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Differential immunomodulation with long-chain *n*-3 PUFA in health and chronic disease

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The balance of intake of *n*-6 and *n*-3 PUFA, and consequently their relative incorporation into immune cells, is important in determining the development and severity of immune and inflammatory responses. Some disorders characterised by exaggerated inflammation and excessive formation of inflammatory markers have become among the most important causes of death and disability in man in modern societies. The recognition that long-chain *n*-3 PUFA have the potential to inhibit (excessive) inflammatory responses has led to a large number of clinical investigations with these fatty acids in inflammatory conditions as well as in healthy subjects. The present review explores the presence of dose-related effects of long-chain *n*-3 PUFA supplementation on immune markers and differences between healthy subjects and those with inflammatory conditions, because of the important implications for the transfer of information gained from studies with healthy subjects to patient populations, e.g. for establishing dose levels for specific applications. The effects of long-chain *n*-3 PUFA supplementation on *ex vivo* lymphocyte proliferation and cytokine production by lymphocytes and monocytes in healthy subjects have been studied in twenty-seven, twenty-five and forty-six treatment cohorts respectively, at intake levels ranging from 0·2 g EPA + DHA/d to 7·0 g EPA + DHA/d. Most studies, particularly those with the highest quality study design, have found no effects on these immune markers. Significant effects on lymphocyte proliferation are decreased responses in seven of eight cohorts, particularly in older subjects. The direction of the significant changes in cytokine production by lymphocytes is inconsistent and only found at supplementation levels $\geq$ 2·0 g EPA + DHA/d. Significant changes in inflammatory cytokine production by monocytes are decreases in their production in all instances. Overall, these studies fail to reveal strong dose–response effects of EPA + DHA on the outcomes measured and suggest that healthy subjects are relatively insensitive to immunomodulation with long-chain *n*-3 PUFA, even at intake levels that substantially raise their concentrations in phospholipids of immune cells. In patients with inflammatory conditions cytokine concentrations or production are influenced by EPA + DHA supplementation in a relatively large number of studies. Some of these studies suggest that local effects at the site of inflammation might be more pronounced than systemic effects and disease-related markers are more sensitive to the immunomodulatory effects, indicating that the presence of inflamed tissue or 'sensitised' immune cells in inflammatory disorders might increase sensitivity to the immunomodulatory effects of long-chain *n*-3 PUFA. In a substantial number of these studies clinical benefits related to the inflammatory state of the condition have been observed in the absence of significant effects on immune markers of inflammation. This finding suggests that condition-specific clinical end points might be more sensitive markers of modulation by EPA + DHA than cytokines. In general, the direction of immunomodulation in healthy subjects (if any) and in inflammatory conditions is the same, which indicates that studies in healthy subjects are a useful tool to describe the general principles of immunomodulation by *n*-3 PUFA. However, the extent of the effect might be very different in inflammatory conditions, indicating that studies in healthy subjects are not particularly suitable for establishing dose levels for specific applications in inflammatory conditions. The reviewed studies provide no indications that the immunomodulatory effects of long-chain *n*-3 PUFA impair immune function or infectious

Abbreviations: ARA, arachidonic acid; Con A, concanavalin A; COPD, chronic obstructive pulmonary disease; IFN-γ, interferon-γ; LT, leukotrienes; LPS, lipopolysaccharide; NK, natural killer; PHA, phytohaemagglutinin; Th, T-helper.

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disease resistance. In contrast, in some conditions the immunomodulatory effects of EPA + DHA might improve immune function.

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PUFA have an important role in shaping and regulating inflammatory processes and responses (Calder, 2006). The balance of *n*-6 and *n*-3 PUFA might be important in determining the development and severity of inflammatory responses (Calder, 2006). Thus, a high intake of *n*-6 PUFA, particularly arachidonic acid (ARA; 20:4*n*-6), could potentiate inflammatory processes and so could predispose to or exacerbate inflammatory diseases. Alterations in the food chain and increased consumption of vegetable oils over the past century have led to alterations in *n*-6 and *n*-3 PUFA intakes, in favour of *n*-6 PUFA (Simopoulos, 1998). This development is likely to have increased the proportion of ARA in inflammatory cell phospholipids, as cell and tissue levels partly reflect dietary intake. Unfortunately, however, historical data to confirm this outcome are not available. On the other hand, increased consumption of *n*-3 PUFA, particularly the long-chain *n*-3 PUFA EPA (20:5*n*-3) and DHA (22:6*n*-3), increases the proportions of these fatty acids in inflammatory cell phospholipids (Calder, 2001*a*). The recognition that EPA and DHA have anti-inflammatory properties (Calder, 2006) suggests that increasing their intake corrects the *n*-6 and *n*-3 PUFA balance, and so may act to decrease the risk of inflammatory conditions and be of benefit to patients with inflammatory diseases.

Some of the most important causes of death and disability in man are characterised by exaggerated inflammation and excessive formation of inflammatory eicosanoids and cytokines. These conditions and diseases include chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, osteoarthritis, cachexia and inflammatory bowel diseases. In addition, many other diseases and conditions that are important causes of death and disability in developed regions have an inflammatory component that may be less pronounced. The latter include CVD, cerebrovascular disease, unipolar major depression, neurodegenerative disease, Alzheimer's disease, allergy, asthma, type 1 and type 2 diabetes and obesity. The onset of some of these disorders has been associated with low consumption of fish (which contain long-chain *n*-3 PUFA) and long-chain *n*-3 PUFA; in particular CVD (Daviglus *et al*. 1997; Oomen *et al*. 2000; Hallgren *et al*. 2001), depression (Hibbeln, 1998, 2002) and Alzheimer's disease (Morris *et al*. 2003). The role of excess inflammation in neural disorders is poorly understood. The essentiality of long-chain *n*-3 PUFA, particularly DHA as a structural component, for normal neural function may explain much of their importance. Some disorders with a more-pronounced and clearly-defined inflammatory character such as COPD (Murray & Lopez, 1997), asthma (Bach, 2002) and Crohn's disease (Bach, 2002) have become more prevalent in most Western societies in the past decades, in parallel with greatly increased intake of *n*-6 PUFA. Clear associations between the risk of their onset and a shifted balance of *n*-6 PUFA and *n*-3 PUFA intake are lacking, but many investigators have recognised the potential of long-chain *n*-3 PUFA in dampening excessive inflammation in most of the inflammatory diseases and conditions listed earlier (Calder, 2006).

