

# Immunoregulatory and anti-inflammatory effects of *n*-3 polyunsaturated fatty acids

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## Abstract

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1. Fish oils are rich in the long-chain *n*-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic (20:5*n*-3) and docosahexaenoic (22:6*n*-3) acids. Linseed oil and green plant tissues are rich in the precursor fatty acid,  $\alpha$ -linolenic acid (18:3*n*-3). Most vegetable oils are rich in the *n*-6 PUFA linoleic acid (18:2*n*-6), the precursor of arachidonic acid (20:4*n*-6).
2. Arachidonic acid-derived eicosanoids such as prostaglandin E<sub>2</sub> are pro-inflammatory and regulate the functions of cells of the immune system. Consumption of fish oils leads to replacement of arachidonic acid in cell membranes by eicosapentaenoic acid. This changes the amount and alters the balance of eicosanoids produced.
3. Consumption of fish oils diminishes lymphocyte proliferation, T-cell-mediated cytotoxicity, natural killer cell activity, macrophage-mediated cytotoxicity, monocyte and neutrophil chemotaxis, major histocompatibility class II expression and antigen presentation, production of pro-inflammatory cytokines (interleukins 1 and 6, tumour necrosis factor) and adhesion molecule expression.
4. Feeding laboratory animals fish oil reduces acute and chronic inflammatory responses, improves survival to endotoxin and in models of autoimmunity and prolongs the survival of grafted organs.
5. Feeding fish oil reduces cell-mediated immune responses.
6. Fish oil supplementation may be clinically useful in acute and chronic inflammatory conditions and following transplantation.
7. *n*-3 PUFAs may exert their effects by modulating signal transduction and/or gene expression within inflammatory and immune cells.

### Key words

- Lymphocyte
- Macrophage
- Immune system
- Inflammation
- Fish oil
- Polyunsaturated fatty acid
- Cytokine
- Eicosanoid

## Biosynthesis and sources of different fatty acids

All mammals can synthesise fatty acids *de novo* from acetyl coenzyme A. The end product of the fatty acid synthetase 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 nor-

mally 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 carbon atoms 9 and 10 is catalysed by the enzyme  $\Delta^9$ -desaturase, which is universally present in both plants and animals. This enzyme catalyses the con-

version 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 9 position and the methyl terminus of the carbon chain; a  $\Delta^{12}$ -desaturase converts oleic acid into linoleic acid (18:2 *n*-6) while a  $\Delta^{15}$ -desaturase converts linoleic acid into  $\alpha$ -linolenic acid (18:3 *n*-3). Since animal tissues are unable to synthesise linoleic and  $\alpha$ -linolenic acids, these fatty acids must be consumed in the diet and so are termed essential fatty acids. Using the pathway outlined in Figure 1, animal cells can convert dietary  $\alpha$ -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  $\gamma$ -linolenic (18:3 *n*-6) and dihomo- $\gamma$ -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 (PUFAs) are not metabolically interconvertible in mammals. Many marine plants, especially the unicellular algae in phytoplankton, also carry out chain elongation and further desaturation of  $\alpha$ -linolenic acid to yield the long-chain *n*-3 PUFAs eicosapentaenoic and docosahexaenoic acids. It is the formation of these long chain *n*-3 PUFAs by marine algae and their transfer through the food chain to fish that accounts for their abundance in some marine fish oils.

### **Influence of dietary *n*-3 PUFAs 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 upon immune cell functions, particularly lymphocyte proliferation in response to mitogens. These studies have been reviewed several times in recent years (1-9); the effects of *n*-3 PUFAs are the most well documented and will be summarised here.

### **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 mitogen (PWM) 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 animals (rats, mice, chickens, rabbits) diets containing high levels (70 to 200 g/kg) of fish oil (rich in eicosapentaenoic and docosahexaenoic acids) has been shown to result in suppressed proliferation of lymphocytes stimulated with T- or B-cell mitogens compared with feeding diets rich in other fats such as lard, coconut oil, corn oil, safflower oil or linseed oil (10-15). Recently, it was reported that eicosapentaenoic and docosahexaenoic acids are equipotent in reducing murine spleen lymphocyte proliferation (16). Diets containing large amounts of linseed oil (rich in  $\alpha$ -linolenic acid) decrease lymphocyte proliferation compared with saturated fatty acid- or *n*-6 PUFA-rich diets (12,17,18). Recently, it was shown that increasing the amount of  $\alpha$ -linolenic acid in the rat diet at the expense of linoleic acid decreases spleen lymphocyte proliferation (19).

Sometimes, the effects of *n*-3 PUFA-rich diets on lymphocyte proliferation were demonstrated when the cells were cultured in autologous serum, but were lost if the cells were cultured in foetal calf serum (11-13). Culture of cells in foetal calf serum may explain the lack of effect upon spleen lym-

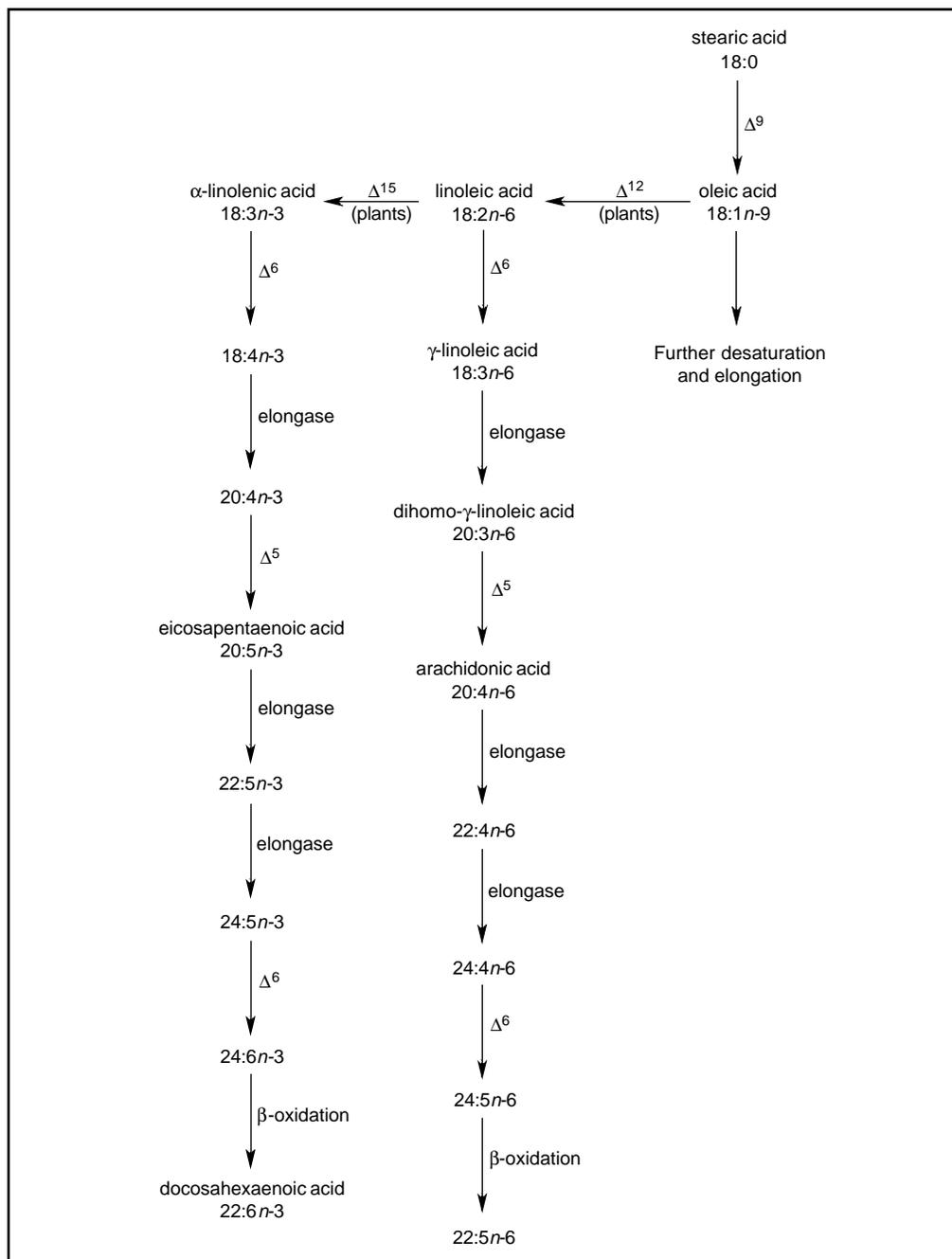


Figure 1 - PUFA metabolism.  $\Delta^5$ ,  $\Delta^6$ ,  $\Delta^9$ ,  $\Delta^{12}$  and  $\Delta^{15}$  indicate desaturase enzymes.

phocyte proliferation of feeding fish and linseed oils to mice reported in some studies (20,21). 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 foetal calf serum (22).

Twelve weeks of supplementation of the

diets of healthy women (51 to 68 years of age) with encapsulated *n*-3 PUFAs (approximately 2.4 g/day) resulted in a lowered mitogenic response of peripheral blood mononuclear cells (PBMNCs) to PHA (23). More recently, a decreased response of PBMNCs to Con A and PHA following supplementation of the diet of volunteers on a low fat, low

cholesterol diet with 1.23 g *n*-3 PUFAs/day was reported (24), while 18 g of fish oil/day (approximately 6 g *n*-3 PUFAs/day) for 6 weeks resulted in lowered PHA-stimulated proliferation of PBMNCs 10 weeks after supplementation had ended (but not at the end of the supplementation period) (25).

#### **Cytotoxic T lymphocyte-mediated cytotoxicity**

The cytotoxic T lymphocyte activity of spleen lymphocytes was reported to be lower after feeding mice 100 g/kg fish oil for up to 10 weeks than after feeding 100 g/kg linseed oil (26). Feeding chickens diets containing 70 g/kg fish or linseed oil significantly reduced spleen cytotoxic T-cell activity compared with diets containing 70 g/kg lard or corn oil (27).

