The Effect of Dietary Fatty Acids on the Immune Response and Susceptibility to Infection

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All mammals can synthesize fatty acids de novo from acetyl coenzyme A. The end product of the fatty acid synthetase enzyme is palmitic acid (16:0), which can be elongated to stearic acid (18:0). Little need is seen for the synthesis of saturated fatty acids in individuals in the Western world as the diet normally supplies adequate amounts. However, cell membranes require unsaturated fatty acids to maintain their structure, fluidity, and function. Therefore, a mechanism, termed desaturation, exists for the introduction of double bonds into acetyl chains. The introduction of a single double bond between carbon atoms 9 and 10 is catalyzed by the enzyme Δ⁹-desaturase, which is universally present in both plants and animals. This enzyme results in the conversion of stearic acid to oleic acid (18:1n-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 Δ¹²-desaturase converts oleic acid into linoleic acid (18:2n-6), whereas a Δ¹⁵-desaturase converts linoleic acid into α-linolenic acid (18:3n-3). Using the pathway outlined in Figure 1, animal cells can convert dietary α-linolenic acid into eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3); by a similar series of reactions dietary linoleic acid is converted via 7-linolenic (18:3n-6) and dihomo-7-linolenic (20:3n-6) acids to arachidonic acid (20:4n-6). Because their tissues do not contain the Δ¹²- or Δ¹⁵-desaturases, animals are unable to interconvert the n-9, n-6, and n-3 families of polyunsaturated fatty acids (PUFA). Many marine plants, especially the unicellular algae in phytoplankton, also carry out chain elongation and further desaturation of α-linolenic acid to yield the long chain n-3 PUFA EPA and DHA. It is the formation of these long chain n-3 PUFA by marine algae and their transfer through the food chain to fish that accounts for
FIG. 1. Metabolism of polyunsaturated fatty acids. $\Delta^6$, $\Delta^9$, $\Delta^{12}$, and $\Delta^{15}$, desaturase enzymes; COX, cyclo-oxygenase; LOX, lipoxygenase enzymes; PG, prostaglandins; LT, leukotrienes; TX, thromboxanes; LX, lipoxins; HETE, hydroxyeicosatetraenoic acids; HPETE, hydroperoxyeicosatetraenoic acids.
EFFECT OF DIETARY FATTY ACIDS

TABLE 1. Principal fatty acids in various dietary fats and oils

<table>
<thead>
<tr>
<th>Fat or oil</th>
<th>Principal fatty acids</th>
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<tbody>
<tr>
<td>Lard</td>
<td>Palmitic (16:0) comprises 20% to 35% of fatty acids</td>
</tr>
<tr>
<td></td>
<td>Oleic (18:1n-9) comprises 35% to 65% of fatty acids</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>Palmitic comprises 20% to 37% of fatty acids</td>
</tr>
<tr>
<td></td>
<td>Oleic comprises 25% to 50% of fatty acids</td>
</tr>
<tr>
<td>Coconut oil</td>
<td>Medium chain saturated [capric (10:0), lauric (12:0), myristic (14:0)] comprise 65% to 80% of fatty acids</td>
</tr>
<tr>
<td>Palm oil</td>
<td>Palmitic comprises 40% to 50% of fatty acids</td>
</tr>
<tr>
<td></td>
<td>Oleic comprises 35% to 45% of fatty acids</td>
</tr>
<tr>
<td>Olive oil</td>
<td>Oleic comprises 55% to 85% of fatty acids</td>
</tr>
<tr>
<td>Maize oil</td>
<td>Linoleic (18:2n-6) comprises 40% to 65% of fatty acids</td>
</tr>
<tr>
<td>Sunflower oil</td>
<td>Linoleic comprises 50% to 75% of fatty acids</td>
</tr>
<tr>
<td>Safflower oil</td>
<td>Linoleic comprises 65% to 85% of fatty acids</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>Linoleic comprises 50% to 60% of fatty acids; α-linolenic (18:3n-3) comprises 5% to 10% of fatty acids</td>
</tr>
<tr>
<td>Evening primrose oil</td>
<td>Linoleic comprises 70% of fatty acids; γ-linolenic (18:3n-6) comprises 5% to 10% of fatty acids</td>
</tr>
<tr>
<td>Flaxseed oil</td>
<td>α-Linolenic comprises 35 to 65% of fatty acids</td>
</tr>
<tr>
<td>Fish (e.g., menhaden) oil</td>
<td>EPA (20:5n-3) comprises 10% to 15% of fatty acids</td>
</tr>
<tr>
<td></td>
<td>DHA (22:6n-3) comprises 5% to 12% of fatty acids</td>
</tr>
</tbody>
</table>

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

their abundance in some marine fish oils. Table 1 lists the principal fatty acids found in various fats and oils that have been used experimentally.

Interest in the effects of fatty acids and dietary lipids on the immune system dates back many years, as reviewed by Meade and Mertin (1), but this interest has intensified with the elucidation of the roles of eicosanoids derived from arachidonic acid in modulating inflammation and immunity (2,3) and with the knowledge that the metabolism of arachidonic acid to yield these mediators can be inhibited by EPA and DHA (4,5). Despite its long history, the field remains a controversial one. Many cell culture and animal feeding experiments have been performed but relatively few good studies have been done in humans. Nevertheless, some key points have emerged:

- Essential fatty acid deficiency impairs cell-mediated immunity
- Cell-mediated immunity decreases as the fat content of the diet increases
- Within a high fat diet, different fatty acids can exert different effects

Various aspects of lipids and immunity have been reviewed (5–21) and this chapter will focus on more recent studies involving mononuclear cells (monocytes, macrophages, lymphocytes) and their functions ex vivo and in vivo. It will not cover in detail in vitro studies involving culture of isolated cells with purified fatty acids or fatty acid-containing lipids; these have been covered extensively elsewhere (7,9–11,16,20,21). Likewise, it will not deal with possible mechanisms of action of fatty acids in detail; these have also been discussed elsewhere (11,17,19).
WHY SHOULD FATTY ACIDS EXERT EFFECTS WITHIN THE IMMUNE SYSTEM?

Various reasons are found to expect that different fatty acids might exert different effects within the immune system (Fig. 2). First, the fatty acid composition of membrane phospholipids changes according to the fatty acid composition of the diet, with some evidence indicating that this can alter the physical characteristics of the membrane (e.g., its fluidity). This, in turn, can affect the functioning of some of the proteins within the membranes (e.g., receptors, ion channels, and enzymes) that will alter the ability of the cell to respond to stimuli. Second, the phospholipids of the cell membrane are used to generate certain signaling molecules when an immune cell is stimulated and evidence suggests that altering the fatty acid composition of the phospholipids can alter the concentration and biological potency of these second messengers. Third, these signaling molecules include some such as prostaglandins and leukotrienes that are formed directly from arachidonic acid in the cell membrane; thus, lowering the concentration of this fatty acid, as happens when the level of n-3 PUFA in the diet is increased, will decrease the capacity to form these important immunoregulatory molecules. Fourth, it is now suspected that particular fatty acids act directly as signaling molecules within cells and so can directly influence their function. Finally, incorporation of PUFA into cells can alter the cellular redox status, so affecting cell function.
IMMUNE CELL FATTY ACID COMPOSITION AND EICOSANOID PRODUCTION

Modification of the fatty acid composition of immune cells such as macrophages and lymphocytes is readily achieved in culture simply by changing the fatty acid composition of the medium. Culture of these cells with n-3 PUFA results in replacement of arachidonic acid in phospholipids by the n-3 PUFA provided (22,23). Changing the fatty acid composition of the diet also results in significant modification of the fatty acid composition of macrophages (24,25) and lymphocytes (25–27). Again, one key change is the replacement of arachidonic acid by n-3 PUFA provided in the diet (Table 2).

Eicosanoids are a family of oxygenated derivatives of dihomo-γ-linolenic, arachidonic, and eicosapentaenoic acids. Eicosanoids include prostaglandins (PG), thromboxanes, leukotrienes (LT), lipoxins, hydroperoxy-eicosatetraenoic acids, and hydroxyeicosatetraenoic acids. In most conditions, the principal precursor for these compounds is arachidonic acid (Fig. 1). Prostaglandins (PG) are involved in modulating the intensity and duration of inflammatory and immune responses. PGE\(_2\) has various proinflammatory effects: inducing fever and erythema; increasing vascular permeability and vasodilatation; and enhancing pain and edema caused by other agents such as bradykinin and histamine. In chronic inflammatory conditions, increased rates of PGE\(_2\) production are observed and increased PGE\(_2\) production has been observed in patients suffering from infections, burns, sepsis, and other traumas. Some leukotrienes act as chemoattractants, promoting movement of leukocytes to sites of inflammatory or immune activity, whereas others are involved in causing smooth muscle contraction and so have been implicated in diseases such as asthma where there is severe constriction of the airways.

