Fatty Acids and Gene Expression Related to Inflammation

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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 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 leukocytes 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. The movement of cells into the inflammatory/infected site is induced by the upregulation of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin on the surface of endothelial cells allowing leukocyte binding. The earliest cells appearing at inflamed sites are granulocytes, with monocyte/macrophages and lymphocytes appearing later. Granulocytes and monocyte/macrophages are involved in pathogen killing, in clearing up cellular and tissue debris, and in tissue repair. The activity of these cells is induced by certain triggers. One important exogenous trigger is bacterial endotoxin (also known as lipopolysaccharide or LPS), a component of the cell wall of Gram-negative bacteria. LPS can trigger complement activation (resulting in vasodilation and increased vascular permeability), blood coagulation, and the kinin cascade. LPS can directly activate monocyte/macrophages inducing them to form cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and IL-8; eicosanoids, such as
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Insult (e.g. infection, trauma, burns, surgery)

Antigen presentation
Cytokines

Acquired immune system
Cytokines

Macrophages
Endothelial cells

Inflammatory mediators
(TNF, IL-1, IL-6, IL-8, PGE₂, reactive oxygen species, nitric oxide, kinins)

Macrophages
Neutrophils

Complement

Local tissue damage
Systemic effects e.g. brain (fever, reduced appetite); skeletal muscle (proteolysis); adipose tissue (lipolysis); liver (acute phase protein synthesis)

Pathogen destruction

Pathogen elimination
Tissue repair
Immunological memory

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

prostaglandin (PG) E₂; nitric oxide; matrix metalloproteinases (MMPs); and other inflammatory mediators. LPS also induces adhesion molecule expression on the surface of endothelial cells and leukocytes. The cytokines produced by monocyte/macrophages serve to regulate the whole body response to infection and injury (Fig. 1). Thus, inflammation and the inflammatory response are part of the normal, innate immune response. However, when inflammation occurs in an uncontrolled or inappropriate manner disease ensues. The importance of a hyperinflammatory response, characterized by overproduction of TNF-α, IL-1β, IL-6 and IL-8, in the progression of trauma patients towards sepsis is now recognized [1]. Markedly elevated circulating concentrations of these mediators are seen in sepsis [2, 3], and Vervloet et al. [4] state that ‘these mediators are largely, if not completely, responsible for
the clinical signs and symptoms of the septic response to a bacterial infection. Pro-inflammatory cytokines can upregulate arachidonic acid metabolism and enhanced production of arachidonic acid-derived eicosanoids such as PGE₂ is also associated with trauma and burns [5, 6]. The inflammatory effects of infection can be mimicked by administration of LPS, which causes an elevation of circulating concentrations of inflammatory cytokines [7, and references therein]. Laboratory animals can be protected against bacterial- and LPS-induced shock by neutralizing these cytokines [for references see 7] and mice deficient in the 55-kD TNF-α receptor are resistant to endotoxic shock [8]. LPS also causes upregulation of adhesion molecule expression [9, 10], and deficiency or blocking of VCAM-1 or ICAM-1 induces resistance to LPS [9–12].

Polyunsaturated Fatty Acids and Eicosanoid Synthesis

Polyunsaturated fatty acids (PUFAs) are fatty acids containing two or more double bonds in their hydrocarbon chain. In recent years, there has been significant interest in the health benefits of two structurally distinct families of PUFAs: the n-6 and the n-3 PUFAs. These fatty acids are named from the position of the first double bond in the hydrocarbon chain, when numbering the carbons from the methyl end (i.e. the non-carboxyl end). Thus, in n-6 PUFA the first double bond is on carbon number 6, while in n-3 PUFA the first double bond is on carbon number 3. The simplest members of these PUFA families are linoleic acid and α-linolenic acid, respectively. These two fatty acids cannot be synthesized in mammals since mammalian tissues lack the enzymes which insert the n-6 bond (so forming linoleic acid from oleic acid) and the n-3 bond (so forming α-linolenic acid from linoleic acid). However, linoleic and α-linolenic acids can be synthesized in plants, and plant seed oils and tissues are the major sources of these two so-called essential fatty acids in most human diets. Because they lack the enzyme Δ\textsuperscript{15}-desaturase that inserts the n-3 bond into fatty acids, n-6 and n-3 fatty acids are not interconvertible in mammals (i.e. they remain metabolically distinct). However, there is direct competition between n-6 and n-3 PUFAs for further metabolism. Once consumed in the diet, linoleic acid is further desaturated and elongated by a series of desaturase and elongase enzymes found mainly in the liver (Fig. 2). Thus, linoleic acid is converted to arachidonic acid, which has a longer hydrocarbon chain and more double bonds, although the methyl terminal double bond remains in the n-6 position (Fig. 2). Using the same series of enzymes, α-linolenic acid is converted to eicosapentaenoic acid (EPA; Fig. 2).

