Modulation of immune function by dietary fatty acids

Elizabeth A. Miles and Philip C. Calder*

Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, UK

Biosynthesis and sources of different fatty acids

All mammals can synthesize fatty acids *de novo* from acetyl-CoA. The endproduct of the fatty acid synthetase (*EC* 2.3.1.85) enzyme is palmitic acid (16 : 0), which in turn can be elongated to stearic acid (18 : 0). There is little need for the synthesis of saturated fatty acids in Western man, since the diet normally supplies adequate amounts. However, cell membranes require unsaturated fatty acids to maintain their structure, fluidity and function; therefore, a mechanism for the introduction of double bonds (i.e. desaturation) exists. The introduction of a single double bond between C-9 and C-10 is catalysed by the enzyme Δ^9-desaturase, which is universally present in both plants and animals. This enzyme results in the conversion of stearic acid to oleic acid (18 : 1*n*-9). Plants, unlike animals, can insert additional double bonds into oleic acid between the existing double bond at the C-9 position and the methyl terminus of the C chain; a Δ^{12}-desaturase converts oleic acid into linoleic acid (18 : 2*n*-6) while a Δ^{15}-desaturase converts linoleic acid into α-linolenic acid (18 : 3*n*-3). Since animal tissues are unable to synthesize linoleic and α-linolenic acids, these fatty acids must be consumed in the diet and so are termed essential fatty acids. Using the pathway outlined in Fig. 1, animal cells can convert dietary α-linolenic acid into eicosapentaenoic acid (20 : 5*n*-3) and docosahexaenoic acid (22 : 6*n*-3); by a similar series of reactions dietary linoleic acid is converted via γ-linolenic (18 : 3*n*-6) and dihomo-γ-linolenic (20 : 3*n*-6) acids to arachidonic acid (20 : 4*n*-6). The *n*-9, *n*-6 and *n*-3 families of polyunsaturated fatty acids (PUFA) are not metabolically interconvertible in mammals. Many marine plants, especially the unicellular algae in phytoplankton, also carry out chain elongation and further desaturation of α-linolenic acid to yield the long-chain *n*-3 PUFA eicosapentaenoic and docosahexaenoic. It is the formation of these long-chain *n*-3 PUFA by marine algae and their transfer through the food chain to fish that accounts for their abundance in some marine fish oils.

Cells of the immune system

Animals possess several barriers for protection against disease and infection. Some of these are present before exposure to infections, microbes or other foreign molecules and are not enhanced by such exposures. These are components of innate (or natural) immunity, and include phagocytic cells in the blood and in tissues, a class of lymphocytes called natural killer (NK) cells and various blood-borne chemicals. Other defence mechanisms are induced or stimulated by exposure to foreign substances, are specific for distinct macromolecules and increase in magnitude with each successive exposure to a particular macromolecule. These mechanisms constitute specific (or acquired) immunity.

The specific immune system has developed to include a number of cooperative functions between effector cells and molecules, allowing amplification of immune responses. Specific immune responses are classified into two types, humoral and cell-mediated immunity, based on the components of the immune system that are involved in the response. Humoral immunity is mediated by antibodies that are released by B-lymphocytes into the bloodstream and are responsible for specific recognition and elimination of antigens. Cell-mediated immunity involves specific antigen recognition by T-lymphocytes.

The cells of the immune system are normally present as circulating cells in blood and lymph, as anatomically-defined collections in lymphoid organs (thymus, spleen, lymph nodes) or as scattered cells in virtually all tissues. The principal cells of the immune system are T- and B-lymphocytes, NK cells, dendritic cells, mononuclear phagocytes (monocytes and macrophages) and granulocytes (these include neutrophils, eosinophils and basophils); together these cells are termed leucocytes.

Influence of dietary *n*-3 polyunsaturated fatty acids on the functions of cells of the immune system

Many studies have investigated the effects of the amount and type of fat in the diet on immune cell functions, particularly lymphocyte proliferation in response to mitogens. These studies have been reviewed several times in recent years (Kinsella *et al.* 1990; Yaqoob & Calder, 1993; Peck, 1994; Calder, 1995, 1996*a, b, c*, 1998; Calder & Yaqoob, 1997); the effects of *n*-3 PUFA are the most well documented and will be summarized here.

Abbreviations: Con A, concanavalin A; DAG, diacylglycerol; IL, interleukin; IP_3, inositol-1,4,5-trisphosphate; LPS, lipopolysaccharide; LT, leukotriene; MHC, major histocompatability complex; NF, nuclear transcription factor; NFκB, NF kappa B; NK, natural killer; PBMNC, peripheral blood mononuclear cells; PG, prostaglandins; PHA, phytohaemagglutinin; PKC, protein kinase C; PLC, phospholipase C; PPAR, peroxisomal proliferator-activated receptors; PUFA, polyunsaturated fatty acids; TNF, tumour necrosis factor.

***Corresponding author:** Dr Philip Calder, fax: 01703 594383, email pcc@soton.ac.uk

https://doi.org/10.1079/PNS19980042 Published online by Cambridge University Press

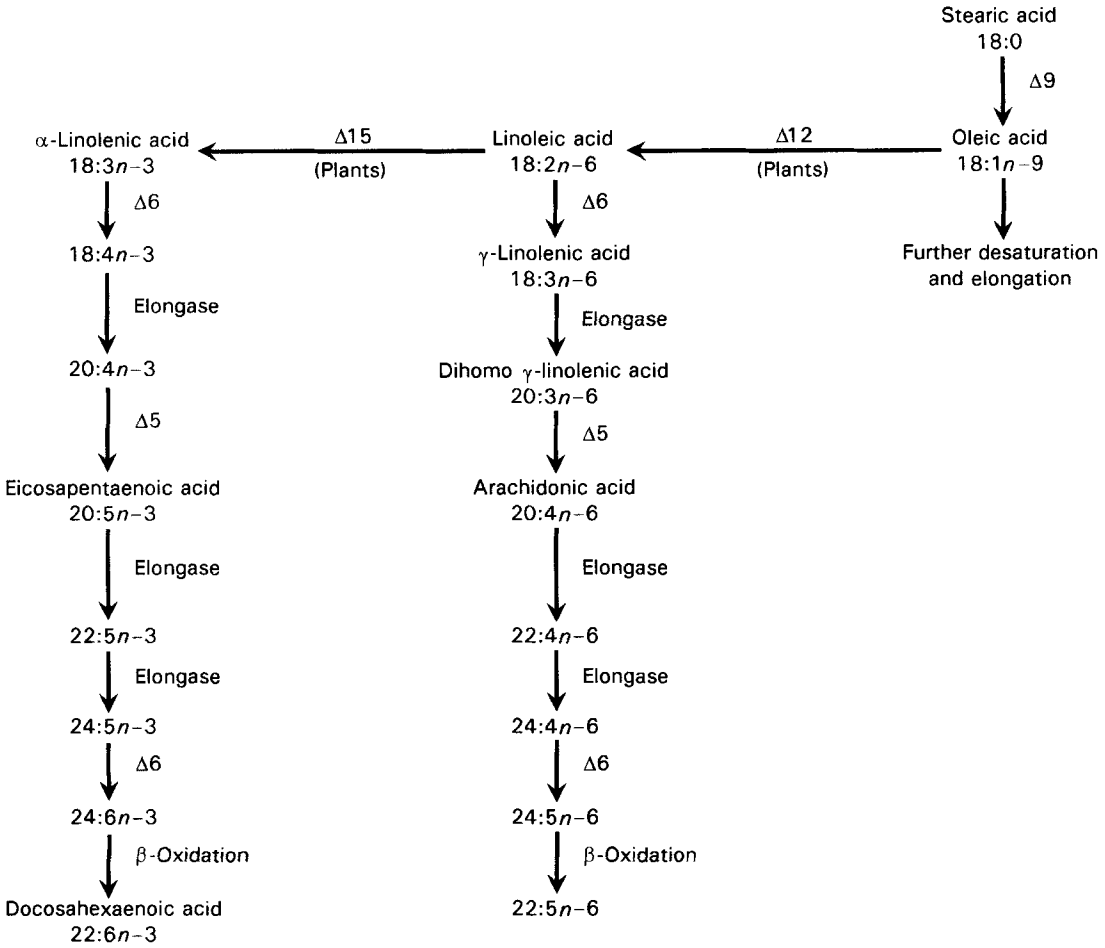

Fig. 1. Polyunsaturated fatty acid metabolism. Δ5, Δ6, Δ9, Δ12 and Δ15, Desaturase enzymes.

Lymphocyte proliferation

Lymphocyte proliferation is usually measured as the incorporation of radioactively-labelled precursors (e.g. thymidine) into DNA. A suitable stimulus (termed a mitogen) to activate the lymphocytes is required; mitogens used most frequently are concanavalin A (Con A) and phytohaemagglutinin (PHA) which stimulate T-lymphocytes specifically, bacterial lipopolysaccharide (LPS) which stimulates B-lymphocytes and pokeweed (*Phytolacca americana*) mitogen which stimulates a population of both T- and B-lymphocytes. Monoclonal antibodies to lymphocyte surface structures (e.g. CD3) can also be used to stimulate proliferation.

Feeding laboratory animals (rats, mice, chickens, rabbits) on diets containing high levels (70–200 g/kg) of fish oil (rich in eiocosapentaenoic and docosahexaenoic acids) has been shown to result in suppressed proliferation of spleen lymphocytes stimulated with T- or B-cell mitogens compared with feeding diets rich in other fats such as lard, coconut oil, maize oil, safflower oil or linseed oil (Alexander & Smythe, 1988; Kelley *et al.* 1988; Fritsche *et al.* 1991; Yaqoob *et al.* 1994*a*; Sanderson *et al.* 1995*a*; Yaqoob & Calder, 1995*a*). Recently it was reported that eicosapentaenoic and docosahexaenoic acids are equipotent in reducing murine spleen lymphocyte proliferation (Jolly *et al.* 1997). Feeding diets containing 76–200 g linseed oil (rich in α-linolenic acid)/kg has been shown to decrease proliferation compared with feeding saturated fatty acid- or *n*-6 PUFA-rich diets (Marshall & Johnston, 1985; Fritsche *et al.* 1991; Jeffery *et al.* 1996). Sometimes these effects were demonstrated when the cells were cultured in autologous serum, but were lost if the cells were cultured in fetal calf serum (Kelley *et al.* 1988; Fritsche *et al.* 1991; Yaqoob *et al.* 1994*a*). Culture of cells in fetal calf serum may explain the lack of effect on spleen lymphocyte proliferation of feeding fish and linseed oils to mice reported in some studies (Cathcart *et al.* 1987; Berger *et al.* 1993). It has been shown that the changes in lymphocyte fatty acid composition induced by dietary manipulations are better maintained if the cells are cultured in autologous rather than fetal calf serum (Yaqoob *et al.* 1995).

Supplementation of the diets of healthy women (51–68 years of age) for 12 weeks with encapsulated *n*-3 PUFA (approximately 2·4 g/day) resulted in a lowered mitogenic response of peripheral blood mononuclear cells (PBMNC) to PHA (Meydani *et al.* 1991). More recently, a decreased response of PBMNC to Con A and PHA following

https://doi.org/10.1079/PNS19980042 Published online by Cambridge University Press

supplementation of the diet of volunteers on a low-fat, low-cholesterol diet with 1·23 g *n*-3 PUFA/d was reported (Meydani *et al.* 1993), while 18 g fish oil/d (approximately 6 g *n*-3 PUFA/d) for 6 weeks resulted in lowered PHA-stimulated proliferation of PBMNC 10 weeks after supplementation had ended (but not at the end of the supplementation period) (Endres *et al.* 1993).

Cytotoxic T-lymphocyte-mediated cytolysis

The cytotoxic T-lymphocyte activity of spleen lymphocytes was reported to be lower after feeding mice on 100 g fish oil/kg for up to 10 weeks than after feeding 100 g linseed oil/kg (Fritsche & Johnston, 1990). Feeding chickens on diets containing 70 g fish or linseed oil/kg significantly reduced spleen cytotoxic T-cell activity compared with diets containing 70 g lard or maize oil/kg (Fritsche & Cassity, 1992).