#### **Natural killer cell-mediated cytotoxicity**

Feeding mice diets containing 100 g/kg fish oil caused a decrease in spleen natural killer (NK) cell activity compared with feeding chow or 100 g/kg corn oil (28,29). In the study of Berger et al. (21) female mice were fed for 5 months on diets containing 100 g/kg olive, safflower, linseed or fish oil 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. (30) fed weanling rats for 10 weeks on a low fat diet or on diets containing 200 g/kg hydrogenated coconut, olive, safflower, evening primrose or fish oil 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 (15). It was reported that a 200 g/kg linseed oil diet decreased rat spleen lymphocyte NK cell activity compared with feeding a 200 g/kg

sunflower oil diet (18). More recently, it was shown that increasing the amount of  $\alpha$ -linolenic acid in the rat diet at the expense of linoleic acid decreases spleen NK cell activity (19).

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 results in suppression of peripheral blood NK cell activity 24 h later (31).

#### **Macrophage-mediated cytotoxicity**

Dietary fish oil (100 g/kg) has been reported to significantly suppress lysis of target tumour cells by mouse-elicited peritoneal macrophages (32-35). The target cell lines used in these studies are sensitive to killing by tumour necrosis factor (TNF)- $\alpha$  (L929 cells; 33,34) or nitric oxide (P815 cells; 32,35). Thus, the suppressed macrophage-mediated cytotoxicity observed after fish oil feeding suggests that fish oil reduces the production of nitric oxide and TNF (see later).

#### **Neutrophil and monocyte chemotaxis**

Chemotaxis of human peripheral blood neutrophils and monocytes towards a variety of chemoattractants including leukotriene (LT) B<sub>4</sub>, platelet-activating factor, formyl-methionyl-leucyl-phenylalanine and autologous serum is suppressed following the supplementation of the human diet with *n*-3 PUFAs (36-40).

#### **MHC expression and antigen presentation**

Inclusion of *n*-3 PUFAs in the diet of mice or rats results in a diminished percentage of peritoneal exudate cells bearing the major histocompatibility class (MHC) II antigens on their surface (41-44). The level of MHC II expression on positive cells is also

suppressed by fish oil feeding (43). In accordance with these animal studies, supplementation of the diet of human volunteers with *n*-3 PUFAs (approximately 1.56 g/day) for 3 weeks resulted in a decreased level of MHC II (HLA-DP, -DQ and -DR) expression on the surface of peripheral blood monocytes (45). These observations suggest that diets rich in *n*-3 PUFAs will result in diminished antigen presentation. Indeed, feeding mice the ethyl ester of eicosapentaenoic acid for a period of 4 to 5 weeks resulted in diminished presentation of antigen (keyhole limpet haemocyanin; KLH) by spleen cells *ex vivo* (46). 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/kg safflower oil, feeding rats a diet containing 200 g/kg fish oil significantly diminishes MHC II expression on dendritic cells and *ex vivo* antigen (KLH) presentation by dendritic cells (obtained by cannulation of the thoracic duct) to KLH-sensitised spleen lymphocytes (47).

### Influence of dietary *n*-3 PUFAs 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, nitric oxide (NO)) and by direct cell-to-cell contact mediated by adhesion molecules. *n*-3 PUFAs have been found to influence each of these communication links.

#### *n*-3 PUFAs and eicosanoid production

Eicosanoids are a family of oxygenated derivatives of dihomo- $\gamma$ -linolenic, arachidonic and eicosapentaenoic acids. Eicosanoids include prostaglandins (PGs), thromboxanes (TXs), LTs, lipoxins (LXs), hydroperoxyeicosatetraenoic acids (HPETEs) and hydroxyeicosatetraenoic acids (HETEs). In

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<sub>2</sub> or from membrane phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>) by the actions of phospholipase C (PLC) and a diacylglycerol (DAG) lipase. The pathways of eicosanoid synthesis begin with cyclooxygenase, which yields the PGs and TXs, or with the 5-, 12- or 15-lipoxygenases, which yield the LTs, HPETEs, HETEs and LXs (Figure 2). The amounts and types of eicosanoids synthesised are determined by the availability of arachidonic acid, by the activities of phospholipase A<sub>2</sub> and PLC, 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 (48-51); the most well-documented effects are those of PGE<sub>2</sub>. *In vivo*, PGs are involved in modulating the intensity and duration of inflammatory and immune responses; PGE<sub>2</sub> has a

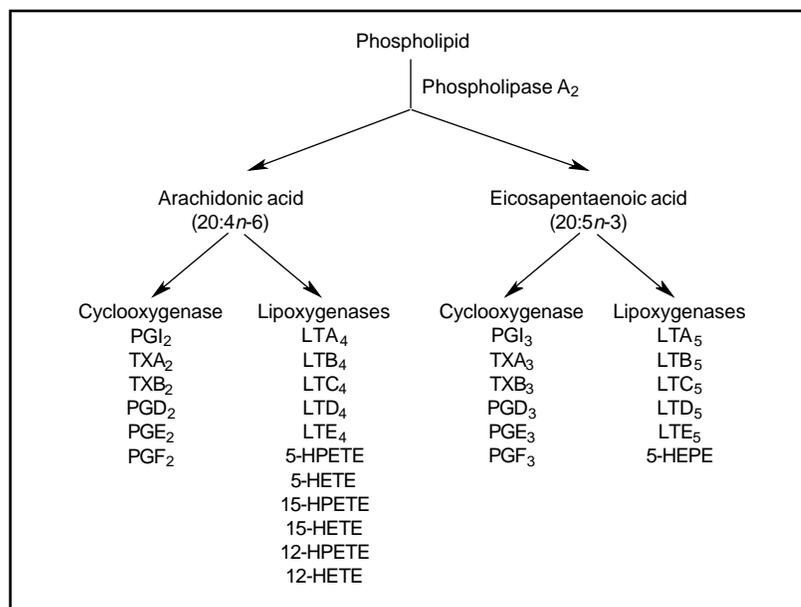


Figure 2 - Synthesis of eicosanoids from arachidonic and eicosapentaenoic acids. HEPE indicates hydroxyeicosapentaenoic acid; see text for explanation of other abbreviations.

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<sub>2</sub> also regulates the production of both monocyte/macrophage- and lymphocyte-derived cytokines (see 52). In chronic inflammatory conditions increased rates of PGE<sub>2</sub> production are found, and elevated PGE<sub>2</sub> production has been observed in patients suffering from infections. LTs have chemotactic properties and are involved in the regulation of inflammatory and immune processes; 4-series LTs regulate cytokine production.

The *n*-3 PUFAs, eicosapentaenoic and docosahexaenoic acids, 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 PUFAs will result in a decrease in membrane arachidonic acid levels and a concomitant decrease in the capacity to synthesise eicosanoids from arachidonic acid (e.g., 53); eicosapentaenoic acid gives rise to the 3-series PGs and TXs and the 5-series LTs (Figure 2). The eicosanoids produced from eicosapentaenoic acid do not always have the same biological properties as the analogues produced from arachidonic acid.

### ***n*-3 PUFAs and cytokine production**

Since cytokine production is regulated by eicosanoids (see 52) and since dietary lipids affect eicosanoid production, it might be expected that dietary lipids, especially those containing *n*-3 PUFAs, will affect cytokine production. The effects of *n*-3 PUFAs on cytokine production have been reviewed several times in recent years (7,54-59).

*Animal studies.* All studies which have

used murine resident peritoneal macrophages (60-65), one study using rat resident alveolar macrophages (66) and one study using rat resident peritoneal macrophages (67) report an enhancing effect of *n*-3 PUFAs upon TNF production; only one study which used rat resident peritoneal macrophages has reported reduced TNF production following fish oil feeding (68). Three studies report that *n*-3 PUFA-rich diets do not affect TNF production by CFA-elicited peritoneal macrophages from rats or mice (62,63,67). The effect of dietary *n*-3 PUFAs upon TNF production by thioglycollate-elicited peritoneal macrophages is unclear, with studies reporting no effect (35,63,69,70), reduction (34, 53,71) or enhancement (35,69). 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 (35,69) in which there was no effect of diet upon 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 PBMCs showed decreased production following the infusion of a 10% (v/v) fish oil emulsion (72); this is an interesting observation since it agrees with the findings of a number of studies using human PBMCs (see below). In addition to studies measuring TNF production *ex vivo*, Black and Kinsella (33) and Renier et al. (34) 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 upon circulating TNF levels which would perhaps reflect *in vivo* production of the cytokine. Watanabe et al. (63)

found that TNF levels were significantly higher in the plasma of LPS-injected mice fed a diet containing perilla oil than in the plasma of those fed a safflower oil-rich diet. Similarly, Chang et al. (65) reported higher serum TNF levels 1 and 1.5 h following intraperitoneal injection of LPS into fish oil-fed mice compared with those fed coconut or corn 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 IL-1 (34,53,70,71,73). In contrast, two studies have reported that fish oil enhances IL-1 production by murine resident macrophages (60,64). In addition, Ertel et al. (74) showed that the reduction in *ex vivo* IL-1 production by resident peritoneal macrophages which accompanies haemorrhagic shock in corn 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 these three diets (74).

There are no studies reporting the effect of dietary fatty acids upon 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 (53); production following stimulation of the cells with TNF was also significantly reduced (Yaqoob P and Calder PC, unpublished observations). Rat PBMNCs showed reduced IL-6 production following fish oil infusion for 4 days (72).

