*n*-6) and 15–25 arachidonic acid (Yaqoob *et al*. 2000; Thies *et al*. 2001). In contrast, the proportions of *n*-3 fatty acids are low; α-linolenic acid (18:3*n*-3) is rare and EPA and docosahexaenoic acid (22:6*n*-3 DHA) comprise only 0·1–0·8 and 2–4 % total fatty acids respectively (Yaqoob *et al*. 2000; Thies *et al*. 2001).

EPA and DHA are found in high proportions in oily fish and fish oils. Animal and human studies show that feeding fish oil results in increased proportions of EPA and DHA in inflammatory cell phospholipids (for references, see Calder 1998, 2001*a*). These fatty acids are incorporated largely at the expense of arachidonic acid (see Calder 1998, 2001*a*). Similar effects occur in neutrophils, monocytes, T lymphocytes and B lymphocytes (Gibney & Hunter, 1993).

Arachidonic acid as an eicosanoid precursor

The principal functional role for arachidonic acid is as a substrate for synthesis of the family of bioactive mediators known as eicosanoids (PG, thromboxanes (TX), leukotrienes (LT), hydroxy-eicosatetraenoic acids etc.; Fig. 2). In fact, several C_{20} PUFA are able to serve as precursors of eicosanoids. However, because the membranes of most cells contain large amounts of arachidonic acid, compared with other potential eicosanoid precursors (including EPA), arachidonic acid is usually the principal precursor of eichosanoid synthesis. Arachidonic acid in cell membranes can be mobilised by various phospholipase enzymes, most notably phospholipase A_2, and the free arachidonic acid can subsequently act as a substrate for the enzymes which synthesise eicosanoids (Fig. 2). Metabolism of arachidonic acid by cyclo-oxygenase (COX) enzymes gives rise to the 2-series PG and TX (Fig. 2). There are two isoforms of COX: COX-1 is a constitutive enzyme; COX-2 is induced in inflammatory cells as a result of stimulation, and is responsible for the markedly elevated production of PG which occurs on cellular activation. There are at least sixteen different 2-series PG, and these PG are formed in a cell-specific manner. For example, monocytes and macrophages produce large amounts of PGE_2 and PGF_2, neutrophils produce moderate amounts of PGE_2 and mast cells produce PGD_2. Metabolism of arachidonic acid by the 5-lipoxygenase pathway gives rise to hydroxy and hydroperoxy derivatives (5-hydroxyeicosatetraenoic acid and 5-hydroperoxyeicosatetraenoic acid respectively), and the 4-series LT (LTA_4, B_4, C_4, D_4 and E_4; Fig. 2). 5-Lipoxygenase is found in mast cells, monocytes, macrophages and granulocytes.

Eicosanoids are involved in modulating the intensity and duration of inflammatory responses (for reviews, see Kinsella *et al*. 1990; Lewis *et al*. 1990; Tilley *et al*. 2001). The effects of PGE_2 and LTB_4 have been studied most widely. PGE_2 has a number of pro-inflammatory effects, including inducing fever, increasing vascular permeability and vasodilation, and enhancing pain and oedema caused by other agents such as bradykinin and histamine. PGE_2 suppresses lymphocyte proliferation and natural killer cell activity, and inhibits production of IL-2 and IFN-γ; thus, in these respects PGE_2 is immunosuppressive. PGE_2 also promotes immunoglobulin E production by B lymphocytes; immunoglobulin E is a mediator of allergic inflammation. LTB_4 increases vascular permeability, enhances local blood flow, is a potent chemotactic agent for leucocytes, induces release of lysosomal enzymes, enhances generation of reactive oxygen species, enhances production of TNF-α, IL-1 and IL-6, and inhibits lymphocyte proliferation. In inflammatory conditions increased rates of production of arachidonic acid-derived eicosanoids are found, and elevated levels of these eicosanoids are found in blood and tissues from patients with a variety of inflammatory disorders (Kinsella *et al*. 1990; Lewis *et al*. 1990).

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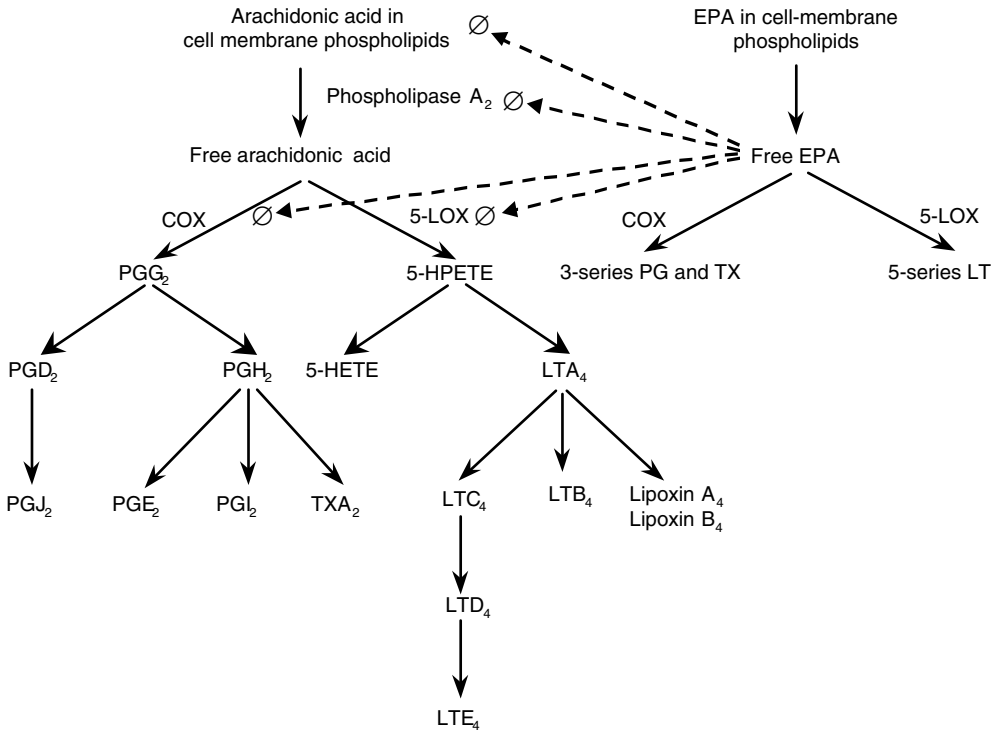

