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Fatty acids and the immune system: from basic science to clinical applications

Parveen Yaqoob
Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, UK

Over the last 25 years, the effects of fatty acids on the immune system have been characterized using *in vitro*, animal and human studies. Advances in fatty acid biochemistry and molecular techniques have recently suggested new mechanisms by which fatty acids could potentially modify immune responses, including modification of the organization of cellular lipids and interaction with nuclear receptors. Possibilities for the clinical applications of *n*-3 PUFA are now developing. The present review focuses on the hypothesis that the anti-inflammatory properties of *n*-3 PUFA in the arterial wall may contribute to the protective effects of *n*-3 PUFA in CVD, as suggested by epidemiological and secondary prevention studies. Studies are just beginning to show that dietary *n*-3 PUFA can be incorporated into plaque lipid in human subjects, where they may influence the morphology and stability of the atherosclerotic lesion.

Fatty acid: Fish oil: Immunity: Inflammation: Lymphocyte: Macrophage

The very first review addressing the roles of fatty acids in the immune system was published by Meade & Mertin (1978), who, in their opening paragraph, comment that it is 'early yet to know whether fatty acid research may finally find a niche in immunology'. They explain that their aim is to provide a new perspective rather than to summarize an established field and by 'gathering together threads from the fields of immunology, biochemistry and nutrition', they discuss 'in a deliberately one-sided way', whether there might be specific roles for fatty acids in the immune system in health and disease. While much of their review is highly speculative, Meade & Mertin (1978) show remarkable foresight by suggesting that there may be immunological explanations for data relating to the relationship between dietary fat and disease, which had commonly been interpreted without any reference to immunology. This idea, placed in the context of atherosclerosis in particular, was well ahead of its time. Of course, not all Meade & Mertin's (1978) predictions were accurate. They speculated, for example, that the immunological basis of atherosclerosis might be a reaction to milk proteins! However, there is no doubt that they gave rise to a field that has seen major developments over the last 25 years. In the present review some of the key developments since the publication of the Meade & Mertin (1978) review will be described and, hopefully, readers will be convinced that fatty acids might finally have found the niche in immunology that they envisaged.

Fatty acid structure and nomenclature

Fatty acids are hydrocarbon chains, which can be saturated, MUFA or PUFA. Unsaturated fatty acids contain double bonds between pairs of adjacent C atoms; MUFA contain one double bond, whereas PUFA contain more than one double bond. There are two essential fatty acids, linoleic and α-linolenic acid, that cannot be synthesized *de novo* in animal cells and, therefore, must be obtained from the diet. Linoleic acid is an *n*-6 PUFA, described by its shorthand notation of 18 : 2*n*-6, which refers to an C_{18} fatty acid with two double bonds, the first of which is on C-6 from the methyl end. α-Linolenic acid is an *n*-3 PUFA with a shorthand notation of 18 : 3*n*-3, describing an C_{18} fatty acid with three double bonds, the first being positioned at C-3 from the methyl end. Both essential fatty acids can be further elongated and desaturated in animal cells forming

Abbreviations: AA, arachidonic acid; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ICAM, intercellular adhesion molecules; LT, leukotrienes; NK, natural killer; VCAM, vascular cell adhesion molecules.

Corresponding author: Dr P. Yaqoob, fax +44 118 931 0800, email P.Yaqoob@reading.ac.uk

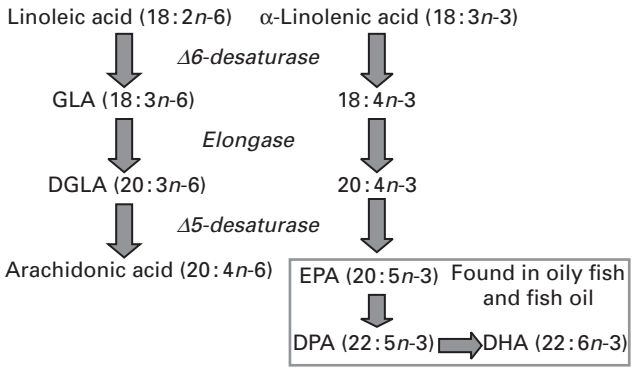

Fig. 1. Metabolism of PUFA. Both the essential fatty acids linoleic acid and α-linolenic acid can be elongated and desaturated in animal cells, forming the *n*-6 and *n*-3 families of PUFA. The metabolism of the *n*-6 and *n*-3 fatty acids is competitive, since both pathways employ the same set of enzymes. The major end product of the *n*-6 pathway is arachidonic acid. This pathway is quantitatively the most important pathway of PUFA metabolism in man, because linoleic acid is abundant in vegetable oils and vegetable oil-based products, and is therefore consumed in greater quantities than α-linolenic acid, which is present in green leafy vegetables and some seed and vegetable oils. The major end products of the *n*-3 pathway are eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA); very little α-linolenic acid proceeds along the entire metabolic pathway to give rise to docosahexaenoic acid (DHA). However, oily fish contain lipid that has a high proportion of the long-chain *n*-3 PUFA, EPA and DHA, and are the chief sources of these fatty acids. GLA, γ-linoleic acid; DGLA, dihomo-γ-linolenic acid.

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Fatty acids and the immune system: a historical perspective

The Meade & Mertin (1978) review was largely based on studies investigating the effects of fatty acids on the proliferation of lymphocytes *in vitro*. These types of studies continue to be published to some extent and demonstrate that saturated fatty acids have little effect on *in vitro* lymphocyte proliferation, while unsaturated fatty acids inhibit lymphocyte proliferation (see Calder *et al*. 1991). The most potent inhibitory effects tend to be observed when lymphocytes are incubated in the presence of AA or EPA at concentrations of ≥50 μM (Calder *et al*. 1991). Since the proliferation of lymphocytes plays an important role in the response of the immune system to a challenge, these results were interpreted to suggest that PUFA were immunosuppressive and that they may affect other aspects of the immune response. The *in vitro* studies inevitably led to investigations of the effects of dietary fatty acids on immune function, initially in animal models, and subsequently in human subjects.

Fatty acids and the immune system: evidence from animal studies

Studies investigating the effects of dietary fats on immune function in laboratory animals (usually rodents) have generally shown that high-fat diets suppress lymphocyte functions compared with low-fat diets, but the nature and extent of the impairment depends on the level and type of fat (see Calder *et al*. 2002). Saturated fatty acids and *n*-6 PUFA have little effect on lymphocyte proliferation (Yaqoob *et al*. 1994*a*, 1995*a*), cytokine production (Yaqoob & Calder, 1995*a*,*b*) or natural killer (NK) cell activity (Yaqoob *et al*. 1994*b*). In contrast, oleic acid (delivered in the form of olive oil) and *n*-3 PUFA (delivered as fish oil) have been demonstrated to inhibit both lymphocyte activation (Yaqoob *et al*. 1994*a*, 1995*a*; Jeffery *et al*. 1996; Jolly *et al*. 1997; Arrington *et al*. 2001) and NK cell activity (Yaqoob *et al*. 1994*b*; Jeffery *et al*. 1996) in animal studies. In addition, fish oil has been demonstrated to inhibit the production of inflammatory cytokines by lymphocytes (Yaqoob & Calder, 1995*b*; Jolly *et al*. 1997, 1998; Wallace *et al*. 2001) and macrophages (Billiar *et al*. 1988; Renier *et al*. 1993; Yaqoob & Calder, 1995*a*; Wallace *et al*. 2000*a*), to decrease the expression of adhesion molecules by lymphocytes (Sanderson *et al*. 1995*a*) and to decrease adhesion of lymphocytes to macrophage monolayers and to endothelial cells (Sanderson *et al*. 1998).