Natural killer cell-mediated cytolysis

Feeding mice on diets containing 100 g fish oil/kg caused a decrease in spleen NK cell activity compared with feeding chow or 100 g maize oil/kg (Meydani *et al.* 1988; Lumpkin *et al.* 1993). In the study of Berger *et al.* (1993), female mice were fed on diets containing 100 g olive, safflower, linseed or fish oil/kg for 5 months and the spleen NK cell activity of the pups was determined before they were weaned; the activity was lower in the fish-oil group than in the safflower- or olive-oil groups. Yaqoob *et al.* (1994*b*) fed weanling rats on a low-fat diet or on diets containing 200 g hydrogenated coconut, olive, safflower, evening primrose (*Oenothera biennis*) or fish oil/kg for 10 weeks before measuring spleen NK cell activity. It was found that feeding each of the high-fat diets resulted in lower NK cell activity compared with feeding the low fat-diet; feeding the fish-oil diet resulted in the lowest activity. Similar results have been found in more mature rats fed on these diets for 12 weeks (Sanderson *et al.* 1995*a*). Recently it was reported that a 200 g linseed oil/kg diet decreased rat spleen lymphocyte NK cell activity compared with feeding a 200 g sunflower oil/kg diet (Jeffery *et al.* 1996).

No studies have investigated the effect of dietary lipids on human NK cell activity, although it was reported that intravenous injection of a triacylglycerol containing eicosapentaenoic acid into healthy human volunteers resulted in suppression of peripheral blood NK cell activity 24 h later (Yamashita *et al.* 1991).

Macrophage-mediated cytotoxicity

Dietary fish oil (100 g/kg) has been reported to suppress significantly the lysis of target tumour cells by mouse elicited peritoneal macrophages (Somers *et al.* 1989; Black & Kinsella, 1993; Renier *et al.* 1993; Hubbard *et al.* 1994). The target cell lines used in these studies are sensitive to killing by tumour necrosis factor (TNF)-α (L929 cells; Black & Kinsella, 1993; Renier *et al.* 1993) or NO (P815 cells; Somers *et al.* 1989; Hubbard *et al.* 1994). Thus, the suppressed macrophage-mediated cytolysis observed after fish-oil feeding suggests that fish oil reduces the production of NO and TNF (see pp. 280–283).

Neutrophil and monocyte chemotaxis

Chemotaxis of human peripheral blood neutrophils and monocytes towards a variety of chemo-attractants, including leukotriene (LT) B_4, platelet-activating factor, formyl-methionine-leucine-phenylalanine and autologous serum, is suppressed following the supplementation of the human diet with *n*-3 PUFA (Lee *et al.* 1985; Endres *et al.* 1989; Schmidt *et al.* 1989, 1992; Sperling *et al.* 1993).

Major histocompatibility complex expression and antigen presentation

Inclusion of *n*-3 PUFA in the diet of mice or rats results in a dimished percentage of peritoneal exudate cells bearing the major histocompatibility complex (MHC) class II antigens on their surface (Kelley *et al.* 1985; Mosquera *et al.* 1990; Huang *et al.* 1992; Sherrington *et al.* 1995). The level of MHC II expression on positive cells is also suppressed by fish-oil feeding (Huang *et al.* 1992). In accordance with these animal studies, supplementation of the diet of human volunteers with *n*-3 PUFA (approximately 1·56 g/d) for 3 weeks resulted in a decreased level of MHC II (human leucocyte antigen-DP, -DQ and -DR) expression on the surface of peripheral blood monocytes (Hughes *et al.* 1996*a*). These observations suggest that diets rich in *n*-3 PUFA will result in diminished antigen presentation. Indeed, feeding mice on the ethyl ester of eicosapentaenoic acid for a period of 4–5 weeks resulted in diminished presentation of antigen (keyhole limpet (*Megathura crenulata*) haemacyanin) by spleen cells *ex vivo* (Fujikawa *et al.* 1992). Dendritic cells are the key antigen-presenting cells *in vivo*. We have recently observed that, compared with a low-fat diet or a diet containing 200 g safflower oil/kg, feeding rats on a diet containing 200 g fish oil/kg significantly diminishes MHC II expression on dendritic cells and *ex vivo* antigen (keyhole limpet haemocyanin) presentation by dendritic cells (obtained by cannulation of the thoracic duct) to keyhole limpet haemocyanin-sensitized spleen lymphocytes (Sanderson *et al.* 1997).

Influence of dietary *n*-3 polyunsaturated fatty acids on the interactions between cells of the immune system

Communication between cells of the immune system is achieved by virtue of the production of chemical mediators (e.g. eicosanoids, cytokines, NO) and by direct cell-to-cell contact mediated by adhesion molecules (Fig. 2). *n*-3 PUFA have been found to influence each of these communication links.

n-3 *Polyunsaturated fatty acids and eicosanoid production*

Eicosanoids are a family of oxygenated derivatives of dihomo-γ-linolenic, arachidonic and eicosapentaenoic

https://doi.org/10.1079/PNS19980042 Published online by Cambridge University Press

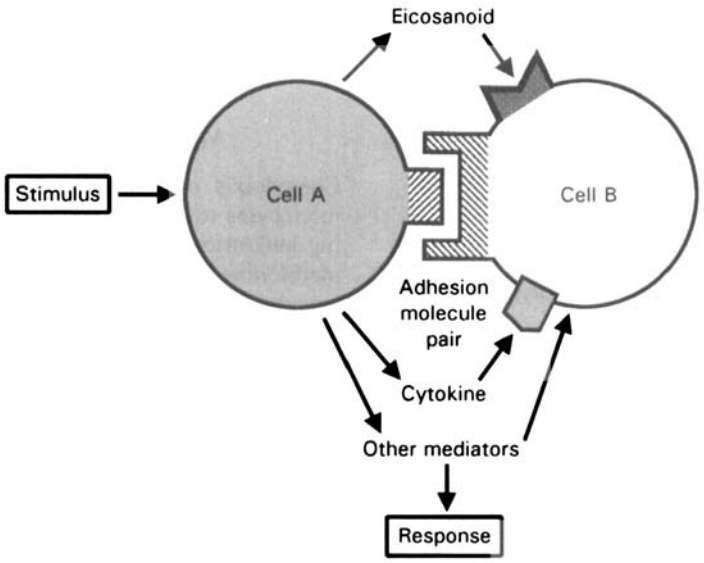

Fig. 2. Schematic representation of the different means by which cells of the immune system communicate with one another in order to respond appropriately to stimuli.

acids. Eicosanoids include prostaglandins (PG), thromboxanes, LT, lipoxins, hydroperoxyeicosatetraenoic acids and hydroxyeicosatetraenoic acids. Under most conditions the principal precursor for these compounds is arachidonic acid. The precursor PUFA is released from membrane phosphatidylcholine by the action of phospholipase A_2 (*EC* 3.1.1.4) or from membrane phosphatidylinositol-4,5-bisphosphate by the actions of phospholipase C (*EC* 3.1.4.3; PLC) and a diacylglycerol (DAG) lipase (*EC* 3.1.1.34). The pathways of eicosanoid synthesis begin with cyclooxygenase (*EC* 1.14.99.1), which yields the PG and thromboxanes, or with the 5-, 12- or 15-lipoxygenases (*EC* 1.13.11.34, *EC* 1.13.11.31 and *EC* 1.13.11.33 respectively), which yield the LT, hydroperoxy-eicosatetraenoic acids, hydroxyeicosatetraenoic acids and lipoxins (Fig. 3). The amounts and types of eicosanoids synthesized are determined by the availability of arachidonic acid, by the activities of phospholipase A_2 and phospholipase C, by the activities of cyclooxygenase and the lipoxygenases, by the cell type and by the nature of the stimulus.

Cells of the immune system are an important source of eicosanoids and they are subject to their regulatory effects (Goldyne & Stobo, 1982; Goodwin & Cueppens, 1983; Hwang 1989; Roper & Phipps, 1994); the most-well-documented effects are those of PGE_2. *In vivo*, PG are involved in modulating the intensity and duration of inflammatory and immune responses; PGE_2 has a number of pro-inflammatory effects, including induction of fever and erythema, increased vascular permeability and vasodilation and enhancement of pain and oedema caused by other agents such as bradykinin and histamine. PGE_2 also regulates the production of monocyte-, macrophage- and lymphocyte-derived cytokines (see Rola-Pleszczynski & Stankova, 1992). In chronic inflammatory conditions increased rates of PGE_2 production are found, and elevated PGE_2 production has been observed in patients suffering from infections. LT have chemotactic properties and are involved in the regulation of inflammatory and immune processes; 4-series LT regulate cytokine production.

The *n*-3 PUFA, eicosapentaenoic and docosahexaenoic, competitively inhibit the oxygenation of arachidonic acid by cyclooxygenase. In addition, eicosapentaenoic acid (but not docosahexaenoic acid) is able to act as a substrate for both cyclooxygenase and 5-lipoxygenase. Thus, ingestion of fish oils which contain *n*-3 PUFA will result in a decrease in membrane arachidonic acid levels and a concomitant decrease in the capacity to synthesize eicosanoids from arachidonic acid (for example, see Yaqoob & Calder, 1995*b*); eicosapentaenoic acid gives rise to the 3-series PG and thromboxanes and the 5-series LT (Fig. 3). The eicosanoids produced from eicosapentaenoic acid do not always have the same biological properties as the analogues produced from arachidonic acid.

n-*3 Polyunsaturated fatty acids and cytokine production*

Since cytokine production is regulated by eicosanoids (see Rola-Pleszczynski & Stankova, 1992) and since dietary lipids affect eicosanoid production, it might be expected that dietary lipids, especially those containing *n*-3 PUFA, will affect cytokine production. The effects of *n*-3 PUFA on cytokine production have been reviewed several times in recent years (Meydani 1992, 1996; Blok *et al.* 1996; Endres, 1996; Endres & von Schacky, 1996; Calder, 1997).

Animal studies. A number of studies have reported that feeding rodents on *n*-3 PUFA-containing oils results in enhanced production of TNF by macrophages *ex vivo* (Lokesh *et al.* 1990; Hardardottir & Kinsella, 1991, 1992; Turek *et al.* 1991; Watanabe *et al.* 1991; Blok *et al.* 1992; Chang *et al.* 1992; Chaet *et al.* 1994; Hubbard *et al.* 1994; Somers & Erickson, 1994), although there are reports of

https://doi.org/10.1079/PNS19980042 Published online by Cambridge University Press

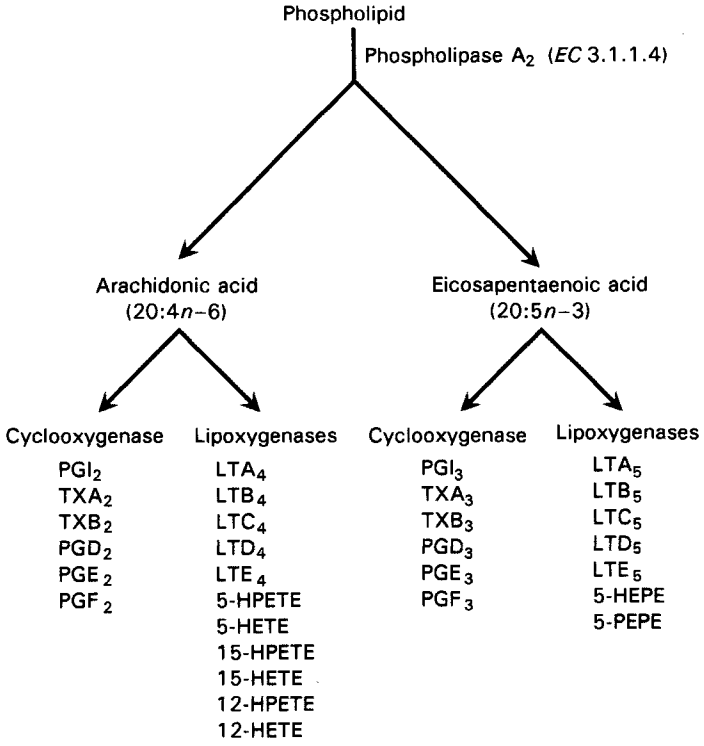

Fig. 3. Synthesis of eicosanoids from arachidonic and eicosapentaenoic acids. PG, prostaglandin, TX, thromboxane; LT, leukotriene; HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid.

