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ORIGINAL COMMUNICATION

Polyunsaturated fatty acids, inflammation and immunity

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Consumption of *n*-6 polyunsaturated fatty acids greatly exceeds that of *n*-3 polyunsaturated fatty acids. The *n*-6 polyunsaturated fatty acid arachidonic gives rise to the eicosanoid family of inflammatory mediators (prostaglandins, leukotrienes and related metabolites) and through these regulates the activities of inflammatory cells, the production of cytokines and the various balances within the immune system. Fish oil and oily fish are good sources of long chain *n*-3 polyunsaturated fatty acids. Consumption of these fatty acids decreases the amount of arachidonic acid in cell membranes and so available for eicosanoid production. Thus, *n*-3 polyunsaturated fatty acids act as arachidonic acid antagonists. Components of both natural and acquired immunity, including the production of key inflammatory cytokines, can be affected by *n*-3 polyunsaturated fatty acids. Although some of the effects of *n*-3 fatty acids may be brought about by modulation of the amount and types of eicosanoids made, it is possible that these fatty acids might elicit some of their effects by eicosanoid-independent mechanisms. Such *n*-3 fatty acid-induced effects may be of use as a therapy for acute and chronic inflammation, and for disorders which involve an inappropriately activated immune response.
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Fatty acids in the human diet

Fatty acids have systematic names but most also have common names and are described by a shorthand nomenclature, eg 18:2*n*-6. This nomenclature indicates the number of carbon atoms in the chain, the number of double bonds in the chain and the position of the first double bond from the methyl terminus of the chain. It is the position of the first double bond in the hydrocarbon chain which is indicated by the *n*-7, *n*-9, *n*-6 or *n*-3 part of the shorthand notation for a fatty acid. Thus, an *n*-6 fatty acid has the first double bond on carbon number 6 counted from the methyl terminus and an *n*-3 fatty acid has the first double bond on carbon number 3 counted from the methyl terminus. Note that the *n*- notation is sometimes referred to as ω- or omega-.

Saturated fatty acids and most monounsaturated fatty acids can be made in mammalian tissues from non-fat precursors like glucose or amino acids, but this does not usually occur in humans eating a Western diet since the consumption of fat in general, and of saturated and monounsaturated fatty acids in particular, is high. However, mammals cannot insert double bonds between the methyl terminus and carbon number 9 in oleic acid (18:1*n*-9). Thus, mammals cannot convert oleic acid into linoleic acid (18:2*n*-6). The enzyme which does this is called 12-desaturase and this is found only in plants. Likewise, mammals cannot convert linoleic acid into α-linolenic acid (18:3*n*-3). The enzyme which does this is called 15-desaturase and again this is found only in plants. Because these two fatty acids cannot be made by mammals they are termed essential fatty acids. Also, because mammalian tissues do not contain the 15-desaturase they cannot interconvert *n*-6 and *n*-3 fatty acids. Plant tissues and plant oils tend to be rich sources of linoleic and α-linolenic acids. For example, linoleic acid contributes over 50% and often up to 80% of the fatty acids found in corn, sunflower, safflower and soybean oils. Rapeseed and soybean oils are also good sources of α-linolenic acid since this fatty acid contributes between 5 and 15% of the fatty acids present. However, the richest source of α-linolenic acid is linseed oil (also called flaxseed oil) in which this fatty acid can contribute as much as 60% of the

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fatty acids. The main polyunsaturated fatty acid in most human diets is linoleic acid (intake is approximately 14 g/day for adult males in the UK) with α-linolenic acid contributing approximately 2 g/day.

Synthesis and sources of long chain polyunsaturated fatty acids

Once consumed in the diet linoleic acid can be converted via γ-linolenic (18:3*n*-6) and dihomo-γ-linolenic (20:3*n*-6) acids to arachidonic acid (20:4*n*-6) by the pathway outlined in Figure 1. γ-Linolenic is a constituent of borage oil (also known as starflower oil) and evening primrose oil, but intake of this fatty acid from habitual diets is very low, probably <20 mg/day. Estimates of the intake of arachidonic acid in Western populations vary between 50 and 300 mg/day for adults. Using the same pathway dietary α-linolenic acid can be converted into eicosapentaenoic acid (EPA; 20:5*n*-3), docosapentaenoic acid (22:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3). Thus, there is competition between the *n*-6 and *n*-3 fatty acids for the enzymes which metabolize them. The intake of longer chain polyunsaturated fatty acids is not clearly known but it appears that the average adult in the UK consumes about 250 mg EPA plus DHA per day. EPA and DHA are found in relatively high proportions in the tissues of so-called 'oily fish' (eg herring, mackerel, tuna, sardines) and in the commercial products called 'fish oils' which are a preparation of the body oils of oily fish; EPA and DHA are also found in high proportions in the oils extracted from the livers of other species of fish which live in warmer waters (eg cod). EPA and DHA comprise 20–30% of the fatty acids in a typical preparation of fish oil. In the absence of significant consumption of oily fish, α-linolenic acid is the major dietary *n*-3 fatty acid.