Fig. 2. Synthesis of eicosanoids from arachidonic acid and sites of inhibition by eicosapentaenoic acid. COX, cyclo-oxygenase; EPA, eicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxy-eicosatetraenoic acid; LOX, lipoxygenase; LT, leukotriene; PG, prostaglandin; TX, thromboxane.

Eicosapentaenoic acid as an alternative eicosanoid precursor

Since feeding animals or human subjects increased amounts of fish oil results in a decrease in the amount of arachidonic acid in the membranes of inflammatory cells, there will be less substrate available for synthesis of eicosanoids from arachidonic acid. Furthermore, EPA inhibits arachidonic acid release from phospholipids by phospholipase A_2 and competitively inhibits the oxygenation of arachidonic acid by COX (Obata *et al*. 1999). Thus, fish oil feeding results in a decreased capacity of inflammatory cells to synthesise eicosanoids from arachidonic acid. For example, after fish oil supplementation of the human diet a 50–65 % decrease in PGE_2 production by mononuclear cells has been reported (Endres *et al*. 1989; Meydani *et al*. 1993; Caughey *et al*. 1996). Detailed examinations of the effect of dietary fish oil on production of 5-lipoxygenase products were made by Lee *et al*. (1985) and Sperling *et al*. (1993). These authors demonstrated that fish oil providing 5·4 or 14·4 g EPA + DHA/d leads to a 40–70 % decrease in generation of LTB_4, 6-*trans* LTB_4, 20-hydroxy LTB_4 and 5-hydroxyeicosatetraenoic acid by neutrophils and monocytes stimulated by various agents including Ca ionophore, zymosan, and platelet-activating factor.

In addition to inhibiting metabolism of arachidonic acid, EPA is able to act as a substrate for both COX and 5-lipoxygenase (Fig. 2), giving rise to derivatives which have a structure different from those produced from arachidonic acid (i.e. 3-series PG and TX and 5-series LT). Thus, the EPA-induced suppression in the production of arachidonic acid-derived eicosanoids is accompanied by an elevation in the production of EPA-derived eicosanoids. Again this response has been clearly demonstrated by Lee *et al*. (1985) and Sperling *et al*. (1993), who showed that dietary fish oil results in significantly increased generation of LTB_5, 6-*trans* LTB_5 and 5-hydroxyeicosapentaenoic acid by stimulated neutrophils and monocytes. The eicosanoids produced from EPA are considered to be less biologically potent than the analogues synthesised from arachidonic acid, although the full range of biological activities of these compounds has not been investigated. The best example of differential inflammatory potencies of eicosanoids produced from arachidonic acid and EPA is that of LTB_4 *v*. LTB_5. LTB_5 is at least 10-fold less potent as a neutrophil chemoattractant than LTB_4, and on this basis can be considered to be less pro-inflammatory. One other aspect of the formation of alternative eicosanoids to those produced from arachidonic acid is that they will share the same receptor on target cells and, therefore, will act to antagonise the arachidonic acid-derived mediators. The reduction in generation of arachidonic acid-derived mediators which accompanies fish oil consumption has led to the idea that fish oil is anti-inflammatory.

The isolated perfused rabbit lung has been used as a model to study the pathophysiological effects of arachidonic acid- and EPA-derived eicosanoids. Infusion with *Escherichia coli* haemolysin was shown to induce vasoconstriction and hypertension, mediated by TXB_2, and vascular permeability and/or leakage, mediated by 4-series LT (Grimminger *et al*. 1995, 1997). Inclusion of free arachidonic acid in the perfusate increased TXB_2 and 4-series

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LT generation, arterial pressure and vascular leakage (Grimminger *et al*. 1995, 1997). In contrast, inclusion of EPA decreased TXB_2 and 4-series LT generation, arterial pressure and vascular leakage, and increased generation of TXB_3 and 5-series LT (Grimminger *et al*. 1995). Perfusion of isolated rabbit lungs with a fish oil-containing emulsion markedly attenuated the vascular inflammatory reaction (hypertension) induced by Ca ionophore (Breil *et al*. 1996). Compared with perfusion with a soyabean oil-rich emulsion, fish oil decreased the concentration of LTC_4 in the perfusate by >50 % and increased the concentration of LTC_5 from barely detectable (<10 pg/ml) to a concentration very similar to that of LTC_4 (approximately 150 pg/ml; Breil *et al*. 1996). These observations indicate that *n*-3 PUFA can inhibit the acute inflammatory responses induced, or at least marked, by production of arachidonic acid-derived eicosanoids.