The experiments described above represent '*ex vivo*' effects of fatty acids on immune function, since the tests of immune response are conducted *in vitro* following a period of dietary manipulation in the animal. Thus, NK cell activity is assessed by the ability of lymphocytes (from animals subjected to specific dietary regimens) to lyse tumour cells *ex vivo*. While these *ex vivo* tests are a useful tool for examining the influence of dietary fatty acids on immune function, some assays (such as lymphocyte proliferation) involve rather extended periods of cell culture, during which, it could be argued, any changes in the fatty acid composition of the cells brought about by dietary manipulation, might be lost. There does indeed appear to be some loss, which can be prevented by culturing cells in autologous serum rather than foetal calf serum (Yaqoob *et al*. 1994*a*, 1995*b*) or by conducting lymphocyte proliferation assays in whole-blood cultures (Yaqoob *et al*. 1995*a*, 1999). Thus, cell culture conditions may be at least partly responsible for the fact that some studies report no effect or even an enhancement in lymphocyte proliferation as a result of feeding olive oil to rodents (Berger *et al*. 1993; De Pablo *et al*. 1998*a*).

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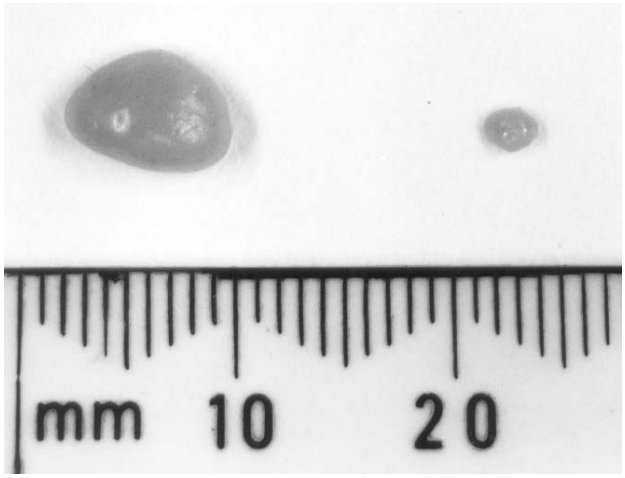

Fig. 2. The popliteal lymph node assay: a graft *v.* host model. This *in vivo* graft *v.* host model is based on the subcutaneous injection of lymph node lymphocytes from adult male Lewis rats into the footpad of weanling male DA/Lewis rats, with the control leg for each rat being injected with saline (9 g NaCl/l). The animals were killed 7 d after injection and the popliteal lymph nodes dissected and weighed. The popliteal lymph node from the experimental leg increases dramatically in size and weight as a result of the accumulation of immune cells from the circulation, increasing from a few mg in weight to approximately 100 mg. (Photograph kindly supplied by Professor P. Calder, Institute of Human Nutrition, University of Southampton, Southampton, UK).

The effects of dietary fatty acids on immune function in laboratory animals have also been investigated using *in vivo* tests of immune response, which overcomes the criticism associated with the loss of dietary-induced changes during cell culture. Both olive oil and fish oil decreased the graft *v.* host response in rats (see Fig. 2) compared with a low-fat diet or high-fat diets containing saturated fatty acids or *n*-6 PUFA (Table 1; Sanderson *et al.* 1995*b*). Dietary fish oil has also been demonstrated to decrease the delayed-type hypersensitivity response (Taki *et al.* 1992) and to prolong the survival of skin, kidney and heart transplants in rodents (Calder, 1998).

A further criticism levelled at the animal studies described earlier is that they have tended to employ very large amounts of fish oil in the diet; often these diets contain as much as 200 g fish oil/kg, equating to approximately 12 % of dietary energy being contributed by *n*-3 PUFA. However, in order to overcome this criticism, studies have tested the effects of *n*-3 PUFA in rats at approximately 1·7 % dietary energy and demonstrated that even at this low level of intake dietary *n*-3 PUFA inhibit lymphocyte proliferation (Jolly *et al.* 1997; Peterson *et al.* 1998*b*).

Fatty acids and the immune system: evidence from human studies

The first human studies to investigate the effects of dietary fatty acids on immune function tested the effects of fish oil on a number of immune variables (Endres *et al.* 1989, 1993). However, these studies were open uncontrolled trials on small numbers of subjects and did not unequivocally support the animal data. Some of the more recent double-blind placebo-controlled studies do support the animal data to some extent. Thies *et al.* (2001*a*,*b*) demonstrated that fish oil suppressed both lymphocyte proliferation and NK cell activity compared with a placebo treatment in healthy subjects aged 55–75 years and several studies have shown that fish oil supplementation decreased the *ex vivo* production of the inflammatory cytokines, TNF-α, IL-1 and IL-6 (for example, see Gallai *et al.* 1993; Caughey *et al.* 1996). However, many studies report no effect of fish oil on the production of inflammatory cytokines *ex vivo* (Molvig *et al.* 1991; Cooper *et al.* 1993; Cannon *et al.* 1995; Schmidt *et al.* 1996; Blok *et al.* 1997; Yaqoob *et al.* 2000). The considerable inconsistency in the reported effects of *n*-3 PUFA on *ex vivo* production of inflammatory cytokines was initially thought to be a result of differences in administered doses. However, this explanation does not fully account for the inconsistency, since some studies employing high doses of *n*-3 PUFA showed no effect on cytokine production, whereas others using low doses reported inhibition (for references, see Yaqoob, 2003*b*). Mantzioris *et al.* (2000) adopted the approach of setting target tissue concentrations of EPA, rather than target dietary intakes; they aimed to increase the mononuclear cell EPA content to 1·5 g/100 g total fatty acids by 2 weeks of dietary modification, a strategy based on the observation by Caughey *et al.* (1996) that the EPA content of mononuclear cells is strongly associated with *ex vivo* production of IL-1β and TNF-α and that 1·5 g EPA/100 g total fatty acids results in maximum suppression of cytokine synthesis. However, a study using a high dose of 2·1 g EPA/d plus 1·1 g DHA/d showed no effect of fish oil supplementation on *ex vivo* production of cytokines, despite achieving mononuclear cell EPA levels of 2·5 g/100 g total fatty acids after 4 weeks and 3·3 g/100 g total fatty acids at 12 weeks (Yaqoob *et al.* 2000). Similarly, Soyland *et al.* (1994) and Molvig *et al.* (1991) reported no effect of 5 or 3·2 g *n*-3 PUFA/d respectively on *ex vivo* cytokine production and although fatty acid composition data for mononuclear cells were not reported, it is likely by comparison with the study by Yaqoob *et al.* (2000) that the EPA content was >1·5 g/100 g total fatty acids in those studies. Thus, while the approach suggested by Mantzioris *et al.* (2000) is interesting, it does not adequately explain

Table 1. Effects of dietary fatty acids on the graft *v.* host response† (data from Sanderson *et al.* 1995*b*)

	Popliteal lymph node wt (mg)	
Diet	Mean	SE
Low fat	102·7	8·2
Coconut oil	101·8	14·9
Olive oil	77·3*	7·5
Safflower oil	92·3	6·3
Fish oil	67·8*	5·7

Mean values were significantly different from that for the low-fat diet: *$P < 0·05$.

†The *in vivo* graft *v.* host model used is based on the subcutaneous injection of lymph node lymphocytes from adult male Lewis rats into the footpad of weanling male DA/Lewis rats, with the control leg for each rat being injected with saline (9 g NaCl/l). The animals were killed 7 d after injection and the popliteal lymph nodes dissected and weighed (see Fig. 2).