The polyunsaturated fatty acid content of cells of the immune system

The exact proportion of arachidonic acid in human immune cells varies according to cell type and the lipid fraction examined (see Calder, 2001a for details). The phospholipids of human mononuclear cells (an approximate 70:20:10 mixture of T lymphocytes, B lymphocytes and monocytes purified from human blood) contain 6–10% linoleic acid, 1–2% dihomo-γ-linolenic and 15–25% arachidonic acid (Gibney & Hunter, 1993; Yaqoob *et al*, 2000). In contrast, the proportions of *n*-3 fatty acids are low: α-linolenic acid is rare and EPA and DHA comprise only 0.1 to 0.8% and 2 to

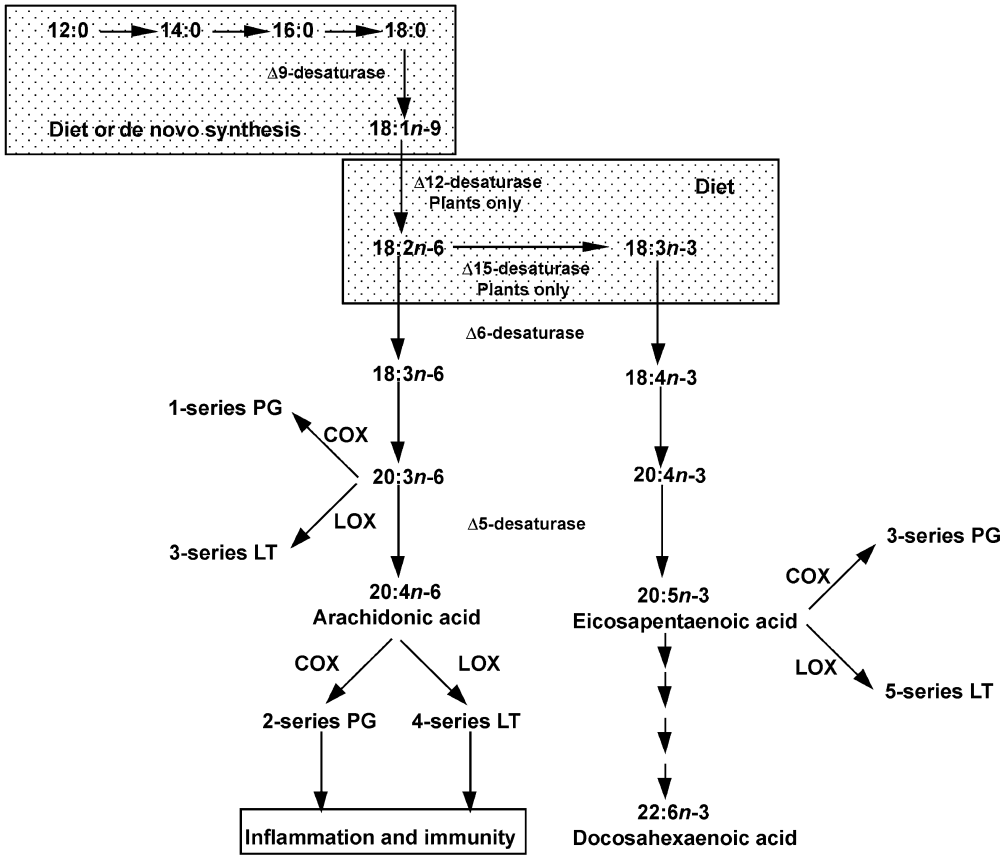

Figure 1 Outline of the pathway of biosynthesis and metabolism of polyunsaturated fatty acids.

4%, respectively (Gibney & Hunter, 1993; Yaqoob *et al*, 2000).