A large number of clinical investigations with long-chain *n*-3 PUFA in chronic inflammatory disorders are now available, particularly in patients with rheumatoid arthritis, inflammatory bowel diseases and asthma (Calder, 2006). Generally, these studies have focused on disease-specific clinical outcomes rather than on markers of immunomodulation. In contrast, a large number of investigations with long-chain *n*-3 PUFA focusing on markers of immunomodulation have been performed with healthy subjects (Calder, 2001*a*). However, a common feature of intervention studies involving healthy subjects and some studies of subjects with inflammatory disorders is the measurement of similar immune markers. It is possible that the sensitivity of the immune system to interventions such as *n*-3 PUFA may be different in subjects with an inflammatory condition as compared with healthy subjects for whom the immune system may be buffered to a larger extent against modulation by such intervention. A difference in sensitivity would have important implications for the transfer of information gained from studies of healthy subjects to patient populations with inflammatory conditions, e.g. for establishing dose levels for specific applications. Thus, the current report reviews the immunomodulatory effects of long-chain *n*-3 PUFA in healthy human subjects as well as in patients with chronic disorders. The aim of the present review is to explore the presence of dose-related effects and differences between populations with different immune states. In order to make this comparison the present review will focus on the measurements that have been reported in studies involving healthy subjects as well as those reported in some studies of subjects with inflammatory disorders; i.e. lymphocyte proliferation, natural killer (NK) cell activity and production of cytokines by monocytes and lymphocytes.

Dietary fatty acids, the inflammatory response and T-cell-mediated immunity

The key link between fatty acids, inflammation and immunity is that eicosanoids, which are among the most important mediators and regulators of inflammation and immune responses, are generated from C_{20} PUFA. Since inflammatory cells typically contain a high proportion of the *n*-6 PUFA ARA and low proportions of other C_{20} PUFA (Calder, 2001*a*), ARA is usually the major substrate for eicosanoid synthesis. Eicosanoids, which include PG, thromboxanes, leukotrienes (LT) and other oxidised

derivatives, are generated from ARA by the action of cyclooxygenase and lipoxygenase enzymes. These mediators are involved in modulating the intensity and duration of inflammatory responses (for reviews, see Lewis *et al.* 1990; Tilley *et al.* 2001), have cell- and stimulus-specific sources and frequently have opposing effects (Calder, 2006). Thus, the overall physiological (or pathophysiological) outcome will depend on the cells present, the nature of the stimulus, the timing of eicosanoid generation, the concentrations of different eicosanoids generated and the sensitivity of target cells and tissues to the eicosanoids generated. In general, pro-inflammatory roles have been ascribed to PGE_2 and 4-series LT derived from ARA. For example, PGE_2 induces fever, pain, vasodilation and vascular permeability, while LTB_4 is chemotactic for leucocytes and induces the release of reactive oxygen species by neutrophils and inflammatory cytokines (TNFα, IL-1β, IL-6) by macrophages (Lewis *et al.* 1990; Tilley *et al.* 2001). In relation to cell-mediated immunity, PGE_2 inhibits T-cell proliferation, the production of T-helper (Th)-1 type cytokines (IL-2 and interferon-γ (IFN-γ)) by T-cells and promotes IgE production by B-cells. In contrast, LTB_4 promotes the production of IL-2 and IFN-γ by T-cells, and enhances NK cell activity.

Animal feeding studies have shown a strong positive relationship between the amount of ARA in inflammatory cells and the ability of those cells to produce eicosanoids such as PGE_2 (Peterson *et al.* 1998). In turn, the amount of ARA in inflammatory cells can be increased by including ARA in the diet of rats (Peterson *et al.* 1998) or by increasing the amount of ARA in the diet of human subjects (Thies *et al.* 2001*c*). The amount of ARA in inflammatory cells may also be influenced by the dietary intake of its precursor linoleic acid (18 : 2*n*-6), although the range of linoleic acid intake over which this relationship occurs has not been defined for man. The role of ARA as a precursor for the synthesis of eicosanoids indicates the potential for dietary *n*-6 PUFA (linoleic acid or ARA) to influence inflammatory and immune processes. The influence of increased dietary ARA has been investigated in two studies in healthy human subjects. In one study (Kelley *et al.* 1998*a*) healthy young males supplemented their diets with 1·5 g ARA/d for 7 weeks. This treatment was found to result in a marked increase in the production of PGE_2 and LTB_4 by bacterial lipopolysaccharide (LPS)-stimulated mononuclear cells. However, production of TNFα, IL-1β and IL-6 by the latter was not found to be significantly altered (Kelley *et al.* 1998*a*). Similarly, ARA was found to have no effect on mitogen-stimulated T-cell proliferation (Kelley *et al.* 1997), NK cell activity (Kelley *et al.* 1997) or IL-2 production by mitogen-stimulated T-cells (Kelley *et al.* 1998*a*). Thus, increased ARA intake may result in changes indicative of selectively increased inflammation or inflammatory responses in man. In another study (Thies *et al.* 2001*a*,*b*,*c*) elderly subjects supplemented their diets with 0·7 g ARA/d for 12 weeks. This treatment was found to have no effect on LPS-stimulated production of TNFα, IL-1β or IL-6 by mononuclear cells (Thies *et al.* 2001*a*), mitogen-stimulated T-cell proliferation (Thies *et al.* 2001*c*), production of IL-2 and IFN-γ by T-cells in response to mitogen (Thies *et al.* 2001*c*) or NK cell activity (Thies *et al.* 2001*b*). Taken together these studies suggest that moderately-increased intake of ARA by healthy subjects results in the incorporation of ARA into cells involved in inflammatory responses (Thies *et al.* 2001*c*) but does not affect the production of inflammatory cytokines, T-cell responses or NK cell activity (Thies *et al.* 2001*a*,*b*,*c*), although the production of inflammatory eicosanoids is increased (Kelley *et al.* 1998*a*).

Increased consumption of the long-chain *n*-3 PUFA EPA and DHA results in increased proportions of these fatty acids in inflammatory cell phospholipids (Lee *et al.* 1985; Endres *et al.* 1989; Gibney & Hunter, 1993; Sperling *et al.* 1993; Caughey *et al.* 1996; Healy *et al.* 2000; Yaqoob *et al.* 2000). The incorporation of EPA and DHA into human inflammatory cells occurs in a dose–response fashion (Healy *et al.* 2000) and is partly at the expense of ARA. Since there is less substrate available for synthesis of eicosanoids from ARA, fish oil supplementation of the human diet has been shown to result in decreased production of ARA-derived eicosanoids by inflammatory cells (for references, see Calder, 2006). Whereas these studies used fish oil providing both EPA and DHA, Kelley *et al.* (1999) have demonstrated that 6 g DHA/d results in decreased production of PGE_2 (by 60%) and LTB_4 (by 75%) by LPS-stimulated mononuclear cells.

EPA is also able to act as a substrate for both cyclooxygenase and lipoxygenase enzymes, giving rise to eicosanoids with a slightly different structure from those formed from ARA. Thus, fish oil supplementation of the human diet has been shown to result in increased production of 5-series LT and 5-hydroxyeicosapentaenoic acid by inflammatory cells (for references, see Calder, 2006). The functional importance of this difference is that the mediators formed from EPA are frequently less potent than those formed from ARA (Goldman *et al.* 1983; Lee *et al.* 1984; Bagga *et al.* 2003), although it is not always the case (Dooper *et al.* 2002; Miles *et al.* 2002, 2003). The reduction in generation of ARA-derived mediators that accompanies fish oil consumption has led to the notion that fish oil is anti-inflammatory and immunomodulatory.