Anti-inflammatory effects of *n*-3 polyunsaturated fatty acids other than altered eicosanoid production

Although their action in antagonising arachidonic acid metabolism is a key anti-inflammatory effect of *n*-3 PUFA, these fatty acids have a number of other anti-inflammatory effects which might occur downstream of altered eicosanoid production or might be independent of this activity. For example, animal and human studies have shown that dietary fish oil results in suppressed production of pro-inflammatory cytokines and can modulate adhesion molecule expression.

n-3 Polyunsaturated fatty acids and inflammatory cytokine production

Since inflammatory cytokine production is regulated by arachidonic acid-derived eicosanoids (see Rola-Pleszczynski & Stankova, 1992) and since dietary *n*-3 PUFA affect production of these eicosanoids, it is expected that *n*-3 PUFA will affect cytokine production. Indeed this is the case (for reviews, see Calder 1997, 1998, 2001*a*). Cell-culture studies demonstrated the ability of EPA and DHA to inhibit the production of IL-1β and TNF-α by human monocytes (Sinha *et al*. 1991; Baldie *et al*. 1993), and the production of IL-6 and IL-8 by human venous endothelial cells (de Caterina *et al*. 1994; Khalfoun *et al*. 1997). Culture of a monocytic cell line with EPA or DHA resulted in decreased production of tissue factor, TNF-α and IL-1β (Chu *et al*. 1999). Fish oil feeding decreased *ex vivo* production of TNF-α, IL-1β and IL-6 by rodent macrophages (Billiar *et al*. 1988; Renier *et al*. 1993; Yaqoob & Calder, 1995; Sasaki *et al*. 2000; Wallace *et al*. 2000) and splenocytes (Xi *et al*. 1998). Infusion of fish oil into horses resulted in decreased production of TNF-α by isolated blood monocytes (McCann *et al*. 2000), while infusion of fish oil into rats decreased production of TNF-α and IL-6 by blood monocytes (Grimm *et al*. 1994). Feeding fish oil to mice infected with the murine AIDS virus decreased production of TNF-α and IL-1β by LPS-stimulated splenocytes (Xi *et al*. 1998). Compared with feeding safflower oil, fish oil feeding resulted in lower peak plasma TNF-α IL-1β and IL-6 concentrations after intraperitoneal injection of LPS in mice (Sadeghi *et al*. 1999). Furthermore, parenteral nutrition supplemented with fish oil decreased serum TNF-α, IL-6, and IL-8 concentrations in burned rats compared with *n*-6 PUFA-rich parenteral nutrition (Hayashi *et al*. 1998; Tashiro *et al*. 1998).

Supplementation of the diet of human volunteers with fish oil providing more than 2·4 g EPA + DHA/d has been shown to decrease production of TNF (Endres *et al*. 1989; Meydani *et al*. 1991; Gallai *et al*. 1993; Caughey *et al*. 1996), IL-1 (Endres *et al*. 1989; Meydani *et al*. 1991; Gallai *et al*. 1993; Caughey *et al*. 1996) and IL-6 (Meydani *et al*. 1991) by mononuclear cells. One other study in which subjects consumed a low-fat diet including oily fish daily (providing 1·2 g EPA + DHA/d) showed decreased production of TNF, IL-1 and IL-6 (Meydani *et al*. 1993). Parenteral nutrition supplemented with fish oil has also been shown to affect circulating inflammatory cytokine concentrations; patients received either a medium-chain triacylglycerol (MCT)–long chain triacylglycerol mix or this mix also containing fish oil for 5 d post-abdominal surgery (Wachtler *et al*. 1997). Patients received 50 g fat/d on the first 2 d and then 100 g fat/d on days 3, 4 and 5; thus patients in the fish oil group received 3 g (days 1 and 2) and 6 g (days 3, 4 and 5) *n*-3 PUFA/d. Serum TNF-α and IL-6 concentrations were lower in patients receiving fish oil (Wachtler *et al*. 1997).

n-3 Polyunsaturated fatty acids and adhesion molecule expression

Although it was shown some years ago that culture of murine macrophages with *n*-3 PUFA decreased their ability to bind to various surfaces (Calder *et al*. 1990), the first demonstration that these fatty acids could affect the expression of adhesion molecules on the cell surface was by de Caterina *et al*. (1994). These authors showed that culture of human venous endothelial cells with DHA decreased cytokine-induced surface expression of E-selectin, ICAM-1 and VCAM-1 (de Caterina *et al*. 1994), and impaired the ability of ligand-bearing monocytes to adhere (de Caterina & Libby, 1996). Although de Caterina *et al*. (1994) reported that EPA was without effect, Kim *et al*. (1995) showed that EPA also inhibited LPS-induced expression of these three adhesion molecules on human venous endothelial cells, and again this action had the functional effect of decreasing binding of monocytes. Khalfoun *et al*. (1996) went on to show that both EPA and DHA could decrease the expression of VCAM-1 on the surface of cytokine-activated human endothelial cells. In another cell-culture study Hughes *et al*. (1996*b*) reported that EPA decreased surface expression of ICAM-1 on monocytes stimulated with IFN-γ.