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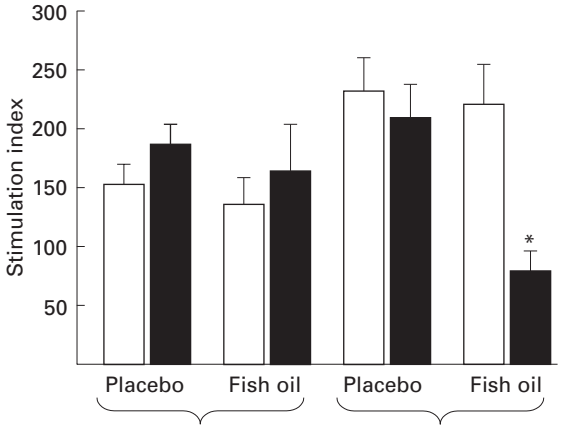

Fig. 3. Older subjects may be more susceptible to the immunomodulatory effects of fish oil than young subjects. There is some inconsistency in the literature regarding the effects of fish oil on immune function. Several studies have demonstrated that older subjects appear to be more susceptible to the immunomodulatory effects of fish oil than younger subjects. The study by Yaqoob *et al.* (2000) demonstrated no effect of 3·2 g *n*-3 PUFA/d on lymphocyte proliferation in healthy subjects aged <60 years (*n* 8), whereas Thies *et al.* (2001*b*) demonstrated a significant inhibitory effect of only 1 g *n*-3 PUFA/d in subjects aged >55 years (*n* 8). (□), Before supplementation; (■), after supplementation. Stimulation index, uptake of ^{3}[H] thymidine by stimulated cells divided by that of unstimulated cells. Values are means with their standard errors represented by vertical bars. Mean value after supplementation was significantly different from that for the corresponding placebo: $^{*}P < 0{\cdot}05$.

the discrepancies in the literature. Differences in dosage also do not fully explain the inconsistencies in the reported effects of fish oil supplementation on lymphocyte functions (Meydani *et al*. 1991; Gallai *et al*. 1993; Yaqoob *et al*. 2000; Thies *et al*. 2001*a*,*b*). However, some of these studies suggest that older subjects may be more susceptible to the immunomodulatory effects of fish oil than young or middle-aged subjects (Meydani *et al*. 1991; Thies *et al*. 2001*a*,*b*; see Fig. 3). It is also possible that the majority of the human studies conducted so far have been insufficiently powered to take into account the enormous variation in indices of immune function, for example *ex vivo* cytokine production, which are now recognized to be influenced by genotypic variation (see Grimble *et al*. 2002). Clarification of the effects of fish oil on immune function in human subjects is therefore still required, perhaps through the design of adequately powered studies using a range of doses of *n*-3 PUFA and assessment of the EPA content of mononuclear cells.

The animal studies described in the previous section suggested that olive oil, containing oleic acid, as well as fish oil, was able to modulate immune responses. However, at least one double-blind study, in which food products were enriched with olive oil or a control oil, demonstrated that dietary olive oil has only limited influence on immune function in healthy middle-aged men, since it does not affect lymphocyte proliferation or NK cell activity, but does

Table 2. Effect of olive oil on the expression of intercellular adhesion molecule-1 (ICAM-1) by human peripheral blood mononuclear cells (data are taken from Yaqoob *et al.* 1998)

	ICAM-1 (% positive cells)					
	Baseline		1 month		2 months	
	Mean	SE	Mean	SE	Mean	SE
Control	19·0	1·3	19·1	1·2	20·0	1·5
Olive oil	20·8	1·4	16·4	1·4	15·9*	1·1

Mean value was significantly different from that at baseline and from that of the control group: $^{*}P < 0{\cdot}05$.

reduce the expression of intercellular adhesion molecule (ICAM)-1 (Yaqoob *et al*. 1998; see Table 2). The reason for the lack of effect of olive oil on immune function in human subjects has been attributed to the lower level of intake in human studies relative to animal studies (Yaqoob, 2002).

Effects of eicosapentaenoic acid *v*. docosahexaenoic acid on immune function

Although some studies demonstrate immunomodulatory effects of *n*-3 PUFA, it is not yet clear whether they are associated with EPA or DHA, or a combined effect of these two *n*-3 PUFA. Animal studies tend to suggest that both EPA and DHA have immunomodulatory effects. Both EPA and DHA, fed to rats at 4·4 g/100 g total fatty acids, inhibited lymphocyte proliferation, although only EPA inhibited NK cell activity (Peterson *et al*. 1998*a*). In a study conducted in mice both EPA and DHA suppressed the proliferation and production of IL-2 by splenic lymphocytes (Jolly *et al*. 1997). However, two animal models of inflammation demonstrate differential effects of EPA and DHA, one suggesting reduced inflammation by DHA (Tomobe *et al*. 2000) and the other suggesting that EPA is the most anti-inflammatory (Volker *et al*. 2000).

In human subjects, a comparison of the effects of 3·8 g EPA/d or 3·6 g DHA/d, with a control treatment of linoleic acid, reported no differential effects of the *n*-3 PUFA on the phagocytic activity of monocytes (Halvorsen *et al*. 1997). Thies *et al*. (2001*b*) compared the effects of supplementation with fish oil (<1 g/d), highly-purified DHA (<1 g/d) and a placebo on lymphocyte proliferation in healthy subjects and demonstrated that fish oil suppressed lymphocyte proliferation, whereas DHA had no effect. This finding could be taken to suggest that either EPA is responsible for the inhibitory effect or that both EPA and DHA are required. In the same study fish oil, but not DHA, decreased NK cell activity (Thies *et al*. 2001*a*). Kelley *et al*. (1998, 1999) examined the effects of a much higher dose of 6 g DHA/d, which replaced 200 mg/g linoleic acid in the diet, on a number of immune responses. They reported no effect of DHA on lymphocyte proliferation, production of IL-2, antibody production or delayed-type hypersensitivity (Kelley *et al*. 1998). In contrast, DHA did appear to decrease NK cell activity and production of the inflammatory cytokines, TNF-α and IL-1β (Kelley *et al*. 1999). In a recent study comparing the effects of

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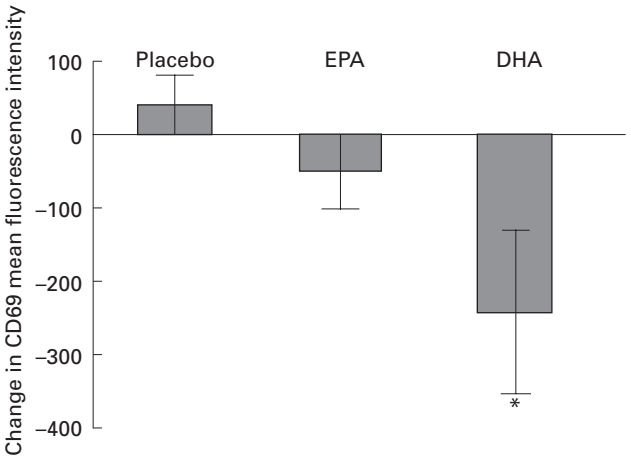

Fig. 4. Differential effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on expression of CD69, an early marker of T-lymphocyte activation. Healthy subjects were supplemented with either olive oil or an EPA-rich or DHA-rich oil for 4 weeks. The EPA- and DHA-rich oils provided approximately 5 g *n*-3 PUFA/d. Lymphocyte activation was determined by measurement of the expression of CD69 in blood diluted 1:1 (v/v) with culture medium and cultured for 24 h with concanavalin A at a final concentration of 6·25 mg/l. Monoclonal antibodies used were anti-CD69 and anti-CD3 (to distinguish T-lymphocytes). Cell preparations were analysed by flow cytometry and fluorescence data were collected on 2×10^4 cells. The change in median fluorescence intensity (related to the no. of CD69 molecules expressed per T-lymphocyte) in post- *v.* pre-supplementation samples is illustrated for each group. Data are means with their standard errors for ten to fourteen subjects and were analysed using a one-factor ANOVA. Mean values were significantly different from those for both the placebo and the EPA treatment: $^*P < 0{\cdot}05$. (Data are taken from Kew *et al.* 2004.)