In addition to long-chain *n*-3 PUFA modulating the generation of eicosanoids from ARA and to EPA acting as substrate for the generation of alternative eicosanoids, recent studies have identified a novel group of mediators, the E- and D-series resolvins formed from EPA and DHA respectively, that appear to exert anti-inflammatory and immunomodulatory actions (for reviews, see Serhan *et al.* 2004; Serhan, 2005).

Through the changed profiles of production of eicosanoids and other mediators, *n*-3 PUFA are expected to influence inflammatory processes and immune responses (Calder, 2003, 2006). Cell-culture and animal experiments have confirmed this expectation, although they have often involved high levels of exposure to the fatty acids under study and other experimental conditions that are not transferable to the human setting. Data from cell-culture and animal models reporting inflammatory and immune outcomes have been reviewed in detail elsewhere (Calder, 2001*a*, 2003, 2006; Calder *et al.* 2002) and will not be further discussed here.

Effects of long-chain *n*-3 PUFA on immune markers in healthy subjects

Introductory comments

There is a great deal of diversity in the measurements reported in the large number of studies investigating the effects of fish oil and other sources of EPA and DHA on markers of immune function in healthy subjects. The objective of the present review is to explore the extent to which long-chain *n*-3 PUFA influence immune outcomes in healthy subjects and how the effects in healthy subjects relate to those seen in patients with disorders characterised by the presence of (chronic) inflammation. Thus, the review is limited to immune markers reported in a substantial number of studies of healthy subjects as well as in studies of subjects with inflammatory disorders. The markers selected according to these criteria are *ex vivo* mitogen-induced T lymphocyte proliferation, NK cell activity, mitogen-induced cytokine production by lymphocytes and cytokine production by monocytes. Some of the best-quality markers of immunomodulation, e.g. delayed-type hypersensitivity response and vaccine-specific antibody response (Albers *et al*. 2005), are reported in too few studies to contribute to the objective of the present overview. Thus, the reviewed data do not provide a complete overview of all available data on the effects of dietary supplementation with EPA and DHA on immune function in healthy subjects.

Selected data are presented in Tables 1–3, in which studies are listed according to ascending EPA and DHA intake. This form of presentation was chosen to provide a clearer picture of potential dose-dependent effects. In addition, the percentage change associated with increased intake of *n*-3 PUFA reported in each study is depicted in Figs. 1–3 to help explore the presence of potential trends in results that were not shown to be significant, as studies could potentially lack the statistical power to find significance because of their small sample size and the large inter-individual variance in outcomes that is generally reported (see Albers *et al*. 2005). The current review covers studies with oral supplementation of fish oil capsules, purified EPA and/or DHA supplements, fish oil-enriched foods or diets with well-defined long-chain *n*-3 PUFA enrichment.

Effects of EPA and DHA on ex vivo *lymphocyte proliferation*

The effects of EPA and DHA on *ex vivo* lymphocyte proliferation in healthy subjects have been reported in fourteen publications at twenty-seven different dose levels ranging from 0·2 to 7 g EPA + DHA/d (Table 1). Seven of these studies had a double-blind randomised placebo-controlled study design. Stimulants used to induce lymphocyte proliferation were concanavalin A (Con A) or phytohaemagglutinin (PHA), which are both mitogens for T-cells. In all cases, cells were from peripheral blood and they were frequently studied as a purified preparation of mononuclear cells, which comprise a mixture of lymphocytes and monocytes in an approximate ratio of 85 : 15.

Significantly increased lymphocyte proliferation ($P<0{\cdot}05$) was found in only one cohort, at a dose level of 2·0 g EPA + DHA/d (Trebble *et al*. 2003*b*). In this open study, which investigated effects of increasing dose levels from baseline to 0·3, 1·0 and 2·0 g/d, a trend towards a dose–response relationship was found (Trebble *et al*. 2003*b*) that was accompanied by a dose-dependent decrease in PGE_2 production and an increased IFN-γ production at the highest intake level. Decreased lymphocyte proliferation was reported in seven treatment cohorts at dose levels of 0·2–7·0 g EPA + DHA/d. Five of these seven studies used an open trial design. In one of the two blinded studies the reported decrease in lymphocyte proliferation was only significantly different ($P<0{\cdot}05$) compared with baseline values but not compared with the placebo values (Bechoua *et al*. 2003). In nineteen treatment cohorts no significant effect on lymphocyte proliferation was reported; fourteen of these cohorts were in studies with a double blind placebo-controlled randomised design. In one of these studies the lymphocyte proliferation response to Con A was found to decrease significantly in all treatments ($P<0{\cdot}05$), including the placebo, illustrating the importance of an adequate placebo control (Wallace *et al*. 2003). This finding emphasises that caution should be exercised in the interpretation of the results of open studies, since the studies with better-quality designs and similar dose levels generally do not confirm their findings.

Fig. 1 illustrates that those studies that reported significant ($P<0{\cdot}05$) effects of *n*-3 PUFA were those that found the largest percentage changes in lymphocyte proliferation from baseline (i.e. before *n*-3 PUFA treatment). The cohorts with significantly decreased lymphocyte proliferation received doses of *n*-3 PUFA that seem randomly spread over the complete dose range studied, indicating the absence of a clear dose–response relationship.

Thus, while there is evidence in the literature to suggest that long-chain *n*-3 PUFA decrease the proliferative capacity of T lymphocytes, even more evidence suggests that there is no significant effect; there is relatively little evidence from healthy human volunteer studies to suggest that fish oil enhances T lymphocyte proliferation. The reasons for these different findings are not apparent. Many confounding factors have been suggested, such as gender, age, different vitamin E content of the capsules used and variations in cell-culture conditions (see Calder, 2001*a*). However, most of the factors that could potentially contribute to the variance between cohorts do not consistently influence the EPA and DHA effects in more than one study. For instance, Kramer and co-workers (Kramer *et al*. 1991) have found a significant decrease in lymphocyte proliferation following a high dose of fish oil, and this effect is completely reversed by concurrent supplementation of vitamin E. In contrast, Trebble *et al*. (2003*b*) have found no effect of co-administering an antioxidant mix with a fish oil supplement, and all other studies that have found decreased lymphocyte proliferation used capsules enriched with vitamin E (apart from one study that used fish; Meydani *et al*. 1993). On the other hand, the observation by Meydani *et al*. (1991) that older (female) subjects are more susceptible to the effects of long-chain *n*-3 PUFA is further supported by three other studies in elderly

Table 1. Effects of EPA and DHA supplementation on *ex vivo* lymphocyte proliferation in healthy human subjects*