Studies of dietary fatty acids and adhesion molecule expression are few. Dietary fish oil decreased expression of certain adhesion molecules, including ICAM-1, on the surface of rat lymphocytes (Sanderson *et al*. 1995*a*,*b*; Sanderson & Calder, 1998*a*) and murine macrophages (Miles *et al*. 2000). Supplementing the diet of healthy human subjects with fish oil providing about 1·5 g EPA + DHA/d resulted in a lower level of expression of ICAM-1

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on the surface of blood monocytes stimulated *ex vivo* with IFN-γ (Hughes *et al*. 1996*a*). Recently, dietary fish intake was found to decrease circulating levels of soluble VCAM-1 in elderly subjects (Miles *et al*. 2001), but it is not clear if this response represents decreased surface expression of VCAM-1.

Effects of *n*-3 polyunsaturated fatty acids on expression of genes involved in inflammation

n-3 Polyunsaturated fatty acids and inflammatory gene expression

Many of the anti-inflammatory effects of *n*-3 PUFA appear to be exerted at the level of altered gene expression. However, these effects have been demonstrated only a limited number of times, and often in artificial *in vitro* settings, and thus the extent of these effects *in vivo* is not yet clear. Nevertheless, cell-culture, and animal feeding, studies indicate potentially very potent effects of *n*-3 PUFA on expression of a range of inflammatory genes.

A recent study demonstrated that culturing bovine chondrocytes with α-linolenic acid, EPA or DHA markedly decreases cytokine-mediated induction of expression of the COX-2 (but not COX-1), TNF-α and IL-1α genes (Curtis *et al*. 2000). The same study investigated the influence of *n*-3 PUFA on the expression of aggrecanase-1 and -2 genes. Aggrecanase-1 and -2 degrade cartilage proteoglycan, and their expression in cartilage is up regulated in response to the pro-inflammatory cytokines TNF-α and IL-1. *n*-3 PUFA, but not other fatty acids, inhibited cytokine-mediated up-regulation of expression of the aggrecanase-1 and aggrecanase-2 genes, and of aggrecanase activity (Curtis *et al*. 2000). McCabe *et al*. (1999) reported that culture of renal carcinoma cells with EPA decreased the level of mRNA for matrix metalloproteinase-2. In an earlier study de Caterina *et al*. (1994) had demonstrated that the down-regulation of 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. Culture of murine peritoneal macrophages with DHA, but not with EPA, decreased the level of mRNA for inducible NO synthase after stimulation with LPS and IFN-γ (Khair-El-Din *et al*. 1996). This effect correlated with decreased production of NO, and was due to decreased transcription of the inducible NO synthase gene (Khair-El-Din *et al*. 1996).

Several animal feeding studies have demonstrated an effect of dietary fish oil on inflammatory gene expression. 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 (Chandrasekar & Fernandes, 1994). Feeding wild-type mice a fish oil-rich diet decreased the level of IL-1β mRNA in LPS- or phorbol ester-stimulated spleen lymphocytes (Robinson *et al*. 1996); 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 (Renier *et al*. 1993). Wallace *et al*. (2001) reported that feeding fish oil to mice resulted in lower levels of IFN-γ mRNA in mitogen-stimulated spleen lymphocytes. ICAM-1 mRNA levels were lower in fresh peritoneal macrophages from mice fed fish oil (Miles *et al*. 2000).

The mechanism(s) by which n-3 polyunsaturated fatty acids might down regulate inflammatory gene expression

Eicosanoids derived from arachidonic acid are able to regulate inflammatory gene expression. Thus, the effects of *n*-3 PUFA described earlier might come about through antagonism of the effects of arachidonic acid-derived mediators. However, at least some (if not all) of these effects seem to occur in an eicosanoid-independent manner (for example, see de Caterina *et al*. 1994). It is now emerging that *n*-3 PUFA might exert their effects through direct actions on the intracellular signalling pathways which lead to activation of one or more transcription factors such as nuclear factor kappa B (NFκB).

NFκB plays a role in inducing a range of inflammatory genes including COX-2, ICAM-1, VCAM-1, E-selectin, TNF-α, IL-1β, IL-6, inducible NO synthase, acute-phase proteins and matrix metalloproteinases in response to inflammatory stimuli (Christman *et al*. 1998; Chen *et al*. 1999; Fig. 3). The signalling pathway leading to NFκB activation is becoming better understood (Karin & Ben-Neriah, 2000; Karin & Delhase, 2000). NFκB exists as an inactive heterotrimer in the cytosol of resting inflammatory cells; one of the subunits is called inhibitory subunit of NFκB (IκB). On stimulation, a signalling cascade activates a protein complex known as IκB kinase. Activated IκB kinase phosphorylates IκB on two N-terminal serine residues, thus promoting its dissociation from the rest of the inactive NFκB trimer. The remaining NFκB heterodimer is rapidly translocated to the nucleus where it binds to response elements in target genes, so regulating their transcription. The phosphorylated IκB undergoes polyubiquination, targeting it for degradation by the 26S proteasome. Recent studies have shown that *n*-3 PUFA can down regulate the activity of the nuclear transcription factor NFκB. Xi *et al*. (2001) reported that feeding mice fish oil resulted in a lower level of NFκB in the nucleus (i.e. activated NFκB) of LPS-stimulated spleen lymphocytes compared with feeding maize oil. Infecting the mice with the murine AIDS virus resulted in increased NFκB in the nucleus, but the level was lower in fish oil-fed mice (Xi *et al*. 2001). The mechanism by which *n*-3 PUFA decreases the activation of NFκB is not clear. However, Chen & Zhao (2001) report, in an abstract, that incubating human monocytes with EPA decreased LPS-induced activation of NFκB and that this response was associated with decreased phosphorylation of IκB. This finding suggests an effect of *n*-3 PUFA on the signalling process leading to activation of IκB kinase. Ross *et al*. (1999) reported that incubation of a pancreatic cell line with TNF-α markedly up regulated degradation of IκB, and that this activity could be totally abolished by previous incubation of the cells with EPA. This effect could be due to inhibition of phosphorylation of IκB, so preventing it from being targeted for degradation, or to inhibition of the degradation process itself.