### TABLE 2. Fatty acid composition of spleen leukocytes taken from rats fed diets containing different levels of long chain PUFA*

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>GLA</th>
<th>ARA</th>
<th>EPA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>24.8 ± 1.7</td>
<td>29.6 ± 1.1</td>
<td>26.6 ± 1.6</td>
<td>29.4 ± 0.5</td>
<td>27.1 ± 1.8</td>
</tr>
<tr>
<td>18:0</td>
<td>15.8 ± 1.4</td>
<td>18.3 ± 0.9</td>
<td>18.5 ± 1.0</td>
<td>18.1 ± 0.5</td>
<td>18.2 ± 1.0</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>10.9 ± 0.3</td>
<td>12.1 ± 0.2</td>
<td>11.1 ± 0.7</td>
<td>12.5 ± 0.3</td>
<td>12.2 ± 0.7</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>11.9 ± 0.8</td>
<td>10.7 ± 0.1</td>
<td>7.9 ± 0.2</td>
<td>14.6 ± 1.7</td>
<td>16.4 ± 1.2</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>0.8 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>16.2 ± 0.5</td>
<td>18.7 ± 0.7</td>
<td>23.5 ± 1.0</td>
<td>11.6 ± 0.5</td>
<td>12.8 ± 0.4</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.20 ± 0.06</td>
<td>0.8 ± 0.5</td>
<td>0.26 ± 0.01</td>
<td>2.9 ± 0.2</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>0.7 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>1.3 ± 0.4</td>
<td>3.8 ± 0.3</td>
</tr>
</tbody>
</table>

* Rats were fed for 6 weeks on a control diet (178 g fat/kg containing 31 g linoleic acid and 4.4 g α-linolenic acid/100 g total fatty acids) or on diets that replaced 4.4 g of linoleic acid with either γ-linolenic acid (GLA) or arachidonic acid (ARA) or which replaced 4.4 g α-linolenic acid with eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA). Spleen leukocytes were prepared and their fatty acid composition determined. Data are mean ± SEM.

PUFA, polyunsaturated fatty acids.

From Peterson LD et al. (27).
FIG. 3. Effects of dietary lipids on PGE\(_2\) production. Mice were fed on a low fat (25 g/kg maize oil) diet or on diets containing 200 g/kg of either hydrogenated coconut oil, olive oil, safflower oil, or fish oil. Thioglycollate-elicited peritoneal macrophages were prepared and cultured with lipopolysaccharide (10 \(\mu\)g/ml) for 24 hours. PGE\(_2\) concentrations were determined by enzyme-linked immunosorbent assay. Data are means; error bars = SEM. From Yaqoob P, Calder PC (30).

Not only do EPA and DHA replace arachidonic acid in cell membranes (Table 2), but they can competitively inhibit the oxygenation of arachidonic acid by cyclo-oxygenase. Thus, ingestion of n-3 PUFA results in a decreased capacity of cells to synthesize eicosanoids from arachidonic acid (24,25,27–31) (Fig. 3). In addition, EPA (but not DHA) is able to act as a substrate for both cyclo-oxygenase and 5-lipoxygenase (Fig. 1). Thus, the suppression of the production of arachidonic acid-derived eicosanoids is mirrored by an increase in the production of EPA-derived eicosanoids (32), such as the 3-series prostaglandins and thromboxanes and the 5-series leukotrienes. The eicosanoids produced from EPA are often less biologically potent than the analogues synthesized from arachidonic acid. For example, LTB\(_5\) is 10 times less potent than LTB\(_4\) as a neutrophil chemoattractant and PGE\(_3\) is less proinflammatory than PGE\(_2\). Thus, fish oil consumption is accompanied by a move toward the production of less inflammatory eicosanoids than those produced from arachidonic acid.

CHEMOTAXIS, PHAGOCYTOSIS, AND THE PRODUCTION OF REACTIVE OXYGEN AND NITROGEN SPECIES

Chemotaxis

Chemotaxis of blood monocytes toward the chemoattractants LTB\(_4\) and formylmethionyl leucylphenylalanine was suppressed following supplementation of the hu-
man diet with approximately 5.5 g EPA plus DHA for 6 weeks (28,29,33). A recent study reported no effect of a lower dose of n-3 PUFA (0.65 g/d for 12 weeks) on monocyte chemotaxis toward pooled human serum (34).

**Phagocytosis**

It is likely that the process of phagocytosis will be influenced by membrane structure, in particular by the fluidity of the membrane; the latter property can be influenced by the fatty acid composition of membrane phospholipids. The phagocytic capacity of macrophages and monocytes is altered by the fatty acid composition of the medium in which they are cultured (21,22,35). A positive correlation is found between phagocytosis and percent PUFA, the ratio of unsaturated to saturated fatty acids, and the index of unsaturation of the cellular phospholipids (22).

Reports have shown that dietary fish oil does not affect phagocytosis of sheep erythrocytes or yeast particles by murine thioglycollate-elicited macrophages (36), or of latex beads by porcine alveolar macrophages (37). However, Eicher and McVey (38) found that fish oil feeding reduced the ability of murine Kupffer cells to phagocytose *Salmonella typhimurium*, although this was not associated with a reduced capacity of the cells to kill the bacteria. If the Kupffer cells were taken from mice infected with *S. typhimurium*, no apparent effect was seen of previous diet on either phagocytosis or bacterial killing. These observations contrast with those of D’Ambola et al. (39), who showed that fish oil given by gastric tube significantly diminished the ability of neonatal rabbits to clear a challenge of *Staphylococcus aureus*. A recent human study indicated no effect of 3.8 g EPA or DHA daily for 7 weeks on phagocytosis of opsonized or unopsonized *Escherichia coli* by human monocytes (40).

**Production of Reactive Oxygen Species and Nitric Oxide**

The enzymes that result in the synthesis of superoxide, hydrogen peroxide, and nitric oxide are regulated by eicosanoids, cytokines, and protein kinase C. As n-3 PUFA affect the production of eicosanoids (see above) and cytokines (see below) and might modulate protein kinase C activity (19), they might affect the production of reactive oxygen species and nitric oxide by macrophages and so regulate the cytotoxic activities of these cells. However, investigations of the effects of diets rich in n-3 PUFA on the production of hydrogen peroxide, superoxide, and nitric oxide have yielded contradictory results. Studies have reported that production of one or more of these mediators is enhanced, diminished, or not affected following fish oil feeding to laboratory animals (21). Likewise n-3 PUFA supplementation of the human diet has been reported to decrease zymosan-induced superoxide production by monocytes (41) but not to affect that induced by *E. coli* (40). The reasons for such significantly different experimental observations might include the different species of origin of the cells studied, the anatomic site of origin of the cells, the state of cellular differentiation, the state of activation of the cell, the stimulus used to elicit mediator pro-
duction, the nature of the culture conditions used (presence or absence of serum, serum source, time of culture), the level of n-3 PUFA in the diet, the duration of feeding, the level of antioxidants in the diet, and so on.

**EXPRESSION OF SURFACE MARKERS**

**Major Histocompatibility Antigens**

Incubation with DHA inhibited interleukin (IL)-4- or γ-interferon–induced cell surface expression of major histocompatibility class II antigen (MHC II; in the mouse, these antigens are termed Ia) on mouse peritoneal macrophages (42); DHA was more inhibitory than EPA and other 20-carbon fatty acids and acted by inhibiting the increase in Ia mRNA, which occurs after stimulation of macrophages with cytokines (42). Hughes et al. (43) examined the effect of incubation of purified human monocytes with either EPA or DHA on expression of MHC II, which is termed human leukocyte antigen (HLA): both EPA and DHA reduced the proportion of HLA-DR or HLA-DP positive monocytes following incubation with γ-interferon and the level of expression of these molecules on the monocyte surface (43). In accordance with this, the ability of monocytes cultured with EPA and DHA to present antigen (tetanus toxoid) to autologous lymphocytes was diminished (44).

Inclusion of EPA plus DHA in the diet of mice or rats results in a diminished percentage of peritoneal exudate cells bearing MHC II antigens on their surface and level of MHC II expression on positive cells (45). Feeding fish oil to rats decreased the level of MHC II expression on thioglycollate-elicited peritoneal macrophages (46) and on dendritic cells obtained by cannulation of the thoracic duct (47). In accordance with these animal studies, supplementation of the diet of human volunteers with 1.6 g EPA plus DHA for 3 weeks resulted in a decreased level of MHC II (HLA-DP, HLA-DQ, and HLA-DR) expression on the surface of peripheral blood monocytes (48).

These observations suggest that diets rich in fish oil will result in diminished antigen presentation. Indeed, feeding mice the ethyl ester of EPA for a period of 4 to 5 weeks resulted in diminished ex vivo presentation of antigen (keyhole limpet hemocyanin; KLH) by spleen cells (49). Compared with feeding a low fat diet or a diet containing 200 g/kg safflower oil, feeding rats a diet containing 200 g/kg fish oil significantly diminished ex vivo KLH presentation by dendritic cells obtained by cannulation of the thoracic duct to KLH-sensitized spleen lymphocytes (47).

**Adhesion Molecules**

Incubation of human lymphocytes with EPA or DHA decreased the level of expression of various adhesion molecules, including leukocyte function associated molecule-1 (LFA-1) and L-selectin, without affecting expression of very late antigen 1; arachidonic acid did not influence surface expression of these molecules (50). In accordance with the effects on expression of some cell surface adhesion molecules,
incubation of human lymphocytes with EPA or DHA reduced adhesion between the lymphocytes and untreated endothelial cells or endothelial cells stimulated by cytokine or bacterial lipopolysaccharide (LPS) (50). The proportion of purified human monocytes expressing intercellular adhesion molecule-1 (ICAM-1) and the level of ICAM-1 expression were reduced by incubation with EPA; DHA did not affect ICAM-1 or LFA-1 expression on resting monocytes (43). Both EPA and DHA reduced the proportion of ICAM-1 positive monocytes and the level of ICAM-1 expression following incubation with γ-interferon (43). DHA also reduced the expression of LFA-1 on γ-interferon–stimulated monocytes.