In contrast to the large number of studies in animals of the effects of dietary lipids, especially fish oil, upon the *ex vivo* production of macrophage-derived cytokines (see above), there have been relatively few studies on lymphocyte-derived cytokines. Decreased IL-2 production by alveolar lymphocytes from pigs fed diets containing fish or

linseed oil was reported (75). Recently, it was observed that feeding mice a diet containing 10 g/kg of ethyl esters of eicosapentaenoic or docosahexaenoic acid for 10 days significantly decreased *ex vivo* IL-2 production by spleen lymphocytes stimulated with Con A; both *n*-3 PUFAs were equally effective (16). In another study weanling mice were fed for 8 weeks on a low fat diet or on diets containing 200 g/kg hydrogenated coconut, olive, safflower or fish oil; the spleen lymphocytes were subsequently stimulated with Con A (14). The concentration of IL-2 was higher in the medium of spleen lymphocytes from mice fed olive or safflower oil than in the medium of cells from mice fed the low fat diet or hydrogenated coconut oil; fish oil feeding had no effect upon the IL-2 concentration in the medium. In contrast, higher IL-2 production by Con A-stimulated spleen lymphocytes taken from autoimmune disease-prone mice fed 100 g/kg fish oil compared with those fed 100 g/kg corn oil has been reported (76); 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  $\alpha$ -linolenic acid or eicosapentaenoic plus docosahexaenoic acid in the diet of monkeys resulted in enhanced *ex vivo* production of IL-2 (77). The latter study related the discrepancies in the literature to the levels of vitamin E included in the diets used.

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 upon production of IL-4, IL-10 and interferon (IFN)- $\gamma$  (14,76), but more studies need to be performed to confirm this.

*Human studies.* Endres et al. (37) were the first to show that supplementation of the diet of healthy volunteers with *n*-3 PUFAs diminishes *ex vivo* production of IL-1 $\alpha$ , IL-1 $\beta$  and TNF by human PBMNCs. Interest-

ingly, the production of these cytokines remained suppressed for 10 weeks after 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 PUFAs vary according to the stimulus used to induce cytokine production. A later study by Endres et al. (25) using the same supplementation regime 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. (78) reported that 6 weeks of supplementation of the diet with 2.4 g eicosapentaenoic acid per day resulted in lowered production of IL-2 by PBMNCs stimulated with PHA or PWM; in agreement with the long “washout” period suggested by Endres et al. (25,37), IL-2 production remained low 8 weeks after the supplementation had ended but returned to pre-supplementation levels 22 weeks post-supplementation. Meydani et al. (23) 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 PUFAs per day 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-1 $\beta$ , IL-2, TNF and IL-6 by PBMNCs 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-1 $\beta$  and IL-2 by these cells. The same workers have studied the effect of including fish (providing 1.23 g *n*-3 PUFAs/day) in a low fat, low cholesterol diet for 24 weeks; the *ex vivo* production of IL-1 $\beta$ , IL-6 and TNF by PBMNCs was significantly reduced and there was a nonsignificant reduction in IL-2 production (24). The production of IL-1 and IL-6 in whole blood cultures was decreased if the subjects had consumed 1.1 to 1.6 g *n*-3 PUFAs per day for 6 to 8 weeks but only if the cultures

were stimulated with “low” concentrations ( $\leq 0.001$   $\mu\text{g/ml}$ ) of LPS (79). Interestingly, if higher LPS concentrations were used there was no effect of the supplementation upon production of these two cytokines; TNF production was unaffected by *n*-3 PUFAs in this study. Recently, the effects of dietary  $\alpha$ -linolenic acid upon IL-1 $\beta$  and TNF- $\alpha$  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  $\alpha$ -linolenic acid which was provided by linseed oil capsules and linseed oil-based spreads and cooking oils (80). In this way the linseed oil consumption increased to a mean of 13.7 g/day. *Ex vivo* production of both IL-1 $\beta$  and TNF- $\alpha$  by PBMNCs was decreased by the linseed oil diet. If the subjects then supplemented their diet with encapsulated eicosapentaenoic plus docosahexaenoic acids (2.7 g/day) production of both cytokines was further decreased. These authors showed a correlation between mononuclear cell eicosapentaenoic acid content and production of IL-1 $\beta$  and TNF- $\alpha$ .

Supplementation of the diet of healthy volunteers or multiple sclerosis patients with encapsulated fish oil providing approximately 5 g *n*-3 PUFAs per day for 24 weeks resulted in lower *ex vivo* production of IL-1 $\beta$ , TNF- $\alpha$ , IL-2 and IFN- $\gamma$  by PBMNCs (81). An earlier study reported lower production of IL-1 by PBMNCs taken from rheumatoid arthritis patients who had consumed *n*-3 PUFAs for 24 weeks (82); interestingly, cells from the patients who consumed an olive oil placebo (9 g/day) in this trial also showed diminished IL-1 production. There was increased production of IL-2 by PBMNCs from rheumatoid arthritis patients who supplemented their diet with olive oil or a “low” level of *n*-3 PUFAs for 24 weeks; this increase did not occur in patients consuming a “high” level of *n*-3 PUFAs (82).

Recently, circulating cytokine levels in patients receiving intravenous infusions of

lipid emulsions post-surgery were reported (83); patients received either a medium chain/long chain triacylglycerol mix (50:50 v/v) or this mix also containing fish oil (50:30:20 v/v). Patients received 50 g fat per day on days 1 and 2 post-surgery and 100 g fat per day 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 PUFAs per day. Plasma TNF- $\alpha$  levels were significantly lower in the fish oil group at 6 days post-surgery; plasma IL-6 levels were lower (but not significantly) 10 days post-surgery and the post-surgery increase in plasma IL-10 levels was reduced in this group.

#### ***n*-3 PUFAs and cytokine receptor expression**

Feeding rats a diet containing 200 g/kg fish oil lowered the proportion of spleen and thymic lymphocytes bearing the IL-2 receptor (IL-2R; CD25) following Con A stimulation (13). Spleen lymphocytes from rats fed fish oil also showed a lower level of expression of the IL-2R following mitogenic stimulation (15). 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/day) caused a significant reduction in the percentage of IL-2R<sup>+</sup> blood lymphocytes following PHA stimulation (84); the level of expression of the IL-2R on the positive cells was also significantly reduced.

#### ***n*-3 PUFAs and nitric oxide production by macrophages**

Two studies using rat resident peritoneal macrophages reported that NO production is significantly diminished by fish oil feeding (68,85). In contrast, Hubbard et al. (35) found no effect of feeding mice 100 g/kg linseed or fish oil upon *ex vivo* NO production by thioglycollate-elicited macrophages; nevertheless these workers, as well as Somers et

al. (32), reported that *n*-3 PUFA feeding diminishes macrophage cytotoxicity towards NO-sensitive P815 cells, suggestive of reduced NO production. Yaqoob and Calder (53) found that several high fat diets, including 200 g/kg fish oil, 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 different high fat diets. Fish oil has been reported to enhance NO production by murine peritoneal (34), rat lung (66) and pig lung (75) macrophages.

Recently, Harris et al. (86) reported increased appearance of NO metabolites in the urine of healthy human volunteers who supplemented their diet with 5 g fish oil concentrate per day (providing 3 g eicosapentaenoic acid plus docosahexaenoic acid) for 3 weeks; interestingly, 3 g of eicosapentaenoic acid alone did not alter urinary NO metabolite levels. The levels measured by Harris et al. (86) were assumed to indicate whole body NO production; as such, a variety of sources of NO are likely to exist and the authors concluded that much of it originated from endothelial cells. There is much direct and circumstantial evidence that *n*-3 PUFAs enhance NO production by endothelial cells (see 86 for references).

#### ***n*-3 PUFAs 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/CD18:CD54, CD11a/CD18:CD102 and CD2:CD58 (for reviews, see 87-89). 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/CD18:CD54, CD54:CD11a/CD18,

CD49d/CD29:CD106, CD2:CD58, CD62L: MAdCAM-1 and CD44:hyaluronate (for reviews, see 87-89). Thus, the 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 a review, see 90), and antibodies against certain adhesion molecules can reduce chronic inflammatory disease (90).

*In vitro studies of n-3 PUFAs and adhesion molecule expression.* It has become apparent that *n-3* PUFAs can affect adhesion molecule expression by some cell types, at least *in vitro*. Calder et al. (91) 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/CD18) than those cultured with some other fatty acids. This observation suggests that *n-3* PUFAs decrease expression of either CD11a or CD18 or both proteins. More recently, De Caterina et al. (92) 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 (92). Since the binding between monocytes and endothelial cells partially depends upon CD106 expression on the endothelial cells, the reduced expression of CD106 caused by docosahexaenoic acid appears to have a functional effect. Kim et al. (93) 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 (93). 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 (94); both *n-3* PUFAs 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* PUFAs 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 (94). 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 IFN- $\gamma$ -stimulated human monocytes (95); docosahexaenoic acid did not alter expression of these molecules. Thus, it appears that culture of macrophages, monocytes, lymphocytes or endothelial cells with *n-3* PUFAs can decrease adhesion molecule expression resulting in diminished ability to bind to other cell types.

*Effects of dietary n-3 PUFAs on adhesion molecule expression.* There are few studies of the effects of inclusion of *n-3* PUFAs in the diet upon adhesion molecule expression, although it was recently shown that supplementation of the human diet with *n-3* PUFAs results in significantly lower levels of expression of CD11a and CD54 on peripheral blood monocytes (45). Feeding rats a diet containing 200 g fish oil/kg significantly reduced (by 20 to 35%) the levels of expression of CD2 and CD11a on freshly

prepared lymphocytes and of CD2, CD11a and CD54 on Con A-stimulated lymphocytes (15). Furthermore, the levels of CD2, CD11a and CD54 were reduced on popliteal lymph node lymphocytes following localised graft vs host or host vs graft responses *in vivo* (96). Reduced adhesion molecule expression suggests that cells will be less able to interact with receptor-bearing cells. In accordance with this, we have recently observed that lymph node lymphocytes obtained from fish oil-fed rats adhere less well to macrophage and endothelial cell monolayers (Sanderson P and Calder PC, unpublished observations). 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.