decreased production (Renier *et al.* 1993; Boutard *et al.* 1994; Yaqoob & Calder, 1995*b*; Wallace *et al.* 1998) or no effect (Turek *et al.* 1991; Watanabe *et al.* 1991; Hardardottir & Kinsella, 1992; Hubbard *et al.* 1994; Somers & Erickson, 1994; Tappia & Grimble, 1994). These differences between studies appear not to relate to species, duration of feeding or type or amount of *n*-3 PUFA in the diet (see Calder, 1997). However, there may be some relationship with the state of activation of the macrophages used and with the stimulus used for activation. Several studies have used resident peritoneal or alveolar macrophages or Kupffer cells, while other studies have used elicited peritoneal macrophages; the agents used to elicit macrophages include thioglycollate, casein and complete Freund's adjuvant. All studies which have used murine resident peritoneal macrophages (Lokesh *et al.* 1990; Hardardottir & Kinsella, 1991, 1992; Watanabe *et al.* 1991; Blok *et al.* 1992; Chang *et al.* 1992), one study using rat resident alveolar macrophages (Chaet *et al.* 1994) and one study using rat resident peritoneal macrophages (Turek *et al.* 1991) report an enhancing effect of *n*-3 PUFA on TNF production; only one study which used rat resident peritoneal macrophages has reported reduced TNF production following fish-oil feeding (Boutard *et al.* 1994). Three studies report that *n*-3 PUFA-rich diets do not affect TNF production by complete Freund's adjuvant-elicited peritoneal macrophages from rats or mice (Turek *et al.* 1991; Watanabe *et al.* 1991; Hardardottir & Kinsella, 1992). The effect of dietary *n*-3 PUFA on TNF production by thioglycollate-elicited peritoneal macrophages is unclear, with studies reporting no effect (Watanabe *et al.* 1991; Hubbard *et al.* 1994; Somers & Erickson, 1994; Tappia & Grimble, 1994), reduction (Renier *et al.* 1993; Yaqoob & Calder, 1995*b*; Wallace *et al.* 1998) or enhancement (Hubbard *et al.* 1994; Somers & Erickson, 1994). Comparison of the outcome of these studies is complicated by the different procedures used for *ex vivo* culture of the cells. That such details are important in determining outcome is shown by the studies of Erickson's group (Hubbard *et al.* 1994; Somers & Erickson 1994) in which there was no effect of diet on TNF production when thioglycollate-elicited macrophages were stimulated with LPS for 1 or 8 h, but if they were stimulated for 24 h there was increased TNF production by cells from fish oil-fed mice. The only animal study which has investigated TNF production by PBMNC showed decreased production following the infusion of a 100 ml fish oil/l emulsion (Grimm *et al.* 1994); this is an interesting observation since it agrees with the findings a number of studies using human PBMNC (see pp. 282–283). In addition to studies measuring TNF production *ex vivo*, Black & Kinsella (1993) and Renier *et al.* (1993) showed that feeding mice *n*-3 PUFA-rich diets resulted in reduced ability of elicited peritoneal macrophages to kill L929 cells; L929 cells are killed by TNF and so the reduced cytotoxicity of macrophages towards these cells suggests a reduced ability to produce TNF. Two animal studies have investigated the effects of dietary lipids on circulating TNF levels which would perhaps reflect *in vivo* production of the cytokine. Watanabe *et al.* (1991) found that TNF levels were

https://doi.org/10.1079/PNS19980042 Published online by Cambridge University Press

significantly higher in the plasma of LPS-injected (10 mg/kg body weight) mice fed on diets containing 100 g perilla oil/kg (high in α-linolenate) than in the plasma of those fed on 100 g safflower oil/kg (high in α-linoleate). Similarly, Chang *et al.* (1992) reported higher serum TNF levels 1 and 1·5 h following intraperitoneal injection of LPS (10 μg) into fish oil-fed mice compared with those fed on coconut or maize oil.

All studies which have used thioglycollate-elicited peritoneal macrophages and the only study to use Kupffer cells report that dietary fish oil results in decreased *ex vivo* production of interleukin (IL)-1 (Billiar *et al.* 1988; Renier *et al.* 1993; Tappia & Grimble, 1994; Yaqoob & Calder 1995*b*; Wallace *et al.* 1998). In contrast, two studies have reported that fish oil enhances IL-1 production by murine resident macrophages (Lokesh *et al.* 1990; Blok *et al.* 1992). In addition, Ertel *et al.* (1993) showed that the reduction in *ex vivo* IL-1 production by resident peritoneal macrophages which accompanies haemorrhagic shock in maize oil- or safflower oil-fed mice was prevented by fish-oil feeding. This study also showed no difference in IL-1 production by resident peritoneal macrophages taken from sham-operated mice fed on these three diets (Ertel *et al.* 1993).

There are no studies reporting the effect of dietary fatty acids on IL-6 production by resident peritoneal macrophages. One study using murine thioglycollate-elicited peritoneal macrophages showed a significant reduction in LPS-stimulated IL-6 production following fish-oil feeding (Yaqoob & Calder, 1995*b*); production following stimulation of the cells with TNF was also significantly reduced (P Yaqoob and PC Calder, unpublished results). Rat PBMNC showed reduced IL-6 production following fish-oil infusion for 4 d (Grimm *et al.* 1994).

Since PGE_2 inhibits the production of TNF, IL-1 and IL-6 (Kunkel *et al.* 1982; Knudsen *et al.* 1986; Renz *et al.* 1988; Endres *et al.* 1991) and since fish-oil feeding significantly diminishes PGE_2 production (for example, see Yaqoob & Calder, 1995*b*), the observations of some workers that fish-oil feeding results in enhanced production of macrophage-derived cytokines suggests that the mechanism of action of *n*-3 PUFA is via a decrease in PGE_2 production. However, addition of PGE_2 to cultures of peritoneal macrophages from fish oil-fed mice did not lower TNF production to the level of cells from animals fed on other diets (Hardardottir & Kinsella, 1991), suggesting a less straightforward explanation. Indeed, the situation will be more complex, because 4-series LT enhance TNF, IL-1 and IL-6 production (Kunkel *et al.* 1982; Rola-Pleszczynski & Lemaire, 1985; Dubois *et al.* 1989; Gagnon *et al.* 1989; Schade *et al.* 1989; Poubelle *et al.* 1991) and fish-oil feeding will decrease 4-series LT production (for example, see Lee *et al.* 1985; Chapkin *et al.* 1990); thus, *n*-3 PUFA feeding will decrease the production of both inhibitory and stimulatory factors. Thus, the precise effect of *n*-3 PUFA feeding on production of macrophage-derived cytokines might relate to the changed balance in production of PGE_2 and 4-series LT and also to the balance in production between these mediators and the analogues produced from eicosapentaenoic acid. It is possible that the contradictory reports in the literature relate simply to the relative changes *n*-3 PUFA-containing diets have induced in production of PGE_2, LTB_4, LTC_4 and eicosapentaenoic acid-derived PG and LT. Furthermore, the apparently contradictory effects observed for resident and elicited macrophages and with different types of eliciting agent might relate to different capacities of the different types of cells to produce PG and LT.

In contrast to the large number of studies in animals of the effects of dietary lipids, especially fish oil, on the *ex vivo* production of macrophage-derived cytokines there have been relatively few studies on lymphocyte-derived cytokines. Decreased IL-2 production by alveolar lymphocytes from pigs fed on diets containing 105 g fish or linseed oil/kg was reported (Turek *et al.* 1994). In contrast, higher IL-2 production by Con A-stimulated spleen lymphocytes taken from autoimmune-disease-prone mice fed on 100 g fish oil/kg compared with those fed on 100 g maize oil/kg has been reported (Fernandes *et al.* 1994); the intracellular levels of mRNA for IL-2 were also elevated in the fish oil-fed mice, although these levels could not be quantified. A recent study reported that inclusion of α-linolenic acid or eicosapentaenoic plus docosahexaenoic acids in the diet of monkeys resulted in enhanced *ex vivo* production of IL-2 (Wu *et al.* 1996). In another study weanling mice were fed for 8 weeks on a low-fat diet or on diets containing 200 g hydrogenated coconut, olive, safflower or fish oil/kg; the spleen lymphocytes were subsequently stimulated with Con A (Yaqoob & Calder, 1995*a*). The concentration of IL-2 was higher in the medium of spleen lymphocytes from mice fed on olive or safflower oil than in the medium of cells from mice fed on the low-fat diet or hydrogenated coconut oil; fish-oil feeding had no effect on the IL-2 concentration in the medium. Recently it was observed that feeding mice on a diet containing 10 g ethyl esters of eicosapentaenoic or docosahexaenoic acid/kg for 10 d significantly decreased *ex vivo* IL-2 production by spleen lymphocytes stimulated with Con A; both *n*-3 PUFA were equally effective (Jolly *et al.* 1997).

There have been few animal studies of the effects of dietary lipids on lymphocyte-derived cytokines other than IL-2. The studies which have been reported suggest minimal effects of dietary fat on production of IL-4, IL-10 and interferon-γ (Fernandes *et al.* 1994; Yaqoob & Calder, 1995*a*), but more studies need to be performed to confirm this.

Human studies. A large number of studies have now investigated the effect of supplementation of the diet of healthy subjects with *n*-3 PUFA on cytokine production by PBMNC *ex vivo* (Endres *et al.* 1989, 1993; Meydani *et al.* 1991, 1993; Molvig *et al.* 1991; Virella *et al.* 1991; Cooper *et al.* 1993; Gallai *et al.* 1993; Caughey *et al.* 1996); studies have also been performed in patients with rheumatoid arthritis (Kremer *et al.* 1990), inflammatory skin diseases (Soyland *et al.* 1994), type-1 diabetes (Molvig *et al.* 1991) and multiple sclerosis (Gallai *et al.* 1993). Endres *et al.* (1989) were the first to show that *n*-3 PUFA diminish *ex vivo* production of IL-1α, IL-1β and TNF. Interestingly, the production of these cytokines remained suppressed for 10 weeks once the supplementation had ended, indicating the long period of 'washout' required to reverse the effects of fish-oil supplementation. It is also worth noting that this study showed that the precise effects of dietary *n*-3 PUFA

vary according to the stimulus used to induce cytokine production. A later study by Endres *et al.* (1993) using the same supplementation regimen reported a decrease in *ex vivo* IL-2 production in response to Con A; the reduction was greater 10 weeks after supplementation had ended than at the end of supplementation. Virella *et al.* (1991) reported that 6 weeks of supplementation of the diet with 2·4 g eicosapentaenoic acid/d resulted in lowered production of IL-2 by PBMNC stimulated with PHA or pokeweed mitogen; in agreement with the long 'washout' period suggested by Endres *et al.* (1989, 1993), IL-2 production remained low during 8 weeks after the supplementation had ended but returned to pre-supplementation levels 22 weeks after supplementation. Meydani *et al.* (1991) supplemented the diet of healthy young (20–33 years of age) and older (51–68 years of age) women with 2·4 g *n*-3 PUFA/d and examined *ex vivo* production of a range of cytokines after 4, 8 and 12 weeks. There were time-dependent decreases in the production of IL-1β, IL-2, TNF and IL-6 by PBMNC from the older women; production of TNF and IL-6 by cells from the young women was also significantly decreased and there were trends towards decreased production of IL-1β and IL-2 by these cells. The same workers have studied the effect of including fish (providing 1·23 g *n*-3 PUFA/d) in a low-fat low-cholesterol diet for 24 weeks; the *ex vivo* production of IL-1β, IL-6 and TNF by PBMNC was significantly reduced and there was a non-significant reduction in IL-2 production (Meydani *et al.* 1993). The production of IL-1 and IL-6 in whole blood cultures was decreased if the subjects had consumed 1·1–1·6 g *n*-3 PUFA/d for 6–8 weeks, but only if the cultures were stimulated with 'low' concentrations ($\leq$0·001 μg/ml) of LPS (Cooper *et al.* 1993). Interestingly, if higher LPS concentrations were used there was no effect of the supplementation on production of these two cytokines; TNF production was unaffected by *n*-3 PUFA in this study. Recently the effects of dietary α-linolenic acid on IL-1β and TNFα production by human cells have been reported; subjects consumed a sunflower oil-rich diet (which was very similar to their typical diet) or a diet rich in α-linolenic acid which was provided by linseed-oil capsules and linseed oil-based spreads and cooking oils (Caughey *et al.* 1996). In this way the linseed-oil consumption increased to a mean of 13·7 g/d. *Ex vivo* production of both IL-1β and TNFα by PBMNC was decreased by the linseed-oil diet. If the subjects then supplemented their diet with encapsulated eicosapentaenoic plus docosahexaenoic acid (2·7 g/d) production of both cytokines was further decreased. These authors showed a correlation between mononuclear-cell eicosapentaenoic acid content and production of IL-1β and TNFα.