A second group of transcription factors currently undergoing scrutiny for their role in inflammation are

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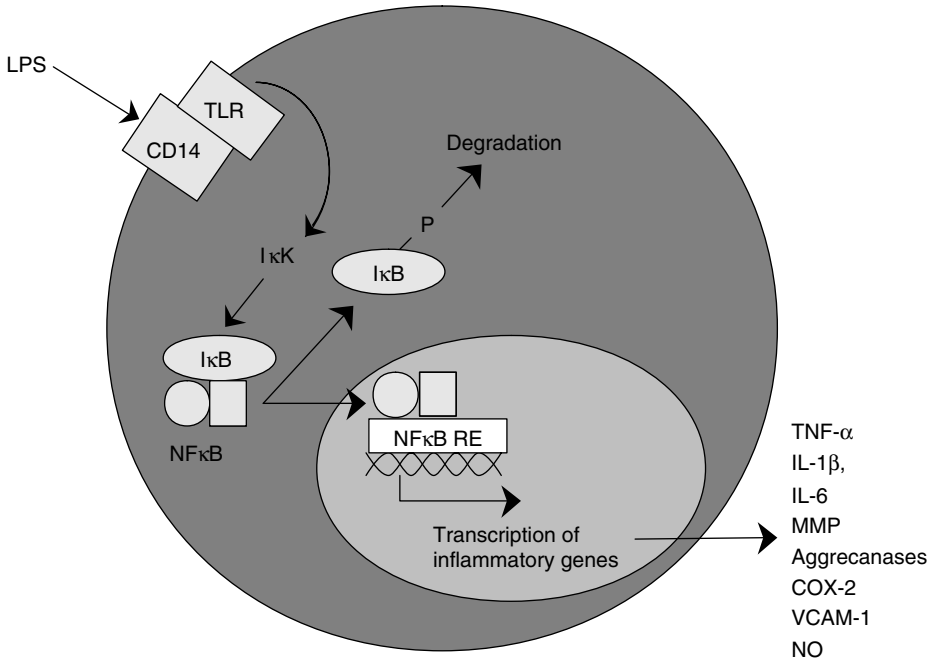

Fig. 3. Outline of the pathway of up-regulation of inflammatory gene expression via nuclear factor kappa B. CD14, cluster of differentiation 14 (the LPS receptor); COX, cyclo-oxygenase; IκB, inhibitory subunit of NFκB; IκK, IκB kinase; IL, interleukin; LPS, lipopolysaccharide; MMP, matrix metalloproteinases; NFκB, nuclear factor kappa B; RE, response element; TLR, toll-like receptor; TNF, tumour necrosis factor; VCAM, vascular cell adhesion molecule.

the peroxisome proliferator-activated receptors (PPAR). The main members of this family are PPARα and PPARγ. PPARα and PPARγ play important roles in liver and adipose tissue respectively (Schoonjans *et al*. 1996). However, they are also found in inflammatory cells (Chinetti *et al*. 1998; Ricote *et al*. 1998). PPAR dimerise with the retinoid-X-receptor to regulate gene expression, and they can bind, and appear to be regulated by, PUFA and eicosanoids (Kliewer *et al*. 1995; Devchand *et al*. 1996). PPARα-deficient mice have a prolonged response to inflammatory stimuli (Devchand *et al*. 1996), suggesting that PPARα activation might be 'anti-inflammatory'. More recently, activators of both PPARα and PPARγ have been shown to inhibit the activation of inflammatory genes, including TNF-α, IL-1β, IL-6, IL-8, COX-2, VCAM-1, inducible NO synthase, matrix metallo-proteinases and acute-phase proteins (Jiang *et al*. 1998; Poynter & Daynes, 1998; Ricote *et al*. 1998; Jackson *et al*. 1999; Marx *et al*. 1999; Takano *et al*. 2000; Wang *et al*. 2001; Xu *et al*. 2001). Two mechanisms for the anti-inflammatory actions of PPAR have been proposed (for reviews, see Chinetti *et al*. 2000; Delerive *et al*. 2001). The first mechanism is that PPAR might stimulate the breakdown of inflammatory eicosanoids through induction of peroxisomal β-oxidation. The second mechanism is that PPAR might interfere with or antagonise the activation of other transcription factors, including NFκB. Feeding mice fish oil increases the expression of PPARα and PPARγ in liver and adipose tissue respectively (Donnellan *et al*. 2000), and increases the expression of PPAR-inducible genes in these tissues (for example, see Berthou *et al*. 1995). Although the effect of fish oil on PPAR expression in inflammatory cells has not been reported, these studies suggest that *n*-3 PUFA might act by increasing the level of these anti-inflammatory transcription factors in such cells.