Feeding rats a fish oil rich diet caused a 30% reduction in the level of expression of CD18 (the β chain of LFA-1) on thioglycollate-elicited peritoneal macrophages (46), and significantly reduced levels of expression of CD2, CD11a, ICAM-1, CD18, CD44, and L-selectin on the surface of lymphocytes in various states of activation (51–53). Diets rich in evening primrose oil or olive oil also decreased the expression of some lymphocyte adhesion molecules (51–53). The fish oil or olive oil diets decreased the adhesion of both freshly prepared and concanavalin A-stimulated lymphocytes to macrophage monolayers or untreated endothelial cells (53). Furthermore, the fish oil diet resulted in a 50% reduction in concanavalin A-stimulated lymphocyte adhesion to endothelial cells stimulated by tumor necrosis factor-α (TNF-α) (53). Supplementation of the human diet with 1.6 g EPA plus DHA daily resulted in significantly lower levels of expression of ICAM-1 and LFA-1 on peripheral blood monocytes (48). These studies show that dietary lipids affect the expression of functionally important adhesion molecules on the surface of various cells of the immune system (and also on endothelial cells) (54). Furthermore, the study of Sanderson and Calder (53) suggests that such diet-induced effects on adhesion molecule expression alter the ability of leukocytes to bind to one another and suggests that fish oil feeding will affect the movement of leukocytes between body compartments and perhaps into sites of inflammatory or immune activity.

In accordance with the effect of olive oil feeding to rats, Yaqoob et al. (55) have shown that increasing the proportion of oleic acid in the human diet, at the expense of saturated fatty acids, significantly decreased (by 20%) the proportion of blood mononuclear cells expressing ICAM-1; the proportion of mononuclear cells expressing CD11b also declined but this was not statistically significant.

Cytokine Receptors

Feeding rats high fat diets rich in olive oil, evening primrose oil, or fish oil decreased the proportion of lymphocytes bearing the interleukin-2 (IL-2) receptor following concanavalin A stimulation (56) and lowered the level of IL-2 receptor on these cells (51). Jolly et al. (57) recently showed that feeding mice a diet rich in EPA or DHA significantly decreased the appearance of mRNA for the alpha subunit of the IL-2 receptor following concanavalin A stimulation, suggesting that these fatty acids regulate expression of the IL-2 receptor gene. Supplementation of the diet of patients with psoriasis or atopic dermatitis with 6 g EPA plus DHA ethyl esters daily caused a sig-
significant reduction in the percentage of IL-2 receptor-positive blood lymphocytes following phytohemagglutinin stimulation; the level of expression of the IL-2 receptor on the positive cells was also significantly reduced (58).

EFFECTS OF AMOUNT AND TYPE OF FAT IN THE DIET ON LYMPHOCYTE PROLIFERATION

Amount of Fat in the Diet

Many studies have compared the effects of feeding laboratory animals low and high fat diets on lymphocyte proliferation (7,9,16,20). Such studies have often found that high fat diets result in diminished \textit{ex vivo} lymphocyte proliferation compared with low fat diets, but the precise effect depends on the level of fat used in the high fat diet and its source. A reduction in total dietary fat intake (from 40\% to 25\% of total energy) resulted in greatly enhanced human blood lymphocyte proliferation in response to concanavalin A or phytohemagglutinin (59,60), suggesting that high fat diets suppress human lymphocyte proliferation.

Saturated Fatty Acids

High fat diets using lard, beef tallow, palm oil, or hydrogenated coconut oil as the source of fat have been used in many animal studies of lymphocyte proliferation; in some studies, the high saturated fat diet has been compared, along with other high fat diets, to a low fat control, whereas in other studies the high saturated fat diet appears to serve as a high fat control to which the effects of PUFA-rich diets were compared (7,9,16,20). As a result, the precise nature of the effects of high saturated fat diets is difficult to gauge. Some studies have revealed that high saturated fat diets do not affect lymphocyte proliferation compared with feeding low fat diets, whereas others have shown that they are suppressive but less so than PUFA-rich diets. A third series of studies in which saturated fatty acids are used as a comparison with PUFA-rich diets simply shows that they have different effects from the PUFA-rich diets; whether one diet is without effect and the other inhibits the lymphocyte response or whether one is without effect and the other enhances the lymphocyte response is often unclear (7,9,16,20).

Only one study has compared the effects of different individual saturated fatty acids on lymphocyte proliferation (61). In this study, diets containing 178 g/kg fat were fed to rats; the diets differed according to the principal saturated fatty acids they contained (medium chain, lauric, palmitic, or stearic) and according to the position of the palmitic acid on the dietary triacylglycerols (sn-1(3) or sn-2) but the levels of total saturated, oleic, polyunsaturated, linoleic, and \(\alpha\)-linolenic acids were identical. Spleen lymphocyte proliferation in response to concanavalin A was enhanced if the animals were fed the diet with palmitic acid at the sn-2 position of dietary triacylglycerols compared with feeding the other diets (61).
EFFECT OF DIETARY FATTY ACIDS

Oleic Acid

Berger et al. (62) reported that a 100 g/kg olive oil diet did not affect concanavalin A-stimulated proliferation of spleen lymphocytes; that study cultured the lymphocytes in fetal calf serum. In contrast, feeding rats a 200 g/kg olive oil diet resulted in diminished ex vivo lymphocyte proliferation when the cells were cultured in autologous serum (but not when they were cultured in fetal calf serum) (56), a finding confirmed using a 200 g/kg oleic acid rich sunflower oil diet (63). A significant inverse relationship was found between the proliferation of spleen lymphocytes in response to concanavalin A and the ratio of oleic acid to linoleic acid in the diet (64). One study of the effect of dietary intervention with oleic acid on human lymphocyte proliferation has been performed (55); after 2 months, a trend developed toward reduced proliferative responses to concanavalin A of whole blood cultures and of isolated blood lymphocytes, but the effect of diet was not statistically significant.

Linoleic Acid-Rich Oils

Several studies have reported lower concanavalin A- or phytohemagglutinin-stimulated T-lymphocyte proliferation following the feeding of diets rich in maize or safflower oils to laboratory rodents, compared with feeding diets rich in saturated fatty acids (7,9,16,20). In contrast, some studies have reported no effect of feeding linoleic acid rich diets on rodent T-lymphocyte proliferation (7,9,16,20). However, it is now apparent that the outcome of such measures of lymphocyte function is strongly influenced by the conditions used to culture the cells ex vivo (26,56) and this may account for the discrepancies in the literature. No difference was observed in the responses to T-cell mitogens of human blood lymphocytes from volunteers consuming low fat diets that were rich (12.9% of energy) or poor (3.5% of energy) in n-6 PUFA (59,60); the cells were cultured in fetal calf serum.

γ-Linolenic Acid

Rat lymph node and spleen lymphocyte proliferation in response to concanavalin A was decreased following the feeding of a diet rich in evening primrose oil, which provided approximately 7 g γ-linolenic acid/100 g total fatty acids (51,56). Inclusion of 4.4 g γ-linolenic acid/100 g fatty acids in the rat diet did not significantly affect concanavalin A-stimulated spleen lymphocyte proliferation (27).

α-Linolenic Acid

Feeding rats diets containing large amounts of flaxseed oil (rich in α-linolenic acid) suppressed spleen T-lymphocyte proliferation compared with feeding diets rich in hydrogenated coconut oil (65) or sunflower oil (66). Similarly, feeding chickens a flaxseed oil-rich diet suppressed spleen lymphocyte proliferation compared with feeding diets rich in canola or maize oils or lard (67). In a recent study, rats were fed diets
containing 178 g fat/kg but differing in PUFA content (17.5 or 35 g/100 g fatty acids) and linoleic acid to α-linolenic acid ratio (ratios of 100,20,10,5,1 were used); the PUFA content was altered by replacing a proportion of palmitic acid with linoleic and α-linolenic acids (68). Lymphocyte proliferation decreased as the linoleic acid to α-linolenic acid ratio of the low PUFA diet decreased; the linoleic acid to α-linolenic acid ratio of the high PUFA did not significantly affect lymphocyte proliferation (68). This study indicates that both linoleic and α-linolenic acids reduce lymphocyte proliferation, that the effect of the latter is dependent on the total PUFA content of the diet, and that α-linolenic acid is more potent than linoleic acid. Wu et al. (69) fed monkeys for 14 weeks on diets containing 3.5% or 5.3% of energy as α-linolenic acid (PUFA comprised 28 g/100 g total fatty acids and the n-6:n-3 PUFA ratios of the two diets were 1.0 and 0.5). Blood lymphocyte proliferation in response to concanavalin A or phytohemagglutinin was unaffected compared with the basal diet, which had an n-6:n-3 PUFA ratio of 36. The observations of Wu et al. (69) and Jeffery et al. (68) are in agreement: both studies indicate that replacing a proportion of linoleic acid with α-linolenic acid in a high PUFA diet has minimal effect on lymphocyte proliferation.

Arachidonic Acid

Feeding mice a diet containing 20 g safflower oil plus 10 g arachidonic acid/kg did not affect concanavalin A-stimulated spleen lymphocyte proliferation compared with feeding a diet containing safflower oil (30 g/kg) (70). Inclusion of 4.4 g arachidonic acid/100 g fatty acids in the rat diet did not significantly affect concanavalin A-stimulated spleen lymphocyte proliferation (27). These observations agree with the outcome of the first study of dietary arachidonic acid and human lymphocyte function: 1.5 g arachidonic acid daily for 50 days did not affect the proliferative response of blood lymphocytes to concanavalin A, phytohemagglutinin, or pokeweed mitogen (71).