Supplementation of the diet of healthy volunteers or patients with multiple sclerosis with encapsulated fish oil providing approximately 5 g *n*-3 PUFA/d for 24 weeks resulted in lower *ex vivo* production of IL-1β, TNF-α, IL-2 and interferon-γ by PBMNC (Gallai *et al.* 1993). An earlier study reported lower production of IL-1 by PBMNC taken from patients with rheumatoid arthritis who had consumed *n*-3 PUFA for 24 weeks (Kremer *et al.* 1990); interestingly, cells from the patients who consumed an olive-oil placebo (9 g/d) in this trial also showed diminished IL-1 production. There was increased production of IL-2 by PBMNC from patients with rheumatoid arthritis who supplemented their diet with olive oil or a 'low' level of *n*-3 PUFA for 24 weeks; this increase did not occur in patients consuming a 'high' level of *n*-3 PUFA (Kremer *et al.* 1990).

Recently, circulating cytokine levels in patients receiving intravenous infusions of lipid emulsions post-surgery were reported (Wachtler *et al.* 1997); patients received either a medium-chain triacylglycerol–long-chain triacylglycerol mix (50 : 50, v/v) or this mix also containing fish oil (50 : 30 : 20, by vol.). Patients received 50 g fat/d on days 1 and 2 post-surgery and 100 g fat/d on days 3, 4 and 5; thus, patients in the group receiving the fish oil-containing emulsion received approximately 3 (days 1 and 2) or 6 (days 3, 4 and 5) g *n*-3 PUFA/d. Plasma TNF-α levels were significantly lower in the fish-oil group at 6 d post-surgery; plasma IL-6 levels were lower (but not significantly) 10 d post-surgery and the post-surgery increase in plasma IL-10 levels was reduced in this group.

n-3 *Polyunsaturated fatty acids and cytokine receptor expression*

Feeding rats on a diet containing 200 g fish oil/kg lowered the proportion of spleen and thymic lymphocytes bearing the IL-2 receptor (IL-2R; CD25) following Con A stimulation (Yaqoob *et al.* 1994*a*). Spleen lymphocytes from rats fed on fish oil also showed a lower level of expression of the IL-2R following mitogenic stimulation (Sanderson *et al.* 1995*a*). In accordance with these animal studies, supplementation of the diet of patients with psoriasis or atopic dermatitis with *n*-3 PUFA ethyl esters (6 g/d) caused a significant reduction in the percentage of IL-2R$^+$ blood lymphocytes following PHA stimulation (Soyland *et al.* 1994); the level of expression of the IL-2R on the positive cells was also significantly reduced.

n-3 *Polyunsaturated fatty acids and nitric oxide production by macrophages*

Investigations of the effects of diets rich in *n*-3 PUFA on the production of reactive oxygen and nitrogen species by phagocytic cells have yielded contradictory results (for references, see Calder, 1996*c*). Two studies using rat resident peritoneal macrophages reported that NO production is significantly diminished by fish-oil feeding (Boutard *et al.* 1994; Joe & Lokesh, 1994). In contrast, Hubbard *et al.* (1994) found no effect of feeding mice on 100 g linseed or fish oil/kg on *ex vivo* NO production by thioglycollate-elicited macrophages; nevertheless, these workers, as well as Somers *et al.* (1989), reported that *n*-3 PUFA feeding diminishes macrophage cytotoxicity towards NO-sensitive P815 cells, suggestive of reduced NO production. Yaqoob & Calder (1995*b*) found that several high-fat diets, including 200 g fish oil/kg, enhanced NO production by murine thioglycollate-elicited macrophages compared with a low-fat diet; there were no significant differences in NO production by macrophages from mice fed on different high-fat diets. Fish oil has been reported to enhance NO

https://doi.org/10.1079/PNS19980042 Published online by Cambridge University Press

production by murine peritoneal (Renier *et al.* 1993), rat lung (Chaet *et al.* 1994) and pig lung (Turek *et al.* 1994) macrophages.

Recently, Harris *et al.* (1997) reported increased appearance of NO metabolites in the urine of healthy human volunteers who supplemented their diet with 5 g fish-oil concentrate (providing 3 g eicosapentaenoic plus docosahexaenoic acid)/d for 3 weeks; interestingly, 3 g eicosapentaenoic acid alone did not alter urinary NO metabolite levels. The levels measured by Harris *et al.* (1997) were assumed to indicate whole-body NO production; as such, a variety of sources of NO are likely and the authors concluded that much of it originated from endothelial cells. There is much direct and circumstantial evidence that *n*-3 PUFA enhance NO production by endothelial cells (for references, see Harris *et al.* 1997).

n-*3 Polyunsaturated fatty acids and adhesion-molecule expression*

Adhesion molecules are involved in many cell-to-cell interactions. For example, interaction between T-lymphocytes and antigen-presenting cells is in part mediated by the ligand–receptor pairs CD11a and CD18–CD54, CD11a and CD18–CD102 and CD2–CD58 (for reviews, see Stoolman, 1989; Springer, 1990; Hogg & Lands, 1993). Thus, an efficient cell-mediated immune response requires appropriate levels of expression of these molecules on T-lymphocytes. In addition, leucocyte adhesion to the endothelium involves a number of ligand–receptor pairs including CD11a and CD18–CD54, CD54–CD11a and CD18, CD49d and CD29–CD106, CD2–CD58, CD62L–MAdCAM-1 and CD44–hyaluronate (for reviews, see Stoolman, 1989; Springer, 1990; Hogg & Lands, 1993). Thus, movement of leucocytes between body compartments, into and out of lymphoid organs and into sites of immune or inflammatory reactivity requires adhesion-molecule expression. Adhesion-molecule expression appears to be involved in several acute and chronic inflammatory disease processes (for review, see Faull, 1995), and antibodies against certain adhesion molecules can reduce chronic inflammatory disease (see Faull, 1995).

In vitro *studies of* n-*3 polyunsaturated fatty acids and adhesion-molecule expression.* It has become apparent that *n*-3 PUFA can affect adhesion-molecule expression by some cell types, at least *in vitro*. Calder *et al.* (1990) observed that murine thioglycollate-elicited peritoneal macrophages cultured in the presence of eicosapentaenoic or docosahexaenoic acid were less adherent to artificial surfaces (the adhesion to one of these surfaces is mediated by CD11a and CD18) than those cultured with some other fatty acids. This observation suggests that *n*-3 PUFA decrease expression of either CD11a or CD18 or both proteins. More recently, De Caterina *et al.* (1995) reported that culture of human adult saphenous-vein endothelial cells with docosahexaenoic acid significantly decreased the cytokine-induced expression of CD106, CD62E and CD54 in a dose-dependent manner. The adhesion of U937 monocytes or human peripheral blood monocytes to the endothelial cells was diminished following incubation of the latter with docosahexaenoic acid (De Caterina *et al.* 1995). Since the binding between monocytes and endothelial cells partially depends on CD106 expression on the endothelial cells, the reduced expression of CD106 caused by docosahexaenoic acid appears to have a functional effect. Kim *et al.* (1995) reported that incubation of LPS-stimulated pig aortic endothelial cells with eicosapentaenoic acid resulted in diminished binding between these cells and U937 monocytes. Eicosapentaenoic acid was shown to reduce the expression of CD106, CD62E and CD54 on the surface of LPS-stimulated human umbilical-vein endothelial cells (Kim *et al.* 1995). Recently, it was shown that inclusion of eicosapentaenoic or docosahexaenoic acid in the medium of cultured resting or LPS- or cytokine-stimulated human umbilical-vein endothelial cells decreased the ability of peripheral blood lymphocytes to bind (Khalfoun *et al.* 1996); both *n*-3 PUFA were shown to decrease the level of expression of CD106, but not of CD54 or CD62E, on the surface of cytokine-stimulated endothelial cells. This latter study also reported, for the first time, the *in vitro* effect of *n*-3 PUFA on adhesion-molecule expression on lymphocytes; incubation of lymphocytes with either eicosapentaenoic or docosahexaenoic acid reduced the level of expression of CD11a and CD62L but did not affect CD49d expression (Khalfoun *et al.* 1996). In parallel with this reduction, the binding of lymphocytes to untreated or cytokine-stimulated endothelial cells was diminished. In another recent study, incubation with eicosapentaenoic acid was shown to reduce the level of expression of CD54, but not CD11a, on the surface of resting or interferon-γ-stimulated human monocytes (Hughes *et al.* 1996*b*); docosahexaenoic acid did not alter expression of these molecules. Thus, it appears that culture of macrophages, monocytes, lymphocytes or endothelial cells with *n*-3 PUFA can decrease adhesion-molecule expression resulting in diminished ability to bind to other cell types.

Effects of dietary n-*3 polyunsaturated fatty acids on adhesion-molecule expression.* There are few studies of the effects of inclusion of *n*-3 PUFA in the diet on adhesion-molecule expression, although it was recently shown that supplementation of the human diet with *n*-3 PUFA results in significantly lower levels of expression of CD11a and CD54 on peripheral-blood monocytes (Hughes *et al.* 1996*a*). Feeding rats on a diet containing 200 g fish oil/kg significantly reduced (by 20–35 %) the levels of expression of CD2 and CD11a on freshly-prepared lymphocytes and of CD2, CD11a and CD54 on Con A-stimulated lymphocytes (Sanderson *et al.* 1995*a*). Furthermore, the levels of CD2, CD11a and CD54 were reduced on popliteal-lymph-node lymphocytes following localized graft *v.* host or host *v.* graft responses in *vivo* (Sanderson *et al.* 1995*b*). Reduced adhesion-molecule expression suggests that cells will be less able to interact with receptor-bearing cells. In accordance with this suggestion, we have recently observed that lymph-node lymphocytes obtained from fish oil-fed rats adhere less well to macrophage and endothelial cell monolayers (Sanderson & Calder, 1997). These observations suggest that *n*-3 PUFA feeding will affect the movement of lymphocytes and monocytes between body

compartments and perhaps into sites of inflammatory or autoimmune activity.

Mechanisms by which *n*-3 polyunsaturated fatty acids might exert their effects

Clearly *n*-3 PUFA do influence the functional activities of cells of the immune system, although a number of conflicting observations have been made. These fatty acids appear to alter the production of mediators involved in communication between cells of the immune system (eicosanoids, cytokines, NO) and to alter the expression of key cell-surface molecules involved in direct cell-to-cell contact (adhesion molecules). The production of cytokines and of NO is regulated by eicosanoids and, therefore, a *n*-3 PUFA-induced change in the amount and types of eicosanoids formed could, at least partially, explain the effects of *n*-3 PUFA. However, many of the effects of *n*-3 PUFA appear to be exerted in an eicosanoid-independent manner. Thus, other mechanisms of action of *n*-3 PUFA on immune cell function must be considered. One such mechanism could be through regulating expression of key genes involved in immune cell functioning and in the production of immune cell-derived mediators.