highly-purified oils rich in either EPA or DHA (supplemented at approximately 5 g/d for 4 weeks) *v.* an olive oil placebo, none of the treatments affected monocyte or neutrophil phagocytosis, the expression of a number of adhesion molecules or the productions of a range of cytokines (Kew *et al.* 2004). However, the DHA treatment, but not the EPA treatment, reduced the expression of CD69, an early marker of T-lymphocyte activation (Kew *et al.* 2004; see Fig. 4). This observation does not appear to be consistent with the lack of effect of DHA on lymphocyte proliferation reported by Thies *et al.* (2001*b*) or Kelley *et al.* (1998). However, both these studies assessed markers of cell division, whereas the study by Kew *et al.* (2004) assessed the expression of CD69 and it was the intensity of expression, rather than the percentage of cells expressing CD69, that was altered by DHA. While the percentage of CD69-positive cells correlates with the extent of lymphocyte proliferation at different concentrations of mitogen, the fluorescence intensity does not. It could be argued, therefore, that cell division itself is not affected by DHA, but that there is a lower level of expression of CD69 on the cell surface and therefore a lower level of activation of the lymphocyte population. Since the function of CD69 is not known, the implications of this effect are unclear, but it remains possible that DHA could affect lymphocyte function without altering proliferation. However, another important consideration in the interpretation of these data is that at high doses of DHA treatment there is evidence for retroconversion to EPA (Kew *et al.* 2004). This evidence makes it difficult to draw the conclusion that DHA alone exerts effects on T-lymphocyte activation, since there remains a possibility that the increase in the proportion of EPA in these cells might also contribute.

Dietary fatty acids and host defence

If some classes of fatty acid possess immunosuppressive properties, it is reasonable to suggest that they may impair host resistance to infection and therefore have undesirable effects. This issue has been subject to controversy. While there have been a few clinical trials investigating the relationship between enterally-delivered *n*-3 PUFA and infectious disease in surgical and critically-ill patients, the data are equivocal and difficult to interpret because of the inclusion of multiple nutrients rather than *n*-3 PUFA alone (for review, see Heyland *et al.* 2001). Furthermore, since they were conducted in patient groups, it would not be appropriate to extrapolate their findings to the general population. Studies conducted in animals, investigating the influence of dietary fatty acids on host survival and/or pathogen clearance in animals challenged with a live infectious agent are also inconclusive, some studies showing that *n*-3 PUFA improve host defence and others showing impairment (Anderson & Fritsche, 2002). A recent review suggests that these studies lack depth and breadth, and that a direct examination of the influence of *n*-3 PUFA on human infectious disease resistance is warranted (Anderson & Fritsche, 2002).

If olive oil suppresses immune function, then applying the same principles it is possible that it too could have a detrimental effect on host defence. This possibility was investigated by Wallace *et al.* (2000*b*), who examined the influence of a range of dietary fatty acids on macrophage-mediated cytotoxicity towards two tumour cell lines (P815 and L929). Feeding olive oil inhibited the killing of these tumour cells compared with a low-fat diet, but other high-fat diets, including those containing safflower oil and coconut oil, had similar effects. Thus, it is not clear whether the effect of the olive oil diet was in fact a result of the amount of fat (Wallace *et al.* 2000*b*). In the same study the olive oil diet decreased the *ex vivo* production of TNF-α and nitrite by macrophages compared with the low-fat diet, but once again the effect may have been associated with the amount of fat (Wallace *et al.* 2000*b*). Only a fish oil-containing diet appeared to have a specific effect on these responses (Wallace *et al.* 2000*b*). Puertollano *et al.* (2002) examined the effects on *in vitro* cellular responses to *Listeria monocytogenes* of feeding a low-fat diet or high-fat diets containing 200 g hydrogenated coconut oil, olive oil or fish oil/kg to *Balb/c* mice. Feeding olive oil did not affect spleen lymphocyte proliferation, but it enhanced the cytotoxicity of the pathogen towards splenic cells compared with the low-fat diet and the hydrogenated-coconut-oil diet, suggesting a potentially detrimental effect of olive oil (Puertollano *et al.* 2002). However, feeding olive oil did not affect the ability of

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Listeria monocytogenes to adhere to or invade the cells *in vitro* (Puertollano *et al*. 2002). Also, the same group investigated the effects of diets containing hydrogenated coconut oil, sunflower oil and olive oil on phagocytic activity in *Balb/c* mice and demonstrated that the olive oil diet enhanced phagocytic activity and production of IL-1 relative to the other groups (De Pablo *et al*. 1998*b*). Thus, the impact of olive oil on host defence is not yet clear.

Mechanisms underlying the immunomodulatory effects of *n*-3 PUFA

Despite the lack of consistency in the reported effects of *n*-3 PUFA, advances in the understanding of the structural organisation and physiological roles of fatty acids within cells have identified new mechanisms by which fatty acids might modulate immune function. Fatty acids play diverse roles in all cells. They are important as a source of energy, as structural components of cell membranes and as signalling molecules. In addition, AA (*n*-6 PUFA) and EPA (*n*-3 PUFA), can both serve as precursors for the synthesis of eicosanoids, a family of hydroxylated PUFA with a wide range of functions. Over the last few years major developments in the understanding of the organisation of lipids within cells have been demonstrated to be particularly relevant to cells of the immune system. The notion that there are 'lipid domains' in cellular membranes has given way to the recognition of lipid rafts and caveolae, which are highly-organized microenvironments with a number of important functions. The presence of intracellular lipid bodies, putatively containing precursors for the rapid production of eicosanoids within inflammatory cells, has been demonstrated. The characterization of new families of nuclear receptors, which can be activated by fatty acids, has led to speculation about the mechanisms by which intracellular fatty acids might be channelled towards these receptors to influence target genes related to immunity and inflammation.

Lipid rafts as platforms for cell activation in the immune system

Lipid rafts are dynamic microenvironments in the exoplasmic leaflets of the phospholipid bilayer of plasma membranes, which are rich in saturated fatty acids, sphingolipids, cholesterol and glycosylphosphatidylinositol-anchored proteins (Simons & Ikonen, 1997; Simons & Toomre, 2000; Horejsi, 2003). Rafts preferentially group proteins according to their function, e.g. a number of proteins involved in signalling are commonly found in lipid rafts and many of these are palmitoylated (Katagiri *et al*. 2001). Rafts are generally thought to serve as platforms to facilitate apical sorting, the association of signalling molecules and interactions and crosstalk between cell types (Simons & Ikonen, 1997; Simons & Toomre, 2000; Katagiri *et al*. 2001; Horejsi, 2003). To date, a number of methods have been used to study raft composition, most of them based on the fact that rafts contain large complexes of lipids and proteins, which are to some extent resistant to solubilization by non-ionic detergents. The detergent-resistant complexes can, therefore, be floated on sucrose gradients and their composition analysed. However, differences in detergent and extraction conditions can produce different results, and it is not clear how closely the composition of biochemically-isolated rafts corresponds with the presumed native structure (Horejsi, 2003).

Activation of the proteins within rafts by an extracellular ligand can result in rapid clustering, which appears to be important for signal transduction in both T- and B-lymphocytes (Katagiri *et al*. 2001; Pierce, 2002; Horejsi, 2003). The T-cell receptor clusters within lipid rafts on contact with an antigen-presenting cell, forming an 'immunological synapse', or contact zone, where intracellular signalling is thought to be initiated, and for this reason T-lymphocyte activation has become a model for studying lipid rafts.