Animal studies show that increasing the availability of *n*-3 polyunsaturated fatty acids in the diet (eg by feeding fish oil) results in a decreased proportion of arachidonic acid and an increased proportion of n-3 fatty acids in immune cell phospholipids (see Calder, 1998a,b). When fish oil is provided in the human diet the proportions of EPA and DHA in immune cells are significantly elevated, probably in a dose-dependent manner (see Calder, 2001a). Similar effects occur in neutrophils, monocytes, T lymphocytes and B lymphocytes (Gibney & Hunter, 1993). The incorporation of the long chain *n*-3 fatty acids is largely at the expense of arachidonic acid (Gibney & Hunter, 1993; Yaqoob *et al*, 2000).

How might altered fatty acid composition of immune cells affect their function?

Altered fatty acid composition might be expected to influence immune cell function for a variety of reasons, summarised in Figure 2 (see also Grimble, 1998; Miles & Calder, 1998). Firstly, the fluidity of the plasma membrane or of regions of the plasma membrane is likely to be important in the functioning of immune cells. Cell culture experiments have demonstrated that changes in fatty acid composition of immune cells alter membrane fluidity, but this has been less easy to demonstrate after dietary manipulations, probably because the fatty acid composition changes induced by diet are less extreme than those seen in culture and because, in the intact animal, mechanisms to counter the fluidising effect of increasing the polyunsaturated fatty acid content of membranes (eg insertion of cholesterol) can be achieved more readily than in culture. Secondly, a number of cell signalling molecules are generated from membrane phospholipids (eg inositol-1,4,5-trisphosphate, diacylglycerol, phosphatidic acid, choline, ceramide, platelet activating factor, arachidonic acid) and phospholipids or phospholipid-derived mediators (eg diacylglycerol) have important roles in regulating the activity of some proteins involved in cell signalling mechanisms within immune cells. Changing the fatty acid composition of phospholipids may change their affinity as substrates for the enzymes which generate the signalling molecules and so could alter immune cell responsiveness. Thirdly, arachidonic acid is a substrate for synthesis of the family of bioactive mediators known as eicosanoids, and altering the availability of arachidonic acid as a substrate alters the ability of cells to produce eicosanoids, and so potentially alters a range of inflammatory and immune cell responses (see below).

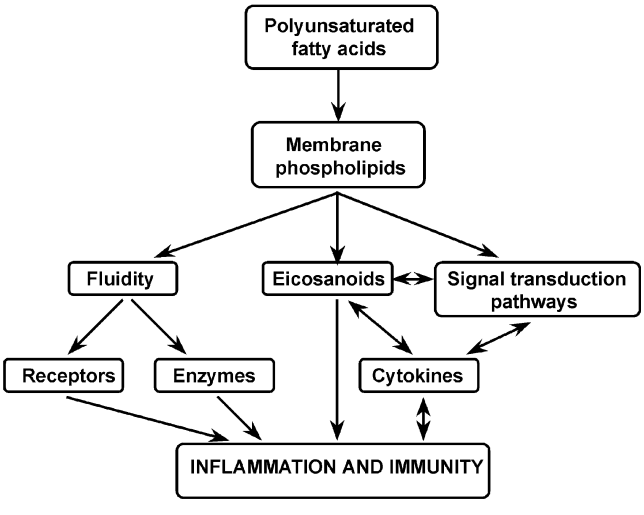

Figure 2 Mechanisms whereby polyunsaturated fatty acids might exert effects on inflammation and immunity.

Eicosanoids: a link between polyunsaturated fatty acids and the immune system

Arachidonic acid as an eicosanoid precursor

The key link between fatty acids and immune function is that a group of mediators termed eicosanoids are synthesised from the 20-carbon polyunsaturated fatty acids (Figure 1). Because the membranes of most cells contain large amounts of arachidonic acid, compared with dihomo-γ-linolenic acid and EPA, arachidonic acid is usually the principal precursor for eicosanoid synthesis. Arachidonic acid in cell membranes can be mobilized by various phospholipase enzymes, most notably phospholipase A_2, and the free arachidonic acid can subsequently act as a substrate for cyclooxygenase (COX), forming 2-series prostaglandins (PG) and related compounds, or for one of the three lipoxygenase (LOX) enzymes, forming 4-series leukotrienes (LT) and related compounds. There are at least 16 different 2-series PG and these 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. The LOX enzymes have different tissue distributions with 5-LOX being found mainly in mast cells, monocytes, macrophages and granulocytes and 12- and 15-LOX being found mainly in epithelial cells.