Evidence that n-*3 polyunsaturated fatty acids affect gene expression in cells of the immune system*

It is now well documented that fatty acids affect the expression of genes involved in hepatic fatty acid and lipoprotein metabolism (for reviews, see Jump *et al.* 1995, 1996; Schoonjans *et al.* 1996) and the genes involved in adipocyte differentiation and development (for reviews, see Ailhaud *et al.* 1995; Jump *et al.* 1996). Dietary *n*-3 PUFA have particularly potent effects on the expression of genes for proteins involved in hepatic peroxisomal proliferation, fatty acid oxidation and lipoprotein assembly (for example, see Berthou *et al.* 1995). Studies of the effects of fatty acids on the expression of genes important in immune cell functioning are few and relatively recent.

n-*3 Polyunsaturated fatty acids and cytokine gene expression.* Inclusion of fish oil in the diet of autoimmune-disease-prone mice resulted in elevated levels of mRNA for IL-2, IL-4 and transforming growth factor-β and reduced levels of mRNA for c-myc and c-ras in the spleen (Fernandes *et al.* 1994). The same workers also showed that dietary fish oil completely abolished mRNA production for IL-1β, IL-6 and TNF-α in the kidneys of these animals (Chandrasekar & Fernandes, 1994). It has been reported that feeding mice on a fish oil-rich diet significantly diminished 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).

n-*3 Polyunsaturated fatty acids and adhesion-molecule gene expression.* De Caterina *et al.* (1995) showed that incubation of human saphenous-vein endothelial cells with docosahexaenoic acid results in reduced levels of mRNA for CD106.

n-*3 Polyunsaturated fatty acids and nitric oxide synthase gene expression.* Khair-El-Din *et al.* (1996) reported that incubation of murine thioglycollate-elicited peritoneal macrophages with docosahexaenoic acid decreased NO production in response to LPS plus interferon-γ (or interferon-γ plus TNFα); the inhibition was concentration dependent. Neither arachidonic nor eicosapentaenoic acids at concentrations up to 100 μM affected NO production. This study found that incubation of the cells with docosahexaenoic acid resulted in a lower level of mRNA for inducible NO synthase, the enzyme responsible for NO production in macrophages stimulated in this way; the lower level of inducible NO synthase mRNA was due to an inhibition of transcription (Khair-El-Din *et al.* 1996).

Mechanisms by which n-*3 polyunsaturated fatty acids might affect gene expression*

Thus, it is now apparent that *n*-3 PUFA might affect immune cell function partly by regulating the expression of genes encoding for proteins involved in cellular responses and in communication between cells. One mechanism by which *n*-3 PUFA could affect gene expression is through changes in the signal transduction pathways which link cell surface receptors to the activation of nuclear transcription factors (NF). Alternatively, *n*-3 PUFA or their derivatives (indeed derivatives of PUFA in general) might bind directly to NF thereby altering their activity.

Fatty acids and signal transduction. Many lipids are involved directly in intracellular signalling pathways; for example, hydrolysis of membrane phospholipids such as phosphatidylinositol-4,5-bisphosphate and phosphatidylcholine by phospholipases generates second messengers such as DAG and inositol-1,4,5-trisphosphate (IP_3). Other phospholipids have roles in activating or stabilizing enzymes involved in intracellular signalling; for example, phosphatidylserine is required for protein kinase C (PKC) activation. DAG activates some isoforms of PKC and also activates sphingomyelin ceramide-phosphohydrolase (*EC* 3.1.4.41) to release the second messenger ceramide. Ceramide in turn may be converted to sphingosine and sphingosine-1-phosphate; sphingosine inhibits PKC and activates some phospholipases. Since phosphatidylinositol-4,5-bisphosphate, phosphatidylcholine, phosphatidylserine and DAG all contain fatty acyl chains attached to the *sn*-1 and -2 positions of the glycerol moiety, it is conceivable that changing the type of fatty acid present may alter the precise properties of these compounds with regard to their functions in signal transduction (Fig. 4). Certainly changing the fatty acid composition of the diet (e.g. by feeding fish oil) markedly alters the phospholipid and DAG molecular species compositions of lymphocytes (Huang & Fritsche, 1992) and macrophages (Marignani & Sebaldt, 1995). That such changes might directly influence signal transduction pathways is shown by the observations that PKC is more active in the presence of dioleoylglycerol or diarachidonoylglycerol than in the presence of DAG containing two saturated fatty acids or one saturated and one unsaturated

https://doi.org/10.1079/PNS19980042 Published online by Cambridge University Press

fatty acid (Kishimoto *et al.* 1980) and that rat spleen PKC is less active in the presence of an *n*-3 PUFA-rich phosphatidylserine compared with a PUFA-poor phosphatidylserine (Bell & Sargent, 1987).

In support of the idea that *n*-3 PUFA influence intracellular signalling pathways, DAG generation was reduced in Con A-stimulated lymphocytes taken from mice fed on the ethyl ester of docosahexaenoic acid compared with those taken from safflower oil-fed mice (Fowler *et al.* 1993). More recently, it was shown that feeding either eicosapentaenoic acid or docosahexaenoic acid to mice resulted in reduced DAG generation by Con A-stimulated spleen lymphocytes (Jolly *et al.* 1997); this study also showed, for the first time, that fish oil-derived *n*-3 PUFA suppress ceramide generation in Con A-stimulated lymphocytes. The DAG in these studies could have been generated by the activity of phosphatidylcholine PLC, phospholipase D or phosphatidylinositol PLC or a combination of these. A recent study which showed reduced calcium ionophore-stimulated DAG production in macrophages from eicosapentaenoic acid-fed mice compared with those fed on an *n*-6 PUFA-rich diet suggested that phosphatidylcholine hydrolysis contributed significantly to the total DAG formed (Marignani & Sebaldt, 1995) implicating phosphatidylcholine PLC and/or phospholipase D in DAG formation. These findings indicate that *n*-3 PUFA in some way affect the activity of one or more phospholipases responsible for generation of key second messengers; this may be via changes in the fatty acid composition of the substrate phospholipids (Fig. 4). Addition of 14·4 g fish oil-derived *n*-3 PUFA/d to the diet for 10 weeks resulted in lower platelet-activating factor- or LTB_4-stimulated IP_3 generation in peripheral blood neutrophils (Sperling *et al.* 1993). The platelet-activating factor and LTB_4 receptors are coupled via G-proteins to PLCβ. In a recent study it was observed that calcium ionophore- or Con A-stimulated IP_3 generation in rat lymphocytes was significantly reduced if the lymphocytes came from animals fed on fish oil (P Sanderson and PC Calder, unpublished results). Furthermore, it was found that, although the amount of PLCγ-1 in rat lymphocytes was unaffected by diet, the ability to phosphorylate, and so activate, the enzyme in response to suitable stimuli was significantly impaired by fish-oil feeding (P Sanderson and PC Calder, unpublished results). Lymphocyte PLCγ-1 is activated by one or more tyrosine kinases (lck, fyn, ZAP-70); thus, these observations are suggestive of lowered activity of certain tyrosine kinases following fish-oil feeding. How *n*-3 PUFA might inhibit tyrosine kinase activity is not clear, but these tyrosine kinases are associated with specific regions of the plasma membrane. This association might require certain phospholipid fatty acid compositions or membrane physical properties which could be markedly altered by *n*-3 PUFA incorporation, thus resulting in an inability of the tyrosine kinase to maintain its optimal activity.

It has been proposed that unsaturated fatty acids themselves may have a direct effect on intracellular signalling pathways (for review, see Sumida *et al.* 1993). This direct modulatory effect of fatty acids has been most extensively documented in relation to *in vitro* PKC activity which was shown to be enhanced by docosahexaenoic acid (Shinomura *et al.* 1991). In contrast, although it was reported that eicosapentaenoic and docosahexaenoic acids increased brain PKC activity in the absence of phosphatidylserine and DAG, in the presence of phosphatidylserine and DAG both *n*-3 PUFA caused up to 60 % inhibition of PKC activity (Speizer *et al.* 1991). Another study has shown that eicosapentaenoic and docosahexaenoic acids inhibit rat lymphocyte PKC activity in the presence of Ca, phosphatidylserine and DAG (May *et al.* 1993); protein kinase A activity was unaffected by these fatty acids. Rat peritoneal macrophage PKC activity was inhibited by eicosapentaenoic and docosahexaenoic acids (Tappia *et al.* 1995); again protein kinase A activity was unaffected. In accordance with both the direct effects of *n*-3 PUFA *in vitro* (May *et al.* 1993; Tappia *et al.* 1995) and the effects of enriching phosphatidylserine with *n*-3 PUFA (Bell & Sargent, 1987), feeding mice on fish oil resulted in diminished spleen lymphocyte PKC activity (van Meter *et al.* 1994).

A change in the concentration of intracellular free Ca is often a key component in the intracellular signalling pathway which follows the stimulation of lymphocytes, macrophages and other cells by growth factors, cytokines and antigens. There is now considerable evidence that free fatty acids influence these changes. For example, it was reported that oleic acid, but not stearic acid, inhibited the target cell- or Con A-stimulated rise in intracellular free Ca concentration in a cytotoxic T-cell line (Richieri & Kleinfeld, 1989). Several unsaturated fatty acids, including α-linolenic, eicosapentaenoic and docosahexaenoic acids, inhibit the anti-CD3-induced increase in intracellular free Ca concentration in the JURKAT T-cell line (Chow *et al.* 1990; Breittmayer *et al.* 1993); the fatty acids appeared to act by blocking Ca entry into the cells and it was concluded that they act directly on receptor-operated Ca channels. Reduced IP_3 levels as a result of diminished phospholipase activity would also contribute to decreased intracellular free Ca concentrations, which in turn would reduce the activity of some isoforms of PKC (Fig. 4).

Fatty acids and transcription factors within cells of the immune system. Lymphocytes and other immune and inflammatory cells contain many transcription factors including NF kappa B (NFκB), NF of activated T-cells, AP-1, various oncogene products (e.g. myc, fos, jun), steroid hormone receptors and specific NF such as NF-IL-2, NF-IL-6 and NF-ICAM-1. NFκB plays a key role in inducing the production of many key mediators within the immune system: NFκB regulates the synthesis of cytokines including IL-1, IL-2, IL-6, TNFα and interferon-β, of cytokine receptors including IL-2R, of adhesion molecules including CD54, CD62E and CD106, of enzymes involved in mediator generation such an inducible NO synthase and of a range of acute-phase proteins (for review, see Kopp & Ghosh, 1995). NFκB is activated by the phosphorylation and subsequent dissociation of one of its three subunits, the so-called inhibitory kappaB; this leaves the remaining dimer free to translocate to the nucleus and bind to appropriate response elements on target genes. It appears that, at least in response to some stimuli, the phosphorylation of inhibitory kappaB is performed by PKC. Given the effects of *n*-3 PUFA outlined previously (e.g. reduced PLC

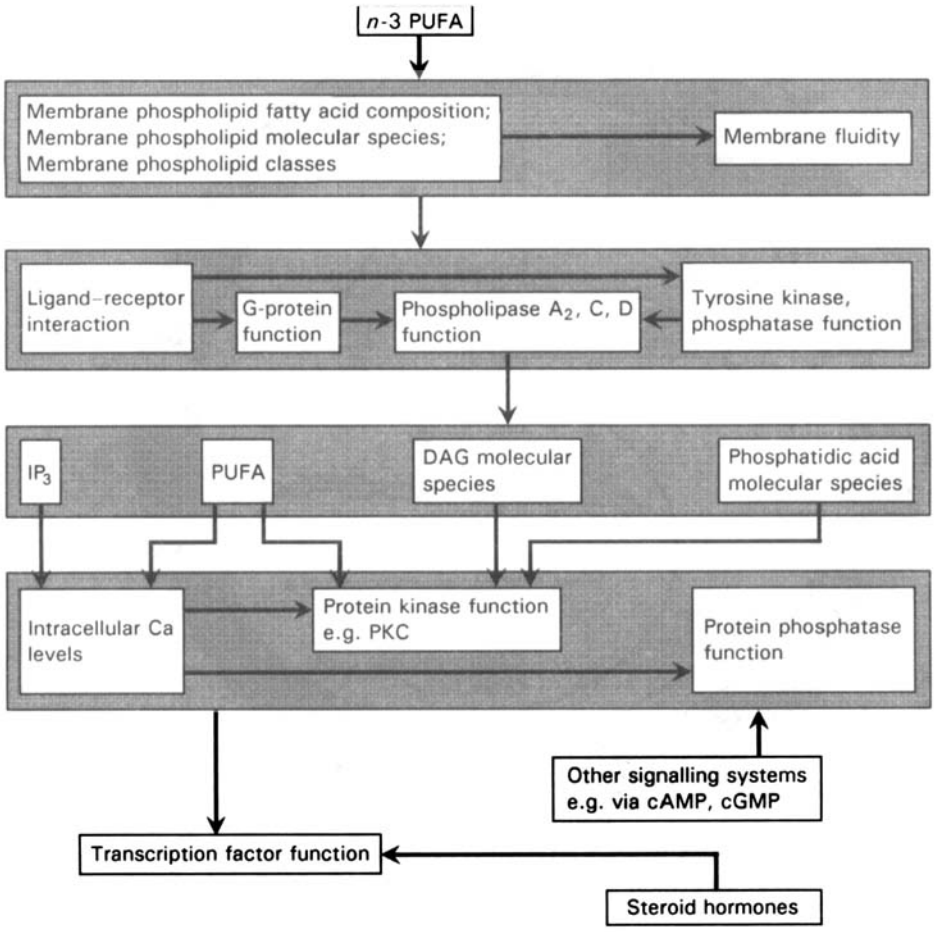

Fig. 4. Mechanisms by which incorporation of *n*-3 polyunsaturated fatty acids (PUFA) into cell membranes might influence downstream signalling events. Phospholipase A_2, C, D, *EC* 3.1.1.4, 3.1.4.3, 3.1.4.4 respectively; IP_3, inositol-1,4,5-trisphosphate; DAG, diacylglycerol; protein phosphatase, *EC* 3.1.3.16.