Reference	Type of study	Dose of EPA + DHA (g/d)	Duration (weeks)	Subjects			Stimulus used	Effect seen†
				n	Gender	Age (years)		
Bechoua *et al.* (2003)	DB, PC, R, parallel	0·2 (0·03 + 0·15)	6	10	M + F	70–83, mean 75·6	Con A	↓ 45%
							PHA	↓ 34%
Trebble *et al.* (2003*b*)	Open, parallel	0·3 (0·2 + 0·1)	4	16	M	mean 30	Con A	None
Wallace *et al.* (2003)	DB, PC, R, parallel	0·4 (0·1 + 0·3)	12	8	M	18–39, mean 25	Con A	None
Thies *et al.* (2001*c*)	DB, PC, R, parallel	0·7 DHA	12	8	M + F	58–71, mean 65	Con A	None
Kew *et al.* (2003)	DB, PC, R, parallel	0·77 (0·3 + 0·47)	24	30	M + F	25–72, mean 52	Con A	None
Miles *et al.* (2004*b*)	DB, PC, R, parallel	0·8 (0·6 + 0·2) + 0·6 GLA + 2·0 ALA + 0·7 STA	12	8–12	M	21–44, mean 32	Con A	None
Wallace *et al.* (2003)	DB, PC, R, parallel	0·9 (0·3 + 0·6)	12	8	M	18–39, mean 25	Con A	None
Thies *et al.* (2001*c*)	DB, PC, R, parallel	1·0 (0·7 + 0·3)	12	7	M + F	60–68, mean 62	Con A	↓ 61%
Trebble *et al.* (2003*b*)	Open, parallel	1·0 (0·7 + 0·3)	4	16	M	mean 30	Con A	None
Miles *et al.* (2004*b*)	DB, PC, R, parallel	1·1 (0·8 + 0·3) + 0·8 GLA + 1·3 ALA + 0·4 STA	12	8–12	M	21–44, mean 32	Con A	None
Meydani *et al.* (1993)	Open, PC, parallel	1·23	24	10	F	>40, mean 65	Con A	↓ 56%
							PHA	↓ 53%
Molvig *et al.* (1991)	Open, PC	1·6 (1·0 + 0·6)	7	9	M	mean 27	PHA	↓ 18%
Miles *et al.* (2004*b*)	DB, PC, R, parallel	1·6 (1·1 + 0·5) + 1·0 GLA	12	8–12	M	21–44, mean 32	Con A	None
Miles *et al.* (2006)	DB, PC, R, parallel	1·65 (1·35 + 0·3)	12	23	M	18–42, mean 24	Con A	None
Kew *et al.* (2003)	DB, PC, R, parallel	1·7 (0·7 + 1·0)	24	30	M + F	25–72, mean 54	Con A	None
Wallace *et al.* (2003)	DB, PC, R, parallel	1·9 (0·5 + 1·4)	12	8	M	18–39, mean 22	Con A	None
Trebble *et al.* (200*3b*)	Open, parallel	2·0 (1·3 + 0·7)	4	16	M	mean 30	Con A	↑ 92%
Meydani *et al.* (1991)	Open, parallel	2·4 (1·7 + 0·7)	12	5	F	23–33, mean 27	Con A	None
							PHA	None
Meydani *et al.* (1991)	Open, parallel	2·4 (1·7 + 0·7)	12	6	F	51–68, mean 61	Con A	None
							PHA	↓ 36%
Miles *et al.* (2004*b*)	DB, PC, R, parallel	3·0 (2·1 + 0·9)	12	10	M	21–44, mean 32	Con A	None
Molvig *et al.* (1991)	Open, PC	3·2 (2 + 1·2)	7	9	M	mean 24	PHA	↓ 33%
Miles *et al.* (2006)	DB, PC, R parallel	3·3 (2·7 + 0·6)	12	23	M	18–42, mean 25	Con A	None
Yaqoob *et al.* (2000)	DB, PC, R, parallel	3·3 (2·1 + 1·1)	12	8	M + F	mean 39	Con A	None
Endres *et al.* (1993)	Open, non-parallel	4·7 (2·8 + 1·9)	6	9	M	21–39, mean 28	PHA	None
Miles *et al.* (2006)	DB, PC, R,parallel	4·95 (4·05 + 0·9)	12	23	M	18–42, mean 24	Con A	None
Kelley *et al.* (1998*b*)	Open, PC, parallel	6 DHA	13	7	M	20–40, mean 33	Con A	None
							PHA	None
Kramer *et al.* (1991)	Open, parallel	7 (5 + 2)	10	40	M	24–57, mean 38	Con A	↓ 80% (effect reversed after FO + vitamin E)

DB, double-blind; PC, placebo-controlled; R, randomised; ALA, α-linolenic acid (18:3*n*-3); STA, stearidonic acid (18:4*n*-3); GLA, γ-linolenic acid (18:3*n*-6); Con A, concanavalin A; PHA, phytohaemagglutinin; ↓, reduction; FO, fish oil.

*The cohorts are listed according to ascending dose of EPA + DHA.

†The effect is shown if it was significant ($P<0·05$).

Table 2. Effects of EPA + DHA supplementation on *ex vivo* cytokine production by lymphocytes in healthy human subjects*