Effects of eicosanoids on inflammation and immunity

Eicosanoids are involved in modulating the intensity and duration of inflammatory and immune responses. 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 histamine. PGE_2 suppresses lymphocyte proliferation and natural killer cell activity and inhibits production of tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-2 and interferon (IFN)-γ; thus in these respects PGE_2 is immunosuppressive and anti-inflammatory. PGE_2 does not affect the production of the Th2-type cytokines IL-4 and IL-

10, but promotes immunoglobulin E (IgE) production by B lymphocytes. 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, inhibits lymphocyte proliferation and promotes natural killer cell activity. 4-series LT also regulate production of pro-inflammatory cytokines; for example LTB_4 enhances production of TNF-α, IL-1, IL-6, IL-2 and IFN-γ. 5-Hydroxyeicosatetraenoic acid enhances, whereas 15-hydroxyeicosatetraenoic acid inhibits, lymphocyte proliferation. Thus, arachidonic acid gives rise to a range of mediators which have opposing effects to one another, so the overall physiological effect will be governed by the concentration of those mediators, the timing of their production and the sensitivities of target cells to their effects.

N-3 polyunsaturated fatty acids as alternative eicosanoid precursors

Since increased consumption of fish oil results in a decrease in the amount of arachidonic acid in the membranes of inflammatory and immune cells, there will be less substrate available for synthesis of eicosanoids from arachidonic acid. Furthermore, EPA competitively inhibits the oxygenation of arachidonic acid by COX. Thus, fish oil feeding results in a decreased capacity of immune cells to synthesise eicosanoids from arachidonic acid (see Calder 1998a,2001a). In addition, EPA is able to act as a substrate for both COX and 5-LOX (Figure 1), giving rise to derivatives which have a different structure from those produced from arachidonic acid (ie 3-series PG and thromboxanes and 5-series LT). Thus, the EPA-induced suppression in the production of arachidonic-acid derived eicosanoids is mirrored by an elevation in the production of EPA-derived eicosanoids (eg Sperling *et al*, 1993). The eicosanoids produced from EPA are often less biologically potent than the analogues synthesised from arachidonic acid (eg LTB_5 is only about 10% as potent as LTB_4 as a chemotactic agent and in promoting lysosomal enzyme release), although the full range of biological activities of these compounds has not been investigated. 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 and might enhance immune function.

Effects of fish oil on inflammation and immune function

A large number of animal studies investigating the effects of fish oil on inflammation and immunity have now been published (see Calder, 1997,1998a,b; Grimble, 1998 for reviews). Most of these studies indicate that, at the levels used in these studies, fish oil is anti-inflammatory and decreases a wide range of immune cell responses (see below for details). However, not all studies agree with this generalisation. Reasons for contradictions in the literature might relate to the species of animal studied, the total fat content of the diets used, the amount of fish oil fed, the comparison being made (eg to a low-fat diet or to another high-fat diet; to saturated fat or to *n*-6 polyunsaturated fat), the amount of vitamin E in the diets, and the conditions used for *ex vivo* cell culture (see Calder, 1997,2001a for discussion).

There appears to be limited impact of fish oil on phagocytosis by rodent macrophages (see Calder, 1998a). Feeding fish oil to laboratory animals has been reported to decrease generation of reactive oxygen species (see Calder, 1998a for references) and production of TNF-α, IL-1β and IL-6 by macrophages (see Calder, 1997,1998a) and to decrease circulating TNF-α, IL-1β and IL-6 concentrations after endotoxin injection or burns. Thus, these studies reveal significant anti-inflammatory effects of dietary fish oil. Animal feeding studies indicate that high levels of fish oil decrease natural killer cell activity, cytotoxic T lymphocyte activity, lymphocyte proliferation and the delayed-type hypersensitivity response (see Calder, 1998b for references). Recent studies report that fish oil decreases production of IL-2 and IFN-γ, but not of IL-4 by rodent lymphocytes (see Wallace *et al*, 2001 and references therein). These observations suggest that fish oil diminishes Th1-type responses. It is clear that the observed effects of fish oil are different from those which are predicted on the basis of a decrease in PGE_2 production being the sole mechanism by which fish oil might act. Thus, there are likely to be other mechanisms of action of fish oil which do not involve eicosanoids (see Grimble, 1998; Miles & Calder, 1998). It is also worth noting that most animal studies investigating the effects of fish oil have used amounts far in excess of those that could be consumed by humans.