activity, resulting in reduced DAG and IP_3 generation, resulting in a reduced intracellular free Ca rise and a reduced activation of PKC isoforms) it is evident how these fatty acids could prevent activation of NFκB and so suppress expression of a range of genes, including those for cytokines, cytokine receptors, adhesion molecules and inducible NO synthase. Ceramide can also activate NFκB independently of PKC activity; thus, the observation of Jolly *et al.* (1997) of reduced ceramide production within lymphocytes from *n*-3 PUFA-fed mice could also partly account for the reduced functional responses (e.g. IL-2 production, proliferation) of these cells.

Some transcription factors are receptors for lipophilic molecules; these include steroid hormone receptors and the family of receptors known as peroxisomal proliferator-activated receptors (PPAR). Long-chain PUFA have been shown to activate PPAR, thereby influencing their action (for reviews, see Jump *et al.* 1996; Schoonjans *et al.* 1996). Recently, derivatives of arachidonic acid have been shown to be ligands for PPARα (LTB_4; Devchand *et al.* 1996) and PPARγ (15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; Forman *et al.* 1995; Kliewer *et al.* 1995), thereby providing a mechanism by which PUFA can directly affect the activity of these transcription factors. The mechanism of action and functional effects of PPAR have been reviewed in detail recently (Jump *et al.* 1996; Schoonjans *et al.* 1996). PPAR isoforms have been identified in lymphoid tissues. Using *in situ* DNA hybridization and immunohistochemical staining, Braissant *et al.* (1996) identified moderate to very strong expression of PPAR-α, -β and -γ in rat spleen (both red and white pulp) and lymph nodes; Kliewer *et al.* (1994) had earlier reported the presence of PPAR-γ and -δ mRNA in murine spleen. These observations suggest that genes within cells of the immune system will be subject to regulation by ligands for PPAR isoforms; these include PUFA derivatives. Cells of the immune system also possess steroid hormone, vitamin D and retinoic acid receptors, making them subject to the effects of ligands of these transcription factors. Incidentally, it is proposed that PUFA regulate the pathway of activation of steroid hormone receptors (for reviews, see Nunez, 1993; Nunez *et al.* 1995; Sumida, 1995), which may account for observations that PUFA, particularly *n*-3 PUFA, sensitize immune cells to the effects of steroid hormones (for example, see Yaqoob & Calder, 1996).

https://doi.org/10.1079/PNS19980042 Published online by Cambridge University Press

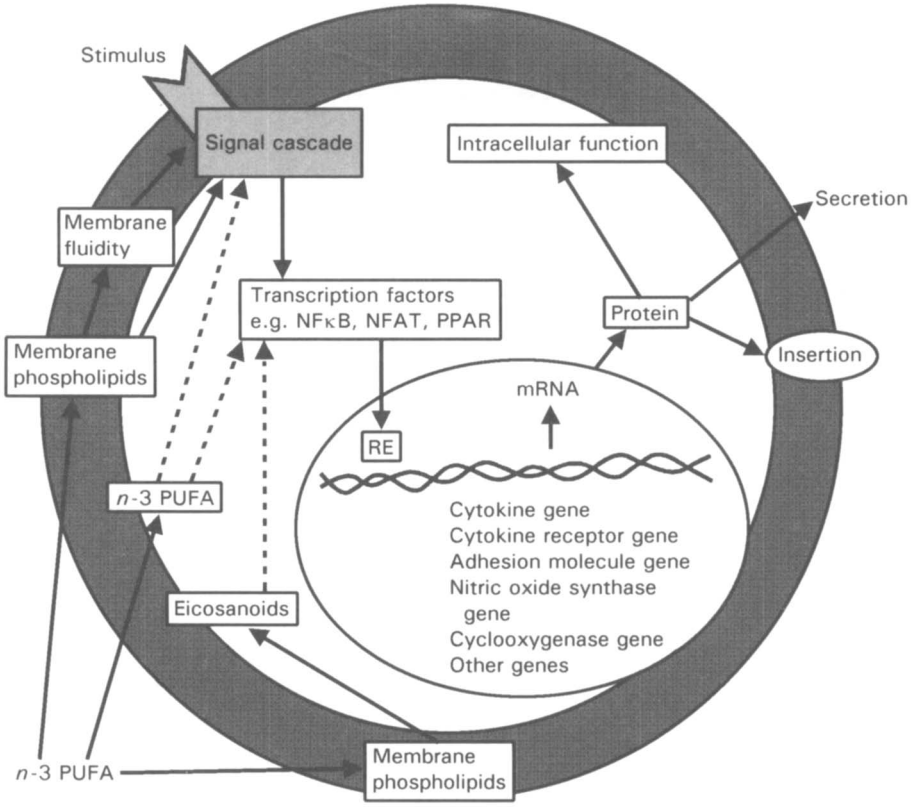

Fig. 5. Schematic representation of the mechanisms by which *n*-3 polyunsaturated fatty acids (PUFA) might influence gene expression. NFκB, nuclear transcription factor kappa B; NFAT, nuclear transcription factor of activated T-cells; PPAR, peroxisomal proliferator-activated receptors; RE, response element.

In addition to effects which influence the activity of transcription factors, *n*-3 PUFA might also regulate the synthesis of transcription factors. Fernandes *et al.* (1994) reported markedly decreased c-myc mRNA in spleens from fish oil-fed mice; c-myc mRNA encodes a transcription factor.

Fig. 5 attempts to give an overview of the sites at which *n*-3 PUFA might act to influence gene expression in cells of the immune system.

References

Ailhaud G, Amri E-Z & Grimaldi P-A (1995) Fatty acids and adipose cell differentiation. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **48**, 113–115.

Alexander NJ & Smythe NL (1988) Dietary modulation of *in vitro* lymphocyte function. *Annals of Nutrition and Metabolism* **32**, 192–199.

Bell MV & Sargent JR (1987) Effects of the fatty acid composition of phosphatidylserine and diacylglycerol on the *in vitro* activity of protein kinase C from rat spleen: influences of (*n*-3) and (*n*-6) polyunsaturated fatty acids. *Comparative Biochemistry and Physiology* **86B**, 227–232.

Berger A, German JB, Chiang BL, Ansari AA, Keen CL, Fletcher MP & Gershwin MR (1993) Influence of feeding unsaturated fats on growth and immune status of mice. *Journal of Nutrition* **123**, 225–233.

Berthou L, Saladin R, Yaqoob P, Branellec D, Calder P, Fruchart J-C, Denefle P, Auwerx J & Staels B (1995) Regulation of rat liver apolipoprotein A-I, apolipoprotein A-II and acyl CoA oxidase gene expression by fibrates and dietary fatty acids. *European Journal of Biochemistry* **232**, 179–187.

Billiar T, Bankey P, Svingen B, Curran RD, West MA, Holman RT, Simmons RL & Cerra FB (1988) Fatty acid uptake and Kupffer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. *Surgery* **104**, 343–349.

Black JM & Kinsella JE (1993) Dietary *n*-3 fatty acids alter mouse peritoneal macrophage cytotoxicity. *Annals of Nutrition and Metabolism* **37**, 110–120.

Blok WL, Katan MB & van der Meer JWM (1996) Modulation of inflammation and cytokine production by dietary (*n*-3) fatty acids. *Journal of Nutrition* **126**, 1515–1533.

Blok WL, Vogels MT, Curfs JH, Eling WM, Buurman WA & van der Meer JWM (1992) Dietary fish oil supplementation in experimental gram-negative infection and in cerebral malaria in mice. *Journal of Infectious Diseases* **165**, 898–903.

Boutard V, Fouquery B, Philippe C, Perez J & Baud L (1994) Fish oil supplementation and essential fatty acid deficiency reduce nitric oxide synthesis by rat macrophages. *Kidney International* **46**, 1200–1286.

Braissant O, Foufelle F, Scotto C, Dauca M & Wahli W (1996) Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat. *Endocrinology* **137**, 354–366.

Breittmayer J-P, Pelassy C, Cousin J-L, Bernard A & Aussel C (1993) The inhibition by fatty acids of receptor-mediated calcium movements in Jurkat T-cells is due to increased calcium extrusion. *Journal of Biological Chemistry* **268**, 20812–20817.

https://doi.org/10.1079/PNS19980042 Published online by Cambridge University Press

Calder PC (1995) Fatty acids, dietary lipids and lymphocyte functions. *Biochemical Society Transactions* **23**, 302–309.

Calder PC (1996*a*) Can *n*-3 polyunsaturated fatty acids be used as immunomodulatory agents? *Biochemical Society Transactions* **24**, 211–220.

Calder PC (1996*b*) Effects of fatty acids and dietary lipids on cells of the immune system. *Proceedings of the Nutrition Society* **55**, 127–150.

Calder PC (1996*c*) Immunomodulatory and anti-inflammatory effects of *n*-3 polyunsaturated fatty acids. *Proceedings of the Nutrition Society* **55**, 737–774.

Calder PC (1997) *n*-3 Polyunsaturated fatty acids and cytokine production in health and disease. *Annals of Nutrition and Metabolism* **41**, 203–234.

Calder PC (1998) Dietary fatty acids and the immune system. *Nutrition Reviews* **41**, 203–234.

Calder PC, Bond JA, Harvey DJ, Gordon S & Newsholme EA (1990) Uptake of saturated and unsaturated fatty acids into macrophage lipids and their effect upon macrophage adhesion and phagocytosis. *Biochemical Journal* **269**, 807–814.

Calder PC & Yaqoob P (1997) *n*-3 Polyunsaturated fatty acids and the immune system. *Recent Research Developments in Lipids Research* **1**, 31–61.

Cathcart ES, Leslie CA, Meydani SN & Hayes KC (1987) A fish oil diet retards experimental amyloidosis, modulates lymphocyte function and decreases macrophage arachidonate metabolism in mice. *Journal of Immunology* **139**, 1850–1854.

Caughey GE, Mantzioris E, Gibson RA, Cleland LG & James MJ (1996) The effect on human tumor necrosis factor α and interleukin 1β production of diets enriched in *n*-3 fatty acids from vegetable oil or fish oil. *American Journal of Clinical Nutrition* **63**, 116–122.

Chaet MS, Garcia VF, Arya G, Ziegler MM (1994) Dietary fish oil enhances macrophage production of nitric oxide. *Journal of Surgical Research* **57**, 65–68.

Chandrasekar B & Fernandes G (1994) Decreased pro-inflammatory cytokines and increased antioxidant enzyme gene expression by ω-3 lipids in murine lupus nephritis. *Biochemical and Biophysical Research Communications* **200**, 893–898.

Chang HR, Arsenijevic D, Pechere JC, Piguet PF, Mensi N, Girardier L & Dulloo AG (1992) Dietary supplementation with fish oil enhances *in vivo* synthesis of tumor necrosis factor. *Immunology Letters* **34**, 13–18.