Eicosapentaenoic and Docosahexaenoic Acids

Feeding diets rich in fish oil to rabbits, chickens, rats, or mice results in suppressed proliferation of T (and in some studies B) lymphocytes compared with feeding hydrogenated coconut, safflower, maize, or flaxseed oils or lard (51,56,67,72–74). A recent study indicates that inclusion of EPA plus DHA in the rat diet at levels of 4.4 or 6.6 g/100 g total fatty acids is sufficient to significantly reduce spleen lymphocyte proliferation in response to concanavalin A (by ~30%) (75). Supplementation of the diets of healthy women (51 to 68 years of age) with encapsulated EPA plus DHA (~2.4 g/d) resulted in a lowered mitogenic response of blood lymphocytes to phytohemagglutinin (76); the mitogenic response of lymphocytes from young women (21 to 33 years of age) supplemented with this level of EPA plus DHA was unaffected. More recently, a decreased response of blood lymphocytes to concanavalin A and phytohemagglutinin following supplementation of the diet of volunteers on a low fat, low cholesterol diet with 1.23 g EPA plus DHA/d was reported (77).
These studies do not indicate whether the suppressive effect of fish oil feeding on lymphocyte proliferation is caused by EPA or DHA, or both; nor is there any indication of the level of long chain \( n-3 \) PUFA required to affect lymphocyte proliferation. These questions have recently been addressed (27,70,78). Feeding mice diets containing 20 g safflower oil plus 10 g of either EPA or DHA/kg reduced concanavalin A-stimulated spleen lymphocyte proliferation compared with feeding a diet containing 30 g/kg safflower oil (70); both \( n-3 \) PUFA were equipotent and reduced proliferation by approximately 80%. Peterson et al. (27) fed rats diets containing 178 g/kg fat and replaced \( \alpha \)-linolenic acid (4.4 g/100 g fatty acids) with either EPA or DHA while keeping the total PUFA content and \( n-6:n-3 \) PUFA ratio of the diet constant. Both EPA and DHA reduced lymphocyte proliferation to the same extent (~30% to 35%) compared with the diet containing \( \alpha \)-linolenic acid. Thus, these studies suggest that both EPA and DHA result in inhibition of lymphocyte proliferation and that relatively low levels are able to exert this effect, compared with the levels present in fish oil. Possible reasons for the quantitative differences in the effects between these studies are discussed elsewhere (27). Kelley et al. (78) reported that supplementation of the human diet with 6 g DHA daily for 90 days did not affect blood lymphocyte proliferation in response to concanavalin A or phytohemagglutinin. This observation suggests that DHA is less potent in humans than in rodents and that the effects of fish oil supplementation in humans (76,77) might result mainly from EPA.

Monkeys were fed for 14 weeks on diets containing 1.3% or 3.3% of energy as EPA plus DHA (PUFA comprised 30 g/100 g total fatty acids and the \( n-6:n-3 \) PUFA ratios of the two diets were 4.4 and 1.1): the proliferative response of blood lymphocytes to concanavalin A or phytohemagglutinin was enhanced (69). This observation is contradictory to previous observations in experimental animals (see earlier) and to previous observations in humans (76,77). The authors provide some evidence that this discrepancy is the result of better maintained levels of vitamin E in comparison with previous studies, thereby suggesting that long chain \( n-3 \) PUFA inhibit lymphocyte proliferation through a process which vitamin E protects against.

EFFECTS OF AMOUNT AND TYPE OF FAT IN THE DIET ON CYTOTOXIC ACTIONS OF LYMPHOCYTES

Cytotoxic T-Lymphocyte Activity

Cytotoxic T-lymphocyte activity is more reduced by feeding laboratory animals high fat diets rich in safflower oil, soybean oil, flaxseed oil, or fish oil than by feeding low fat diets or high fat diets rich in lard or hydrogenated coconut oil; fish oil appears to cause the greatest reduction in cytotoxic T-lymphocyte activity (7,9,16,20).

Natural Killer Cell Activity

A reduced fat intake (to less than 30% or 22% of total energy, respectively) is associated with a significant increase in natural killer (NK) cell activity of human blood
lymphocytes (79,80), suggesting that high fat consumption suppresses NK cell activity in humans. Subsequent supplementation of the diet with 15 g coconut or sunflower oil daily for 2 months reduced NK-cell activity to the prestudy level, both oils being equally effective (79).

Several early studies suggest little effect of high saturated fat or linoleic acid-rich diets on rodent NK-cell activity (7,9,16,20). Feeding a flaxseed oil-rich diet decreased rat spleen lymphocyte NK-cell activity compared with feeding a sunflower oil-rich diet (66). A high evening primrose oil diet also decreased NK-cell activity (51,81). Various studies have shown that feeding rats or mice on fish oil-rich diets results in suppressed spleen lymphocyte NK-cell activity compared with feeding low fat diets or high fat diets rich in saturated fat or n-6 PUFA (52,62,81,82); fish oil appears to be more suppressive than flaxseed oil (83). Although one study reports no effect of an olive oil-rich diet on ex vivo rat spleen NK-cell activity (62), diets containing 200 g/kg of olive oil or oleic acid-rich sunflower oil were found to significantly reduce this activity (52,63,81).

Recent animal studies have endeavored to establish the effects of particular discrete changes in dietary fatty acid composition on NK-cell activity. The type of saturated fatty acid in the rat diet has been reported to influence spleen NK-cell activity, which was greater if the animals had been fed a diet containing palmitic acid as the principal saturated fatty acid than if they had consumed diets rich in medium chain, lauric, or stearic acids (61). Another study reported a significant inverse linear correlation between the level of oleic acid or the ratio of oleic acid to linoleic acid in the rat diet and spleen lymphocyte NK-cell activity (64). One study of the effect of dietary intervention with oleic acid on human NK-cell activity has been performed (55). After 2 months of increased oleic acid intake at the expense of saturated fatty acids, a trend developed toward reduced NK-cell activity, but the effect of diet was not statistically significant (55). In another study, rats were fed diets containing 178 g/kg fat but differing in PUFA content (17.5 or 35 g/100 g fatty acids) and linoleic acid to α-linolenic acid ratio (ratios of 100,20,10,5,1 were used); the PUFA content was altered by replacing a proportion of palmitic acid with linoleic and α-linolenic acids (68). NK-cell activity decreased as the linoleic acid to α-linolenic acid ratio of the low PUFA diet decreased; the linoleic acid to α-linolenic acid ratio of the high PUFA had less impact on NK-cell activity (68). This study indicates that dietary α-linolenic acid reduces NK-cell activity but that its effect is dependent on the total PUFA content of the diet and its level relative to that of linoleic acid. Inclusion of 4.4 g arachidonic acid/100 g fatty acids in the rat diet did not affect NK-cell activity of spleen lymphocytes (27). Similarly, Kelley et al. (71) reported that 1.5 g arachidonic acid daily for 50 days did not affect the human NK-cell activity. Replacing α-linolenic acid (4.4 g/100 g fatty acids) with EPA in a high fat diet (with the total PUFA content and n-6:n-3 PUFA ratio of the diet kept constant) reduced rat spleen NK-cell activity, whereas replacing α-linolenic acid with DHA did not affect NK-cell activity (27). An intravenous injection of a triacylglycerol containing EPA into healthy human volunteers resulted in suppression of peripheral blood NK-cell activity 24 hours later (84). These observations suggest that the effects of fish oil on NK-cell activity might be caused mainly by EPA.
ANTIBODY PRODUCTION

Studies in Experimental Animals

Essential fatty acid deficiency impaired the ability of mice to produce IgG and IgM in response to sheep red blood cells (85); this response was restored by feeding diets containing 130, 500, or 700 g/kg maize oil (85). In contrast to this apparent enhancing effect of linoleic acid on antibody production, dietary linoleic acid was found to reduce the production of antibodies, including IgG and IgM, following antigenic challenges, compared with feeding low fat or high saturated fat diets (beef tallow, coconut oil) (86,87). Enhanced production of IgE to ovalbumin was reported in rats fed a diet high in fish oil compared with those fed a saturated fat diet (88). Total serum IgG and IgM levels were increased (two- and threefold, respectively) in mice fed fish oil compared with those fed maize oil (89).

Studies in Humans and Other Primates

Feeding cebus or squirrel monkeys on diets containing 143 g/kg coconut or maize oil for several years did not result in different antibody responses to measles vaccine (90). Kelley et al. (59,60) reported no effect on circulating IgM, IgG, IgE, or IgA levels of reducing total fat intake (from 40% to 25–30% of total energy) or of varying the amount of PUFA (3.5% or 12.9% of energy) in the human diet. Including flaxseed oil or salmon in the diet did not alter circulating antibody levels (91,92). Feeding healthy volunteers a diet containing 1.5 g arachidonic acid/d for 60 days did not alter serum antibody titers against three strains of the influenza virus (93).

CYTOKINE PRODUCTION

As cytokine production by macrophages is regulated by eicosanoids and as dietary lipids affect eicosanoid production (see earlier), it might be expected that dietary lipids, especially those containing n-3 PUFA, will affect cytokine production.