### **Dietary fatty acids and *in vivo* measures of cell-mediated immunity and inflammation**

The studies outlined above have investigated the effects of dietary manipulations upon *ex vivo* functions of isolated cell populations. Although a number of consistent patterns have emerged from these studies, there are also contradictory reports and it is evident that the outcome of such *ex vivo* measures is strongly influenced by the experimental conditions used. Furthermore, *in vivo* cells exist as part of a network being influenced by other cell types; often such interactions are disturbed by the purification of the particular cell types to be studied. Therefore, it is important to investigate the effect of dietary fats on the intact, fully functioning system in which all normal cellular interactions are in place. The ability to make *in vivo* measures of inflammation and cell-mediated immunity offers the prospect of investigating the effects of dietary manipulations upon the overall responses of these systems.

### **Acute inflammatory responses**

Arachidonic acid-derived eicosanoids are involved in mediating inflammatory responses. Since *n*-3 PUFAs diminish the production of these mediators, they should exert anti-inflammatory activities. Indeed, *n*-3 PUFAs decrease the production of pro-inflammatory mediators such as PGE<sub>2</sub> and LTB<sub>4</sub> during antigen-induced inflammation of the air pouch (97), carrageenan-induced inflammation of the footpad (97) and zymosan-induced peritoneal inflammation (98). The latter study also reported that dietary *n*-3 PUFAs inhibited the influx of neutrophils into the peritoneal cavity which accompanies such treatment. Feeding rats 100 g/kg cod liver oil for 10 weeks significantly lowered (by 40%) the inflammatory response to carrageenan injection into the footpad compared with feeding coconut oil or groundnut oil (99). In accordance with that observation, feeding rats high fat diets containing ethyl esters of eicosapentaenoic or docosahexaenoic acid resulted in a 50% reduction in footpad swelling in response to carrageenan injection compared with feeding safflower oil (100); both *n*-3 PUFAs were equally effective.

### ***In vivo* responses to endotoxin and cytokines**

Intravenous infusions of a 10% (v/v) lipid emulsion rich in fish oil into guinea pigs significantly enhanced survival to intraperitoneally injected LPS compared with infusion of a 10% (v/v) safflower oil emulsion (101); the total amount of lipid infused was 13 g/animal. The same workers later showed that feeding a fish oil-rich diet to guinea pigs for 6 weeks significantly increased survival to an intraperitoneal injection of LPS compared with animals fed a safflower oil-rich diet (102).

In accordance with the diminished susceptibility to the lethal effects of endotoxin in experimental animals, feeding weanling

rats a 100 g/kg fish oil diet for 8 weeks significantly decreased a number of responses to intraperitoneal TNF- $\alpha$ : the rises in liver zinc and plasma C3 concentrations, the fall in plasma albumin concentration and the increases in liver, kidney and lung protein synthesis rates were all prevented by the fish oil diet (103). Fish oil feeding to rats or guinea pigs also diminishes the pyrogenic (104,105) and anorexic effects (103, 106) of IL-1 and TNF- $\alpha$  compared with feeding *n*-6 PUFA-containing oils.

#### Delayed-type hypersensitivity

The delayed-type hypersensitivity (DTH) reaction is the result of a cell-mediated response to challenge with an antigen to which the individual has already been primed. There are several reports that the DTH response in rodents is significantly reduced by *n*-3 PUFAs. For example, intragastric administration of a fish oil concentrate to rats for 50 days lowered the DTH response to bovine serum albumin compared with administering safflower oil, olive oil or water (97) while addition of ethyl esters of either eicosapentaenoic or docosahexaenoic acid to the diet of mice consuming a safflower oil diet reduced the DTH response to tuberculin (107); both *n*-3 PUFAs were equally effective. The DTH response to sheep red blood cells in mice was diminished following tail-vein injections of emulsions of triglycerides rich in eicosapentaenoic or docosahexaenoic acid (108); a soybean oil emulsion was injected in the control animals. Most recently, it was reported that feeding aged beagle dogs a long-chain *n*-3 PUFA-rich diet significantly diminished the DTH response to intradermal KLH compared with feeding diets containing smaller amounts of long-chain *n*-3 PUFAs (109).

Feeding a linseed oil-rich diet to healthy human volunteers for 8 weeks lowered the DTH response to 7 recall antigens, although

this reduction was not statistically significant (110). Supplementation of the diet of volunteers consuming a low fat, low cholesterol diet with 1.25 g *n*-3 PUFA/day diminished the DTH responses to 7 recall antigens (24).

#### Graft vs host and host vs graft responses

The so-called popliteal lymph node (PLN) assay provides a useful experimental model in rodents for measuring graft vs host (GvH) and host vs graft (HvG) responses elicited by injection of allogeneic cells into the footpad of the host. The GvH response primarily involves the polyclonal activation, and subsequent proliferation, of host B-cells, although NK cells may also be involved in the host defence. In contrast, the HvG reaction is a T-cell-mediated response, in which CTLs of the host recognise MHC antigens on the injected cells. In both cases the enlargement in PLN size (more than 15-fold in the GvH response and 4-fold in the HvG response) is due largely to proliferation of activated host cells; most of these originate within the PLN although there is also some recruitment of cells from the bloodstream. A suppressed HvG response was observed in mice fed a 160 g/kg fish oil diet compared with those fed a standard chow diet (111); lower levels of fish oil (25, 50, 100 g/kg) did not significantly affect the response. Significantly diminished GvH and HvG responses (by 34% and 20%, respectively) were observed in rats fed 200 g/kg fish oil compared with those fed a low fat diet or diets containing 200 g/kg coconut, olive, safflower or evening primrose oils (96). Such observations accord with the demonstrations of significantly diminished *ex vivo* T lymphocyte proliferation, NK cell activity and CTL activity following fish oil feeding. Feeding rats a 200 g/kg fish oil diet resulted in fewer IL-2R<sup>+</sup> cells and CD16<sup>+</sup>/CD3<sup>-</sup> cells in the popliteal lymph node following the GvH

response, indicating an inhibition of lymphocyte activation and a decrease in the proportion of NK cells, respectively (96). Recently, a dose-dependent effect of linseed oil compared with sunflower oil upon the GvH response in rats was reported (18).

### **Animal models of organ transplantation**

Graft rejection in transplantation surgery is caused by an immune reaction to the foreign material introduced into the body; T-cells have been implicated in accelerated graft rejection, but antibodies with specificity for the graft donor have also been observed following rejection, implying that both cell-mediated immunity and humoral immunity play a part in the rejection process. Studies investigating the effects of eicosanoids on organ transplantation pre-date those investigating the effects of fatty acids. Some eicosanoids promote graft rejection while others promote graft survival (for a review, see 112). Therefore, since *n*-6 and *n*-3 PUFAs will affect the levels and types of eicosanoids formed, they would be expected to influence graft survival. In addition, PUFAs exert immunomodulatory effects which might be independent of eicosanoids and so these effects might play a role in enhancing graft survival. The effect of feeding *n*-3 PUFAs to recipient or donor or both upon subsequent cardiac allograft survival was investigated in rats (113). It was found that survival was prolonged if the recipients were fed fish oil or ethyl esters of eicosapentaenoic or docosahexaenoic acid regardless of the diet of the donor, or if the donors were fed fish oil or ethyl esters of eicosapentaenoic or docosahexaenoic acid or fish oil regardless of the diet of the recipient or if both donor and recipient were fed fish oil. Greater prolongation of cardiac survival in rats receiving an infusion of fish oil post-transplantation compared with those receiving soybean

oil infusion was reported (114); in turn soybean oil enhanced survival compared with saline infusion. Oral fish oil (4.5 g/day) has also been shown to prolong the survival of islets of Langerhans grafts in mice (115).

### **Effects of *n*-3 PUFAs in animal models of inflammatory and autoimmune diseases**

Dietary fish oil has been shown to have significantly beneficial clinical, immunological and biochemical effects in a number of animal disease models. These effects include increased survival and decreased proteinuria and anti-DNA antibodies in mice with autoimmune glomerulonephritis, a model for systemic lupus erythematosus (41,116-119), decreased joint inflammation in rodents with collagen-induced arthritis (120,121) and less inflammation in rats with various models of colitis (122-125). These observations suggest that diets enriched in *n*-3 PUFAs might be of some therapeutic benefit in these diseases in man.

### **Clinical studies of the effects of dietary fatty acids in inflammatory and autoimmune diseases and in transplantation**

#### **Inflammatory and autoimmune diseases**

The low incidence of cardiovascular disorders amongst populations consuming large quantities of oily fish has been well documented (126-128), but the intense interest in fish oil and heart disease has overshadowed the unusual pattern of the incidence of some other diseases in native Greenland Eskimos. Kromann and Green (127) described a very low incidence of autoimmune and inflammatory disorders, such as psoriasis, asthma and type-I diabetes, and the complete absence of multiple sclerosis in a population of Greenland Es-

kimos compared with sex- and age-matched groups living in Denmark. Thus, the *n*-3 PUFA-containing fish oil in the Eskimo diet could have a protective role towards these types of diseases. Most of these diseases are characterised by inappropriate activation of T-cells resulting in attack on, and ultimately destruction of, host tissues. Typically, the sites of tissue destruction (e.g., joints in rheumatoid arthritis, neural tissue in multiple sclerosis) contain activated T-cells and macrophages and mediators produced by these cells, such as cytokines, eicosanoids and reactive oxygen species. The favourable outcome resulting from dietary fish oil in animal models of inflammatory and autoimmune diseases (see above) indicates that there may be some benefit from supplementation of the diet of suitable patients with *n*-3 PUFAs. There have been a number of clinical trials assessing the benefits of dietary supplementation with fish and other oils in several inflammatory and autoimmune diseases; these studies have been reviewed elsewhere (7,129-134).