There are a number of other transcription factors which are activated by inflammatory signals and which play a role in expression of inflammatory genes (for a review, see Hwang & Rhee, 1999). It is possible that *n*-3 PUFA might affect the activation of these factors, but this possibility has not been studied in detail. However, effects of *n*-3 PUFA on signalling processes which lead to activation of various transcription factors including, but not necessarily restricted to, NFκB have been reported. Several early cell signalling events are affected by *n*-3 PUFA. For example, EPA and DHA inhibited the anti-CD3-induced increase in intracellular free Ca concentration in the JURKAT T-cell line (Chow *et al*. 1990; Breittmayer *et al*. 1993), acting by blocking Ca entry into the cells through a direct effect on receptor-operated Ca channels (Chow *et al*. 1990). Feeding fish oil was shown to decrease the tyrosine phosphorylation state of phospholipase C-γ in rat lymphocytes (Sanderson & Calder, 1998*b*) and to reduce the generation of the signalling molecules inositol-1,4,5-trisphosphate (Sanderson & Calder, 1998*b*), diacylglycerol and ceramide (Jolly *et al*. 1997) in lymphocytes. EPA and DHA inhibited protein kinase C activity in rat lymphocytes (May *et al*. 1993) and peritoneal macrophages (Tappia *et al*. 1995). Recently, incubation of murine macrophages with

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EPA was found to decrease LPS-induced phosphorylation and activation of mitogen-activated protein kinase (Lo *et al*. 2000). Thus, a variety of intracellular signalling steps are partly inhibited by the presence of increased amounts of *n*-3 PUFA in cells.

Clinical applications of the anti-inflammatory effects of *n*-3 polyunsaturated fatty acids

Rheumatoid arthritis: a T-helper 1-type disorder

Chronic inflammatory diseases such as rheumatoid arthritis are characterised by a dysregulated T-helper 1-type response which drives the production of inflammatory cytokines (e.g. TNF-α, IL-1β, IL-6, IL-8) and eicosanoids (e.g. PGE_2, LTB_4; Feldmann & Maini, 1999; Panayi, 1999). Synovial biopsies from patients with rheumatoid arthritis contain high levels of TNF-α, IL-1β, IL-6, IL-8 and granulocyte–macrophage-colony stimulating factor, and synovial cells cultured *ex vivo* produce TNF-α, IL-1β, IL-6, IL-8 and granulocyte–macrophage-colony stimulating factor for extended periods of time without additional stimulus (see Feldmann *et al*. 1996). COX-2 expression is increased in the synovium of patients with rheumatoid arthritis, and in the joint tissues in rat models of arthritis (Sano *et al*. 1992). PGE_2, LTB_4, 5-hydroxyeicosatetraenoic acid and also platelet-activating factor, another phospholipid-derived inflammatory mediator, are found in the synovial fluid of patients with active rheumatoid arthritis (Sperling, 1995). The efficacy of non-steroidal anti-inflammatory drugs in rheumatoid arthritis indicates the importance of pro-inflammatory COX-pathway products in the pathophysiology of the disease. It might be expected that up-regulation of adhesion molecules underpins the infiltration of leucocytes into the synovium. Indeed, increased expression of E-selectin, VCAM-1 and ICAM-1 is found in patients with arthritis, and blocking ICAM-1 or VCAM-1 with antibodies reduces leucocyte infiltration into the synovium and synovial inflammation in animal models (for references, see Faull, 1995).

The effects of fish oil on inflammatory eicosanoid and cytokine production and on adhesion molecule expression suggest that it might have a role in prevention and therapy of rheumatoid arthritis and other chronic inflammatory diseases. Dietary fish oil has been shown to have beneficial clinical, immunological and biochemical effects in various animal models of human chronic inflammatory diseases. For example, Leslie *et al*. (1985) reported that, compared with vegetable oil, feeding mice fish oil delayed the onset and reduced the incidence and severity of type II collagen-induced arthritis. It was recently reported that both EPA and DHA suppress streptococcal cell wall-induced arthritis in rats, but that EPA was more effective (Volker *et al*. 2000); this finding fits with the more potent effects of EPA than DHA on inflammation.

Supplementation of the diet with fish oil has also been shown to decrease the production of IL-1 by mononuclear cells from patients with rheumatoid arthritis (Kremer *et al*. 1990). Fourteen randomised placebo-controlled double-blind studies of fish oil in patients with rheumatoid arthritis have been reported. Each of these trials indicates benefits of fish oil which include 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 non-steroidal anti-inflammatory drugs. Details of these trials are reviewed elsewhere (Volker & Garg, 1996; James & Cleland, 1997; Geusens, 1998; Calder, 2001*a*,*b*; Calder & Zurier, 2001). In an editorial commentary discussing the use of fish oil in rheumatoid arthritis Cleland & James (2000) conclude that 'the findings of benefit from fish oil in rheumatoid arthritis are robust', that 'dietary fish oil supplements in rheumatoid arthritis have treatment efficacy', and that 'dietary fish oil supplements should now be regarded as part of the standard therapy for rheumatoid arthritis'.