Reference	Type of study	Dose of EPA + DHA (g/d)	Duration (weeks)	Subjects			Stimulus used	Cytokines studied	Effect seen†
				n	Gender	Age (years)			
Trebble *et al.* (2003*b*)	Open, parallel	0·3 (0·2 + 0·1)	4	16	M	mean 30	Con A	IFN-γ, IL-4	None
Wallace *et al.* (2003)	DB, PC, R, parallel	0·4 (0·1 + 0·3)	12	8	M	18–39, mean 25	Con A	IL-2, IFN-γ, IL-4, IL-10	None
Thies *et al.* (2001*c*)	DB, PC, R, parallel	0·7 DHA	12	8	M + F	58–71, mean 65	Con A	IL-2, IFN-γ	None
Kew *et al.* (2003)	DB, PC, R, parallel	0·77 (0·3 + 0·47)	24	30	M + F	25–72, mean 52	Con A	IL-2, IFN-γ, IL-4	None
Miles *et al.* (2004*b*)	DB, PC, R, parallel	0·8 (0·6 + 0·2) + 0·6 GLA + 2·0 ALA + 0·7 STA	12	8–12	M	21–44, mean 32	Con A	IL-2, IFN-γ, IL-4, IL-10	None
Wallace *et al.* (2003)	DB, PC, R, parallel	0·9 (0·3 + 0·6)	12	8	M	18–39, mean 25	Con A	IL-2, IFN-γ, IL-4, IL-10	None
Thies *et al.* (2001*c*)	DB, PC, R, parallel	1·0 (0·7 + 0·3)	12	7	M + F	60–68, mean 62	Con A	IL-2, IFN-γ	None
Trebble *et al.* (2003*b*)	Open, parallel	1·0 (0·7 + 0·3)	4	16	M	mean 30	Con A	IFN-γ, IL-4	None
Miles *et al.* (2004*b*)	DB, PC, R, parallel	1·1 (0·8 + 0·3) + 0·8 GLA + 1·3 ALA + 0·4 STA	12	8–12	M	21–44, mean 32	Con A	IL-2, IFN-γ, IL-4, IL-10	None
Miles *et al.* (2004*b*)	DB, PC, R, parallel	1·6 (1·1 + 0·5) + 1·0 GLA	12	8–12	M	21–44, mean 32	Con A	IL-2, IFN-γ, IL-4, IL-10	None
Miles *et al.* (2006)	DB, PC, R, parallel	1·65 (1·35 + 0·3)	12	23	M	18–42, mean 24	Con A	IL-2, IFN-γ, IL-4, IL-10	None
Kew *et al.* (2003)	DB, PC, R, parallel	1·7 (0·7 + 1·0)	24	30	M + F	25–72, mean 54	Con A	IL-2, IFN-γ, IL-4	None
Wallace *et al.* (2003)	DB, PC, R, parallel	1·9 (0·5 + 1·4)	12	8	M	18–39, mean 22	Con A	IL-2, IFN-γ, IL-4, IL-10	None
Trebble *et al.* (2003*b*)	Open, parallel	2·0 (1·3 + 0·7)	4	16	M	mean 30	Con A	IFN-γ, IL-4	IFN-γ, 220% ↑; IL-4, none
Virella *et al.* (1991)	DB, PC	2·4 EPA	6	4	M	24–48	PHA	IL-2	↓ 59%
							PWM	IL-2	↓ 45%
Meydani *et al.* (1991)	Open, parallel	2·4 (1·7 + 0·7)	12	5	F	23–33, mean 27	Con A	IL-2,	None
							PHA	IL-2	None
Meydani *et al.* (1991)	Open, parallel	2·4 (1·7 + 0·7)	12	6	F	51–68, mean 61	Con A	IL-2	None
							PHA	IL-2	None
Miles *et al.* (2004*b*)	DB, PC, R, parallel	3·0 (2·1 + 0·9)	12	10	M	21–44, mean 32	Con A	IL-4, IL-10	None
Miles *et al.* (2006)	DB, PC, R, parallel	3·3 (2·7 + 0·6)	12	23	M	18–42, mean 25	Con A	IL-2, IFN-γ, IL-4, IL-10	IL-4, ↑ 81%; IL-2, IFN-γ, IL-10, none
Yaqoob *et al.* (2000)	DB, PC, R, parallel	3·3 (2·1 + 1·1)	12	8	M + F	mean 39	Con A	IL-2, IFN-γ, IL-10	None
Endres *et al.* (1993)	Open, non-parallel	4·7 (2·8 + 1·9)	6	9	M	21–39, mean 28	PHA	IL-2	None
Kew *et al.* (2004)	DB, PC, R, parallel	4·7 EPA	4	14	M + F	23–65, mean 46	Con A	IL-2, IL-4, IL-5, IL-10, IFN-γ	None
Kew *et al.* (2004)	DB, PC, R, parallel	4·9 DHA	4	14	M + F	23–65, mean 45	Con A	IL-2, IL-4, IL-5, IL-10, IFN-γ	None
Miles *et al.* (2006)	DB, PC, R, parallel	4·95 (4·05 + 0·9)	12	23	M	18–42, mean 24	Con A	IL-2, IFN-γ, IL-4, IL-10	IL-4,↑ 62%; IL-2, IFN-γ, IL-10, none
Gallai *et al.* (1995)	Open	5·2 (3·1 + 2·1)	26	15	M + F	20–50	PHA	IL-2	↓ 20%
							Con A	IFN-γ	↓ 40%

DB, double-blind; PC, placebo-controlled; R, randomised; ALA, α-linolenic acid (18:3*n*-3); STA, stearidonic acid (18:4*n*-3); GLA, γ-linolenic acid (18:3*n*-6); Con A, concanavalin A; PHA, phytohaemagglutinin; PWM, pokeweed mitogen; IFN-γ, interferon-γ; ↓, decrease; ↑, increase.

*The cohorts are listed according to ascending dose of EPA + DHA.

†The effect is shown if it was significant ($P < 0·05$).

subjects (Meydani *et al*. 1993; Thies *et al*. 2001*c*; Bechoua *et al*. 2003) that report decreased lymphocyte proliferation associated with increased EPA and DHA intake. It would be useful to conduct a well-powered study comparing the effects of long-chain *n*-3 PUFA on T lymphocyte proliferation in young and older subjects.

Effects of EPA and DHA on natural killer cell activity

Four studies have examined the effects of long-chain *n*-3 PUFA on NK cell activity in healthy human volunteers. In all cases this variable has been measured as activity within purified peripheral blood mononuclear cells. Thies *et al*. (2001*b*) have reported inhibition of NK cell activity by 1·0 g EPA + DHA/d but no effect of 0·7 g DHA/d in elderly subjects. Kelley *et al*. (1999) have reported inhibition of NK cell activity following 6 g DHA/d in young males. In contrast, Yaqoob *et al*. (2000) have found no effect of 3·3 g EPA + DHA/d on NK cell activity in a mixed group of healthy subjects. Moreover, a recent study (Miles *et al*. 2006) has identified a trend toward increased NK cell activity with increased EPA and DHA consumption in young males. Taken together, the effects of long-chain *n*-3 PUFA on NK cell activity remain unclear and the inconsistency has only increased with this recent report. Previous suggestions that 'high' but not 'low' doses of DHA can decrease NK cell activity (Kelley *et al*. 1999; Thies *et al*. 2001*b*) and that cells from older subjects might be more susceptible to the effects of EPA and DHA than those of young subjects (Calder, 2001*a*) are not rejected by the recent data. Again, it would be useful to conduct a well-powered study comparing the effects of long-chain *n*-3 PUFA on NK cell activity in young and older subjects.

Effects of EPA and DHA on ex vivo *cytokine production by T-cells*

Twelve healthy volunteer studies have reported the effects of long-chain *n*-3 PUFA on *ex vivo* cytokine production by lymphocytes (Table 2). Again the most-frequent stimulus used to elicit cyokine production has been a T-cell mitogen, particularly Con A. The supplementation level in these studies ranged from 0·3 to 5·2 g EPA + DHA/d in a total of twenty-five treatment cohorts. Seven studies with a double-blind placebo-controlled randomised parallel design accounted for seventeen treatment cohorts. Most studies measured concentrations of one or more of the cytokines IL-2, IFN-γ, IL-4 and IL-10 in supernatant fractions from stimulated peripheral blood mononuclear cell cultures. IL-4 is a cytokine produced by Th-2-type lymphocytes, whereas IL-2 and IFN-γ are produced by Th-1-type lymphocytes (Mosmann & Sad, 1996) and IL-10 is the product of regulatory T-cells.