### Transplantation

The animal studies described above indicate that PUFAs, particularly *n*-3 PUFAs, could be used to prolong the survival of organ transplants. Recipients of kidney transplants who received 9 g fish oil/day for one year post-transplant (in conjunction with cyclosporin A and prednisolone) had significantly improved glomerular filtration rate and significantly diminished cyclosporin A nephrotoxicity although there was no effect upon graft survival (135). A similar finding was made by Homan van der Heide et al. (136) who reported that renal transplant patients who received fish oil (6 g/day for the first post-operative year) in combination with cyclosporin A had better kidney function and less rejections over one year compared with patients who received coconut oil and

cyclosporin A. Better kidney function in kidney graft recipients who consumed fish oil (8 g/day for one year post-transplant) was reported, although there was no reduction in rejection episodes compared with controls (137). Bennett et al. (138) reported no rejection incidents in a group of kidney transplant recipients who received 9 or 18 g eicosapentaenoic acid/day for 16 weeks post-transplantation; cyclosporin nephrotoxicity did not occur in this group but did occur in the control group who supplemented their diet with corn oil.

### Mechanisms by which *n*-3 PUFAs might exert their effects

Clearly *n*-3 PUFAs 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 NO is regulated by eicosanoids and so an *n*-3 PUFA-induced change in the amount and types of eicosanoids formed could, at least partially, explain the effects of *n*-3 PUFAs. However, many of the effects of *n*-3 PUFAs appear to be exerted in an eicosanoid-independent manner. Thus, other mechanisms of action of *n*-3 PUFAs upon 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 PUFAs 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 metabo-

lism (for reviews, see 139-141) and the genes involved in adipocyte differentiation and development (for reviews, see 140,142). Dietary *n*-3 PUFAs have particularly potent effects upon the expression of genes for proteins involved in hepatic peroxisomal proliferation, fatty acid oxidation and lipoprotein assembly (e.g., 143). Studies of the effects of fatty acids upon the expression of genes important in immune cell functioning are few and relatively recent.

*n*-3 PUFAs 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 TGF- $\beta$  and reduced levels of mRNA for c-myc and c-ras in the spleen (76). The same authors also showed that dietary fish oil completely abolished mRNA production for IL-1 $\beta$ , IL-6 and TNF- $\alpha$  in the kidneys of these animals (144). It has been reported that feeding mice a fish oil-rich diet significantly diminished the level of IL-1 $\beta$  mRNA in LPS- or phorbol ester-stimulated spleen lymphocytes (145); the lower IL-1 $\beta$  mRNA level was not due to accelerated degradation but to impaired synthesis. Fish oil feeding to mice lowered basal and LPS-stimulated TNF- $\alpha$  mRNA levels in peritoneal macrophages (34).

*n*-3 PUFAs and adhesion molecule gene expression. De Caterina et al. (92) showed that incubation of human saphenous vein endothelial cells with docosahexaenoic acid results in reduced levels of mRNA for CD106.

*n*-3 PUFAs and NO synthase gene expression. Khair-El-Din et al. (146) reported that incubation of murine thioglycollate-elicited peritoneal macrophages with docosahexaenoic acid decreased NO production in response to LPS plus IFN- $\gamma$  (or IFN- $\gamma$  plus TNF- $\alpha$ ) and that the inhibition was concentration dependent. Neither arachidonic nor eicosapentaenoic acids at concentrations of up to 100  $\mu$ M 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 (iNOS), the enzyme responsible for NO production in macrophages stimulated in this way; the lower level of iNOS mRNA was due to an inhibition of transcription (146).

#### **Mechanisms by which *n*-3 PUFAs might affect gene expression**

Thus, it is now apparent that *n*-3 PUFAs 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 PUFAs could affect gene expression is through changes in the signal transduction pathways which link cell surface receptors to the activation of nuclear transcription factors. Alternatively, *n*-3 PUFAs (indeed PUFAs in general) or their derivatives might bind directly to nuclear transcription factors 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 PIP<sub>2</sub> and phosphatidylcholine by phospholipases generates second messengers such as DAG and inositol-1,4,5-trisphosphate (IP<sub>3</sub>). Other phospholipids play a role in activating or stabilising 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 sphingomyelinase 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 PIP<sub>2</sub>, 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. 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 (147) and macrophages (148). 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 DAGs containing two saturated fatty acids or one saturated and one unsaturated fatty acid (149) and that rat spleen PKC is less active in the presence of an *n*-3 PUFA-rich phosphatidylserine compared with a PUFA-poor phosphatidylserine (150).

In support of the idea that *n*-3 PUFAs influence intracellular signalling pathways, DAG generation was reduced in Con A-stimulated lymphocytes taken from mice fed the ethyl ester of docosahexaenoic acid compared with those taken from safflower oil-fed mice (151). More recently, it was shown that feeding either eicosapentaenoic or docosahexaenoic acid to mice results in reduced DAG generation by Con A-stimulated spleen lymphocytes (16); this study also showed, for the first time, that fish oil-derived *n*-3 PUFAs 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 an *n*-6 PUFA-rich diet suggested that phosphatidylcholine hydrolysis contributed significantly to the total DAG formed (148), implicating phosphatidylcholine PLC and/or phospholipase D in DAG formation. These data indicate that *n*-3 PUFAs in some way affect the activity of one or more phospholipases responsible for the generation of key second messengers; this may be via changes in the fatty

acid composition of the substrate phospholipids. Addition of 14.4 g per day of fish oil-derived *n*-3 PUFAs to the diet for 10 weeks resulted in lower platelet activating factor- or LTB<sub>4</sub>-stimulated IP<sub>3</sub> generation in peripheral blood neutrophils (40). The platelet activating factor and LTB<sub>4</sub> receptors are coupled via G-proteins to PLC $\beta$ . In a recent study, it was observed that calcium ionophore- or Con A-stimulated IP<sub>3</sub> generation in rat lymphocytes was significantly reduced if the lymphocytes came from animals fed fish oil (Sanderson P and Calder PC, unpublished observations). Furthermore, it was found that, although the amount of PLC $\gamma$ -1 in rat lymphocytes was unaffected by diet, the ability to phosphorylate, and so to activate, the enzyme in response to suitable stimuli appeared to be significantly impaired by fish oil feeding (Sanderson P and Calder PC, unpublished observations). Lymphocyte PLC $\gamma$ -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 PUFAs 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 upon intracellular signalling pathways (for a review, see 152). This direct modulatory effect of fatty acids has been most extensively documented in relation to PKC activity which was shown to be enhanced by docosahexaenoic acid (153). 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 phospho-

tidylserine and DAG both *n*-3 PUFAs caused up to 60% inhibition of PKC activity (154). Other studies have shown that eicosapentaenoic and docosahexaenoic acids inhibit rat lymphocyte or macrophage PKC activity in the presence of calcium, phosphatidylserine and DAG (155,156), whereas protein kinase A activity was unaffected by these fatty acids. In accordance with both the direct effects of *n*-3 PUFAs *in vitro* (155,156) and the effects of enriching phosphatidylserine with *n*-3 PUFAs (150), feeding mice fish oil resulted in diminished spleen lymphocyte PKC activity (157).

A change in the concentration of intracellular free calcium 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 calcium concentration in a cytotoxic T-cell line (158). Several unsaturated fatty acids including  $\alpha$ -linolenic, eicosapentaenoic and docosahexaenoic acids inhibit the anti-CD3-induced increase in intracellular free calcium concentration in the Jurkat T-cell line (159,160); the fatty acids appeared to act by blocking calcium entry into the cells and it was concluded that they act directly upon receptor-operated calcium channels. Reduced IP<sub>3</sub> levels as a result of diminished phospholipase activity (see above) would also contribute to decreased intracellular free calcium concentrations, which in turn would reduce the activity of some isoforms of PKC.

*Fatty acids and transcription factors within cells of the immune system.* Lymphocytes and other immune and inflammatory cells contain many transcription factors including nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B), nuclear transcription factor of activated T-cells (NFAT), AP-1, various oncogene