TNF, IL-1, and IL-6: Animal Studies

Several animal studies have investigated the effect of dietary lipids on ex vivo production of macrophage-derived cytokines, including TNF, IL-1, and IL-6. These studies have been reviewed in detail elsewhere (21). Several studies have reported that feeding rodents with n-3 PUFA-containing oils results in enhanced production of TNF by macrophages ex vivo, although decreased production or no effect following fish or flaxseed oil feeding has been reported (21). These differences between studies appear not to relate to species, duration of feeding, or type or amount of n-3 PUFA in the diet. However, some relationship may exist with the state of activation of the macrophages used. All studies that have used murine resident peritoneal macrophages, one study using rat resident alveolar macrophages and one study using rat resident peritoneal macrophages report an enhancing effect of n-3 PUFA on TNF
production (21); only one study that used rat resident peritoneal macrophages has reported reduced TNF production following fish oil feeding (21). The effect of dietary n-3 PUFA on TNF production by thioglycollate-elicited peritoneal macrophages is unclear, with studies reporting no effect, reduction, or enhancement (21). Comparison of the outcome of these studies is complicated by the different procedures used for ex vivo culture of the cells (21). The only animal study that has investigated TNF production by peripheral blood mononuclear cells showed decreased production following the infusion of a 10% (vol/vol) fish oil emulsion (94); this is an interesting observation because it agrees with the findings of several studies using human blood mononuclear cells (see below). Fish oil feeding also significantly reduced TNF-α production by cultures of murine splenocytes stimulated with LPS (89). In addition to studies measuring TNF production ex vivo, it was reported that feeding mice n-3 PUFA-rich diets resulted in reduced ability of elicited peritoneal macrophages to kill L929 cells (31,95); L929 cells are killed by TNF and so the reduced cytotoxicity of macrophages toward these cells suggests a reduced ability to produce TNF.

Some studies have investigated the effects of dietary lipids on circulating TNF levels that would reflect in vivo production of the cytokine. TNF levels were significantly higher in the plasma of LPS-injected mice fed diets containing 100 g/kg perilla oil than in the plasma of those fed 100 g/kg safflower oil (96). Similarly, higher serum TNF levels following intraperitoneal injection of LPS were reported in fish oil fed mice compared with those fed coconut or maize oil (97,98). In contrast, compared with safflower oil, coconut or fish oil feeding both reduced peak plasma TNF-α concentrations after intraperitoneal injection of LPS in mice (99). Furthermore, parenteral nutrition supplemented with fish oil reduced serum TNF-α concentrations in burned rats compared with n-6 PUFA rich parenteral nutrition (100).

All studies that 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 (30,101,102). Furthermore, fish oil feeding significantly reduced IL-1β production by cultures of murine splenocytes stimulated with LPS (89). Compared with safflower oil, coconut or fish oil feeding both reduced peak plasma IL-1β concentrations after intraperitoneal injection of LPS in mice (99). In contrast, one study reported that fish oil enhances IL-1 production by murine resident macrophages (103), whereas Blok et al. (104) report no difference in the concentrations of IL-1α or IL-1β in the medium of LPS-stimulated resident peritoneal macrophages taken from mice fed palm, maize, or fish oils. Ertel et al. (105) also showed no difference in IL-1 production by resident peritoneal macrophages taken from mice fed diets rich in maize, safflower, or fish oil.

No studies report the effect of dietary fatty acids on IL-6 production by resident peritoneal macrophages. One study using murine thioglycollate-elicited peritoneal macrophages showed a significant reduction in LPS-stimulated IL-6 production following fish oil feeding (30); production following stimulation of the cells with TNF was also significantly reduced (Yaqoob P and Calder PC. 1995, unpublished observations). Rat blood mononuclear cells showed reduced IL-6 production following fish oil infusion for 4 days (94). Compared with safflower oil, coconut or fish oil feeding
both reduced peak plasma IL-6 concentrations after intraperitoneal injection of LPS in mice (99). These studies are supported by the in vitro observation that EPA and DHA inhibit IL-6 production by rat thioglycollate-elicited peritoneal macrophages (106).

Despite the apparent contradictions in published reports regarding the effects on proinflammatory cytokine production of feeding animals with n-3 PUFA rich diets, some patterns do emerge. It appears that fish oil feeding enhances, or does not affect, TNF and IL-1 production by resident macrophages. In contrast, fish oil feeding reduces TNF, IL-1, and IL-6 production by thioglycollate-elicited macrophages and blood mononuclear cells. Fish oil feeding to mice lowered TNF-α mRNA levels in LPS-stimulated, thioglycollate-elicited murine macrophages (95), completely abolished the appearance of mRNA for IL-1β, IL-6, and TNF-α in the kidneys of autoimmune disease-prone mice (107), and significantly diminished IL-1β mRNA production by LPS or phorbol ester-stimulated spleen lymphocytes (108); the lower IL-1β mRNA level was not caused by accelerated degradation but by impaired synthesis. These studies suggest that n-3 PUFA affect proinflammatory cytokine production by control at the transcriptional level.

Infection with human immunodeficiency virus (HIV) alters cytokine profiles, increasing TNF and IL-1; TNF-α and IL-1β can stimulate HIV expression in infected cells and a correlation between the progression of acquired immunodeficiency syndrome (AIDS) and serum TNF concentration was observed in one study (89). Murine AIDS is also associated with increased production of TNF and IL-1 (89). Feeding fish oil to mice infected with murine AIDS significantly reduced TNF-α and IL-1β production by LPS-stimulated splenocytes compared with feeding maize oil (89). Fish oil was shown to increase survival of mice infected with murine AIDS significantly compared with maize oil (109).

IL-2, IL-4, γ-Interferon, and IL-10: Animal Studies

In contrast with the large number of studies of the effects of dietary lipids, especially fish oils, on the ex vivo production of macrophage-derived cytokines, relatively few studies have been done on lymphocyte-derived cytokines. Fish oil and flaxseed oil have been shown to reduce IL-2 production by pig alveolar lymphocytes (37). Feeding mice diets rich in either EPA or DHA diminished ex vivo IL-2 production by concanavalin A-stimulated spleen lymphocytes (70) by altering the kinetics of the appearance of mRNA for IL-2 (57). In contrast, inclusion of fish oil in the diet of autoimmune disease-prone mice resulted in raised levels of mRNA for IL-2, IL-4, and transforming growth factor-β in the spleen (110). One animal study that compared the effects of dietary lipids on the production of both Th1- (IL-2, γ-interferon) and Th2-derived cytokines (IL-4, IL-10) showed little effect of dietary lipids, including fish oil, in mice (74). This was confirmed recently by the lack of effect of fish oil compared with maize oil on IL-2 and γ-interferon production by murine splenocytes (89). In the study of Wu et al. in monkeys described earlier (69), IL-2 production was increased in the groups fed diets enriched with EPA plus DHA. Although this observation is contradictory to some studies in animals and humans (see below),
the investigators suggest that the difference reflects the level of vitamin E included in the monkey diets. Together, these studies suggest that different effects may be observed in different animal species and in different strains within a species, that fish oil might exert different effects on lymphocytes in healthy or diseased animals, and that an interaction may exist between fish oil and antioxidant vitamins.

### Human Studies

A number of studies have shown that supplementation of the diet of healthy humans with between 1.1 and 5 g EPA plus DHA daily for several weeks (up to 24) leads to a significant reduction in \textit{ex vivo} production of TNF (29,76,77,111,112), IL-1 (29,76,77,111–114), IL-6 (76,113,114), IL-2 (76,77,111,115), and γ-interferon (111) by blood mononuclear cells. A controlled study of fish oil-supplemented parenteral nutrition following major surgery was recently reported (116); the characteristic postoperative rises in the plasma concentrations of TNF-α and IL-6 that occurred in the control group who received a soya bean oil-rich emulsion were significantly blunted in the group receiving fish oil. Supplementation of the diet of healthy subjects with 0.65 g EPA plus DHA daily for 12 weeks did not affect \textit{ex vivo} production of TNF-α, IL-1β, or IL-6 by blood mononuclear cells (34).

The effects of dietary α-linolenic acid on IL-1β and TNF-α production by human cells have been reported: subjects consumed a sunflower oil-rich diet (similar to their typical diet) or a diet rich in α-linolenic acid provided by flaxseed oil capsules and flaxseed oil-based spreads and cooking oils (112). In this way, the flaxseed oil consumption increased to a mean of 13.7 g/d. \textit{Ex vivo} production of both IL-1β and TNF-α by blood mononuclear cells was decreased by the flaxseed oil diet (112). When the subjects then supplemented their diet with encapsulated EPA plus DHA, production of both cytokines was further decreased (112). These investigators showed an inverse correlation between mononuclear cell EPA content and production of IL-1β and TNF-α (112).

Arachidonic acid (1.5 g/d for 60 days) did not alter TNF-α, IL-1β, IL-6, or IL-2 production by human mononuclear cells (93).

### \textit{IN VIVO} MEASURES OF INFLAMMATION AND CELL-MEDIATED IMMUNITY

The studies outlined above have most often investigated the effects of dietary manipulations on \textit{ex vivo} functions of isolated cell populations. Although various consistent patterns have emerged from these studies, contradictory reports were also made and it is evident that the outcome of such \textit{ex vivo} measures is strongly influenced by the experimental conditions used. Furthermore, \textit{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 \textit{in vivo} measures of
inflammation and of cell-mediated immunity offer the prospect of investigating the effects of dietary manipulations on the overall responses of these systems.

**Acute Inflammatory Responses**

Arachidonic acid-derived eicosanoids are involved in mediating inflammatory responses. As n-3 PUFA diminish the production of these mediators following an inflammatory challenge (117,118), they should exert anti-inflammatory activities. The changes in the pattern of inflammatory eicosanoid production after intraperitoneal injection of zymosan in rodents were accompanied by a decreased influx of neutrophils into the peritoneal cavity (118). Furthermore, 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 (119).