In the lower dose ranges (<2·0 g/d) no significant effects of EPA + DHA on the production of any cytokine have been reported (Table 2). However, Trebble *et al*. (2003*b*) have found a dose-responsive increase in IFN-γ production from baseline that reached significance ($P<0·05$) at 2·0 g EPA + DHA/d. A non-significant trend towards increased IL-4 production was also noted in this study, and IL-4 production was found to be positively correlated with IFN-γ production. A positive correlation between the production of both cytokines and concentrations of EPA in plasma phosphatidylcholine was also reported. Miles *et al*. (2006) have reported significantly ($P<0·05$) increased IL-4 production from baseline at EPA + DHA intakes of 3·3 and 4·95 g/d (Fig. 2(c)), but these increases were not different from placebo values (Table 2). In contrast with the finding by Trebble *et al*. (2003*b*), at higher EPA + DHA intakes decreased production of IFN-γ and IL-2, another Th-1 cytokine, has been reported in some studies. Virella *et al*. (1991) have reported decreased IL-2 production following 2·4 g EPA/d. Meydani *et al*. (1991), using a supplement of the same dose level, have found decreased IL-2 production that approaches significance ($P = 0·057$) in older, but not young, women. Gallai *et al*. (1995) have reported a decrease in PHA-induced IL-2 production as well as Con A-induced IFN-γ production following supplementation with 5·2 g EPA + DHA/d. Trebble *et al*. (2003*b*) have proposed a dose–response relationship between EPA and DHA intake and cytokine production by Th-1 type cells; increasing production at intakes of ≤ 2 g/d for ≤ 4 weeks and an inhibitory effect at higher intakes or after longer periods of similar intakes. On the other hand, all studies that used a double-blind placebo-controlled randomised parallel design, covering a dose range from 0·4 to 4·95 g EPA + DHA/d and seventeen dose levels, have failed to show significant effects on the production of Th-1-type cytokines (Table 2). In all but one study post supplementation IFN-γ and IL-2 concentrations were found to be similar to those in controls as well as at baseline (Yaqoob *et al*. 2000; Thies *et al*. 2001*c*; Kew *et al*. 2003, 2004; Wallace *et al*. 2003; Miles *et al*. 2006). Miles *et al*. (2004*a*) have found a generalised and significant ($P<0·05$) increase in the production of all measured T lymphocyte-derived cytokines (IL-2, IL-4, IL-10, IFN-γ) from baseline. This response was also found in the placebo group, implying a causal factor different from EPA and DHA intake. There are no studies that report significant effects of long-chain *n*-3 PUFA supplementation on IL-10 production. Fig. 2(a,b,c,d) indicate that the non-significant percentage changes from baseline observed in these studies were generally small and not consistent in direction. These observations suggest that the observed effects of EPA and DHA on the production of T-cell cytokines may be a result of factors that are uncorrected by the limitations of open studies. For instance, changes in outcomes reported may occur over time. The observation by Kew *et al*. (2004) is noteworthy in that it shows that in the absence of effects on cytokines CD69 expression, a marker of T-cell activation, is reduced by 4·9 g DHA/d but not by 4·7 g EPA/d. This result suggests that *ex vivo* cytokine response to Con A may not be the most-sensitive marker of T-cell function. In addition, this result suggests that DHA may be more potent in modulating T-cell function than EPA.

In conclusion, the studies conducted so far on the effects of dietary long-chain *n*-3 PUFA on lymphocyte function reflected by cytokine production are highly inconsistent and therefore inconclusive. If there is any response at all, a dose level of ≥ 2 g EPA + DHA/d is required to significantly affect cytokine production by T-cells. The

Table 3. Effects of EPA + DHA supplementation on *ex vivo* cytokine production by monocytes in healthy human subjects*

Reference	Type of study	Dose of EPA + DHA (g/d)	Duration (weeks)	Subjects			Cytokines studied†	Effect seen‡
				n	Gender	Age (years)		
Trebble *et al.* (2003*a*)	Open, parallel	0·3 (0·2 + 0·1)	4	16	M	mean 30	IL-6, TNFα	IL-6, ↓ 51%; TNFα, ↓ 48%
Wallace *et al.* (2003)	DB, PC, R, parallel	0·4 (0·1 + 0·3)	12	8	M	18–39, mean 25	IL-1β, TNFα, IL-6	None
Hawkes *et al.* (2002)	DB, PC, R, prospective	0·4 (0·1 + 0·3)	4	26	F	>18, mean 30	IL-1β, IL-6, TNFα	None
Schmidt *et al.* (1996)	DB, PC, R	0·6 (0·34 + 0·19)	12	16	M + F	24–52	IL-1β, IL-6, TNFα	None
Hawkes *et al.* (2002)	DB, PC, R, prospective	0·7 (0·1 + 0·6)	4	29	F	>18, mean 30	IL-1β, IL-6, TNFα	None
Thies *et al.* (2001*a*)	DB, PC, R, parallel	0·7 DHA	12	8	M + F	58–71, mean 65	IL-1β, IL-6, TNFα	None
Kew *et al.* (2003)	DB, PC, R, parallel	0·77 (0·3 + 0·47)	24	30	M + F	25–72, mean 52	IL-1β, IL-6, TNFα, IL-10	None
Miles *et al.* (2004*b*)	DB, PC, R, parallel	0·8 (0·6 + 0·2) + 0·6 GLA + 2·0 ALA + 0·7 STA	12	8–12	M	21–44, mean 32	IL-1β, TNFα	None
Wallace *et al.* (2003)	DB, PC, R, parallel	0·9 (0·3 + 0·6)	12	8	M	18–39, mean 25	IL-1β, TNFα, IL-6	IL-1β, TNFα, none; IL-6, ↓ 45%
Thies *et al.* (2001*a*)	DB, PC, R, parallel	1·0 (0·7 + 0·3)	12	7	M + F	60–68, mean 62	IL-1β, IL-6, TNFα	None
Trebble *et al.* (2003*a*)	Open, parallel	1·0 (0·7 + 0·3)	4	16	M	mean 30	IL-6, TNFα	IL-6, ↓ 84%; TNFα, ↓ 66%
Blok *et al.* (1997)	DB, PC, R, parallel	1·0 (0·8 + 0·2)	52	15	M	21–87, mean 56	IL-1β, TNFα	None
Miles *et al.* (2004*b*)	DB, PC, R, parallel	1·1 (0·8 + 0·3) + 0·8 GLA + 1·3 ALA + 0·4 STA	12	8–12	M	21–44, mean 32	IL-1β, TNFα	None
Meydani *et al.* (1993)	Open, PC, parallel	1·24	24	10	F	>40 mean 65	IL-1β, IL-6, TNFα	IL-1β, ↓ 50%; IL-6, none; TNFα, ↓ 49%
Ciubotaru *et al.* (2003)	DB, PC, R,	1·1 (0·6 + 0·5)	5	10	F	mean 60	IL-6	IL-6, ↓ 10%
Cooper *et al.* (1993)	Open	1·3 (0·8 + 0·5)	6–8	6–8	M + F	18–36	IL1β, IL-6, TNFα	IL1β, ↓ 85%; IL-6, ↓ 90%; TNFα, none
Vega-Lopez *et al.* (2004)	DB, PC, R, parallel	1·5 (0·6 + 0·9)	12	20	M + F	20–55, mean 30	MCP-1-induced IL-1β, IL-6, TNFα	None
Miles *et al.* (2004*b*)	DB, PC, R, parallel	1·6 (1·1 + 0·5) + 1·0 GLA	12	8–12	M	21–44, mean 32	IL-1β, TNFα	None
Molvig *et al.* (1991)	Open, PC	1·6 (1 + 0·6)	7	9	M + F	mean 27	IL-1β, TNFα	None
Rees *et al.* (2006)	DB, PC, R, parallel	1·65 (1·35 + 0·3)	12	23	M	18–42, mean 24	IL-1β, IL-6, TNFα	None
	DB, PC, R, parallel	1·65 (1·35 + 0·3)	12	15	M	53–70, mean 61	IL-1β, IL-6, TNFα	None
Kew *et al.* (2003)	DB, PC, R, parallel	1·7 (0·7 + 1·0)	24	30	M + F	25–72, mean 54	IL-1β, IL-6, IL-10, TNFα	None
Grimble *et al.* (2002)	Open	1·8	12	37	M	20–57, mean 28	TNFα	↓ 63% or ↑ 161% genotype dependent
Wallace *et al.* (2003)	DB, PC, R, parallel	1·9 (0·5 + 1·4)	12	8	M	18–39, mean 22	IL-1β, TNFα, IL-6	IL-1β, TNFα, none; IL-6, ↓ 19%
Blok *et al.* (1997)	DB, PC, R, parallel	1·9 (1·6 + 0·3)	52	15	M	21–87, mean 56	IL-1β, TNFα	None
Trebble *et al.* (2003*a*)	Open, parallel	2·0 (1·3 + 0·7)	4	16	M	mean 30	IL-6, TNFα	IL-6, ↓ 79%; TNFα, ↓ 59%
Ciubotaru *et al.* (2003)	DB, PC, R	2·2 (1·2 + 1·0)	5	10	F	mean 60	IL-6	None
Meydani *et al.* (1991)	Open, parallel	2·4 (1·7 + 0·7)	12	5	F	23–33, mean 27	IL-1β, IL-6, TNFα	IL-1β, ↓ 43%; IL-6, ↓ 22%; TNFα, ↓ 45%
	Open, parallel	2·4 (1·7 + 0·7)	12	6	F	51–68, mean 61	IL-1β, IL-6, TNFα	IL-1β, ↓ 85%; IL-6, ↓ 60%; TNFα, ↓ 66%
Wu *et al.* (2004)	DB, PC, R	2·5 (1·5 + 1·0)	12	10	M + F	>65	IL-1β	None
							Con A- and PHA-induced IL-6, TNFα	IL-6, ↓; IL-6, NS; TNFα, NS; TNFα, ↓ following PHA
Caughey *et al.* (1996)	Open, parallel	2·7 (1·6 + 1·1)	4	13	M + F	24–44	IL-1β, TNFα	IL-1β, ↓ 80%; TNFα, ↓ 74%
Blok *et al.* (1997)	DB, PC, R, parallel	2·9 (2·4 + 0·5)	52	14	M	21–87, mean 56	IL-1β, TNFα	None