The key link between PUFA and inflammation is that the n-6 PUFA, arachidonic acid, is the precursor for synthesis of the eicosanoid family of bioactive mediators: PG, thromboxanes (TXs), leukotrienes (LTs), etc. (Fig. 3).
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Linoleic acid (18:2n-6) → 6-Desaturase → γ-Linolenic acid (18:3n-6) → Stearidonic acid (18:4n-3) → Eicosatetraenoic acid (20:4n-3) → Docosahexaenoic acid (22:6n-3)

α-Linolenic acid (18:3n-3) → Elongase → Dihomo-γ-linolenic acid (20:3n-6) → Eicosapentaenoic acid (20:5n-3) → Docosapentaenoic acid (22:5n-3)

Fig. 2. Metabolism of n-6 and n-3 polyunsaturated fatty acids.

These eicosanoids are involved in modulating the intensity and duration of inflammatory responses [for review see 13–15]. For example, PGE$_2$ induces fever, vascular permeability and vasodilation and enhances pain and edema caused by other agents such as bradykinin and histamine. LTB$_4$ increases vascular permeability, enhances local blood flow, is a potent chemotactic agent for leukocytes, induces release of lysosomal enzymes, enhances generation of reactive oxygen species, and enhances production of TNF-α, IL-1, and IL-6. 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 [13, 14].

The n-3 PUFAs, EPA and docosahexaenoic acid (DHA), are found in high proportions in oily fish and fish oils. Feeding fish oil results in increased proportions of EPA and DHA in inflammatory cell phospholipids [for references see 16, 17]. These fatty acids are incorporated largely at the expense of
Arachidonic acid in cell membrane phospholipids

\[
\begin{align*}
\text{Phospholipase } A_2 \quad &\quad \text{Free arachidonic acid} \\
\quad &\quad \text{COX} \quad \text{5-LOX} \\
\quad &\quad 2\text{-series PG and TX} \quad 4\text{-series LT} \\
\quad &\quad \text{Inflammation}
\end{align*}
\]

**Fig. 3.** General outline of the synthesis of eicosanoids from arachidonic acid. COX = Cyclooxygenase; LOX = lipoxygenase; LT = leukotriene; PG = prostaglandin; TX = thromboxane.

Arachidonic acid [16, 17]. This has two main effects, which contribute to the decreased capacity of inflammatory cells to synthesize eicosanoids from arachidonic acid: (1) there will be less arachidonic acid available for synthesis of inflammatory eicosanoids, and (2) EPA is an inhibitor arachidonic acid release from phospholipids and of its further metabolism (Fig. 4).

In addition, EPA is able to act as a substrate for eicosanoid synthesis (Fig. 4), giving rise to derivatives that have a different structure to 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 [for references see 17]. The eicosanoids produced from EPA are considered to be less biologically potent than the analogues synthesized from arachidonic acid, although the full range of biological activities of these compounds has not been investigated. The reduction in generation of arachidonic acid-derived mediators that accompanies fish oil consumption has lead to the idea that fish oil is anti-inflammatory.

The opposing effects of arachidonic acid- and EPA-derived eicosanoids have been investigated in detail using lung models of hyperinflammation. Infusion with *Escherichia coli* hemolysin into isolated, perfused rabbit lungs was shown to induce vasoconstriction/hypertension, mediated by TXB₂, and vascular permeability/leakage, mediated by 4-series LT [18, 19]. Inclusion of free arachidonic acid in the perfusate increased TXB₂ and 4-series LT
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**Fig. 4.** Theoretical basis of the anti-inflammatory effects of eicosapentaenoic acid. COX = Cyclooxygenase; EPA = eicosapentaenoic acid; LOX = lipoxygenase; LT = leukotriene; PG = prostaglandin; TX = thromboxane.

The diagram illustrates the conversion of arachidonic acid and EPA in cell membranes to inflammatory and anti-inflammatory eicosanoids, respectively. Arachidonic acid is converted to free arachidonic acid by phospholipase A2, which can then be metabolized by COX to 2-series PG and TX or 5-LOX to 4-series LT, leading to inflammation. EPA is converted to free EPA, which is then metabolized by COX to 3-series PG and TX or 5-LOX to 5-series LT, resulting in less inflammation.