Chapkin RS, Hubbard NE & Erickson KL (1990) 5-Series peptido-leukotriene synthesis in mouse peritoneal macrophages: modulation by dietary *n*-3 fatty acids. *Biochemical and Biophysical Research Communications* **171**, 764–769.

Chow SC, Ansotegui IJ & Jondal M (1990) Inhibition of receptor-mediated calcium influx in T cells by unsaturated non-esterified fatty acids. *Biochemical Journal* **267**, 727–732.

Cooper AL, Gibbons L, Horan MA, Little RA & Rothwell NJ (1993) Effect of dietary fish oil supplementation on fever and cytokine production in human volunteers. *Clinical Nutrition* **12**, 321–328.

De Caterina R, Cybulsky MA, Clinton SK, Gimbrone MA & Libby P (1995) Omega-3 fatty acids and endothelial leukocyte adhesion molecules. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **52**, 191–195.

Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ & Wahli W (1996) The PPARα-leukotriene B_4 pathway to inflammation control. *Nature* **384**, 39–43.

Dubois C, Bissonnette E & Rola-Pleszczynski M (1989) Platelet-activating factor (PAF) stimulates tumor necrosis factor production by alveolar macrophages: prevention of PAF receptor antagonists and lipoxygenase inhibitors. *Journal of Immunology* **143**, 964–971.

Endres S (1996) *n*-3 Polyunsaturated fatty acids and human cytokine synthesis. *Lipids* **31**, S239–S242.

Endres S, Fulle HJ, Sinha B, Stoll D, Dinarello CA, Gerzer R & Weber PC (1991) Cyclic nucleotides differentially regulate the synthesis of tumour necrosis factor-alpha and interleukin-1 beta by human mononuclear cells. *Immunology* **72**, 56–60

Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JMW, Cannon JG, Rogers TS, Klempner MS, Weber PC, Schaeffer EJ, Wolff SM & Dinarello CA (1989) The effect of dietary supplementation with *n*-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *New England Journal of Medicine* **320**, 265–271.

Endres S, Meydani SN, Ghorbani R, Schindler R & Dinarello CA (1993) Dietary supplementation with *n*-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. *Journal of Leukocyte Biology* **54**, 599–603.

Endres S & von Schacky C (1996) *n*-3 Polyunsaturated fatty acids and human cytokine synthesis. *Current Opinion in Lipidology* **7**, 48–52.

Ertel W, Morrison MH, Ayala A & Chaudry IH (1993) Modulation of macrophage membrane phospholipids by *n*-3 polyunsaturated fatty acids increases interleukin 1 release and prevents suppression of cellular immunity following hemorrhagic shock. *Archives of Surgery* **128**, 15–21.

Faull RJ (1995) Adhesion molecules in health and disease. *Australian and New Zealand Journal of Medicine* **25**, 720–730.

Fernandes G, Bysani C, Venkatraman JT, Tomar V & Zhao W (1994) Increased TGF-β and decreased oncogene expression by ω-3 fatty acids in the spleen delays onset of autoimmune disease in B/W mice. *Journal of Immunology* **152**, 5979–5987.

Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM & Evans RM (1995) 15-Deoxy-D12,14 prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ. *Cell* **83**, 803–812.

Fowler KH, Chapkin RS & McMurray DN (1993) Effects of purified dietary *n*-3 ethyl esters on murine T lymphocyte function. *Journal of Immunology* **151**, 5186–5197.

Fritsche KL & Cassity NA (1992) Dietary *n*-3 fatty acids reduce antibody-dependent cell cytotoxicity and alter eicosanoid release by chicken immune cells. *Poultry Science* **71**, 1646–1657.

Fritsche KL, Cassity NA & Huang S-C (1991) Effect of dietary fat source on antibody production and lymphocyte proliferation in chickens. *Poultry Science* **70**, 611–617.

Fritsche KL & Johnston PV (1990) Effect of dietary omega-3 fatty acids on cell-mediated cytotoxic activity in BALB/c mice. *Nutrition Research* **10**, 577–588.

Fujikawa M, Yamashita N, Yamazaki K, Sugiyama E, Suzuki H & Hamazaki T (1992) Eiciosapentaenoic acid inhibits antigen-presenting cell function of murine splenocytes. *Immunology* **75**, 330–335.

Gagnon L, Filion LG, Dubois C & Rola-Pleszczynski M (1989) Leukotrienes and macrophage activation: augmented cytotoxic activity and enhanced interleukin 1, tumor necrosis factor and hydrogen peroxide production. *Agents and Actions* **26**, 141–147.

Gallai V, Sarchielli P, Trequattrini A, Franceschini M, Floridi A, Firenze C, Alberti A, Di Benedetto D & Stragliotto E (1993) Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with *n*-3 polyunsaturated fatty acids. *Journal of Neuroimmunology* **56**, 143–153.

Goldyne ME & Stobo JD (1981) Immunoregulatory role of prostaglandins and related lipids. *Critical Reviews in Immunology* **2**, 189–223.

Goodwin JS & Cueppens J (1983) Regulation of the immune response by prostaglandins. *Journal of Clinical Immunology* **3**, 295–315.

Grimm H, Tibell A, Norrlind B, Blecher C, Wilker S & Schwemmle K (1994) Immunoregulation by parenteral lipids: impact of the *n*-3 to *n*-6 fatty acid ratio. *Journal of Parenteral and Enteral Nutrition* **18**, 417–421.

Hardardottir I & Kinsella JE (1991) Tumour necrosis factor production by murine resident peritoneal macrophages is enhanced by dietary *n*-3 polyunsaturated fatty acids. *Biochimica et Biophysica Acta* **1095**, 187–195.

Hardardottir I & Kinsella JE (1992) Increasing the dietary (*n*-3) to (*n*-6) polyunsaturated fatty acid ratio increases tumor necrosis factor production by murine resident peritoneal macrophages without an effect on elicited peritoneal macrophages. *Journal of Nutrition* **122**, 1942–1951.

Harris WS, Rambjor GS, Windsor SL & Diederich D (1997) *n*-3 Fatty acids and urinary excretion of nitric oxide metabolites in humans. *American Journal of Clinical Nutrition* **65**, 459–464.

Hogg N & Lands RC (1993) Adhesion molecules in cell interactions. *Current Opinion in Immunology* **5**, 383–390.

Huang S-C & Fritsche KL (1992) Alteration in mouse splenic phospholipid fatty acid composition and lymphoid cell populations by dietary fat. *Lipids* **27**, 25–32.

Huang S-C, Misfeldt ML & Fritsche KL (1992) Dietary fat influences Ia antigen expression and immune cell populations in the murine peritoneum and spleen. *Journal of Nutrition* **122**, 1219–1231.

Hubbard NE, Chapkin RS & Erickson KL (1994) Effect of dietary linseed oil on tumoricidal activity and eicosanoid production in murine macrophages. *Lipids* **29**, 651–655.

Hughes DA, Pinder AC, Piper Z, Johnson IT & Lund EK (1996*a*) Fish oil supplementation inhibits the expression of major histocompatibility complex class II molecules and adhesion molecules on human monocytes. *American Journal of Clinical Nutrition* **63**, 267–272.

Hughes DA, Southon S & Pinder AC (1996*b*) (*n*-3) Polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes in vitro. *Journal of Nutrition* **126**, 603–610.

Hwang D (1989) Essential fatty acids and the immune response. *FASEB Journal* **3**, 2052–2061.

Jeffery NM, Sanderson P, Sherrington EJ, Newsholme EA & Calder PC (1996) The ratio of *n*-6 to *n*-3 polyunsaturated fatty acids in the rat diet alters serum lipid levels and lymphocyte functions. *Lipids* **31**, 737–745.

Joe B & Lokesh BR (1994) Role of capsaicin, curcumin and dietary *n*-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. *Biochimica et Biophysica Acta* **1224**, 255–263.

Jolly CA, Jiang Y-H, Chapkin RS & McMurray DN (1997) Dietary (*n*-3) polyunsaturated fatty acids suppress murine lymphoproliferation, interleukin-2 secretion and the formation of diacylglycerol and ceramide. *Journal of Nutrition* **127**, 37–43.

Jump DB, Clarke SD, Thelan A, Liimatta M, Ren B & Badin M (1996) Dietary polyunsaturated fatty acid regulation of gene transcription. *Progress in Lipid Research* **35**, 227–241.

Jump DB, Ren B, Clarke S & Thelen A (1995) Effects of fatty acids on hepatic gene expression. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **48**, 107–111.

Kelley DS, Nelson GJ, Serrato CM, Schmidt PC & Branch LB (1988) Effects of type of dietary fat on indices of immune status of rabbits. *Journal of Nutrition* **118**, 1376–1384.

Kelley VE, Ferretti A, Izui S & Strom TB (1985) A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites and suppresses lupus in MRL-lpr mice. *Journal of Immunology* **134**, 1914–1919.

Khair-El-Din T, Sicher SC, Vazquez MA, Chung GW, Stallworth KA, Kitamura K, Miller RT & Lu CY (1996) Transcription of the murine iNOS gene is inhibited by docosahexaenoic acid, a major constituent of fetal and neonatal sera as well as fish oils. *Journal of Experimental Medicine* **183**, 1241–1246.

Khalfoun B, Thibault G, Bardos P & Lebranchu Y (1996) Docosahexaenoic and eicosapentaenoic acids inhibit in vitro human lymphocyte-endothelial cell adhesion. *Transplantation* **62**, 1649–1657.

Kim DN, Schmee J & Thomas WA (1995) Dietary fish oil added to a hyperlipidemic diet for swine results in reduction in the excessive number of monocytes attached to the arterial endothelium. *Atherosclerosis* **81**, 209–216.

Kinsella JE, Lokesh B, Broughton S & Whelan J (1990) Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: An overview. *Nutrition* **6**, 24–44

Kishimoto A, Takai Y, Mori T, Kikkawa U & Nishizuka Y (1980) Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol: Its possible relation to phosphatidylinositol turnover. *Journal of Biological Chemistry* **255**, 2273–2276.

Kliewer SA, Forman BM, Blimberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, Umesono K & Evans RM (1994) Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proceedings of the National Academy of Sciences USA* **91**, 7355–7359.

Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC & Lehman JM (1995) A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. *Cell* **83**, 813–819.

Knudsen PJ, Dinarello C & Strom TB (1986) Prostaglandins post-transcriptionally inhibit monocyte expression of interleukin-1 activity by increasing cyclic adenosine monophosphate. *Journal of Immunology* **137**, 3187–3194.

Kopp EB & Ghosh S (1995) NF-κB and rel proteins in innate immunity. *Advances in Immunology* **58**, 1–27.

Kremer JM, Lawrence DA, Jubiz W, Di Giacomo R, Rynes K, Bartholomew LE & Sherman M (1990) Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. *Arthritis and Rheumatism* **33**, 810–820.

Kunkel SL, Remick DG, Spengler M & Chensue SW (1982) Modulation of macrophage-derived interleukin-1 and tumor necrosis factor by prostaglandin E_2. *Advances in Prostaglandin, Thromboxane and Leukotriene Research* **9**, 331–339.

Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese J, Spur BW, Robinson DR, Corey EJ, Lewis RA & Austen KF (1985) Effects of dietary enrichment with EPA and DHA on *in vitro* neutrophil and monocyte leukotriene generation and neutrophil function. *New England Journal of Medicine* **312**, 1217–1224.

Lokesh BR, Sayers TJ & Kinsella JE (1990) Interleukin-1 and tumor necrosis factor synthesis by mouse peritoneal macrophages is enhanced by dietary *n*-3 polyunsaturated fatty acids. *Immunology Letters* **23**, 281–286.

Lumpkin EA, McGlone JJ, Sells JL & Hellman JM (1993) Modulation of murine natural killer cell cytotoxicity by dietary fish oil, maize oil or beef tallow. *Journal of Nutritional Immunology* **2**, 43–53.

Marignani PA & Sebaldt RJ (1995) Formation of second messenger diradylglycerol in murine peritoneal macrophages is altered after *in vivo* (*n*-3) polyunsaturated fatty acid supplementation. *Journal of Nutrition* **125**, 3030–3040.