Cannon *et al.* (1995)	DB, PC, R, parallel	3·0	16	7	M+F	61–72	IL-1β	None
Miles *et al.* (2004*b*)	DB, PC, R, parallel	3·0 (2·1+0·9)	12	10	M	21–44, mean 32	IL-1β, TNFα	None
Molvig *et al.* (1991)	Open, PC	3·2 (2+1·2)	7	9	M+F	mean 24	IL-1β, TNFα	None
Yaqoob *et al.* (2000)	DB, PC, R, parallel	3·3 (2·1+1·1)	12	8	M+F	mean 39	IL-1β, TNFα	None
Rees *et al.* (2006)	DB, PC, R, parallel	3·3 (2·7+0·6)	12	23	M	18–42, mean 25	IL-1β, IL-6, TNFα	None
	DB, PC, R, parallel	3·3 (2·7+0·6)	12	15	M	53–70, mean 61	IL-1β, IL-6, TNFα	None
Abbate *et al.* (1996)	Open, non-PC	3·4 (2·0+1·4)	18	9	M+F	27–39, mean 32	IL-6	↓ 46%
Endres *et al.* (1989)	Open, non-parallel	4·7 (2·8+1·9)	6	9	M+F	21–39, mean 28	IL-1β, TNFα	IL-1β, ↓ 43%; TNFα, none
Kew *et al.* (2004)	DB, PC, R, parallel	4·7 EPA	4	14	M+F	23–65, mean 46	IL-1β, IL-6, IL-8, IL-10, TNFα	None
	DB, PC, R, parallel	4·9 DHA	4	14	M+F	23–65, mean 45	IL-1β, IL-6, IL-8, IL-10,TNFα	None
Rees *et al.* (2006)	DB, PC, R, parallel	4·95 (4·05+0·9)	12	23	M	18–42, mean 24	IL-1β, IL-6, TNFα	None
	DB, PC, R, parallel	4·95 (4·05+0·9)	12	15	M	53–72, mean 60	IL-1β, IL-6, TNFα	None
Gallai *et al.* (1995)	Open	5·2 (3·1+2·1)	26	15	M+F	20–50	IL-1β, TNFα	IL-1β, ↓ 15%; TNFα, ↓ 26%
Kelley *et al.* (1999)	Open, PC, parallel	6 DHA	13	7	M	20–40, mean 33	IL-1β, TNFα	IL-1β, ↓ 45%; TNFα, ↓ 30%

DB, double-blind; PC, placebo-controlled; R, randomised; ALA, α-linolenic acid (18 : 3*n*-3); STA, stearidonic acid (18 : 4*n*-3); GLA, γ-linolenic acid (18 : 3*n*-6); Con A, concanavalin A; PHA, phytohaemagglutinin; MCP-1, monocyte chemotactic protein-1; ↓, reduction; ↑, increase.
*The cohorts are listed according to ascending dose of EPA+DHA.
†Cells were stimulated with lipopolysaccharide unless stated otherwise.
‡The effect is shown if it was significant ($P<0·05$).

concept that supplementation of EPA might influence the Th-1 *v*. Th-2 balance in favour of the Th-1 phenotype via inhibition of PGE_2 is not supported by the available data. Particularly, the observation of a possible concomitant dose-dependent decrease in PGE_2 and an increase in IL-4 production (Trebble *et al*. 2003*b*) is in contrast with this concept. If any effect on Th cell cytokine production is real, another mechanism is more likely underlying this effect.

Effects of EPA and DHA on ex vivo *cytokine production by monocytes*

The largest body of evidence on immunomodulatory effects of long-chain *n*-3 PUFA in healthy subjects derives from studies on inflammatory cytokines. In general, these studies measured concentrations of one or more of the cytokines IL-1β, TNFα and IL-6 produced by monocytes in response to *ex vivo* stimulation with LPS. LPS selectively stimulates monocyte function because these cells express CD14, the LPS receptor. Studies typically used purified peripheral blood mononuclear cells, although some studies used isolated monocytes. Twenty-four studies covering a dose range from 0·3 to 6 g EPA+DHA/d in forty-six treatment cohorts were identified (Table 3). At least twenty-five cohorts in eleven studies were investigated in a double-blind placebo-controlled randomised parallel study design (Table 3).