In contrast, inclusion of EPA in cell membranes decreases TXB2 and 4-series LT generation, arterial pressure, and vascular leakage [18, 19]. Fish oil perfusion of isolated rabbit lungs markedly attenuated the vascular inflammatory reaction (hypertension) induced by calcium ionophore [20]. Compared with perfusion with a soybean oil-rich emulsion, fish oil decreased the concentration of LTC4 in the perfusate by >50% and increased the concentration of LTC5 from barely detectable (<10 pg/ml) to a concentration very similar to that of LTC4 (approximately 150 pg/ml) [20]. These observations indicate that n-3 PUFA can significantly inhibit the acute inflammatory responses induced, or at least marked, by production of arachidonic acid-derived eicosanoids.

**n-3 PUFA and Inflammatory Cytokine Production**

Cell culture studies demonstrated the ability of EPA and DHA to inhibit the production of IL-1β and TNF-α by human monocytes, and the production of IL-6 and IL-8 by human venous endothelial cells [for references see 16, 21]. Culture of a monocytic cell line with EPA or DHA resulted in decreased production of tissue factor, TNF-α and IL-1β [22]. Fish oil feeding decreased ex vivo production of TNF-α, IL-1β and IL-6 by rodent macrophages [for references see 16, 21, 23] and splenocytes [24]. Infusion of fish oil into horses resulted in decreased production of TNF-α by isolated blood monocytes [25], while infusion of fish oil into rats decreased production of...
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Fig. 5. Effect of vegetable and fish oils on post-endotoxin cytokine concentrations in mice. C57Bl/6 mice were fed for 6 weeks on diets containing 20% by weight safflower oil (rich in linoleic acid; dark bars) or 20% by weight fish oil (rich in long-chain n-3 PUFA; open bars). They were then injected intraperitoneally with E. coli LPS (100 µg/20 g body weight) and blood was collected at 90 or 180 min. The plasma concentrations of TNF-α (at 90 min), IL-1β and IL-6 (both at 180 min) were measured by specific enzyme-linked immunosorbant assays. Data are taken from Sadeghi et al. [7].

TNF-α and IL-6 by blood monocytes [26]. Feeding fish oil to mice infected with the murine AIDS virus significantly decreased production of TNF-α and IL-1β by LPS-stimulated splenocytes [24]. 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 [7] (Fig. 5). 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 [27, 28]. Supplementation of the diet of human volunteers with fish oil providing more than 2.4 g EPA plus DHA per day has been shown to decrease production of TNF, IL-1 and IL-6 by mononuclear cells [for references see 16, 17, 21, 23]. Parenteral nutrition supplemented with fish oil has also been shown to decrease serum TNF-α and IL-6 concentrations in patients following major abdominal surgery [29].

n-3 PUFA and Adhesion Molecule Expression

Culture of human venous endothelial cells with DHA significantly decreased cytokine-induced surface expression of E-selectin, ICAM-1 and VCAM-1 [30], and impaired the ability of ligand-bearing monocytes to adhere [31]. Although
De Caterina et al. [30] reported that EPA was without effect, others showed that EPA also inhibited LPS-induced expression of these three adhesion molecules on human venous endothelial cells, and again this had the functional effect of decreasing binding of monocytes [32]. Khalfoun et al. [33] 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, EPA decreased surface expression of ICAM-1 on monocytes stimulated with interferon (IFN)-γ [34]. Dietary fish oil decreased expression of certain adhesion molecules, including ICAM-1, on the surface of rat lymphocytes [35–37] and murine macrophages [38]. Supplementing the diet of healthy humans with fish oil providing about 1.5 g EPA plus DHA per day, resulted in a lower level of expression of ICAM-1 on the surface of blood monocytes stimulated \textit{ex vivo} with IFN-γ [39]. Recently, dietary fish was found to decrease circulating levels of soluble VCAM-1 in elderly subjects [40], but it is not clear if this represents decreased surface expression of VCAM-1.