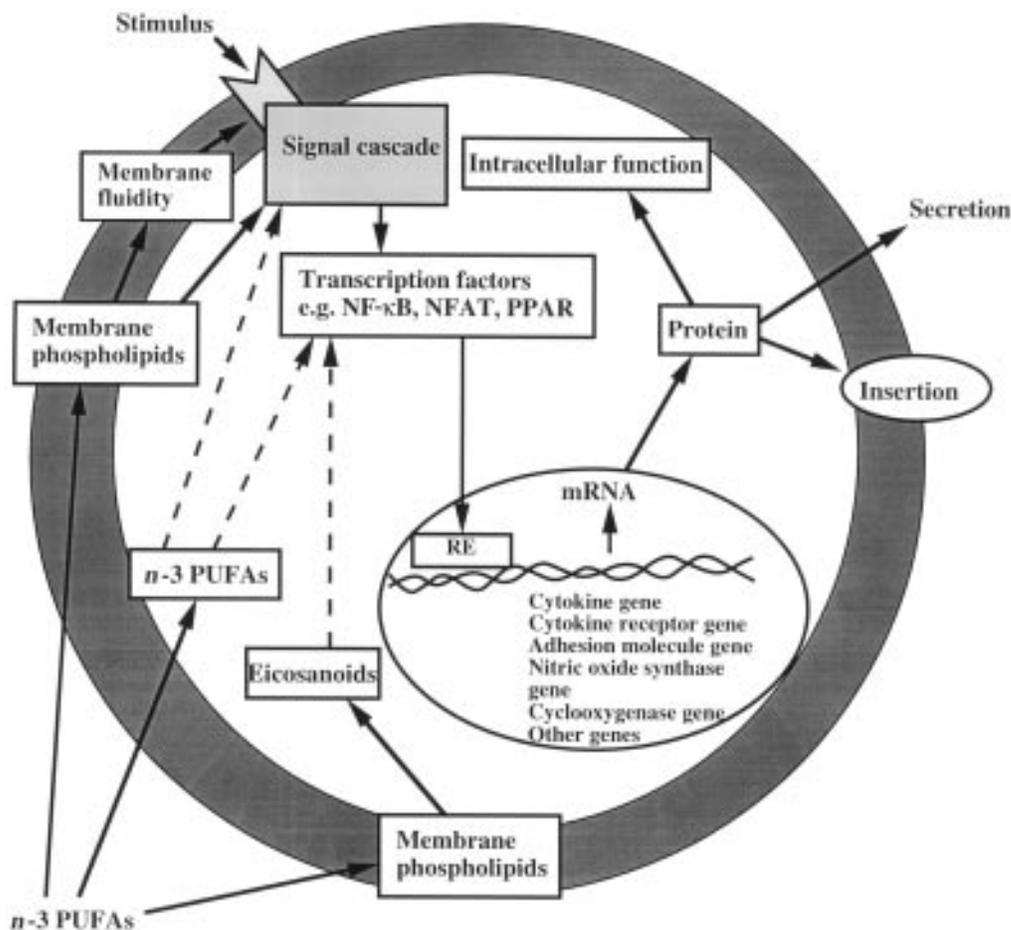
products (e.g., myc, fos, jun), steroid hormone receptors and specific transcription factors such as NF-IL-2, NF-IL-6 and NF-ICAM-1. NF- $\kappa$ B plays a key role in inducing the production of many key mediators within the immune system: NF- $\kappa$ B regulates the synthesis of cytokines including IL-1, IL-2, IL-6, TNF- $\alpha$  and IFN- $\gamma$ , of cytokine receptors including IL-2R, of adhesion molecules including CD54, CD62E and CD106, of enzymes involved in mediator generation such as iNOS and of a range of acute phase proteins (for a review, see 161). NF- $\kappa$ B is activated by the phosphorylation and subsequent dissociation of one of its three subunits, the so-called inhibitory- $\kappa$ B (I $\kappa$ B); 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 I $\kappa$ B is performed by PKC. Given the effects of *n*-3 PUFAs outlined previously (e.g., reduced PLC activity, resulting in reduced DAG and IP<sub>3</sub> generation, resulting in turn in a reduced intracellular free calcium rise and a reduced activation of PKC isoforms), it is evident how these fatty acids could prevent activation of NF- $\kappa$ B and so suppress expression of a range of genes including those for cytokines, cytokine receptors, adhesion molecules and iNOS. Ceramide can also activate NF- $\kappa$ B independently of PKC activity; thus, the observation of Jolly et al. (16) 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 and 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 (PPARs). Eicosanoid derivatives of arachidonic acid including PGA<sub>2</sub>, PGB<sub>2</sub>, PGD<sub>2</sub>, 15-deoxy- $\Delta$ <sup>12,14</sup>-PGJ<sub>2</sub>, but not PGE<sub>2</sub>, PGF<sub>2 $\alpha$</sub>  or PGI<sub>2</sub>, are activators of PPAR $\alpha$ ,  $\gamma$  and  $\delta$  (162); LTB<sub>4</sub>, 8-HETE

and 12-HETE appear to activate PPAR $\alpha$  only (162). In contrast to their role as PPAR activators, most eicosanoids are poor PPAR ligands (162), although 8-HETE is a high affinity ligand for PPAR $\alpha$  (162). The claim that LTB $_4$  is a PPAR $\alpha$  ligand (163) is now disputed (162). It has been claimed that 15-deoxy- $\Delta^{12,14}$ -PGJ $_2$  is a ligand for PPAR $\gamma$  (164-166) and PPAR $\alpha$  (166), although Forman et al. (162) showed it does not bind PPAR $\alpha$  or  $\delta$ . Interestingly, the eicosapentaenoic acid derivative 8-HEPE is a potent activator of, and ligand for, PPAR $\alpha$  (162). The various roles of arachidonic and eicosapentaenoic acid derivatives as activators and ligands of PPAR isoforms provide a mechanism by which these fatty acids can affect the activity of these transcription factors. It has been known for some time that PUFAs them-

selves are activators of PPARs (for reviews, see 140,141). Very recently, and in contrast to earlier reports, it has been found that unsaturated fatty acids (linoleic,  $\alpha$ -linolenic,  $\gamma$ -linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids) bind directly to PPAR $\alpha$  (162,166),  $\delta$  (162) and  $\gamma$  (166). Thus, it appears that PUFAs are both activators of and ligands for some forms of PPARs. The mechanism of action and functional effects of PPARs have been reviewed in detail recently (140,141). PPAR isoforms have been identified in lymphoid tissues. Using *in situ* DNA hybridisation and immunohistochemical staining Braissant et al. (167) identified moderate to very strong expression of PPAR $\alpha$ ,  $\beta$  and  $\gamma$  in rat spleen (both red and white pulp) and lymph nodes; Kliewer et al. (168) had earlier reported the presence of

Figure 3 - Schematic representation of the mechanisms by which *n*-3 PUFAs might influence immune cell function.



PPAR $\gamma$  and  $\delta$  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 PUFAs and 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 PUFAs regulate the pathway of activation of steroid hormone receptors (for reviews, see 169-171), which may account for the observations that PUFAs, particularly *n*-3 PUFAs, sensitise

immune cells to the effects of steroid hormones (e.g., 172).

In addition to the effects which influence the activity of transcription factors, *n*-3 PUFAs might also regulate the synthesis of transcription factors. Fernandes et al. (76) reported markedly decreased *c-myc* mRNA in spleens from fish oil-fed mice; *c-myc* mRNA encodes a transcription factor.

Figure 3 attempts to give an overview of the sites at which *n*-3 PUFAs might influence the functional activities of cells of the immune system.

## References

- 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.
- Yaqoob P & Calder PC (1993). The effects of fatty acids on lymphocyte functions. *International Journal of Biochemistry*, 25: 1705-1714.
- 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.
- Calder PC (1995). Fatty acids, dietary lipids and lymphocyte functions. *Biochemical Society Transactions*, 23: 302-309.
- Calder PC (1996). Can *n*-3 polyunsaturated fatty acids be used as immunomodulatory agents? *Biochemical Society Transactions*, 24: 211-220.
- Calder PC (1996). Effects of fatty acids and dietary lipids on cells of the immune system. *Proceedings of the Nutrition Society*, 55: 127-150.
- Calder PC (1996). Immunomodulatory and anti-inflammatory effects of *n*-3 polyunsaturated fatty acids. *Proceedings of the Nutrition Society*, 55: 737-774.
- Calder PC (1997). Dietary fatty acids and the immune system. *Nutrition Reviews* (in press).
- Miles EA & Calder PC (1997). Modulation of immune function by dietary fatty acids. *Proceedings of the Nutrition Society* (in press).
- Alexander NJ & Smythe NL (1988). Dietary modulation of *in vitro* lymphocyte function. *Annals of Nutrition and Metabolism*, 32: 192-199.
- 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.
- 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.
- Yaqoob P, Newsholme EA & Calder PC (1994). The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. *Immunology*, 82: 603-610.
- Yaqoob P & Calder PC (1995). The effects of dietary lipid manipulation on the production of murine T-cell-derived cytokines. *Cytokine*, 7: 548-553.
- Sanderson P, Yaqoob P & Calder PC (1995). 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.
- 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.
- 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.
- 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.
- Jeffery NM, Newsholme EA & Calder PC (1997). The level of polyunsaturated fatty acids and the *n*-6 to *n*-3 polyunsaturated fatty acid ratio in the rat diet both affect serum lipid levels and lymphocyte functions. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 57: 149-160.
- 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.
- 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.
- 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.
- 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.
24. 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.
  25. 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.
  26. Fritsche KL & Johnstone PV (1990). Effect of dietary omega-3 fatty acids on cell-mediated cytotoxic activity in BALB/c mice. *Nutrition Research*, 10: 577-588.
  27. 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.
  28. Meydani SN, Yogeewaran G, Liu S, Baskar S & Meydani M (1988). Fish oil and tocopherol-induced changes in natural killer cell-mediated cytotoxicity and PGE<sub>2</sub> synthesis in young and old mice. *Journal of Nutrition*, 118: 1245-1252.
  29. Lumpkin EA, McGlone JJ, Sells JL & Hellman JM (1993). Modulation of murine natural killer cell cytotoxicity by dietary fish oil, corn oil or beef tallow. *Journal of Nutritional Immunology*, 2: 43-53.
  30. Yaqoob P, Newsholme EA & Calder PC (1994). Inhibition of natural killer cell activity by dietary lipids. *Immunology Letters*, 41: 241-247.
  31. 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.
  32. 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.
  33. Black JM & Kinsella JE (1993). Dietary n-3 fatty acids alter mouse peritoneal macrophage cytotoxicity. *Annals of Nutrition and Metabolism*, 37: 110-120.
  34. 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 Thrombosis*, 13: 1515-1524.
  35. 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.
  36. 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.
  37. Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonemann 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.
  38. 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.
  39. 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.
  40. Sperling RI, Benincaso AI, Knoell CT, Larkin JK, Austen KF & Robinson DR (1993). Dietary  $\omega$ -3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. *Journal of Clinical Investigation*, 91: 651-660.
  41. 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.
  42. Mosquera J, Rodriguez-Iturbe B & Parra G (1990). Fish oil dietary supplementation reduces la expression in rat and mouse peritoneal macrophages. *Clinical Immunology and Immunopathology*, 56: 124-129.
  43. Huang S-C, Misfeldt ML & Fritsche KL (1992). Dietary fat influences la antigen expression and immune cell populations in the murine peritoneum and spleen. *Journal of Nutrition*, 122: 1219-1231.
  44. Sherrington EJ, Sanderson P & Calder PC (1995). The effect of dietary lipid manipulation on macrophage cell surface molecule expression. *Biochemical Society Transactions*, 23: 272S.
  45. Hughes DA, Pinder AC, Piper Z, Johnson IT & Lund EK (1996). 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.
  46. Fujikawa M, Yamashita N, Yamazaki K, Sugiyama E, Suzuki H & Hamazaki T (1992). Eicosapentaenoic acid inhibits antigen-presenting cell function of murine splenocytes. *Immunology*, 75: 330-335.
  47. Sanderson P, MacPherson GG, Jenkins CH & Calder PC (1997). Dietary fish oil diminishes antigen presentation activity by rat dendritic cells. *Journal of Leukocyte Biology* (in press).
  48. Goldyne ME & Stobo JD (1981). Immunoregulatory role of prostaglandins and related lipids. *Critical Reviews in Immunology*, 2: 189-223.
  49. Goodwin JS & Cueppens J (1983). Regulation of the immune response by prostaglandins. *Journal of Clinical Immunology*, 3: 295-315.
  50. Hwang D (1989). Essential fatty acids and the immune response. *FASEB Journal*, 3: 2052-2061.
  51. Roper RL & Phipps RP (1994). Prostaglandin E<sub>2</sub> regulation of the immune response. *Advances in Prostaglandin, Thromboxane and Leukotriene Research*, 22: 101-111.
  52. Rola-Pleszczynski M & Stankova J (1992). Cytokine gene regulation by PGE<sub>2</sub>, LTB<sub>4</sub> and PAF. *Mediators of Inflammation*, 1: 5-8.
  53. Yaqoob P & Calder PC (1995). Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. *Cellular Immunology*, 163: 120-128.
  54. Meydani SN (1992). Modulation of cytokine production by dietary polyunsaturated fatty acids. *Proceedings of the Society for Experimental Biology and Medicine*, 200: 189-193.
  55. Meydani SN (1996). Effect of (n-3) polyunsaturated fatty acids on cytokine production and their biologic function. *Nutrition*, 12: S8-S14.
  56. 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.
  57. Endres S (1996). n-3 Polyunsaturated fatty acids and human cytokine synthesis. *Lip-*