Asthma: a T-helper 2-type disorder

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 the major mediators of asthmatic bronchoconstriction. 4-Series LT have been detected in the blood, bronchoalveolar lavage fluid and urine of asthmatics (Henderson, 1994). In addition to the direct role of eicosanoids as mediators of allergic inflammation, PGE_2 regulates T lymphocyte differentiation, promoting the development of the T-helper 2-type phenotype which underlies allergic inflammation (Betz & Fox, 1991; Snijdewint *et al*. 1993; Gold *et al*. 1994). Thus, a case has been made for increasing the consumption of *n*-3 PUFA by patients with allergic diseases (Hodge *et al*. 1994; Black & Sharp, 1997). Indeed, there is increasing epidemiological evidence to support a protective role of long-chain *n*-3 PUFA in allergic disease (for references, see Calder & Miles, 2000; see also Dunder *et al*. 2001). A number of trials of fish oil in asthma and related atopic diseases have been performed (for reviews see Knapp, 1995; Calder & Miles, 2000). Several of these studies reveal limited clinical impact, despite biochemical changes (e.g. reduced 4-series LT production); details of these studies are discussed elsewhere (Knapp, 1995; Calder & Miles, 2000). In contrast, some studies have shown clinical improvements, at least in some patient groups, and suggest that this type of approach may be useful in conjunction with other drug- and diet-based therapies (see Calder & Miles, 2000). Broughton *et al*. (1997) found that 'low' *n*-3 PUFA ingestion resulted in increased methacholine-induced respiratory distress in adult patients with asthma. In contrast, 'high' *n*-3 PUFA ingestion resulted in an improved response in > 40 % of subjects; all measures of respiratory function were markedly improved in this group of patients, who also showed elevated appearance of the EPA-derived 5-series LT in their urine. However, some patients did not respond favourably to the high *n*-3 PUFA intake. This study suggests that there are patients who respond positively to fish oil intervention and patients who are non-responders. This finding suggests that such therapies should be approached cautiously until more is understood about the interaction between fatty acid consumption and disease activity.

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Systemic inflammatory response to injury

The importance of a hyperinflammatory response, characterised by overproduction of TNF-α, IL-1β, IL-6 and IL-8, in the progression of trauma patients towards sepsis is now recognised. Markedly elevated circulating concentrations of these mediators are seen in sepsis (for example, see Cannon *et al*. 1990; Arnalich *et al*. 2000), and Vervloet *et al*. (1998) state that 'these mediators are largely, if not completely, responsible for the clinical signs and symptoms of the septic response to a bacterial infection'. Enhanced production of arachidonic acid-derived eicosanoids such as PGE_2 is also associated with trauma and burns (Grbic *et al*. 1991; Ertel *et al*. 1992). The inflammatory effects of infection can be mimicked by administration of LPS, which causes an elevation of circulating concentrations of inflammatory cytokines (Tracey *et al*. 1986; Mathison *et al*. 1988; Okusawa *et al*. 1988; Cannon *et al*. 1990). Laboratory animals can be protected against bacterial- and LPS-induced shock by neutralising these cytokines (Beutler *et al*. 1985; Tracey *et al*. 1987; Alexander *et al*. 1991) and mice deficient in the 55 kDa TNF-α receptor are resistant to endotoxic shock (Pfeffer *et al*. 1993). LPS also causes up-regulation of adhesion molecule expression (Kamochi *et al*. 1999; Raeburn *et al*. 2001), and deficiency or blocking of VCAM-1 or ICAM-1 induces resistance to LPS (Tang *et al*. 1995; Kumasaka *et al*. 1996; Kamochi *et al*. 1999; Raeburn *et al*. 2001). The ability of *n*-3 PUFA to decrease production of inflammatory cytokines and eicosanoids and to decrease adhesion molecule expression suggests that fish oil might be a useful agent to aid the control of endotoxaemia and the so-called systemic inflammatory response syndrome.

Fish oil feeding or infusions enhanced the survival of guinea-pigs following LPS challenge (Mascioli *et al*. 1988, 1989) and decreased the accompanying metabolic perturbations in guinea-pigs and rats (Hellerstein *et al*. 1989; Pomposelli *et al*. 1990; Mulrooney & Grimble, 1993). Mice fed fish oil and then injected with LPS had lower plasma TNF-α, IL-1β and IL-6 concentrations than mice fed safflower oil (Sadeghi *et al*. 1999), while fish oil-containing parenteral nutrition decreased serum TNF-α, IL-6 and IL-8 concentrations in burned rats (Hayashi *et al*. 1998; Tashiro *et al*. 1998). Total parenteral nutrition using fish oil as the lipid source was found to prevent the LPS-induced reduction in blood flow to the gut and to reduce the number of viable bacteria in mesenteric lymph nodes and liver following exposure to live bacteria (Pscheidl *et al*. 2000). Fish oil did not, however, decrease bacterial translocation across the gut, and the authors concluded that fish oil must have improved bacterial killing. Fish oil administration before exposure to live pathogens decreased mortality of rats compared with vegetable oil (Barton *et al*. 1991; Rayon *et al*. 1997). These studies did not measure inflammatory cytokine levels, but they showed that PGE_2 levels were decreased by fish oil (Barton *et al*. 1991; Rayon *et al*. 1997). More recently, fish oil infusion after induction of sepsis by caecal ligation and puncture in rats was shown to decrease mortality (and PGE_2 production) compared with vegetable oil (Lanza-Jacoby *et al*. 2001).

An understanding of the inflammatory changes occurring during sepsis and of the anti-inflammatory effects of fish oil combined with the outcome of these animal experiments has prompted clinical studies investigating the influence of fish oil administered either parenterally or enterally. Patients receiving parenteral fish oil following major abdominal surgery had lower serum concentrations of TNF-α and IL-6 than those receiving an MCT–long-chain triacylglycerol mix (Wachtler *et al*. 1997). This study did not report clinical outcome. In a study reported only in abstract form parenteral administration of an emulsion containing soyabean oil, MCT, olive oil, fish oil and increased amounts of antioxidant vitamins and minerals to patients (*n* 19) following major abdominal, thoracic or urological surgery enhanced *ex vivo* LTB_5 production by leucocytes and decreased hospital stay (13·4 (SD 2·0) d *v*. 20·4 (SD 10·0) d compared with standard soyabean oil-based nutrition (Schulzki *et al*. 1999).