**Effects of n-3 PUFA on Expression of Genes Involved in Inflammation**

\textit{n-3 PUFA 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 (Table 1). An excellent description of this is the recent study by Curtis \textit{et al}. [41]. These authors demonstrated that culturing bovine chondrocytes with α-linolenic acid, EPA or DHA markedly decreases cytokine-mediated induction of expression of the cyclooxygenase (COX)-2 (but not COX-1), TNF-α and IL-1α genes [41]. This suggests that at least part of the effect of n-3 PUFA in lowering the production of arachidonic acid-derived eicosanoids might be mediated via an effect on expression of COX-2. The same study investigated the influence of n-3 PUFA upon the expression of aggrecanase-1 and 2 genes. Aggrecanase-1 and 2 degrade cartilage proteoglycan and their expression in cartilage is upregulated in response to the pro-inflammatory cytokines TNF-α and IL-1. n-3 PUFA, but not other fatty acids, inhibited cytokine-mediated upregulation of expression of the aggrecanase-1 and aggrecanase-2 genes and of aggrecanase activity [41]. In an earlier study, De Caterina \textit{et al}. [30] had demonstrated that the downregulation 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.

Several animal feeding studies have demonstrated an effect of dietary fish oil on inflammatory gene expression (Table 1). 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 [44]. Feeding wild-type mice a fish oil-rich
**Table 1. Influence of n-3 PUFA on expression of inflammatory genes**

<table>
<thead>
<tr>
<th>Inflammatory gene</th>
<th>Effect of n-3 PUFA reported</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2</td>
<td>Decreased mRNA expression in cultured bovine chondrocytes stimulated with IL-1β</td>
<td>Effect seen with α-linolenic acid, EPA and DHA, but detailed results shown for α-linolenic acid only</td>
<td>41</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Decreased mRNA expression in cultured murine peritoneal macrophages stimulated with LPS or interferon-γ</td>
<td>Effect seen with DHA, but not EPA; effect correlated with decreased nitric oxide production; effect due to decreased transcription of iNOS gene</td>
<td>42</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Decreased mRNA expression in cultured human endothelial cells stimulated with cytokines</td>
<td>Effect seen with DHA; effect independent of eicosanoid production and antioxidant status; effect correlated with decreased adhesion</td>
<td>30</td>
</tr>
<tr>
<td>Aggrecanase-1</td>
<td>Decreased mRNA expression in freshly isolated peritoneal macrophages from mice fed fish oil</td>
<td>Effect correlated with decreased cell surface expression of protein</td>
<td>38</td>
</tr>
<tr>
<td>Aggrecanase-2</td>
<td>Decreased mRNA expression in unstimulated and LPS-stimulated peritoneal macrophages from mice fed fish oil</td>
<td>Effect correlated with decreased production of TNF-α protein</td>
<td>43</td>
</tr>
<tr>
<td>iNOS</td>
<td>Decreased mRNA expression in kidneys of autoimmune disease-prone mice fed fish oil</td>
<td>Effect due to decreased mRNA synthesis not increased degradation</td>
<td>44</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Decreased mRNA expression in LPS- or phorbol ester-stimulated splenocytes from mice fed fish oil</td>
<td>Effect shown for EPA; publication in abstract form only</td>
<td>45</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Decreased mRNA expression in cultured renal carcinoma cells</td>
<td></td>
<td>46</td>
</tr>
</tbody>
</table>
diet significantly decreased the levels of IL-1β mRNA in LPS- or phorbol ester-stimulated spleen lymphocytes [45] and of TNF-α mRNA in LPS-stimulated peritoneal macrophages [43]. ICAM-1 mRNA levels were lower in peritoneal macrophages from mice fed fish oil [38].