Human studies have used rather less fish oil in the diet than the amount provided in most animal studies. Nevertheless, a number of studies in healthy humans reveal significant immunomodulatory effects of long chain *n*-3 fatty acids. Providing more than 2.3 g EPA plus DHA per day (and in some studies up to 14.5 g per day) has been reported to decrease chemotaxis and superoxide production by neutrophils and monocytes, to decrease production of TNF, IL-1, IL-2, IL-6 and IFN-γ by mononuclear cells, and to decrease lymphocyte proliferation (see Calder, 2001a for references). Taken together these studies indicate that addition of high levels of fish oil to the human diet exert potent anti-inflammatory effects. However, according to some studies, a high level of dietary fish oil also impairs lymphocyte responses. Other studies indicate that more modest addition of fish oil to the diet does not affect inflammatory or immune activities (see Calder, 2001a). However, there are a large number of studies which fall between the extremes of 'modest addition' and 'high levels' and these studies provide conflicting results. It is unclear what the reasons for these discrepancies are, but they might be related to different experimental protocols used, particularly those involving cell preparation, cell culture and cytokine assays and/or to different subject characteristics (eg gender, age, habitual diet; see Calder, 2001a for a discussion). Although not all studies

which have been performed agree, a number of studies now support the idea that fish oil (at the levels used experimentally in humans and animals) exerts a range of anti-inflammatory effects.

Fish oil and the response to endotoxin

Fish oil significantly increases survival following challenge with lipopolysaccharide (endotoxin: a component of the cell wall of gram-negative bacteria; see Calder, 1997 for references). This might relate to the decreased production of inflammatory mediators, including cytokines, which mediate the pathophysiological response to endotoxin, and to decreased responsiveness to those mediators (see Calder, 1997; Grimble, 1998).

Fish oil and Th-1 skewed immunological diseases

Chronic inflammatory diseases are characterised by a dysregulated Th1-type response and by an inappropriate production of inflammatory cytokines and arachidonic acid-derived eicosanoids. The effects of fish oil outlined above suggest that *n*-3 polyunsaturated fatty acids might have a role in prevention and therapy of chronic inflammatory diseases. In support of this idea, dietary fish oil has been shown to have beneficial clinical, immunological and biochemical effects in various animal models of human diseases, including decreased incidence and severity of collagen-induced arthritis and less inflammation in rats with various models of inflammatory bowel disease (see Calder, 1997 for references). There have been a number of clinical trials assessing dietary supplementation with fish oils in several chronic inflammatory diseases in humans, including rheumatoid arthritis, Crohn's disease, psoriasis and multiple sclerosis. In some of these studies anti-inflammatory effects of fish oil were observed (eg lowered LTB_4, IL-1 and C-reactive protein production), suggesting that it might bring about clinical improvements. Many of the placebo-controlled, double-blind trials of fish oil in chronic inflammatory diseases reveal significant benefit including decreased disease activity and a lowered use of anti-inflammatory drugs; the evidence for a beneficial effect of fish oil is strongest in rheumatoid arthritis (see Calder, 2001b; Calder and Zurier, 2001 for reviews).

Fish oil and Th-2 skewed immunological diseases

Eicosanoids synthesised from arachidonic acid play a role in allergic diseases: PGD_2, LTC_4, D_4 and E_4 are produced by the cells that mediate pulmonary inflammation in asthma such as mast cells and are believed to be the major mediators of asthmatic bronchoconstriction. In addition, PGE_2 regulates the activities of lymphocytes, promoting changes consistent with the development of allergic disease.

Since *n*-3 fatty acids potentially antagonise the effects of arachidonic acid, there may be a role for fish oil in treating, or in protecting against the development of, allergic diseases. A number of trials of fish oil in asthma have been performed. Although these trials often show fish oil-induced changes in production of some inflammatory mediators (eg LTB_4), many reveal limited clinical impact (see Calder & Miles, 2000). In contrast, some studies have shown significant 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) compared the effects of 'low' dose and 'high' dose fish oil in adult atopic asthmatics. With low *n*-3 polyunsaturated fatty acid ingestion, methacholine-induced respiratory distress increased. In contrast, high *n*-3 polyunsaturated fatty acid ingestion resulted in improved lung function in more than 40% of subjects; all measures of respiratory function were improved in this group of patients who also showed markedly elevated appearance of the EPA-derived 5-series LT in their urine. However, some patients did not respond to the high *n*-3 polyunsaturated fatty acid intake, which in some cases worsened respiratory function. This study suggests that there are subjects who respond positively to fish oil intervention and subjects whose response may be worsened by such intervention.