In accordance with that observation, feeding rats high fat diets containing 20 g/kg of ethyl esters of EPA or DHA resulted in a 50% reduction in footpad swelling in response to carrageenan injection compared with feeding safflower oil (120); EPA and DHA were equally effective.

**In vivo Response to Endotoxin and Cytokines**

Two 24-hour intravenous infusions of a 10% (vol/vol) lipid emulsion rich in fish oil into guinea pigs significantly enhanced survival after intraperitoneally injected LPS compared with infusion of a 10% (vol/vol) safflower oil emulsion (121); the total amount of lipid infused was 13 g/animal. The same authors later showed that feeding a 145 g/kg fish oil diet to guinea pigs for 6 weeks significantly increased survival after an intraperitoneal injection of LPS compared with animals fed a 150 g/kg safflower oil diet (122). 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 several responses to intraperitoneal TNF-α: 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 (123). Fish oil feeding to rats or guinea pigs also diminished the pyrogenic (124, 125) and anorexic effects (123,126) of IL-1 and TNF-α compared with feeding linoleic acid-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.

Essential fatty acid deficiency impaired the murine DTH response, which was restored by addition of maize oil (130 or 500 g/kg), to the diet (127). The DTH response in rats or guinea pigs is reduced by feeding high fat diets compared with feeding low fat diets (86,128); linoleic acid-rich diets are more suppressive than high saturated fat diets. Dietary fish oil reduces the DTH response in mice compared with n-6 PUFA
EFFECT OF DIETARY FATTY ACIDS

rich or olive oil-rich diets (117), whereas the addition of ethyl esters of either EPA or DHA to the diet of mice consuming a safflower oil diet reduced the DTH response (129); EPA and DHA were equally effective. The DTH response to sheep red blood cells in mice was diminished following tail vein injections of emulsions of triacylglycerols rich in EPA or DHA (130). Feeding beagle dogs a diet with an n-6:n-3 PUFA ratio of 1.4 resulted in a reduced DTH response to intradermal KLH compared with diets with n-6 to n-3 PUFA ratios of 31 or 5.4 (131); the increased n-3 PUFA content was brought about by replacing linoleic acid with EPA plus DHA. These observations are consistent with many of the reported effects of dietary n-3 PUFA on ex vivo lymphocyte responses (e.g., decreased proliferation and IL-2 production).

A 40-day reduction in fat intake (from 40% to 25–30% of energy) by healthy human volunteers did not alter the DTH responses to seven recall antigens (60); these responses were also unaffected by differences in the PUFA level of the diet (3.2% or 9.1% of energy) (60) or by consuming a salmon rich diet (500 g/d for 40 days) (92). Feeding a flaxseed oil rich diet to healthy human volunteers for 8 weeks lowered the DTH response to seven recall antigens, although this reduction was not statistically significant (91). Arachidonic acid (1.5 g arachidonic acid/d) did not affect the DTH response to seven recall antigens applied intradermally (71). Supplementation of the diet of volunteers consuming a low fat, low cholesterol diet with 1.25 g EPA plus DHA daily, diminished the DTH responses to seven recall antigens (77). Recently, Kelley et al. (78) reported that 6 g DHA/d for 90 days did not alter the DTH response to intradermal application of seven recall antigens.

Graft Versus Host and Host Versus Graft Responses

The so-called popliteal lymph node assay provides a useful experimental model in rodents for measuring graft versus host (GvH) and host versus 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 can also be involved in the host defense. In contrast, the HvG reaction is a T-cell–mediated response, in which cytotoxic lymphocytes of the host recognize MHC antigens on the injected cells. In both cases, the enlargement in popliteal lymph node size is caused largely by proliferation of activated host cells; most of these originate within the popliteal lymph node, although some recruitment of cells also comes from the bloodstream. Using this assay, Mertin et al. (132) reported that both the GvH and HvG responses were suppressed following a single administration of fish oil concentrate (750 mg/kg body weight) by esophageal catheter to mice before or immediately after the inoculation with allogeneic cells. A suppressed HvG response was observed in mice fed a 160 g/kg fish oil diet compared with those fed a standard chow diet (133); lower levels of fish oil (25,50,100 g/kg) did not significantly affect the response. 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 (52). Such observations accord with the finding of significantly
diminished *ex vivo* T-lymphocyte proliferation, NK-cell activity, and cytotoxic lymphocyte activity following fish oil feeding. The fish oil diet resulted in less IL-2 receptor-positive cells and CD16⁺/CD3⁻ cells in the popliteal lymph nodes following the GvH response (52), indicating an inhibition of lymphocyte activation and a decrease in the proportion of NK cells, respectively. A dose-dependent effect of flaxseed oil compared with sunflower oil on the GvH response in rats was reported (66); the level of fat in the diet was 200 g/kg. Replacing α-linolenic acid (4.4 g/100 g fatty acids) with EPA was observed to significantly decrease (by 15% to 20%) the GvH in rats (27); replacement of α-linolenic acid with DHA was without effect.

**Animal Models of Inflammatory and Autoimmune Diseases**

Dietary fish oil has been shown to have significantly beneficial clinical, immunologic, and biochemical effects in various 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, decreased joint inflammation in rodents with collagen-induced arthritis, and less inflammation in rats with various models of ulcerative colitis (16). These observations suggest that diets enriched in EPA plus DHA might be of some therapeutic benefit in these diseases in humans (see below).

**Animal Models of Organ Transplantation**

Linoleic acid given subcutaneously, intraperitoneally, or orally prolonged the survival of skin allografts in mice or rats (134). Renal or cardiac transplants have been shown to survive longer if recipient rats are fed oleic, linoleic, or eicosapentaenoic acids or fish oil (16). Greater prolongation of cardiac survival in rats receiving an infusion of fish oil after transplantation compared with those receiving soybean oil infusion has been reported (135,136); in turn, soybean oil enhanced survival compared with saline infusion. Oral fish oil (4.5 g/d) has also been shown to prolong the survival of islets of Langerhans grafts in mice (137). These observations are in accordance with the reduced lymphocyte responses observed following fish oil feeding in particular, and they indicate that intervention with n-3 PUFA may be useful before and following organ transplantation in humans (see below).

**USE IN CHRONIC INFLAMMATORY DISEASES AND PANCREATIC CANCER**

Various human diseases are characterized by dysregulation of the immune system. Several of these diseases involve inappropriate production of proinflammatory cytokines (*e.g.*, TNF-α) and so are termed *chronic inflammatory diseases*. Many cancers, including pancreatic cancer, and AIDS are also characterized by raised levels of proinflammatory cytokines in the circulation. Studies in healthy animals and humans reveal that fish oil decreases the ability of cells to produce proinflammatory cy-
tokines (see above), suggesting that increasing the amount of long chain n-3 PUFA in the diet of patients with various diseases could be beneficial. This idea is supported by the fish oil-induced amelioration of symptoms observed in animal models of chronic inflammatory diseases (see earlier), which in some cases resulted in increased lifespan (e.g., in mice carrying the murine AIDS virus (89,109), and in tumor-bearing mice (138). Thus, fish oil has been provided to patients with chronic inflammatory and autoimmune diseases, pancreatic cancer, and AIDS. It is beyond the scope of this article to review the effectiveness of this treatment in these diseases. However, reviews have been done of the use of long chain n-3 PUFA in rheumatoid arthritis (139–141), psoriasis (142), ulcerative colitis (143), Crohn’s disease (144), IgA nephropathy (145), and pancreatic cancer (146). Generally, these studies show biochemical changes such as enrichment of plasma or mononuclear cells with EPA and DHA, decreased production of arachidonic acid-derived eicosanoids, and in some studies decreased production of proinflammatory cytokines. In addition, several of these studies show improvement in clinical symptoms and in some cases decreased use of drug treatment (139–146).

USE FOLLOWING ORGAN TRANSPLANTATION IN HUMANS

The animal studies described above indicate that PUFA, particularly long chain n-3 PUFA, could be used to prolong the survival of organ transplants. Recipients of kidney transplants who received 9 g fish oil/d for 1 year after transplantation (in conjunction with cyclosporin A and prednisolone) had significantly improved glomerular filtration rate and significantly diminished cyclosporin A nephrotoxicity, although no effect was seen on graft survival (147). A similar finding was made by van der Heide et al. (148). These investigators reported that renal transplant patients who received fish oil (6 g/d for the first postoperative year) in combination with cyclosporin A had better kidney function and fewer rejection episodes over 1 year compared with patients who received coconut oil and cyclosporin A. Better kidney function was reported in kidney graft recipients who consumed fish oil (8 g/d for the first postoperative year) in combination with cyclosporin A had better kidney function and fewer rejection episodes over 1 year compared with controls (149). Bennett et al. (150) reported no rejection incidents in a group of kidney transplant recipients who received 9 or 18 g EPA/d for 16 weeks after transplantation; cyclosporin nephrotoxicity did not occur in this group but did occur in the control group who supplemented their diet with maize oil.

DO DIETARY LIPIDS DIMINISH HOST DEFENSE?