**The Mechanism(s) by Which n-3 PUFA Might Downregulate Inflammatory Gene Expression**

Eicosanoids derived from arachidonic acid are able to regulate inflammatory gene expression. Therefore, the effects of n-3 PUFA described above 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 [e.g. 30]. It is now emerging that n-3 PUFA might exert their effects through direct actions on the intracellular signaling 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 nitric oxide synthase (iNOS), acute phase proteins and MMPs in response to inflammatory stimuli [47, 48] (Fig. 6). The signaling pathway leading to NFκB activation is becoming better understood [49, 50]. NFκB exists as an inactive heterotrimer in the cytosol of resting inflammatory cells; one of the subunits is called inhibitory subunit of NFκB (IκB). Upon stimulation, a signaling cascade activates a protein complex known as IκB kinase (IκK). Activated IκK 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 downregulate the activity of NFκB. 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 corn oil [51]. 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 [51]. The mechanism by which n-3 PUFA decreases the activation of NFκB is not clear. However, an abstract reports that incubating human monocytes with EPA decreased LPS-induced activation of NFκB and that this was associated with decreased phosphorylation of IκB [52]. This suggests an effect of n-3 PUFA on the signaling process leading to activation of IκK. Incubation of a pancreatic cell line with TNF-α markedly upregulated degradation of IκB, and this could be totally abolished by prior incubation of the cells with EPA [53]. 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 the peroxisome proliferator activated receptors (PPARs). The main members of this family are PPARα and PPARγ. PPARα and γ play important roles in liver and adipose tissue, respectively. However, they are also found in inflammatory cells [54, 55]. PPARs dimerize with the retinoid-X receptor to regulate gene expression, and they can bind, and appear to be regulated by, PUFAs and eicosanoids [56, 57]. PPARα-deficient mice have a prolonged response to inflammatory stimuli [57], suggesting that PPARα activation might be ‘anti-inflammatory’. More recently, activators of both PPARα and γ have been shown to inhibit the activation of inflammatory genes including TNFα, IL-1β, IL-6, IL-8, COX-2, VCAM-1, iNOS, MMPs and acute phase proteins [55, 58–64]. Two mechanisms for the anti-inflammatory actions of PPARs have been proposed. The first is that PPARs might stimulate the breakdown of inflammatory eicosanoids through induction of peroxisomal β-oxidation. The second is that PPARs might interfere with/antagonize the
activation of other transcription factors, including NFκB. Feeding mice fish oil increases the expression of PPARα and γ in liver and adipose tissue, respectively [65], and increases the expression of PPAR-inducible genes in these tissues [e.g. 66]. 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 review see 67]. It is possible that n-3 PUFA might affect the activation of these factors, but this has not been studied to detail. However, effects of n-3 PUFA on signaling processes, which lead to activation of various transcription factors including, but not necessarily restricted to, NFκB have been reported. Several early cell signaling events are affected by n-3 PUFA [for references see 68]. Recently, incubation of murine macrophages with EPA was found to decrease LPS-induced phosphorylation and activation of mitogen-activated protein kinase [69]. Thus, a variety of intracellular signaling steps are partly inhibited by the presence of increased amounts of n-3 PUFA in cells.

**n-3 PUFA and the Systemic Inflammatory Response to Injury**

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 endotoxemia and the so-called systemic inflammatory response syndrome (SIRS).

Fish oil feeding or infusions enhanced the survival of guinea pigs following LPS challenge and decreased the accompanying metabolic perturbations in guinea pigs and rats [for references see 23]. 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 [7], while fish oil-containing parenteral nutrition decreased serum TNF-α, IL-6 and IL-8 concentrations in burned rats [27, 28]. 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 [70]. 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 prior to exposure to live pathogens decreased mortality of rats compared with vegetable oil [71, 72]. These studies did not measure inflammatory cytokine levels but they showed that PGE2 levels were decreased by fish oil. More recently, fish oil infusion after induction of sepsis by cecal ligation and puncture in rats was shown to decrease mortality (and PGE2 production) compared with vegetable oil [73].
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 alternative mix [29]. This study did not report clinical outcome. In a study reported only in abstract form, parenteral administration of an emulsion containing soybean oil, medium-chain triglycerides (MCT), olive oil, fish oil and increased amounts of antioxidant vitamins and minerals to patients (n = 19) following major surgery enhanced ex vivo LTB₅ production by leukocytes and decreased hospital stay (13.4 ± 2.0 vs. 20.4 ± 10.0 days) compared with standard soybean oil-based nutrition [74].

A large number of clinical trials (at least 20) have been performed in intensive care or surgical patients using enteral formulae containing n-3 PUFA. The majority of these trials have used the commercial product IMPACT® 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 SIRS, and decreased length of intensive care unit and/or total hospital stay. A comprehensive meta-analysis of 15 randomized, controlled studies using IMPACT or Immun-Aid® (also rich in arginine, RNA and n-3 PUFA) has been performed [75]. This analysis confirmed significant reductions in infection rate, number of ventilator days 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 [76], while pre-operative IMPACT decreased post-surgery plasma IL-6 concentrations in patients, who underwent surgery to remove malignancies [77, 78]. More recently, plasma IL-6 concentrations after cardiac surgery were lower in patients who received IMPACT pre-operatively than in controls [79]. Wu et al. [80] 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 formulae 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.
One other recent trial performed in patients with moderate and severe acute respiratory distress syndrome has used an enteral preparation apparently differing only in lipid source from the control (32% canola oil + 25% MCT + 20% borage oil + 20% fish oil + 3% soy lecithin vs. 97% corn oil + 3% soy lecithin) [81]. However, as well as the difference in fatty acid composition between the formulae, 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 IU vitamin E and 6.6 mg β-carotene per day for up to 7 days. By 4 days the numbers of leukocytes and neutrophils in the alveolar fluid had significantly 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 requirements for supplemental oxygen, reduced time on ventilation support, and shorter length of intensive care unit stay (12.8 ± 1.1 vs. 17.5 ± 1.7 days). Total length of hospital stay also tended to be shorter (29.4 ± 2.6 vs. 34.6 ± 3.3 days). Fewer patients in the treatment group developed new organ failure (4/51 vs. 13/47). Mortality was 19% in the control group and 12% in the treatment group but this was not a significant difference. Nevertheless, this study suggests the efficacy of n-3 PUFA (in combination with γ-linolenic acid, MCT, antioxidant vitamins, taurine and carnitine) in this group of patients.