The Src kinases play an important role in T-cell activation and their myristoylation or palmitoylation is regarded as essential for targetting them to rafts, since proteins can be artificially targetted to rafts by acylation (Zlatkine *et al*. 1997). Lck and Fyn, which are members of the Src family of kinases, are concentrated on the cytoplasmic side of lipid rafts and become activated in response to stimulation of the T-cell receptor, triggering a number of downstream signalling events (Liang *et al*. 2001). Lipid rafts from Jurkat cells treated with AA *in vitro* have a reduced content of Lck and Fyn, a decline in Ca signalling and a decline in some other downstream events (Stulnig *et al*. 2001). Of major interest is the fact that PUFA treatment results in remodelling of murine T-cell lipid rafts (Fan *et al*. 2003), and may even result in PUFA acylation of Fyn itself. This process is thought to be possible because palmitoyl acyltransferase is a relatively promiscuous enzyme that is able to form covalent attachments between a wide range of fatty acids and proteins (Webb *et al*. 2000; Liang *et al*. 2001). However, it is not clear whether this phenomenon is physiological. The transmembrane adaptor protein, linker for activation of T cells, is another signalling molecule constitutively present in rafts, and when phosphorylated binds to several other molecules, including phospholipase Cγ1, initiating key pathways in T-cell activation (Zhang *et al*. 1998). The functionality of the linker for activation of T cells is dependent on its palmitoylation. Treatment of Jurkat cells with the *n*-3 PUFA EPA, but not stearic acid, diminished the phosphorylation of the linker for activation of T cells and phospholipase Cγ1, and it was suggested that this effect was a result of selective displacement of the linker for activation of T cells from lipid rafts (Zeyda *et al*. 2002). Another example of alteration of lymphocyte function as a result of modulation of raft fatty acid composition is the displacement and subsequent activation of phospholipase D by the *n*-3 PUFA DHA in human T-lymphocytes (Diaz *et al*. 2002). The authors suggest that this activation of phospholipase D might be responsible for the anti-proliferative effects of DHA in lymphoid cells (Diaz *et al*. 2002). However, it would be important to determine whether there is a physiological threshold for these reported disruptive effects of PUFA on lipid rafts, and indeed whether all PUFA exert the same effect.

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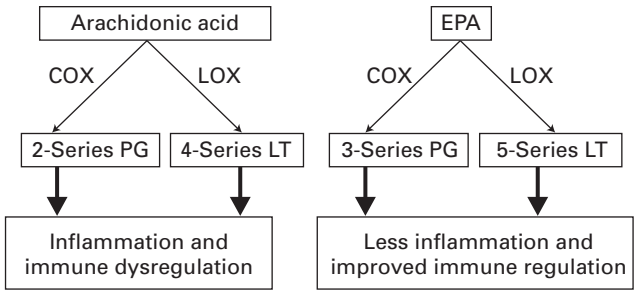

Fig. 5. Proposed modulation of eicosanoid synthesis by eicosapentaenoic acid (EPA). Fish oil results in a decreased capacity of monocytes and macrophages to synthesize eicosanoids from arachidonic acid. In addition to effects on generation of eicosanoids from AA, EPA is potentially able to act as a substrate for both cyclooxygenase (COX) and 5-lipoxygenase (LOX), giving rise to derivatives that have a different structure from those produced from AA (i.e. 3-series PG and 5-series leukotrienes (LT)). Thus, the EPA-induced suppression in the production of AA-derived eicosanoids can potentially be mirrored by an elevation in the production of EPA-derived eicosanoids. However, the generation of EPA-derived COX metabolites following fish oil feeding has not been demonstrated, suggesting that, at the concentrations incorporated into membrane phospholipids, EPA may be a relatively poor substrate for COX.

Eicosanoid generation

Eicosanoids are a family of oxygenated derivatives of AA, dihomo-γ-linolenic acid and EPA. Eicosanoids include PG, thromboxanes, leukotrienes (LT), lipoxins, hydroperoxyeicosatetraenoic acids and hydroxyeicosatetraenoic acids. Monocytes and macrophages are important sources of eicosanoids, and because their membranes typically contain large amounts of AA, compared with dihomo-γ-linolenic acid and EPA, AA is usually the principal precursor for eicosanoid synthesis. AA in the monocyte and macrophage can be mobilized by various phospholipase enzymes, most notably phospholipase A_2, and the free AA can subsequently act as a substrate for cyclooxygenase, forming 2-series PG and related compounds, or for one of the lipoxygenase enzymes, forming 4-series LT and related compounds (Fig. 5).

Eicosanoids are involved in modulating the intensity and duration of inflammatory and immune responses. PGE_2 has a number of pro-inflammatory effects, including inducing fever, increasing vascular permeability and vasodilation, and enhancing pain and oedema caused by other agents such as histamine. However, PGE_2 also inhibits production of TNF-α, IL-1 and IL-6 and this inhibition of synthesis of the pro-inflammatory cytokines by PGE_2 forms an important regulatory loop. LTB_4 increases vascular permeability, enhances local blood flow, is a potent chemotactic agent for leucocytes (including monocytes), induces release of lysosomal enzymes by neutrophils, enhances generation of reactive oxygen species, inhibits lymphocyte proliferation and promotes NK cell activity. The 4-series LT also regulate production of pro-inflammatory cytokines, e.g. LTB_4 enhances production of TNF-α, IL-1 and IL-6. In relation to the latter effect, PGE_2 and LTB_4 are antagonistic. Thus, AA gives rise to mediators that can have opposing effects to one another, so that the overall physiological effect will be governed by the concentrations of those mediators, the timing of their production and the sensitivities of target cells to their effects. The potential importance of eicosanoids in atherosclerosis was recently highlighted in a study demonstrating that antagonism of the receptor for LTB_4 resulted in a reduction in lesion progression as a result of the inhibition of monocyte recruitment (Aiello *et al*. 2002).

Since increased consumption of fish oil results in a decrease in the amount of AA in the membranes of monocytes and macrophages, there will be less substrate available for synthesis of eicosanoids from AA. Furthermore, *n*-3 PUFA inhibit phospholipase A_2 activity in macrophages and competitively inhibit the oxygenation of AA by cyclooxygenase. Thus, fish oil feeding results in a decreased capacity of monocytes and macrophages to synthesize eicosanoids from AA. This effect has been demonstrated many times in a variety of animal models (for example, see Magrum & Johnston, 1983; Brouard & Pascaud, 1990; Chapkin *et al*. 1992; Yaqoob & Calder, 1995*a*) and in human subjects (Lee *et al*. 1985; Sperling *et al*. 1993). In addition to effects on generation of eicosanoids from AA, EPA is potentially able to act as a substrate for both cyclooxygenase and 5-lipoxygenase (Fig. 5), giving rise to derivatives that have a different structure from those produced from AA (i.e. 3-series PG and and 5-series LT). Thus, the EPA-induced suppression in the production of AA-derived eicosanoids can potentially be mirrored by an elevation in the production of EPA-derived eicosanoids. Studies in experimental animals have demonstrated that feeding fish oil results in markedly enhanced production of 5-series LT (Chapkin *et al*. 1990; Whelan *et al*. 1991). Similarly, dietary fish oil (at a high dose) was demonstrated to increase generation of LTB_5, 6-trans LTB_5 and 5-hydroxyeicosapentaenoic acid by stimulated human monocytes (Lee *et al*. 1985; Sperling *et al*. 1993). The generation of EPA-derived cyclooxygenase metabolites following fish oil feeding has not been demonstrated, suggesting that at the concentrations incorporated into membrane phospholipids, EPA may be a relatively poor substrate for cyclooxygenase. However, there is still a great deal concerning the modulation of eicosanoid metabolism that is not well understood. Clarification of these pathways will be important in improving the understanding of the role of eicosanoids in inflammatory disease and of their potential modulation by dietary fatty acids.