Marshall LA & Johnston PV (1985) The influence of dietary essential fatty acids on rat immunocompetent cell prostaglandin

synthesis and mitogen-induced blastogenesis. *Journal of Nutrition* **115**, 1572–1580.
May CL, Southwoth AJ & Calder PC (1993) Inhibition of lymphocyte protein kinase C by unsaturated fatty acids. *Biochemical and Biophysical Research Communications* **195**, 823–828.
Meydani SN (1992) Modulation of cytokine production by dietary polyunsaturated fatty acids. *Proceedings of the Society for Experimental Biology and Medicine* **200**, 189–193.
Meydani SN (1996) Effect of (*n*-3) polyunsaturated fatty acids on cytokine production and their biologic function. *Nutrition* **12**, S8–S14.
Meydani SN, Endres S, Woods MM, Goldin BR, Soo C, Morrill-Labrode A, Dinarello C & Gorbach SL (1991) Oral (*n*-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *Journal of Nutrition* **121**, 547–555.
Meydani SN, Lichtenstein AH, Cornwall S, Meydani M, Goldin BR, Rasmussen H, Dinarello CA & Schaefer EJ (1993) Immunologic effects of national cholesterol education panel step-2 diets with and without fish-derived *n*-3 fatty acid enrichment. *Journal of Clinical Investigation* **92**, 105–113.
Meydani SN, Yogeeswaran G, Liu S, Baskar S & Meydani M (1988) Fish oil and tocopherol-induced changes in natural killer cell-mediated cytotoxicity and PGE_2 synthesis in young and old mice. *Journal of Nutrition* **118**, 1245–1252.
Molvig J, Pociot F, Worsaae H, Wogensen LD, Baek L, Christensen P, Mandrup-Poulsen T, Andersen K, Madsen P, Dyerberg J & Nerup J (1991) Dietary supplementation with ω-3 polyunsaturated fatty acids decreases mononuclear cell proliferation and interleukin-1β content but not monokine secretion in healthy and insulin-dependent diabetic individuals. *Scandanavian Journal of Immunology* **34**, 399–410.
Mosquera J, Rodriguez-Iturbe B & Parra G (1990) Fish oil dietary supplementation reduces Ia expression in rat and mouse peritoneal macrophages. *Clinical Immunology and Immunopathology* **56**, 124–129.
Nunez EA (1993) Free fatty acids as modulators of the steroid hormone message. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **48**, 63–70.
Nunez EA, Haourigui M, Martin ME & Benassayag C (1995) Fatty acids and steroid hormone action. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **52**, 185–190.
Peck MD (1994) Interactions of lipids with immune function II: Experimental and clinical studies of lipids and immunity. *Journal of Nutritional Biochemistry* **5**, 514–521.
Poubelle P, Stankova J, Grassii J & Rola-Pleszczynski M (1991) Leukotriene B4 up-regulates IL-6 rather than IL-1 synthesis in human monocytes. *Agents and Actions* **34**, 42–45.
Renier G, Skamene E, de Sanctis J & Radzioch D (1993) Dietary *n*-3 polyunsaturated fatty acids prevent the development of atherosclerotic lesions in mice: Modulation of macrophage secretory activities. *Arteriosclerosis and Thombosis* **13**, 1515–1524.
Renz H, Gong JH, Schmidt A, Nain M & Gemsa D (1988) Release of tumor necrosis factor-alpha from macrophages. Enhancement and suppression are dose-dependently regulated by prostaglandin E2 and cyclic nucleotides. *Journal of Immunology* **141**, 2388–2393.
Richieri GV & Kleinfeld AM (1989) Free fatty acid perturbation of transmembrane signalling in cytotoxic T lymphocytes. *Journal of Immunology* **143**, 2302–2310.
Robinson DR, Urakaze M, Huang R, Taki H, Sugiyama E, Knoell CT, Xu L, Yeh ETH & Auron PE (1996) Dietary marine lipids suppress continuous expression of interleukin-1β gene expression. *Lipids* **31**, S23–S31.
Rola-Pleszczynski M & Lemaire I (1985) Leukotrienes augment interleukin 1 production by human monocytes. *Journal of Immunology* **135**, 3958–3961.
Rola-Pleszczynski M & Stankova J (1992) Cytokine gene regulation by PGE2, LTB4 and PAF. *Mediators of Inflammation* **1**, 5–8.
Roper RL & Phipps RP (1994) Prostaglandin E_2 regulation of the immune response. *Advances in Prostaglandin, Thromboxane and Leukotriene Research* **22**, 101–111.
Sanderson P & Calder PC (1998) Dietary fish oil diminishes lymphocyte adhesion to macrophage and endothelial cell monolayers. *Immunology* (In the Press).
Sanderson P, MacPherson GG, Jenkins CH & Calder PC (1997) Dietary fish oil diminishes antigen presentation activity by rat lymphoid dendritic cells. *Journal of Leukocyte Biology* **62**, 771–777.
Sanderson P, Yaqoob P & Calder PC (1995*a*) Effects of dietary lipid manipulation upon rat spleen lymphocyte functions and the expression of lymphocyte surface molecules. *Journal of Nutritional and Environmental Medicine* **5**, 119–132.
Sanderson P, Yaqoob P & Calder PC (1995*b*) Effects of dietary lipid manipulation upon graft vs. host and host vs. graft responses in the rat. *Cellular Immunology* **164**, 240–247.
Schade UF, Ernst M, Reinke M & Wolter DT (1989) Lipoxygenase inhibitors suppress formation of tumor necrosis factor in vitro and in vivo. *Biochemical and Biophysical Research Communications* **159**, 748–754.
Schmidt EB, Pederson JO, Ekelund S, Grunnet N, Jersild C & Dyerberg J (1989) Cod liver oil inhibits neutrophil and monocyte chemotaxis in healthy males. *Atherosclerosis* **77**, 53–57.
Schmidt EB, Varming K, Pederson JO, Lervang HH, Grunnet N, Jersild C & Dyerberg J (1992) Long term supplementation with *n*-3 fatty acids. II. Effect on neutrophil and monocyte chemotaxis. *Scandinavian Journal of Clinical and Laboratory Investigation* **52**, 229–236.
Schoonjans K, Staels B & Auwerx J (1996) Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids in gene expression. *Journal of Lipid Research* **37**, 907–925.
Sherrington EJ, Sanderson P & Calder PC (1995) The effect of dietary lipid manipulation on macrophage cell surface molecule expression. *Biochemical Society Transactions* **23**, 272S.
Shinomura T, Asoaka Y, Oka M, Yoshida K & Nishizuka Y (1991) Synergistic action of diacylglycerol and unsaturated fatty acids for protein kinase C activation. *Proceedings of the National Academy of Sciences USA* **88**, 5149–5153.
Somers SD, Chapkin RS & Erickson KL (1989) Alteration of in vitro murine peritoneal macrophage function by dietary enrichment with eicosapentaenoic and docosahexaenoic acids in menhaden fish oil. *Cellular Immunology* **123**, 201–211.
Somers SD & Erickson KL (1994) Alteration of tumor necrosis factor-α production by macrophages from mice fed diets high in eicosapentaenoic and docosahexaenoic acids. *Cellular Immunology* **153**, 287–297.
Soyland E, Lea T, Sandstad B & Drevon A (1994) Dietary supplementation with very long chain *n*-3 fatty acids in man decreases expression of the interleukin-2 receptor (CD25) on mitogen-stimulated lymphocytes from patients with inflammatory skin diseases. *European Journal of Clinical Investigation* **24**, 236–242.
Speizer LA, Watson MJ & Brunton LL (1991) Differential effects of omega-3 fish oils on protein kinase activities in vitro. *American Journal of Physiology* **261**, E109–E114.
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. *Journal of Clinical Investigation* **91**, 651–660.
Springer TA (1990) Adhesion receptors of the immune system. *Nature* **346**, 425-433.
Stoolman LM (1989) Adhesion molecules controlling lymphocyte migration. *Cell* **56**, 907–910.
Sumida C (1995) Fatty acids: Ancestral ligands and modern co-regulators of the steroid hormone receptor cell signalling pathway. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **52**, 137–144.
Sumida C, Graber R & Nunez E (1993) Role of fatty acids in signal transduction: modulators and messengers. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **48**, 117–122.
Tappia PS & Grimble RF (1994) Complex modulation of cytokine induction by endotoxin and tumour necrosis factor from peritoneal macrophages of rats by diets containing fats of different saturated, monounsaturated and polyunsaturated fatty acid composition. *Clinical Science* **87**, 173–178.
Tappia PS, Man WJ & Grimble RF (1995) Influence of unsaturated fatty acids on the production of tumour necrosis factor and interleukin-6 by rat peritoneal macrophages. *Molecular and Cellular Biochemistry* **143**, 89–98.
Turek JJ, Schoenlein IA & Bottoms GD (1991) The effect of dietary *n*-3 and *n*-6 fatty acids on tumor necrosis factor-α production and leucine aminopeptidase levels in rat peritoneal macrophages. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **43**, 141–149.
Turek JJ, Schoenlein IA, Clark LK & van Alstine WG (1994) Dietary polyunsaturated fatty acids effects on immune cells of the porcine lung. *Journal of Leukocyte Biology* **56**, 599–604.
van Meter AR, Ehringer WD, Stillwell W, Blumenthal EJ & Jenski LJ (1994) Aged lymphocyte proliferation following incorporation and retention of dietary omega-3 fatty acids. *Mechanisms of Ageing and Development* **75**, 95–114.
Virella G, Fourspring K, Hyman B, Haskill-Stroud R, Long L, Virella I, La Via M, Gross AJ & Lopes-Virella M (1991) Immunosuppressive effects of fish oil in normal human volunteers: Correlation with the *in vitro* effects of eicosapentaenoic acid on human lymphocytes. *Clinical Immunology and Immunopathology* **61**, 161–176.
Wachtler P, Konig W, Senkal M, Kemen M & Koller M (1997) Influence of a total parenteral nutrition enriched with ω-3 fatty acids on leukotriene synthesis of peripheral leukocytes and systemic cytokine levels in patients with major surgery. *Journal of Trauma* **42**, 191–198.
Wallace FA, Neely SJ, Miles EA & Calder PC (1998) Dietary fish oil decreases the production of pro-inflammatory mediators by murine macrophages. *Proceedings of the Nutrition Society* **57**. 50A.
Watanabe S, Hayashi H, Onozaki K & Okuyama H (1991) Effect of dietary α-linolenate/linoleate balance on lipopolysaccharide-induced tumor necrosis factor production in mouse macrophages. *Life Sciences* **48**, 2013–2020.
Wu D, Meydani SN, Meydani M, Hayek MG, Huth P & Nicolosi RJ (1996) Immunologic effects of marine- and plant-derived *n*-3 polyunsaturated fatty acids in nonhuman primates. *American Journal of Clinical Nutrition* **63**, 273–280.
Yamashita N, Maruyama M, Yamazaki K, Hamazaki T & Yano S (1991) Effect of eicosapentaenoic and docosahexaenoic acid on natural killer cell activity in human peripheral blood lymphocytes. *Clinical Immunology and Immunopathology* **59**, 335–345.
Yaqoob P & Calder PC (1993) The effects of fatty acids on lymphocyte functions. *International Journal of Biochemistry* **25**, 1705–1714.
Yaqoob P & Calder PC (1995*a*) The effects of dietary lipid manipulation on the production of murine T-cell-derived cytokines. *Cytokine* **7**, 548–553.
Yaqoob P & Calder PC (1995*b*) Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. *Cellular Immunology* **163**, 120– 128.
Yaqoob P & Calder PC (1996) Dietary lipids affect the sensitivity of rat thymic lymphocytes to glucocorticoids. *Proceedings of the Nutrition Society* **55**, 160A.
Yaqoob P, Newsholme EA & Calder PC (1994*a*) The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. *Immunology* **82**, 603–610.
Yaqoob P, Newsholme EA & Calder, PC (1994*b*) Inhibition of natural killer cell activity by dietary lipids. *Immunology Letters* **41**, 241–247.
Yaqoob P, Newsholme EA & Calder PC (1995) Influence of cell culture conditions on diet-induced changes in lymphocyte fatty acid composition. *Biochimica et Biophysica Acta* **1255**, 333–340.

https://doi.org/10.1079/PNS19980042 Published online by Cambridge University Press