Concluding Statement

n-3 PUFAs 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 PUFAs are potentially potent anti-inflammatory agents. As such, they may be of therapeutic use in a variety of acute and chronic inflammatory settings. Although clinical studies suggest their efficacy in trauma and surgical patients, strong evidence of this is currently lacking.

References


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**Discussion**

*Dr. Winter:* The NFκB pathway is clearly pivotal in the whole inflammatory cascade process, and the data you present suggest that the n-3 fatty acids seem to interfere with that pathway, as do many other substances including antioxidants, steroids, various
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drugs and compounds, immunosuppressive agents, and so on. How do the n-3 fatty acids compare with these other potentially efficacious agents in the control of the NFκB activation?

Dr. Calder: That is an important question of course. I don’t know of any head to head comparisons in an experimental system comparing these different kinds of agents, and that needs to be done to give us more confidence in the n-3 fatty acids as anti-inflammatory agents. Not being as important as some of the pharmacological agents may not of course be a problem because, as we have heard, we can have too much or too little of some of these mediators. It may be that the nutritional interventions somehow bring the cellular activation state to a downregulated but not completely abolished condition.

Dr. Meguid: Both you and the previous speaker mentioned a dose of 6 or 10 g as the appropriate one to use, although no basis for that was given. Generally we use milligrams per kilogram body weight. How did you derive the 6 g or the 10 g?

Dr. Calder: Almost all the studies that have been done on n-3 fatty acids in relation to inflammation and immune function have been performed in healthy human volunteers. There have been relatively few studies in patient groups. Most of the studies have been performed as supplementation studies rather than as strict dietary intervention studies. Thus one is able to provide as much or as little of the n-3 fatty acids as one can convince the volunteers to take. I think the levels that have been used have been derived pragmatically – for example, in the early studies it was thought that more is better, so the plan was to give as much as possible. In the study I showed on the eicosanoids from human neutrophils in monocytes, the investigators actually gave 50 g of cod liver oil a day, on the basis that by giving as much as they could they would be able to show the greatest possible effect. Obviously we don’t need to give that much, but there is probably a threshold somewhere between 1.5 and 2.5 g of long chain n-3 fatty acids a day.

Dr. Meguid: As a follow-up question, how do you give it? We have been doing studies in the regulation food intake in cancer anorexia, where we have generally been giving 3 g, but we have a problem giving our patients n-3 fatty acids unless they are in capsules.

Dr. Calder: In most of our studies we have used capsules, although we have done some studies incorporating n-3 fatty acids into food. The practical problem is the taste. People don’t like the taste, and there are also people who develop gastrointestinal disturbances. Such individuals always drop out of the studies. There are commercial organizations that have developed microencapsulation processes, but those microcapsules still have to be incorporated into foods. You have highlighted a difficulty of using n-3 fatty acids.

Dr. Labadarios: You suggested 1.5 to 2.5 g of n-3 fatty acids a day. Is that a rule of thumb, or should one take into account the n-6 composition?