Local generation of fatty acid-derived mediators by lipid bodies in inflammatory cells

It is becoming clear that the regulation of eicosanoid formation involves activation of enzymes at specific intracellular sites, and that this local generation of eicosanoids may be facilitated by the presence of lipid bodies present within many (if not all) cell types. Lipid bodies within eosinophils increase in number following an inflammatory stimulus and appear to contain all the enzymes necessary for eicosanoid synthesis (Bandeira-Melo *et al*. 2002). Unlike lipid rafts, these distinct

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intracellular domains are not resistant to detergent solubilization and there are consequently some methodological limitations to their study. However, novel techniques have been used to cross-link newly-synthesized LTC_4 at sites of synthesis within eosinophils and to follow its fate on stimulation (Bandeira-Melo *et al*. 2002). This approach demonstrated that LTC_4 formation does indeed occur in lipid bodies and that, depending on the nature of the stimulus, LTC_4 can be either targetted towards the perinuclear membrane or released extracellularly (Bandeira-Melo *et al*. 2001, 2002). Like lipid rafts, the distribution of lipid bodies can be polarized, but it is not clear whether those producing eicosanoids destined to be secreted are located close to the plasma membrane, while those that are perinuclear produce eicosanoids only for autocrine effects (Bandeira-Melo *et al*. 2002).

Interactions between fatty acids and nuclear transcription factors in cells of the immune system

PPAR are ligand-activated transcription factors present in a variety of cell types, with diverse actions, mainly in lipid metabolism. A range of synthetic PPAR-γ and PPAR-α ligands have been demonstrated to inhibit phorbol ester-stimulated cytokine production by monocytic cells (Jiang *et al*. 1998) and studies using PPAR-α knock-out mice have demonstrated prolonged inflammatory responses (Devchand *et al*. 1996), suggesting that PPAR may be anti-inflammatory. PPAR-α is the predominant isoform expressed in murine T- and B-lymphocytes, whereas PPAR-γ dominates in myeloid cells (Jones *et al*. 2002). Following activation of T cells, PPAR-α expression is decreased, whereas PPAR-γ expression is increased (Jones *et al*. 2002). PPAR-γ ligands have been reported to decrease the production of interferon γ and IL-2 by mitogen-activated splenocytes (Cunard *et al*. 2002), inhibit proliferation of human T cells (Clark *et al*. 2000; Harris & Phipps, 2001) and promote apoptosis in murine helper-T-cell clones (Clark *et al*. 2000). To date, most of the research examining the biological effects of PPAR has employed synthetic agonists at concentrations that are higher than their dissociation constants for binding to PPAR. The reliance on synthetic activators of PPAR has meant that there is still very little information about the physiological roles of the natural ligands of these transcription factors. It is often assumed that because some fatty acids and their metabolites have been demonstrated to act as PPAR ligands in competitive binding and/or reporter assays, they must act as natural ligands. This assumption has led to considerable speculation about the potential for specific classes of fatty acids (particularly the *n*-3 PUFA) to mediate effects on cell function through PPAR. However, there appears to be no distinction between the *n*-3 PUFA and the *n*-6 PUFA in their binding affinity and/or activating capacity, and no relationship with chain length or number of double bonds (Forman *et al*. 1997; Kliewer *et al*. 1997; Krey *et al*. 1997; Lin *et al*. 1999; Wolfrum *et al*. 2001; Xu *et al*. 2001). Thus, there is a lack of plausible evidence to support the idea that any particular class of fatty acids has a superior capacity to act as ligands for PPAR in the immune system *in vivo*. However, it has been suggested that fatty acid-binding proteins are able to interact physically with both PPAR-α and PPAR-γ to direct ligands to their responsive genes, in what has been described as a 'cytosolic gateway' (Tan *et al*. 2002). If the presence of this gateway can be established, it would represent an elegant mechanism by which intracellular fatty acids could be directed to interact with a target gene. A recent review suggests that fatty acids act as gatekeepers in immune cell regulation, in the sense that their location and organization within cellular lipids have a direct influence on the behaviour of a number of proteins involved in immune cell activation (Yaqoob, 2003*a*).

Clinical applications

The suggestion that *n*-3 PUFA might possess anti-inflammatory properties has generated considerable interest in their potential application as therapeutic agents in chronic inflammatory disorders. Unfortunately, for most inflammatory disorders, evidence for therapeutic effects of *n*-3 PUFA is very weak (see Yaqoob, 2003*b*). The only condition for which the evidence is consistently positive is rheumatoid arthritis. At least thirteen double-blind placebo-controlled trials of fish oil supplementation have been conducted to date, all of which demonstrate at least modest improvements in clinical symptoms and severity of disease in the treatment groups (for review, see Calder & Zurier, 2001). In recent years, the *n*-3 PUFA have been suggested as therapeutic agents in another chronic inflammatory disease that affects many thousands of individuals worldwide, atherosclerosis. This condition, which describes the gradual process by which lesions form in arterial walls, has only relatively recently been recognized as an inflammatory disease. Even when Meade & Mertin (1978) speculated that there might be an immunological basis for data relating to dietary fat and chronic disease, they could not have imagined the importance of their predictions in relation to atherosclerosis.

Inflammation in atherosclerosis

Atherosclerosis is characterized by the accumulation of monocytes and lymphocytes through all stages of its pathogenesis, beginning with the formation of fatty streaks underlying the endothelium of large arteries. The infiltration of monocytes and lymphocytes occurs as a result of the secretion of chemoattractant molecules (e.g. monocyte chemoattractant protein-1) and the expression of adhesion molecules by endothelial cells lining the artery wall in a manner identical to that observed in the inflammatory response to an infection. Several stimuli for the inflammatory response in atherosclerosis have been proposed, including oxidized LDL, homocysteine, free radicals and infectious micro-organisms. However, the nature of the immune response towards these stimuli is not clear. While the precise inflammatory nature of oxidized LDL is not entirely clear, it is accepted that monocytes that have infiltrated the arterial intima and differentiated into macrophages take up oxidized LDL through scavenger receptors in an unregulated manner, accumulating large amounts of cholesterol and becoming foam cells. Although homeostatic responses exist to remove cholesterol from

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macrophages, they progressively fail in atherosclerosis, and when the macrophages eventually die, through necrosis or apoptosis, the lipid is deposited within the core of the developing plaque. Cytokines secreted by both lymphocytes and macrophages within the plaque exert pro- and anti-atherogenic effects on components of the vessel wall (Ross, 1999; Glass & Witztum, 2001). Smooth muscle cells migrate from the medial portion of the arterial wall towards the intima and secrete extracellular matrix proteins that form a fibrous cap. The cap separates the highly thrombogenic contents of the plaque lipid core from the potent coagulation system contained within the circulating blood. Analysis of advanced human plaques suggests that they undergo repetitive cycles of microhaemorrhage and thrombosis, which predominantly occur at the shoulder regions (Glass & Witztum, 2001). Matrix metalloproteinases secreted by macrophages degrade extracellular matrix proteins and contribute to the weakening of the fibrous cap, which can lead to plaque rupture (Libby *et al*. 1996). The resulting thrombosis can lead to a fatal occlusion of the artery.

While much of the inflammatory activity in atherosclerosis is located in the arterial intima, there is compelling evidence to suggest that it is reflected by a persistent low-grade inflammation in the circulation. This chronic low-grade inflammation is likely to be the result of a 'spilling over' of inflammatory molecules (cytokines secreted by monocytes and soluble adhesion molecules shed from the surface of endothelial cells) from the vessel wall into the circulation, where they subsequently act on the liver to induce the secretion of acute-phase proteins, including C-reactive protein (CRP), fibrinogen and serum amyloid A.