The diminished cellular responses observed after feeding diets rich in n-3 PUFA result in suppressed cell-mediated immune responses (see above), suggesting that these fatty acids could affect the host response to infection. Some animal studies support this suggestion. Mice fed a diet containing 200 g fish oil/kg showed lower survival over 15 days (48%) to orally administered Salmonella typhimurium than those fed maize oil (62.5%), coconut oil (87.5%), or a low fat diet (88%) (152); spleens from
the fish oil-fed animals had a greater number of bacteria than those from animals fed the other diets. Similarly, a study of experimental tuberculosis in guinea pigs reported an increased number of bacteria in the spleen of fish oil-fed animals and it was concluded that this represented persistence of the experimental infection (152). Compared with safflower oil, fish oil decreased the clearance of bacteria (inspired *Staphylococcus aureus*) in neonatal rabbits (39). Recently, it was reported that 170 g/kg fish oil decreased survival of mice to an intraperitoneal injection of *Listeria monocytogenes* compared with feeding 200 g/kg of lard, but not compared with feeding 200 g/kg of soybean oil, which also resulted in lower survival (153). The spleens from the fish oil-fed mice contained significantly more bacteria than those from the other two groups (153). As the response to microbial infections is predominantly a Th1-mediated response, the reduced survival of rodents fed large amounts of fish oil to bacterial challenges suggests that fish oil suppresses the Th1 response; this is consistent with many of the observed effects of fish oil on cytokine (IL-2, γ-interferon) and antibody production. In contrast to these observations, some studies show that fish oil feeding does not affect resistance of laboratory rodents to bacterial (*L. monocytogenes, Pseudomonas aeruginosa*) and viral (murine cytomegalovirus) challenges (154,155). Furthermore, some studies have shown that dietary fish oil enhances survival during some infections. For example, Blok et al. (104) reported increased survival of fish oil-fed mice challenged by intramuscular injection with *Klebsiella pneumoniae*; 90% of fish oil-fed mice survived, compared with 30%, 40%, and 0 in groups fed maize oil, palm oil, or chow, respectively. Furthermore, cerebral malaria induced by intraperitoneal injection of erythrocytes infected with *Plasmodium berghei* occurred in only 23% of fish oil-fed mice compared with 61%, 81%, and 78% of mice fed maize oil, palm oil, or chow, respectively (104).

No reports have been made of compromised immunity in humans supplementing their diet with n-3 PUFA. In contrast, one study has reported decreased numbers of respiratory infections, decreased days of fever, and reduced absence from school in children 3 to 4 years of age who supplemented their diet with linoleic and α-linolenic acids (600 plus 855 mg/d) for 4 months over winter compared with children who used a placebo (156).

**OUTSTANDING QUESTIONS**

Given the potential for clinical use of fish oil-derived n-3 PUFA (see above) and the speculation that the ratio of n-6 to n-3 PUFA in the diet might be a predisposing factor in some diseases with an immunologic basis (157), it is surprising that so little is still known about the immunologic impact of these fatty acids. Several key questions remain unresolved:

- Are both EPA and DHA active within the immune system or are the effects of fish oil principally caused by one of these fatty acids? Recent animal experiments suggest that both fatty acids alter rodent lymphocyte proliferation but that NK-cell activity is influenced only by EPA (27). Another recent study indicates that even a
large amount of DHA (6 g/d for 60 days) has very little immunologic impact in healthy humans (78). As some studies have reported marked effects of fish oil supplementation providing as little as 1.5 g EPA plus DHA daily, this finding suggests that most of the effects of fish oil in humans are caused by EPA. However, no study has been done of the dietary effects of EPA alone on immune function in healthy humans.

- What is the level of fish oil required to exert immunomodulatory effects? Related to this is the question of whether some immune functions (e.g., production of proinflammatory cytokines) are more sensitive to n-3 PUFA than others (e.g., lymphocyte proliferation). Surprisingly, no studies have been published on dose-response to fish oil or its component fatty acids with respect to their impact on human immune function. Again, recent animal studies suggest that EPA (alone or in combination with DHA) is effective at influencing lymphocyte functions at levels far below those provided in fish oil-rich diets and at levels which can be achieved by supplementation of the human-diet (27,75).

- Is the ratio of EPA to DHA an important factor in determining their potency?

- What is the equivalence of α-linolenic acid to EPA with respect to immunomodulation? This is an important issue to some because α-linolenic acid is more readily incorporated into foodstuffs than its long chain derivatives.

- Are there age, sex, or ethnic group related sensitivities to fish oil supplementation? Meydani et al. (76) showed more potent effects in older women than younger, but few if any other studies have made such comparisons, and no studies have determined whether the immune systems of men or women are equally sensitive to fish oil.

- What is the immunologic influence of the relation between the levels of n-3 PUFA and α-tocopherol in the diet? The study of Wu et al. (69) in monkeys highlights this as a key area of uncertainty.

- Is the immune response in vivo altered by the levels of n-3 PUFA that can be provided to humans, and how does this relate to effects on individual components of that response that can be examined ex vivo?

- Are the effects of n-3 PUFA the same in healthy and diseased subjects?

Another issue worth highlighting is that few good human studies have been performed: many of the studies of fish oil supplementation have not been controlled or blinded and most have used small numbers of subjects.

It seems important to address each of the above questions in well-controlled animal and human studies. Only then will the true immunologic impact of n-3 PUFA be known.

CONCLUSION

It seems likely that a significant reduction in fat consumption will enhance cell-mediated immune functions. Within the current Western style diet, it is unlikely that small to moderate changes in the amounts of saturated, monounsaturated, or
linoleic acids consumed will alter immunity, although the ratios between these types of fatty acids and between these and the n-3 PUFA might have a hitherto little appreciated importance. Perhaps, surprisingly, recent animal and human studies indicate that even large amounts of arachidonic acid in the diet do not affect immunity. Thus, among the fatty acids, it is the n-3 PUFA that possess immunomodulatory activity, and among the n-3 PUFA those from fish oil (EPA and DHA) are more biologically potent than α-linolenic acid (notwithstanding the questions outlined above). The production of macrophage-derived proinflammatory eicosanoids such as PGE$_2$ is markedly reduced by feeding diets rich in n-3 PUFA. Furthermore, inclusion in the diet of high levels of EPA plus DHA significantly reduces the movement of human monocytes toward chemotactic agents and the production of proinflammatory cytokines by human mononuclear cells. Several contradictory observations can be made regarding the effects of dietary n-3 PUFA on production of proinflammatory cytokines by animal macrophages and lymphocytes; these most likely relate to the different experimental models and protocols used. EPA and DHA also appear to reduce adhesion molecule expression and, thus, might influence the movement of leukocytes between body compartments. Several studies indicate a reduction of MHC II expression on antigen presenting cells following fish oil feeding; this would suggest a diminished ability to present antigen. No clear consensus is found regarding the effects of n-3 PUFA consumption on the generation of reactive oxygen and nitrogen species and on macrophage-mediated phagocytosis. Inclusion in the diet of high levels of n-3 PUFA markedly alters the functions of lymphocytes subsequently tested ex vivo. Components of both natural and acquired immunity are affected. Recent studies have sought to identify the effects of lower levels of particular fatty acids in the diet; these studies reveal complex interactions between fatty acids. In vivo tests are perhaps the most appropriate approach for determining the effect of different dietary fatty acids on immune function. Several studies indicate that diets rich in EPA plus DHA are anti-inflammatory and immunosuppressive in vivo, although relatively few studies have been done in humans. Although some of the effects of n-3 PUFA may be brought about by modulation of the amount and types of eicosanoids made, it is possible that these fatty acids might elicit some of their effects by eicosanoid-independent mechanisms, including actions on intracellular signaling pathways and transcription factor activity (11,17,19). Such n-3 PUFA-induced effects may be of use as treatment for acute and chronic inflammation, disorders that involve an inappropriately activated immune response, and the enhancement of graft survival.

REFERENCES


EFFECT OF DIETARY FATTY ACIDS


**DISCUSSION**

Dr. Haschke: The anti-inflammatory properties of n-3 fatty acids could also be used in therapeutic interventions in enteral nutrition. Are you aware of any use in Crohn’s disease, for example, and do you know the outcome?

Dr. Calder: Much literature exists on the use of fish oil in Crohn’s disease, ulcerative colitis, and other chronic inflammatory diseases. Many of these studies show changes in biochemical and immunologic variables. Some of them show clinical improvements, although not all. Perhaps the best study is one by Beluzzi (1), where he used a special enterically coated preparation of fish oil and showed very marked maintenance of patients in remission compared with a placebo group, where the patients relapsed.

Dr. Haschke: Is it possible that fish oil might act locally within the gut?

Dr. Calder: I am sure it does. I am sure it acts locally on the gut immune system, on epithelial cells, and on a variety of other cells.

Dr. Haschke: If fish oil is given to premature infants with their very immature immune systems, what could happen if they have a bacterial infection? Many of them do have such infections.

Dr. Calder: This is an important question, but I do not feel confident to answer it because very little information is available on the subject apart from experiments done in animal models, mainly in mature animals. These show a variety of effects, depending on the model and the pathogen. When one gives oils rich in very unsaturated fatty acids, it is important to ensure that the antioxidant protective mechanisms are maintained. In the scenario you are talking about, where there could be diminished reserves or insufficient reserves of antioxidant protectants, it might be very dangerous to give fish oil.

Dr. Meydani: We have shown in humans that a marked decrease in T-cell–mediated immune function occurs following consumption of fish oil (2,3). I think this is important, because these effects are seen particularly in older people who are also more likely to have impaired immunity. We have done studies showing that some of the effect is related to antioxidant status. This could be very important in the case of premature infants. Currently, much interest is expressed in the use of long chain polyunsaturated fatty acids in infant formulas for brain function and so on, but I think we need to be cautious that this use has no adverse effects on immune function.