Dr. Calder: These are key questions and it is a focus of much activity in western Europe and North America. When I have been talking about n-3 fatty acids, I have been referring to eicosapentaenoic acid (EPA), which is the end of the metabolic pathway, so I am talking about giving individuals a preformed end product. Now we don’t eat much EPA. We eat the precursor α-linolenic acid and we require the body to convert that to EPA. As there is competition between metabolism of the n-6 and n-3 pathways, if one wants to maximize conversion of α-linolenic acid one would give a lot of α-linolenic acid at the same time as reducing the intake of linoleic acid. So when people talk about the n-6 to n-3 ratio, they really mean the linoleic to α-linolenic acid ratio. In most of western Europe and in North America, that ratio is 10 or more, and the thought is that it should be 5 or less, perhaps even as low as 2, in order to maximize conversion of α-linolenic acid. However, there are no guidelines as yet.
Dr. Waitzberg: I enjoyed the talks by both Dr. Grimble and Dr. Calder. I would like you to expand your ideas. I’d like Dr. Calder to comment on the results obtained using intravenous n-3 fatty acids in psoriasis. This is a chronic disease of the skin but the results have not been uniform. How do you explain this? Then Dr. Grimble said that nutritional therapy should be a general approach, which may be effective in some patients but would not harm the rest. In view of the wide genetic variability in humans, I wonder whether this is really true for all patients in intensive care. Is there some way of targeting those who could benefit?

Dr. Calder: A few patients with psoriasis have received infusions of fish oil preparations and these have been shown to be of some benefit, but as soon as the infusions were stopped the patients relapsed. Thus this may not be a very practical approach to such patients and longer term oral supplementation might be better, or even topical application.

In relation to your question to Dr. Grimble, I think the essence of the question was whether nutrition is a useful type of therapy for all patients, or whether we might be able to target patients according to their genotype. It is clear that we are in the early days of our ability to target, and we need to understand the system much better. But there is certainly potential for targeting particular at-risk individuals and at-risk patient groups. This doesn’t detract from the idea that some form of nutritional therapy is going to be useful across the board in a wide range of patient groups, although it may not be necessarily the same type of nutritional therapy.

Dr. Grimble: I totally agree with that. We need to think about what we are trying to do in general terms. Is our objective simply to reduce the strength of the inflammatory response, while supporting antioxidant defense? My view is yes, that is indeed what we are trying to achieve. We have seen the anti-inflammatory effects of fish oil in the studies we’ve done already, but we have only looked at this in healthy individuals. We don’t know whether there are different ground rules for individuals who have chronic inflammation or severe trauma. Those studies need to be done. So I still stick to my original statement: to my knowledge there is no evidence of any harmful effects from nutritional interventions, provided of course that you don’t feed vast amounts of calories that stress the body, so we should continue to provide this type of nutritional support, but we should also explore how to predict which patients are likely to benefit from fish oil.

Dr. Segal: I have three comments. First, there is work that shows a highly significant association between the genotype for interleukin-1β and gastric carcinoma, owing to inhibition of gastrin and the secretion of gastric acid. Second, as clinicians we find a dramatic improvement in patients with severe Crohn’s disease given anti-tumor necrosis factor α (anti-TNFα) antibodies, particularly in patients with fistulas. And third, now that there is free availability of cyclooxygenase (COX)-2 inhibitors, perhaps the indications for these will expand; at the moment they are mainly used in acute inflammatory conditions.

Dr. Calder: The particular problem with nonspecific COX inhibitors was gastrointestinal disturbances. It is clear that COX-2 inhibitors offer much greater efficacy and specificity, without the side effects. I’m not really the right person to comment on COX-2 drugs, but they seem to offer greater potential than their predecessors.

Dr. Chioléro: I would like to come back to the very important question that was addressed earlier – that is, treatment that can be beneficial in one patient category can be harmful in another. A meta-analysis published in the August issue of *JAMA* clearly showed that immunonutrition improved survival in postoperative patients who were not critically ill, but increased the mortality of intensive care unit patients. Similarly growth hormone therapy has been shown to be harmful in some circumstances,
whereas in others – in burned children for example – the outcome can be improved. These aspects need careful consideration.

**Dr. Calder:** I think you are right. The situation is highly complex in that a response which is beneficial in one setting can be detrimental in another. However, we need to be clear what we mean by immunonutrition. Almost all the published studies have been done with a mixed bag of nutrients – arginine, nucleotides, n-3 fatty acids, antioxidants, with or without glutamine. The problem is to identify whether there are particular nutrients that may be more or less useful in different patient groups.

**Dr. Carpentier:** I would like you to comment on that word ‘immunonutrition’. The term suggests that nutrients like n-3 fatty acids enhance the immune defenses, but what your work has shown is that, while there can be very potent effects in decreasing inflammation and its consequences, there may also be immunosuppressive effects, which can be beneficial in some patients and not in others. How can we resolve this contradiction?