The protective effects of *n*-3 PUFA in atherosclerosis may involve effects on inflammation

There is epidemiological evidence that consumption of fish or long-chain *n*-3 PUFA found in oily fish and fish oils protects against CVD in Western populations (Miettinen *et al*. 1982; Kromhout *et al*. 1985, 1995; Shekelle *et al*. 1985; Norell *et al*. 1986; Feskens *et al*. 1993; Siscovick *et al*. 1995; Daviglus *et al*. 1997; Albert *et al*. 1998, 2002; Hu *et al*. 2002). Long-chain *n*-3 PUFA lower fasting plasma triacylglycerol concentrations (Harris, 1996) and reduce the postprandial lipidaemic response (Williams, 1997). Several secondary prevention studies, providing long-chain *n*-3 PUFA to patients who had already suffered a myocardial infarction, demonstrate substantial benefit (for example, see Burr *et al*. 1989; Singh *et al*. 1997; GISSI Prevenzione, 1999; Marchioli *et al*. 2002), although one study demonstrated a higher risk of cardiac death, which could not be explained (Burr *et al*. 2003). A recent meta-analysis of randomized controlled trials, which compared dietary or non-dietary intake of *n*-3 PUFA with a control diet or placebo in patients with CVD, identified eleven trials fitting specific criteria relating to length of study, clinical outcomes etc., and concluded that *n*-3 PUFA reduce total mortality, fatal myocardial infarction and sudden death (Bucher *et al*. 2002). This reduction in mortality might be a result of anti-thrombotic and anti-arrhythmic actions of *n*-3 PUFA (Leaf *et al*. 1998), although *n*-3 PUFA might also contribute to the stabilization of atherosclerotic plaques by reducing inflammation.

Effects of n-*3 PUFA on systemic markers of inflammation*

CRP is an acute-phase reactant synthesized by the liver and regulated principally by the cytokine IL-6. High serum concentrations of CRP correlate with the presence of subclinical CVD and the risk of acute cardiovascular events. Several large-scale prospective epidemiological studies have shown that the plasma level of CRP is a strong independent predictor of future myocardial infarction, stroke and pulmonary vascular disease among individuals without known CVD (Kuller *et al*. 1996; Tracy *et al*. 1997; Ridker *et al*. 1998, 2000; Koenig *et al*. 1999; Danesh *et al*. 2000; Mendall *et al*. 2000). Some studies have suggested that the addition of 'high-sensitivity' CRP to lipid screening improves the estimation of vascular risk over the use of lipid screening alone, since 'high-sensitivity' CRP has been shown to be an important predictor of risk, even in individuals with normal LDL-cholesterol levels (Ridker *et al*. 2000). This finding is pertinent, since a high proportion of myocardial infarctions occur in individuals with normal plasma lipid levels. It is interesting to note in this context that the relationship between LDL-cholesterol and 'high-sensitivity' CRP is weak, which has led to the suggestion that hyperlipidaemia and enhanced inflammation are separate but interactive processes (Ridker *et al*. 2000). It is unclear at present whether CRP is simply a marker of the inflammatory process associated with atherosclerosis, or whether it plays an aetiological role in atherogenesis. It is possible that both are partially true and that serum concentrations of CRP (as well as other acute-phase proteins) reflect the inflammatory response to atherosclerotic damage, but in addition enhance clot formation, lipid oxidation and cell activation (Tracy, 1998).

The question of whether alleviation of the inflammatory component of CVD may provide additional benefits to other treatments has not been studied to a great extent in the context of CRP. DeMaat *et al*. (1994) have reported that short-term treatment (1 week) with fish oil at a high dose of 30 g/d had no significant effect on CRP levels in healthy young subjects. Furthermore, Chan *et al*. (2002) demonstrated that treatment for 6 weeks with statin, but not fish oil (4 g/d), reduced CRP and IL-6 concentrations in individuals with visceral obesity. Thus, there is currently no evidence to suggest that *n*-3 PUFA are able to modulate plasma levels of CRP.

The hepatic synthesis of CRP is largely under the control of the pro-inflammatory cytokine, IL-6. Leucocytes are thought to be an important source of circulating IL-6, although it has been estimated that as much as one-third of total circulating IL-6 can originate from adipose tissue, depending on the extent of adiposity (Yudkin *et al*. 2000). A limited number of animal and human studies report the effects of dietary *n*-3 PUFA on circulating inflammatory cytokine concentrations (in contrast to the numerous

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studies examining the effects of *n*-3 PUFA on *ex vivo* cytokine production by leucocytes, described earlier). Mice fed fish oil had lower plasma concentrations of TNF-α, IL-1β and IL-6 following endotoxin injection than did mice fed safflower oil (Sadeghi *et al*. 1999). This observation might be linked to better survival of fish oil-fed animals when exposed to endotoxin (for references, see Calder, 2001). Fish oil-containing parenteral nutrition decreased serum TNF-α, IL-6 and IL-8 concentrations in burned rats compared with *n*-6 PUFA-rich parenteral nutrition (Hayashi *et al*. 1998; Tashiro *et al*. 1998). Likewise, surgical patients infused with a fish oil-rich emulsion showed lower TNF-α concentrations in the bloodstream at some time points compared with patients receiving a control infusion (Wachtler *et al*. 1997). However, the influence of *n*-3 PUFA on subclinical levels of circulating inflammatory cytokines is not known.

Adhesion molecules mediate the attachment of leucocytes to the endothelium, their transmigration into the subendothelial space and their retention and accumulation within the artery wall. Several families of adhesion molecules are known to exist. The key adhesion molecules, in terms of atherosclerosis, are the selectins, ICAM and vascular cell adhesion molecules (VCAM). The surface expression of these molecules can be up regulated very rapidly because they exist within an intracellular pool and translocate to the plasma membrane following cell activation. Here they engage with their complementary adhesion molecule or are recycled. At the cell surface these adhesion molecules may also be cleaved to form soluble fragments that enter the circulation. Soluble forms of ICAM-1 and VCAM-1 are found in the plasma, probably as a result of shedding from the surface of activated endothelial cells (Rothlein *et al*. 1991).

Plasma concentrations of soluble ICAM-1 and soluble-VCAM-1 have been reported in some studies to be higher in individuals with CVD and pulmonary vascular disease than in controls (Blann & McCollum, 1994; Haught *et al*. 1996; Morisaki *et al*. 1997; Caulin-Glaser *et al*. 1998). However, the results of such studies are not entirely consistent and a recent meta-analysis has demonstrated that soluble adhesion molecules are unlikely to add much predictive information to that provided by established risk factors (Malik *et al*. 2001).

Studies investigating the effects of supplementation with fish oil on serum soluble adhesion molecule levels have also reported equivocal findings (Abe *et al*. 1998; Seljeflot *et al*. 1998; De Caterina *et al*. 2000). Several studies have shown no effect on levels of soluble VCAM-1, while one study reported an increase (Johansen *et al*. 1999) and one study reported that supplementation with a moderate dose of fish oil (1·2 g EPA + DHA/d) for 12 weeks decreased plasma levels of soluble VCAM-1 in older subjects, but did not have any effect in young males (Miles *et al*. 2001). Most studies show no effect on plasma soluble ICAM-1 levels (Abe *et al*. 1998; Miles *et al*. 2001). Two studies showed that fish oil increased plasma soluble E-selectin (Johansen *et al*. 1999; Miles *et al*. 2001); in one of these studies soluble E-selectin was increased by fish oil in young subjects, but not in older subjects (Miles *et al*. 2001).

Effects of n-*3 PUFA on monocyte and macrophage chemotaxis*

Chemotaxis of monocytes and macrophages could be affected by changes in the fatty acid composition of membrane phosopholipids that might influence the binding of chemotactic agents to their receptors, the subsequent signalling pathways or the cytoskeletal rearrangements that occur. Modulation of chemotaxis by *n*-3 PUFA could potentially influence the extent of infiltration of monocytes into the arterial intima. Chemotaxis of blood monocytes towards the chemo-attractants LTB_4 and formyl-methionylleucyl-phenylalanine was found to be suppressed following supplementation of the human diet with approximately 5·5 g EPA + DHA/d for 6 weeks (Lee *et al*. 1985; Schmidt *et al*. 1992). However, there was no effect of a much lower (and more nutritionally relevant) dose of *n*-3 PUFA (0·65 g/d for 12 weeks) on monocyte chemotaxis towards pooled human serum (Schmidt *et al*. 1996).