- ids, 31: S239-S242.
58. Endres S & von Schacky C (1996). n-3 Polyunsaturated fatty acids and human cytokine synthesis. *Current Opinion in Lipidology*, 7: 48-52.
  59. Calder PC (1997). N-3 polyunsaturated fatty acids and cytokine production in health and disease. *Annals of Nutrition and Metabolism* (in press).
  60. 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.
  61. Hardardottir I & Kinsella JE (1991). Tumor necrosis factor production by murine resident peritoneal macrophages is enhanced by dietary n-3 polyunsaturated fatty acids. *Biochimica et Biophysica Acta*, 1095: 187-195.
  62. 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.
  63. Watanabe S, Hayashi H, Onozaki K & Okuyama H (1991). Effect of dietary  $\alpha$ -linolenate/linoleate balance on lipopolysaccharide-induced tumor necrosis factor production in mouse macrophages. *Life Sciences*, 48: 2013-2020.
  64. 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.
  65. 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.
  66. 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.
  67. Turek JJ, Schoenlein IA & Bottoms GD (1991). The effect of dietary n-3 and n-6 fatty acids on tumor necrosis factor- $\alpha$  production and leucine aminopeptidase levels in rat peritoneal macrophages. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 43: 141-149.
  68. 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.
  69. Somers SD & Erickson KL (1994). Alteration of tumor necrosis factor- $\alpha$  production by macrophages from mice fed diets high in eicosapentaenoic and docosahexaenoic acids. *Cellular Immunology*, 153: 287-297.
  70. Tappia PS & Grimble RF (1994). Complex modulation of cytokine induction by endotoxin and tumor 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.
  71. Wallace FA, Neely SJ, Miles EA & Calder PC (1997). Dietary fish oil decreases the production of pro-inflammatory mediators by murine macrophages. *Proceedings of the Nutrition Society* (Abstract) (in press).
  72. Grimm H, Tibell A, Norrlind B, Blecher C, Wilker S & Schwemmler 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.
  73. 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.
  74. 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.
  75. 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.
  76. Fernandes G, Bysani C, Venkatraman JT, Tomar V & Zhao W (1994). Increased TGF- $\beta$  and decreased oncogene expression by  $\omega$ -3 fatty acids in the spleen delays onset of autoimmune disease in B/W mice. *Journal of Immunology*, 152: 5979-5987.
  77. 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.
  78. 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.
  79. 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.
  80. Caughey GE, Mantzioris E, Gibson RA, Cleland LG & James MJ (1996). The effect on human tumor necrosis factor  $\alpha$  and interleukin 1 $\beta$  production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. *American Journal of Clinical Nutrition*, 63: 116-122.
  81. 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.
  82. 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.
  83. Wachtler P, Konig W, Senkal M, Kemen M & Koller M (1997). Influence of a total parenteral nutrition enriched with  $\omega$ -3 fatty acids on leukotriene synthesis of peripheral leukocytes and systemic cytokine levels in patients with major surgery. *Journal of Trauma*, 42: 191-198.
  84. 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.
  85. 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.
  86. 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.
  87. Stoolman LM (1989). Adhesion molecules controlling lymphocyte migration. *Cell*, 56: 907-910.

88. Springer TA (1990). Adhesion receptors of the immune system. *Nature*, 346: 425-433.
89. Hogg N & Lands RC (1993). Adhesion molecules in cell interactions. *Current Opinion in Immunology*, 5: 383-390.
90. Faull RJ (1995). Adhesion molecules in health and disease. *Australian and New Zealand Journal of Medicine*, 25: 720-730.
91. 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.
92. 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.
93. 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.
94. 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.
95. Hughes DA, Southon S & Pinder AC (1996). (n-3) Polyunsaturated fatty acids modulate the expression of functionally associated molecules on human monocytes *in vitro*. *Journal of Nutrition*, 126: 603-610.
96. Sanderson P, Yaqoob P & Calder PC (1995). Effects of dietary lipid manipulation upon graft vs host and host vs graft responses in the rat. *Cellular Immunology*, 164: 240-247.
97. Yoshino S & Ellis EF (1987). Effect of a fish oil-supplemented diet on inflammation and immunological processes in rats. *International Archives of Allergy and Applied Immunology*, 84: 233-440.
98. Lefkowitz JB, Morrison A, Lee V & Rogers M (1990). Manipulation of the acute inflammatory response by dietary polyunsaturated fatty acid modulation. *Journal of Immunology*, 145: 1523-1529.
99. Reddy ACB & Lokesh BR (1994). Studies on anti-inflammatory activity of spice principles and dietary n-3 polyunsaturated fatty acids on carrageenan-induced inflammation in rats. *Annals of Nutrition and Metabolism*, 38: 349-358.
100. Nakamura N, Hamazaki T, Kobayashi M & Yazawa K (1990). The effect of oral administration of eicosapentaenoic and docosahexaenoic acids on acute inflammation and fatty acid composition in rats. *Journal of Nutritional Science and Vitamins*, 40: 161-170.
101. Mascioli EA, Leader L, Flores E, Trimbo S, Bistrain B & Blackburn G (1988). Enhanced survival to endotoxin in guinea pigs fed *in vivo* fish oil emulsion. *Lipids*, 23: 623-625.
102. Mascioli EA, Iwasa Y, Trimbo S, Leader L, Bistrain BR & Blackburn GL (1989). Endotoxin challenge after menhaden oil diet: effects on survival of guinea pigs. *American Journal of Clinical Nutrition*, 49: 277-282.
103. Mulrooney HM & Grimble RF (1993). Influence of butter and of corn, coconut and fish oils on the effects of recombinant human tumour necrosis factor- $\alpha$  in rats. *Clinical Science*, 84: 105-112.
104. Pomposelli J, Mascioli EA, Bistrain BR & Flores SM (1990). Attenuation of the febrile response in guinea pigs by fish oil enriched diets. *Journal of Parenteral and Enteral Nutrition*, 13: 136-140.
105. Cooper AL & Rothwell NJ (1993). Inhibition of the thermogenic and pyrogenic responses to interleukin-1 beta in the rat by dietary n-3 fatty acid supplementation. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 49: 615-626.
106. Hellerstein MK, Meydani SN, Meydani M, Wu K & Dinarello CA (1989). Interleukin-1-induced anorexia in the rat: Influence of prostaglandins. *Journal of Clinical Investigation*, 84: 228-235.
107. 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.
108. Taki H, Morinaga S-I, Yamazaki K, Hamazaki T, Suzuki H & Nakamura N (1992). Reduction of delayed-type hypersensitivity by the injection of n-3 polyunsaturated fatty acids in mice. *Transplantation*, 54: 511-514.
109. Wander RC, Hall JA, Gradin JL, Du S-H & Jewell DE (1997). The ratio of dietary (n-6) to (n-3) fatty acids influences immune system function, eicosanoid metabolism, lipid peroxidation and vitamin E status in aged dogs. *Journal of Nutrition*, 127: 1198-1205.
110. Kelley DS, Branch LB, Love JE, Taylor PC, Rivera YM & Iacono JM (1991). Dietary alpha-linolenic acid and immunocompetence in humans. *American Journal of Clinical Nutrition*, 53: 40-46.
111. Hinds A & Sanders TAB (1993). The effect of increasing levels of dietary fish oil rich in eicosapentaenoic and docosahexaenoic acids on lymphocyte phospholipid fatty acid composition and cell-mediated immunity in the mouse. *British Journal of Nutrition*, 69: 423-429.
112. Feogh ML, Alijani MR, Helfrich GB, Khirabadi BS & Ramwell PW (1986). Fatty acids and eicosanoids in organ transplantation. *Progress in Lipid Research*, 25: 567-572.
113. Otto DA, Kahn DR, Hamm MW, Forrest DE & Wooten JT (1990). Improved survival of heterotopic cardiac allografts in rats with dietary n-3 polyunsaturated fatty acids. *Transplantation*, 50: 193-198.
114. Grimminger F, Grimm H, Fuhrer D, Papavassilis C, Lindemann G, Blecher C, Mayer K, Tabesch F, Kramer HJ, Stevens J & Seeger W (1996). Omega 3 lipid infusion in a heart allotransplant model: shift in fatty acid and lipid mediator profiles and prolongation of transplant survival. *Circulation*, 93: 365-371.
115. Linn K, Lux W, Woehrl M, Zekorn T, Hering B, Bretzel R & Federlin K (1990). Fish oil diet prevents insulinitis in transplanted islets of Langerhans. *Transplantation Proceedings*, 22: 871-872.
116. Prickett JD, Robinson DR & Steinberg AD (1982). Effects of dietary enrichment with eicosapentaenoic acid upon autoimmune nephritis in female NZB x NZW/F1 mice. *Arthritis and Rheumatism*, 26: 133-139.
117. Robinson DR, Prickett JD, Polisson R, Steinberg AD & Levine L (1985). The protective effect of dietary fish oil on murine lupus. *Prostaglandins*, 30: 51-75.
118. Robinson DR, Prickett JD, Makoul GT, Steinberg AD & Colvin RB (1986). Dietary fish oil reduces progression of established renal disease in (NZB x NZW)F1 mice and delays renal disease in BXSB and MRL/1 strains. *Arthritis and Rheumatism*, 29: 539-546.
119. Robinson DR, Xu L, Tateno S, Guo M & Colvin RB (1993). Suppression of autoimmune disease by dietary n-3 fatty acids. *Journal of Lipid Research*, 34: 1435-1444.
120. Leslie CA, Conte JM, Hayes KC & Cathcart ES (1988). A fish oil diet reduces the severity of collagen induced arthritis after onset of disease. *Clinical and Experimental Immunology*, 73: 328-332.
121. Cathcart ES & Gonnerman WA (1991). Fish oil fatty acids and experimental ar-