Dr. Marini: There is a big difference between Europe and the United States in relation to the clinical use of fish oil derivatives in preterm babies. We feel that formulas for preterm ba-
bries should contain DHA because such babies are not able to synthesize DHA in sufficient quantity. I think it is also very important to maintain the balance between arachidonic acid and DHA intake in these babies. A recent study by Carlson in St. Louis showed that the addition of DHA plus arachidonic acid to a formula reduced the incidence of necrotizing enterocolitis significantly in the treated group versus a placebo group (4). This may be the answer to Dr. Haschke’s question about the local effect of DHA in the intestine.

I have a naive question. At the turn of the 20th Century, people were talking about cholesterol as a defensive factor, as cholesterol is an integral part of the membrane of phospholipids. Now people think that we should lower our cholesterol intake to protect against atherosclerosis. However, during the first years of life maybe a low level of cholesterol could in some way predispose to infection.

Dr. Klish: I feel a need to respond to Dr. Marini’s comment about the width of the Atlantic ocean! I think most investigators in the United States are probably not concerned about the addition of these polyunsaturated fatty acids to preterm formulas, as you commented, although the issue with regard to term formula is still unsettled.

Dr. Marini: I am glad that the United States has changed its opinion about supplementation of preterm formula.

Dr. Klish: Let me clarify that. There are no recommendations yet.

Dr. Kotchabhakdi: I would like to raise a few points. No doubt malnutrition and deficiencies of particular nutritional components, including micronutrients, can affect the immune system directly. We are also all aware that the immune system is closely involved with the brain, and that nutrition, whether it be protein, energy, or lipids, also directly affects structure and functional development of the brain, particularly in children. To what extent do you think this effect is caused directly by immune function and how much of it is indirect, via brain immune control? The reason for my question is that in our experience when children suffering from early malnutrition are given nutritional supplements alone, this does not bring about a very rapid improvement, but when food is given together with sensory stimulation the improvement is much more rapid. The use of sensory stimulation in preterm and low birthweight infants appears to improve both the ability to thrive and immune response in these infants.

The second interesting point you brought up was in relation to homeostasis, and how the baby tries to adapt to nutritional deficiency, and how nutrients could be redistributed to the various compartments where their effects are most needed. I remember reading in Myron Winnick’s classical text that the brain is relatively spared even in severe malnutrition, but how does it compare with the immune system?

Dr. Calder: In relation to the immune system versus the brain, I think this takes us back to the intimate association between the endocrine system and the immune system. I am sure that situations of stress versus calm can play a role in changing the sensitivity of the immune system to nutritional interventions. For example, it may not be possible for a nutrient to be effective against a very large concentration of a particular stress hormone, whereas if that concentration decreased, it might be possible for the nutrient to exert its effect. I cannot answer specifically on how much these factors are caused by direct or indirect effects, although I think that many of the effects of dietary manipulations can be mimicked in cell culture systems.

Dr. Farthing: I think we accept that some of the studies on inflammatory bowel disease have been beneficial, but fish oil has not found a place in the routine management of these diseases, I think mainly because the effect is not important enough to be clinically useful. Can you tell us what has been done in other inflammatory disorders such as asthma, eczema,
and hay fever; and secondly, does feeding fish oil modify the host response to developing a fever?

Dr. Calder: In relation to other inflammatory disorders, much literature is found on rheumatoid arthritis, with results similar to those in inflammatory bowel disease: all the expected biochemical changes, some immunologic changes, and some demonstration of clinical benefit, which include reduced use of other drugs (5). You asked about allergies and asthma. One study that I would draw to your attention to was published in the American Journal of Clinical Nutrition in 1997 (6), which involved a very nice fish oil intervention in atopic asthma. The authors found some individuals who responded with dramatic clinical improvement, and others whose condition worsened. They were unable to explain the difference between responders and nonresponders, but the responders had markedly increased urinary 5-series leukotriene production, indicating very marked incorporation of EPA and reduced arachidonic acid-derived mediator production. The nonresponders had much less 5-series leukotriene in the urine than the responders. So, some patients can and some cannot respond to this intervention, but the basis of this is not known.

In relation to fever, animal studies quite clearly show that fish oil does modify the fever response. If you feed fish oil and then give the animal a challenge that is normally associated with fever induction, fever is not induced (7). Studies in humans by Rothwell, involving a fish oil intervention, then looked at the induction of fever following injection with a tetanus toxoid vaccine, and showed a reduced fever response (8).

Dr. Meydani: There was also an animal study in guinea pigs looking at anaphylactic response, where they showed worsening of the symptoms with fish oil (9).

Dr. Socha: In relation to the discussion on the treatment of inflammatory bowel diseases in comparison with the use of fish oil supplements in infants, I would like to draw your attention to some differences. First there was a difference in dosage: we use quite high doses of fish oil for the treatment of inflammatory bowel disease, and the effect, therefore, would be very different from supplementation in infants. The other difference is in relation to the type of fatty acid: EPA is the main fatty acid used in the treatment of inflammatory bowel disease, and DHA for supplementing infants.

Dr. Calder: That is a very important point. It seems now from both animal work and human studies that DHA does not have particularly dramatic effects on the immune system, although we know it has important effects on brain development. In contrast, EPA, which as you say is not included in infant formulas, seems to be the immunologically active fatty acid in fish oil. So perhaps functional separation exists there that can be taken advantage of in the preparation of products for different settings.

Dr. Suskind: I think it has been well recognized that children who are severely malnourished have essential fatty deficiency, but the impact of that deficiency on the immune function has not been explored and deserves to be looked at more fully. It could explain some of the rapid reversal in cellular immunity that occurs with refeeding. Have you come across any studies in children dealing with this relationship?

Dr. Calder: Not in humans.

Dr. Tantibhedhayangkul: All mammalian milk contains very large amounts of fat. From the immunologic standpoint, it appears that this is not a good thing. But why would Nature do this if it is not beneficial to the infant?

Dr. Calder: This perhaps raises an important point about differences between adults and infants. The studies I described were carried out in adult volunteers, and they quite clearly showed this effect of high fat feeding. However, it may be that the effect is not exerted earlier in life. This may reflect the composition of human milk, which contains fatty acids at specific
positions on the triglyceride molecule. The positional isomerism of the fatty acids and triglycerides may have an important role in maintaining host defenses.

Dr. Woodward: I am wondering how confident it is possible to be that the immunologic effects seen of feeding fish oil versus other types of oil are, in fact, attributable to the EPA or DHA in the fish oil, given the nature of fish oil as a product?

Dr. Calder: If you had asked this question a couple of years ago, I would say that you could not be 100% confident, because what you are alluding to as fish oil contains a variety of other components—vitamin E at different levels from other oils and other fat soluble vitamins at levels that are different from other oils. But I think now, we have very tightly controlled feeding studies on rats fed EPA or DHA with normalization of the other fatty acids and vitamin E, as well as the other vitamins in the diet. So I am reasonably confident that the immunologic effect of fish oil is mainly caused by EPA.

Dr. Woodward: Has that kind of work been done with methyl esters of fatty acids?

Dr. Calder: I think Hamazaki in Japan has done some work with ethyl esters of EPA and DHA and triglycerides of EPA and DHA.

Dr. Griffin: Are there any epidemiologic data looking at different ethnic groups with different dietary fatty acid ingestion? For example, Eskimos compared to Mediterraneans?

Dr. Calder: Epidemiologic observations have been made from the point of view of the incidence of particular disorders that involve a dysfunctional immune response. No associations between fatty acid composition of the diet or serum or cells and immune cell function were found in different ethnic groups. But certainly, the basis of the interest in fish oils and inflammation was the observation that Eskimos have a very low incidence of chronic inflammatory disease, and this was attributed to the change in eicosanoid balance.

Dr. Marini: I would like to comment on the Eskimo diet. On this diet, an increase in serious infectious disease has been shown—not disease in general but the infectious diseases such as sepsis. I do not know whether this is because of the fatty acids or because of other factors in the diet. Another effect of this kind of fish diet is reduced platelet activation. This is probably the reason why Eskimos have less coronary artery disease. DHA is also a very powerful agent against ventricular fibrillation. A study recently presented by the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardio (GISSI)-Prevenzione at the American College of Cardiology (New Orleans, LA, USA March 7–10, 1999), showed that supplementing patients with fish oil after the myocardial infarction can reduce the incidence of ventricular fibrillation by 25%.

Dr. Calder: Fish oil, by virtue of the fatty acids it contains, has a variety of effects that might be responsible for protection against cardiovascular disease. You mentioned at least two of these: platelet aggregation and ventricular fibrillation; a blood lipid lowering effect is also seen. You also mentioned the increase in infectious disease in Eskimos. Tuberculosis in Eskimos is a particular problem, and in one of the animal models I showed, fish oil feeding was associated with increased susceptibility to tuberculosis.

Dr. Meydani: Another observation in Eskimos is that the incidence of diarrheal diseases in children is said to be increased. This might also be related to the effects of the fatty acids in fish oil on immune function.

Dr. Gershwin: I may be the only one here who has lived in Alaska. I should tell you that one of the major staples in the diet of Eskimos, unfortunately, is alcohol, and that plays a significant role in their tuberculosis. I should also say the incidence of rheumatoid arthritis in Eskimos is no different from any other population. Finally, rheumatologists say that feeding fish oils in animal models of systemic lupus erythematosus is effective, but it is only effective if given before the animals get sick. Once the animals have developed lupus, fish oils are relatively ineffective.
Dr. Calder: I think that is a very important point. Maybe using this as a strategy for intervention once the disease has developed is too late, but perhaps these interventions can play a role in the prevention of disease.

REFERENCES