Concluding statement

Arachidonic acid gives rise to inflammatory mediators (prostaglandins, leukotrienes and related metabolites) and through these regulates the activities of inflammatory cells, the production of cytokines and the Th1 *vs* Th2 balance. It is generally considered that *n*-3 polyunsaturated fatty acids act as arachidonic acid antagonists. Amongst the *n*-3 fatty acids those from fish oil (EPA and DHA) are more biologically potent than α-linolenic acid. Components of both natural and acquired immunity, including the production of key inflammatory mediators, can be affected by *n*-3 polyunsaturated fatty acids. Although some of the effects of *n*-3 fatty acids may be brought about by modulation of the amount and types of eicosanoids made, it is possible that these fatty acids might elicit some of their effects by eicosanoid-independent mechanisms, including actions upon intracellular signalling pathways and transcription factor activity (see Grimble, 1998; Miles & Calder, 1998; Yaqoob, 1998). Such *n*-3 fatty acid-induced effects may be of use as a therapy for acute and chronic inflammation, and for disorders which involve an inappropriately activated immune response.

References

Broughton KS, Johnson CS, Pace BK, Liebman M & Kleppinger KM (1997): Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. *Am. J. Clin. Nutr.* **65**, 1011–1017.

Calder PC (1997): n-3 polyunsaturated fatty acids and cytokine production in health and disease. *Ann. Nutr. Metab.* **41**, 203–234.

Calder PC (1998a): *n*-3 fatty acids and mononuclear phagocyte function. In *Medicinal Fatty Acids in Inflammation*, ed. JM Kremer, pp 1–27. Basel: Birkhauser.

Calder PC (1998b): Dietary fatty acids and lymphocyte functions. *Proc. Nutr. Soc.* **57**, 487–502.
Calder PC (2001a): *n*-3 polyunsaturated fatty acids, inflammation and immunity: pouring oil on troubled waters or another fishy tale? *Nutr. Res.* **21**, 309–341.
Calder PC (2001b): The scientific basis for fish oil supplementation in rheumatoid arthritis. In *Food and Nutritional Supplements — Their Role in Health and Disease*, ed. JK Ransley, JK Donnelly & NW Read, pp 175–197. Berlin: Springer.
Calder PC & Miles EA (2000): Fatty acids and atopic disease. *Ped. All. Immunol.* **11**(Suppl), 29–36.
Calder PC & Zurier RB (2001): Polyunsaturated fatty acids and rheumatoid arthritis. *Curr. Opin. Clin. Nutr. Metab. Care* **4**, 115–121.
Gibney MJ & Hunter B (1993): The effects of short- and long-term supplementation with fish oil on the incorporation of n-3 polyunsaturated fatty acids into cells of the immune system in healthy volunteers. *Eur. J. Clin. Nutr.* **47**, 255–259.
Grimble RF (1988): Dietary lipids and the inflammatory response. *Proc. Nutr. Soc.* **57**, 535–542.
Miles EA & Calder PC (1998): Modulation of immune function by dietary fatty acids. *Proc. Nutr. Soc.* **57**, 277–292.
Sperling RI, Benincaso AI, Knoell CT, Larkin JK, Austen KF & Robinson DR (1993): Dietary ω-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. *J. Clin. Invest.* **91**, 651–660.
Wallace FA, Miles EA, Evans C, Stock TE, Yaqoob P & Calder PC (2001): Dietary fatty acids influence the production of Th1- but not Th2-type cytokines. *J. Leuk. Biol.* **69**, 449–457.
Yaqoob P (1998): Lipids and the immune response. *Curr. Opin. Clin. Nutr. Metab. Care* **1**, 153–161.
Yaqoob P, Pala HS, Cortina-Borja M, Newsholme EA & Calder PC (2000): Encapsulated fish oil enriched in α-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. *Eur. J. Clin. Invest.* **30**, 260–274.