Effects of n-*3 PUFA on adhesion molecule expression*

As described earlier, adhesion molecules are involved in interactions between leucocytes and endothelial cells, which may facilitate movement of leucocytes into the arterial wall. *In vitro* studies have highlighted the potential for *n*-3 PUFA to modulate the expression of adhesion molecules by some cell types. Calder *et al*. (1990) observed that murine peritoneal macrophages cultured in the presence of EPA or DHA were less adherent to artificial surfaces than those cultured with other fatty acids. Incubation of human monocytes with EPA has also been shown to result in reduced expression of ICAM-1, while DHA had no effect (Hughes *et al*. 1996*b*). The reduction in ICAM-1 expression on human monocytes was also observed following dietary supplementation with *n*-3 PUFA (Hughes *et al*. 1996*a*). In animal feeding studies Sanderson *et al*. (1995*b*, 1998) demonstrated that fish oil reduced the expression of specific adhesion molecules on concanavalin A-stimulated lymphocytes and their adhesion to macrophage and endothelial cell monolayers.

Effects of n-*3 PUFA on the expression of scavenger receptors*

Scavenger receptors take up modified forms of LDL in an unregulated manner, leading to foam cell formation. A few studies have examined the effects of *n*-3 PUFA on scavenger receptor expression by monocytes or macrophages. An animal study demonstrated that feeding a fish oil-rich diet to mice resulted in down-regulation of macrophage scavenger receptors AI and AII, while coconut oil and sunflower oil had no effect, when compared with the standard diet fed to the animals (Miles *et al*. 2000). Pietsch *et al*. (1995) reported a down-regulation of the expression of CD36 by the human monocytic U937 cell line after incubation with EPA (5 μM) or DHA, but not with linoleic acid or AA. In another study EPA (30–240 μM) was shown to inhibit the proliferation of the same cell line in a dose-dependent manner and, at the

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highest concentrations, induced apoptosis (Finstad *et al*. 1998). Expression of CD36 was lower in cells treated with EPA (60 μM) or oleic acid compared with untreated cells (Finstad *et al*. 1998). However, EPA unexpectedly caused greater accumulation of lipid droplets in the cells than oleic acid, although the effects were reversed when cells were re-incubated in EPA-free medium. This finding leaves the question of the precise nature of the effects of fatty acids on foam cell formation unresolved.

Effects of n-*3 PUFA on thrombogenic potential of macrophages*

Macrophages present in atherosclerotic lesions produce tissue factor, a highly thrombogenic agent which, when released as a result of plaque rupture, activates platelet aggregation and thrombosis. Analysis of human lesions in individuals with advanced atherosclerosis suggests that repetitive cycles of microhaemorrhage and non-fatal thrombosis occur (Glass & Witztum, 2001). Human monocytes that have been differentiated and transformed into foam cells *in vitro* have been demonstrated to express tissue factor (Colli *et al*. 1999). AA, but not EPA or DHA, enhanced both the expression of tissue factor and the procoagulant activity of human monocyte-derived macrophages by a mechanism suggested to involve the cyclo-oxygenase pathway (Cadroy *et al*. 1998).

Effects of n-*3 PUFA on atherosclerotic plaque morphology and stability*

The propensity of atherosclerotic plaques to rupture is influenced by their lipid content and the distribution of lipid within the plaque, by the extent of infiltration of inflammatory macrophages at the shoulder regions of the plaque and by the thickness of the fibrous cap (Davies *et al*. 1993; Libby *et al*. 1996; Plutzky, 1999). A greater lipid content, a high presence of inflammatory macrophages and a thin fibrous cap reflect a plaque that is vulnerable and likely to rupture. Macrophages secrete metalloproteinases (and induce smooth muscle cells to secrete them), which weaken the fibrous cap and, once rupture has occurred, can induce thrombosis by expressing tissue factor (Libby *et al*. 1996). The effects of specific fatty acids on plaque morphology and progression are not clear (Felton *et al*. 1997). However, given the evidence for the anti-coagulatory, anti-thrombotic and anti-inflammatory properties of *n*-3 PUFA, it is possible that alteration of the PUFA composition of the diet could affect plaque progression, stability and thrombus formation. If *n*-3 PUFA are to affect plaque stability it is likely that they must first be incorporated into the plaque. In a study by Rapp *et al*. (1991) patients destined to undergo carotid endarterectomy consumed fish oil for a period before surgery and the levels of EPA and DHA in the plaques removed at surgery were higher than those in plaques removed from control patients. However, the study used a very high dose of fish oil, 48–64 g/d providing 16–21 g EPA + DHA/d (Rapp *et al*. 1991). In comparison, habitual consumption of long-chain *n*-3 PUFA in most Western diets is <0·3 g/d, while secondary prevention studies demonstrate protective effects of <1·8 g EPA + DHA/d (Burr *et al*. 1989; GISSI Prevenzione, 1999; Marchioli *et al*. 2002). A recent study in patients awaiting carotid endarterectomy (fifty-nine or more patients per treatment group), investigated the effects of moderate doses of *n*-3 PUFA on plaque composition, morphology and stability (Thies *et al*. 2003). Patients were randomly assigned to either placebo (palm oil + soybean oil), sunflower oil or fish oil, with those in the fish oil group consuming an extra 1·4 g *n*-3 PUFA (EPA and DHA)/d, while those in the sunflower oil group consumed an extra 3·6 g linoleic acid/d (Thies *et al*. 2003). The duration of oil treatment was 7–189 (median 42) d, which represented the waiting time before surgical removal of the carotid plaques. The proportions of EPA and DHA were higher in carotid plaque phospholipids, cholesteryl esters and triacylglycerols in patients receiving fish oil compared with patients in the control group (Thies *et al*. 2003). Fewer plaques from patients being treated with fish oil had thin fibrous caps and signs of inflammation and more plaques had thick fibrous caps and no signs of inflammation, compared with the other two groups; these differences were significant in patients who had been treated with fish oil for >42 d ($P < 0{\cdot}05$; Thies *et al*. 2003). The number of macrophages in the plaques from patients receiving fish oil for >42 d was lower than that in the other two groups (Thies *et al*. 2003). These results suggest that advanced atherosclerotic plaques are dynamic and readily incorporate *n*-3 PUFA, even when provided at relatively modest doses. Furthermore, incorporation of *n*-3 PUFA into carotid plaques was associated with a reduced number of macrophages and fewer signs of inflammation, suggesting that *n*-3 PUFA induce changes that may increase the stability of atherosclerotic plaques.

Conclusion

When Meade & Mertin (1978) published their highly speculative review, it is unlikely that they could have imagined the subsequent interest in the effects of fatty acids on the immune system. Over the last 25 years fatty acid research has gradually established its niche in immunology. Although there are still many questions regarding the exact nature of the modulation of immune responses by fatty acids, research is increasingly being conducted in human subjects and study design is gradually improving to overcome some of the criticisms of earlier studies. Advances in fatty acid biochemistry and molecular techniques are suggesting new mechanisms by which fatty acids could potentially alter cellular responses, many of them being particularly relevant to the immune system. Finally, there are exciting possibilities for the clinical applications of *n*-3 PUFA. The present review has focused on the hypothesis that the anti-inflammatory properties of *n*-3 PUFA in the arterial wall may contribute to the protective effects of *n*-3 PUFA in CVD, as suggested by epidemiological and secondary prevention studies. Studies are just beginning to show that dietary *n*-3 PUFA can be incorporated into plaque lipid in human subjects, where they may influence the morphology and stability of the atherosclerotic lesion.

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