A large number of clinical trials (at least twenty) have been performed in intensive care or surgical patients using enteral formulas containing *n*-3 PUFA. The majority of these trials have used the commercially-available product IMPACT® (Novartis Nutrition, Berne, Switzerland) which contains arginine, yeast RNA and *n*-3 PUFA. Many of these trials report beneficial outcomes, including decreased numbers of infections and infectious or wound complications, decreased severity of infection, decreased need for mechanical ventilation, decreased progression to systemic inflammatory response syndrome and decreased length of intensive care unit and/or total hospital stay. A comprehensive meta-analysis of fifteen randomised controlled studies using IMPACT or Immun-Aid® (McGaw, Irvine, CA, USA; also rich in arginine, RNA and *n*-3 PUFA) has been performed (Beale *et al*. 1999). This analysis confirmed reductions in infection rate, number of d on a ventilator and length of hospital stay, but not in overall mortality. Few of the studies reviewed measured circulating cytokine levels or *ex vivo* cytokine production. However, some other studies of IMPACT, not included in the meta-analysis, did so. Plasma IL-6 concentrations were lower in patients given IMPACT following major abdominal surgery than in those receiving standard enteral nutrition (Braga *et al*. 1996), while preoperative IMPACT decreased post-surgery plasma IL-6 concentrations in patients who underwent surgery to remove malignancies (Braga *et al*. 1999; Gianotti *et al*. 1999). More recently, post-cardiac surgery plasma IL-6 concentrations were lower in patients who received IMPACT pre-operatively than in controls (Tepaske *et al*. 2001). Wu *et al*. (2001) showed that patients who received an enteral formula containing glutamine, arginine and *n*-3 PUFA post-operatively exhibited lower TNF-α and IL-6 concentrations. Although each of these observations fits with the predicted effects of *n*-3 PUFA and could be used as evidence of their efficacy in the trauma and post-surgery settings, the complex nature of the formulas prevents such a clear interpretation. The effects could be due to any one of the specified nutrients (i.e. arginine, RNA, *n*-3 PUFA) or to the combination of these nutrients. Indeed, the positive outcomes from the use of IMPACT and Immun-Aid have often been used as evidence for the benefit of arginine in these settings.

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One other recent trial performed in patients with moderate and severe acute respiratory distress syndrome has used an enteral preparation apparently differing from the control only in lipid source (% total lipid (w/w); 32 rapeseed oil + 25 MCT + 20 borage (*Borago officinalis*) oil + 20 fish oil + 3 soyabean lecithin *v*. 97 maize oil + 3 soyabean lecithin; Gadek *et al*. 1999). However, as well as the difference in fatty acid composition between the formulas, the *n*-3 PUFA-rich formula contained more vitamin C and E than the control and contained β-carotene, taurine and carnitine, which the control did not. Nevertheless, as the authors state, this study allows the direct assessment of the effects of *n*-3 PUFA plus γ-linolenic acid (from the borage oil) as a replacement for linoleic acid without the confounding effects of different levels of macronutrients and different types of amino acid. Patients received about 7 g EPA, 3 g DHA, 6 g γ-linolenic acid, 1·1 g vitamin C, 400 mg vitamin E and 6·6 mg β-carotene/d for up to 7 d. The control and experimental groups were well matched and included a number of patients with surgical trauma, sepsis and pneumonia; all patients had respiratory failure and about one-third had failure of at least one other organ system. By 4 d the numbers of leucocytes and neutrophils in the alveolar fluid had declined in the fish oil + γ-linolenic acid group and were lower than in the control group. Furthermore, arterial oxygenation and gas exchange were improved in the treatment group. Patients in the treatment group had decreased requirement for supplemental O_2, reduced time on ventilation support and shorter length of intensive care unit stay (12·8 (SE 1·1) d *v*. 17·5 (SE 1·7 d). Total length of hospital stay also tended to be shorter (29·4 (SE 2·6) d *v*. 34·6 (SE 3·3) d). Fewer patients in the treatment group developed new organ failure (four of fifty-one *v*. thirteen of forty-seven). Mortality was 19 % in the control group and 12 % in the treatment group, but this difference was not significant. Nevertheless, this study suggests efficacy of *n*-3 PUFA (in combination with γ-linolenic acid, MCT, antioxidant vitamins, taurine and carnitine) in this group of patients.

Concluding statement

Inflammation is a component of a range of acute and chronic human diseases, and is characterised by the production of inflammatory cytokines, arachidonic acid-derived eicosanoids, other inflammatory mediators (e.g. platelet-activating factor) and adhesion molecules. *n*-3 PUFA decrease the production of inflammatory mediators and the expression of adhesion molecules. They act both directly (e.g. by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism) and indirectly (e.g. by altering the expression of inflammatory genes through effects on transcription factor activation). Thus, *n*-3 PUFA are potentially potent anti-inflammatory agents. As such they may be of therapeutic use in a variety of acute and chronic inflammatory settings. Evidence of their clinical efficacy is strong in some settings (e.g. in rheumatoid arthritis), but generally weak in others (e.g. in asthma, in trauma patients).

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