- thritis. *Rheumatoid Disease Clinics of North America*, 17: 235-242.
122. Wallace JL, Keenan CM & Finn NJ (1989). Anti-inflammatory effects of a fish oil diet in a rat model of chronic colitis. *Gastroenterology*, 96: 535 (Abstract).
  123. Vilaseca J, Salas A, Guarner F, Rodriguez R, Martinez M & Malagelada J-R (1990). Dietary fish oil reduces progression of chronic inflammatory lesions in a rat model of granulomatous colitis. *Gut*, 31: 539-544.
  124. Empey LR, Jewell LD, Garg ML, Thomson AB, Clandinin MT & Fedorak RN (1991). Fish oil-enriched diet is mucosal protective against acetic acid-induced colitis in rats. *Canadian Journal of Physiology and Pharmacology*, 69: 480-487.
  125. Guarner F, Vilaseca J & Malagelada JR (1992). Dietary manipulation in experimental inflammatory bowel disease. *Agents and Actions*, Special number: C10-C14.
  126. Dyerberg J, Bang HO & Hjorne N (1975). Fatty acid composition of the plasma lipids in Greenland Eskimos. *American Journal of Clinical Nutrition*, 28: 958-966.
  127. Kromann N & Green A (1980). Epidemiological studies in the Upernavik District, Greenland. *Acta Medica Scandinavica*, 208: 401-406.
  128. Kromhout D, Bosschieter EB & de Lezenne Coulander C (1985). The inverse relationship between fish consumption and 20-year mortality from coronary heart disease. *New England Journal of Medicine*, 312: 1205-1208.
  129. Belch JJJ (1990). Essential fatty acids and rheumatoid arthritis. In: Horrobin DF (Editor), *Omega-6 Essential Fatty Acids: Pathophysiology and Roles in Clinical Medicine*. Wiley-Liss, New York, 223-237.
  130. Kremer JM (1993). Studies on the effects of omega-3 fatty acids in the treatment of patients with rheumatoid arthritis. In: Drevon CA, Baksaas I & Krokan HE (Editors), *Omega-3 Fatty Acids: Metabolism and Biological Effects*. Birkhäuser, Basel, 271-283.
  131. Soyland E & Drevon CA (1993). The effect of very long-chain n-3 fatty acids on immune-related skin diseases. *European Journal of Clinical Nutrition*, 47: 381-388.
  132. Grimminger F & Maysen P (1995). Lipid mediators, free fatty acids and psoriasis. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 52: 1-15.
  133. Bates D (1989). Lipids and multiple sclerosis. *Biochemical Society Transactions*, 17: 289-291.
  134. Burton JL (1990). Essential fatty acids in atopic eczema: clinical studies. In: Horrobin DF (Editor), *Omega-6 Essential Fatty Acids: Pathophysiology and Roles in Clinical Medicine*. Wiley-Liss, New York, 67-73.
  135. Berthoux FC, Guerin C, Berthoux P, Burgard G & Alamartine E (1992). One-year randomized controlled trial with omega-3 fatty acid-rich fish oil in clinical renal transplant. *Transplantation Proceedings*, 24: 2578-2582.
  136. Homan van der Heide JJ, Bilo HJG, Donker JM, Wilmink JM & Tegzess AM (1993). Effect of dietary fish oil on renal function and rejection in cyclosporine-treated recipients of renal transplants. *New England Journal of Medicine*, 329: 769-773.
  137. Maachi K, Berthoux P, Burgard G, Alamartine E & Berthoux F (1995). Results of a 1-year randomized controlled trial with omega-3 fatty acid fish oil in renal transplantation under triple immunosuppressive therapy. *Transplantation Proceedings*, 27: 846-849.
  138. Bennett WM, Carpenter CB, Shapiro ME, Strom TB, Hefty D, Tillman M, Abrams J, Ryan D & Kelley VA (1995). Delayed omega-3 fatty acid supplements in renal transplantation. *Transplantation*, 59: 352-356.
  139. 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.
  140. 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.
  141. 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.
  142. Ailhaud G, Amri E-Z & Grimaldi P-A (1995). Fatty acids and adipose cell differentiation. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 48: 113-115.
  143. Berthou L, Saladin R, Yaqoob P, Branellec D, Calder P, Fruchart J-C, Deneffe 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.
  144. Chandrasekar B & Fernandes G (1994). Decreased pro-inflammatory cytokines and increased antioxidant enzyme gene expression by  $\omega$ -3 lipids in murine lupus nephritis. *Biochemical and Biophysical Research Communications*, 200: 893-898.
  145. 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-1 $\beta$  gene expression. *Lipids*, 31: S23-S31.
  146. 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.
  147. 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.
  148. Marignani PA & Sebaldt RJ (1995). Formation of a second messenger diacylglycerol in murine peritoneal macrophages is altered after *in vivo* (n-3) polyunsaturated fatty acid supplementation. *Journal of Nutrition*, 125: 3030-3040.
  149. 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.
  150. 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.
  151. 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.
  152. 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.
  153. 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.
154. 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.
  155. May CL, Southworth AJ & Calder PC (1993). Inhibition of lymphocyte protein kinase C by unsaturated fatty acids. *Biochemical and Biophysical Research Communications*, 195: 823-828.
  156. Tappia PS, Man WJ & Grimbale 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.
  157. van Meter AR, Ehringer WD, Stillwell W, Blumenthal EJ & Janski LJ (1994). Aged lymphocyte proliferation following incorporation and retention of dietary omega-3 fatty acids. *Mechanisms of Ageing and Development*, 75: 95-114.
  158. Richieri GV & Kleinfeld AM (1989). Free fatty acid perturbation of transmembrane signalling in cytotoxic T lymphocytes. *Journal of Immunology*, 143: 2302-2310.
  159. 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.
  160. 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.
  161. Kopp EB & Ghosh S (1995). NF- $\kappa$ B and related proteins in innate immunity. *Advances in Immunology*, 58: 1-27.
  162. Forman BM, Chen J & Evans RM (1997). Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors  $\alpha$  and  $\delta$ . *Proceedings of the National Academy of Sciences*, 94: 4312-4317.
  163. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ & Wahli W (1996). The PPAR $\alpha$ -leukotriene B4 pathway to inflammation control. *Nature*, 384: 39-43.
  164. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM & Evans RM (1995). 15-Deoxy-D12,14 prostaglandin J2 is a ligand for the adipocyte determination factor PPAR $\gamma$ . *Cell*, 83: 803-812.
  165. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC & Lehmann JM (1995). A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor  $\gamma$  and promotes adipocyte differentiation. *Cell*, 83: 813-819.
  166. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM & Lehmann JM (1997). Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors  $\alpha$  and  $\gamma$ . *Proceedings of the National Academy of Sciences*, 94: 4318-4323.
  167. Braissant O, Fougelle F, Scotto C, Dauca M & Wahli W (1996). Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR- $\alpha$ , - $\beta$ , and - $\gamma$  in the adult rat. *Endocrinology*, 137: 354-366.
  168. 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*, 91: 7355-7359.
  169. Nunez EA (1993). Free fatty acids as modulators of the steroid hormone message. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 48: 63-70.
  170. Nunez EA, Haourigui M, Martin ME & Benassayag C (1995). Fatty acids and steroid hormone action. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 52: 185-190.
  171. 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.
  172. Yaqoob P & Calder PC (1996). Dietary lipids affect the sensitivity of rat thymic lymphocytes to glucocorticoids. *Proceedings of the Nutrition Society*, 55: 160 (Abstract).