**Dr. Calder:** You are right. Animal studies show that, if you feed a lot of fish oil, not only do you get the effects we describe as anti-inflammatory but you also get immunosuppressive effects. This is evident in healthy animals fed large amounts of fish oil. There are also human studies that show depression of cell-mediated immune function with high intakes of fish oil, and this clearly wouldn’t be good. Part of the reason may be an imbalance between the provision of n-3 fatty acids and the provision of antioxidants to protect against any damage, either in vivo or ex vivo. There is a further level of complexity in that our data are mainly derived from studies in healthy animals and humans and there aren’t many studies in good animal models of disease or in human patients. It seems that the effects of n-3 fatty acids in animals depend upon the model being used. For example, there are studies showing improved cell-mediated immunity in animals fed fish oil and subjected to traumatic insults such as hemorrhagic shock. Thus I think the response depends on the exact model being used and we must be careful about drawing conclusions from studies in healthy animals.

**Dr. Winter:** You focused mainly on n-3 fatty acids and EPA. What about docosahexaenoic acid (DHA)? I am interested in the possible neurological effects of DHA. Does it have any role in the inflammatory cascade?

**Dr. Calder:** It is quite clear that DHA is absolutely essential for brain and visual function, though EPA is probably a more important anti-inflammatory agent – perhaps because of its antagonism of arachidonic acid. There are also important differences from a nutritional viewpoint, because we can synthesize EPA moderately efficiently from α-linolenic acid, provided our linoleic acid intake isn’t too high, while recent studies with stable isotopes have shown that humans are very poor at synthesizing DHA. Some would regard DHA as an essential fatty acid, particularly in the newborn infant. Thus they have different roles physiologically and, in the present setting, EPA is probably more important than DHA.

**Dr. Labadarios:** While work in the n-3 field has actually improved our understanding of some disease processes – the role of fish oil in rheumatoid arthritis for instance – there is still a great deal of controversy about the role of n-3 fatty acids in many clinical settings. My question is, when we do these studies, do we ask the right questions in terms of end points, against the background of the genetic predisposition and genetic handling that you outlined?

**Dr. Calder:** That is a relevant question, because in the sorts of clinical settings you are thinking of there have been very few studies of n-3 fatty acids per se in good randomized controlled trials. In fact there haven’t been any studies with enteral n-3 fatty acids and very few with intravenous, and even those are complicated by methodological differences. So the first thing we need to do is to define what the intervention is going to be. In terms of defining end points, in the clinical situation the
relevant end points are related to patient outcome – length of stay, mortality, and so on. While people are probably quite good at measuring circulating cytokines or *ex vivo* leukotriene production from neutrophils, these are not good markers of the ultimate outcome.

*Dr. Meguid:* I have a practical question. I visit Japan from time to time and I never cease to be impressed by how much fish the Japanese eat. Was your study population composed purely of white north Europeans, and if you were to take your studies to Japan would you anticipate a different outcome?

*Dr. Calder:* We have always defined our study populations precisely. We only use white north Europeans and we always exclude people who eat more than one oily fish meal a week. I showed you the inverse correlation between EPA status and the production of inflammatory cytokines. Bearing that in mind, in Japan giving people more n-3 fatty acids is not going to change their cytokine production. You are right that you have to know about the characteristics of your study population and its n-3 fatty acids status, just as you would want to know about any other aspects of nutritional status if you were going to do a nutritional intervention.

*Dr. Soeters:* I have a comment about whether n-3 fatty acids are useful or not. There are several confusing factors: one is genetics, another is the difference between acute disease and chronic disease, a third is the problem of multimodal treatment (unfortunately all the studies have been done with different modalities), and then the whole question revolves around the use of the acute phase response. Forgetting about all those problems, I would like to look at this subject from the viewpoint of acute versus chronic disease. Acute disease by definition is something that can be overcome – you die of it or you heal, and under those conditions you need the inflammatory response and you shouldn’t downregulate it. (For simplicity’s sake I’m forgetting about genetics.) In chronic disease, by definition you are not going to be cured of the disease, you can only alleviate the symptoms. In that situation, downregulation of the acute phase response or the inflammatory response may be useful. That is why there are many papers now suggesting modest but beneficial effects in chronic diseases such as psoriasis, rheumatoid arthritis, Crohn’s disease, and so on, and also end-stage cancer. In these conditions, the symptoms of the acute phase response are alleviated and patients feel better, even if they don’t survive longer. So in summary, my view is that in chronic disease this form of therapy is useful, but in acute disease we must be very careful.