The results of the large number of studies are not fully consistent. There are no studies that report that long-chain *n*-3 PUFA significantly increase production of IL-1β, TNFα or IL-6 in response to *ex vivo* stimulation with LPS. A substantial number of studies have found decreased production of one or more inflammatory cytokine, but a larger number of studies have failed to find significant effects (Table 3). In ten of twenty-eight treatment cohorts reporting IL-6 production a significant ($P<0·05$) decrease was reported (Fig. 3(c)). A significant ($P<0·05$) decrease in IL-6 responses to LPS stimulation was found at EPA+DHA doses ranging from 0·3 to 3·4 g/d. Trebble *et al*. (2003*a*) have reported a negative but 'U-shaped' dose–response relationship between long-chain *n*-3 PUFA intake and IL-6 production in an open uncontrolled study, with a maximum inhibition demonstrated at a supplementary intake of 1·0 g/d. Wallace *et al*. (2003) have studied similar dose levels in a controlled blinded parallel study and have found a very similar result. On the other hand, other blinded parallel studies (Thies *et al*. 2001*a*; Kew *et al*. 2003, 2004; Rees *et al*. 2006) have not found decreased IL-6 production within the EPA+DHA dose range of 1–2 g/d or at higher doses. IL-1β production was found to be decreased at EPA+DHA intake levels from 1·24 g/d to 6 g/d in eight of thirty-five of the cohorts in which it was measured,. A striking similarity of these treatment cohorts is that all were part of open studies. A similar picture emerges from the studies on TNFα production; in nine of thirty-nine treatment cohorts a significant ($P<0·05$) decrease in LPS-induced TNFα production was reported, all were part of open studies. The EPA+DHA intake levels that were associated with inhibition of TNFα production varied from 0·3 g/d to 6 g/d.

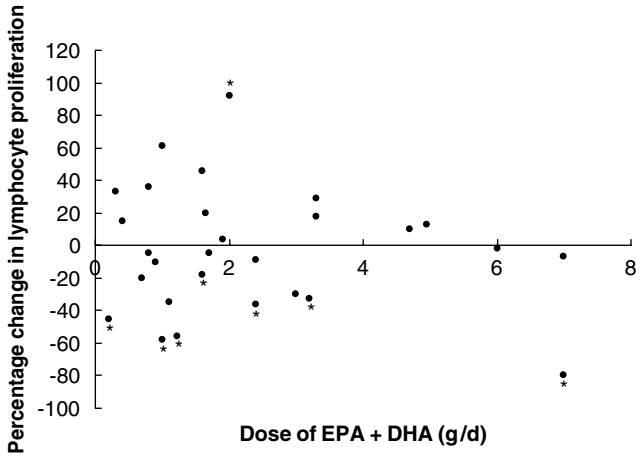

Fig. 1. Relationship between the daily intake of EPA and DHA and *ex vivo* lymphocyte proliferation response in healthy human subjects. Each data point represents the percentage change in mitogen-induced lymphocyte proliferation observed in a cohort in an EPA + DHA supplementation study. Percentage changes were reported to be significantly different from their controls: $^{*}P<0{\cdot}05$. Data are taken from the studies listed in Table 1.

The study by Caughey *et al*. (1996) indicates a strong inverse dose–response relationship between mononuclear cell EPA content and production of IL-1β and TNFα. This earlier report was followed by several more recent reports of studies that have failed to find the same direct correlations between increased EPA concentrations in mononuclear cells and production of IL-1β and TNFα. Trebble *et al*. (2003*a*) have reported a negative but 'U-shaped' dose–response relationship between long-chain *n*-3 PUFA intake and TNFα production, with a maximum inhibition demonstrated at a supplementary EPA + DHA intake of 1·0 g/d. Unfortunately, no EPA concentrations in peripheral blood mononuclear cells were reported in this study. Miles *et al*. (2004*a*) have found a dose-dependent rise in EPA concentrations in blood mononuclear cells with intake levels ≤ 3 g EPA + DHA/d but with no concomitant change in IL-6 and TNFα production. Yaqoob *et al*. (2000) have found a fourfold rise in blood mononuclear cell EPA concentration following an intake of 3·3 g EPA + DHA/d resulting in only a small non-significant decrease in IL-6 and TNFα production by those cells. A recent study (Rees *et al*. 2006) that used four intake levels of ≤ 4·95 g EPA + DHA/d has found a dose-dependent increase in EPA concentrations and in EPA : ARA in mononuclear cell phospholipids. This shift in eicosanoid precursors was shown to be correlated with a stepwise decline in PGE_2 production by mononuclear cells stimulated with LPS and in neutrophil respiratory burst, but was not found to be correlated with changes in IL-1β and TNFα production by mononuclear cells. Fig. 3(a,b) show the percentage changes from baseline in IL-1β and TNFα production from all studies and illustrate the distribution of the significant ($P<0{\cdot}05$) changes over the entire EPA + DHA dose range studied. The results do not confirm the presence of a dose–response relationship between long-chain *n*-3 PUFA intake and inhibition of IL-1β and TNFα production. The different observations made by Caughey *et al*. (1996) and in some later reports may be explained by differences in the age of study subjects, greater amounts of α-tocopherol in the capsules used in the more recent studies and differences in the precise nature of samples in which cytokine measurements were made (Calder, 2001*a*), but the reason for the differences is not yet clear (Rees *et al*. 2006). One other factor recently identified by Grimble *et al*. (2002) is polymorphisms in genes affecting cytokine production. These authors have shown that the effect of dietary fish oil on TNFα production by human mononuclear cells is dependent on the nature of the −308 TNFα and the +252 TNFβ polymorphisms.

In conclusion, a large body of available studies provides substantial evidence that the production of inflammatory cytokines in healthy subjects can be decreased by increasing intake of EPA + DHA. However, this evidence is not conclusive, as these reports are outnumbered by studies that find no significant effects of EPA + DHA, and the reason for this inconsistency is unclear. The proposed dose dependence of anti-inflammatory actions of long-chain *n*-3 PUFA is not consistently reflected in cytokine concentrations, perhaps because of low susceptibility of *ex vivo* cytokine production in healthy subjects to changed EPA concentrations in membrane phospholipids of monocytes and mononuclear cells. That supplemental long-chain *n*-3 PUFA do not increase the production of inflammatory cytokines in healthy subjects is highly consistent; no studies have reported such an increase. Thus, these findings in healthy subjects may serve as a rationale to supplement long-chain *n*-3 PUFA to subjects with inflammatory disorders that are (partly) caused by excessive formation of inflammatory cytokines.

Intervention studies in (chronic) inflammatory conditions and disorders

Introductory comments

Inflammatory cytokines produced by monocytes and macrophages have an important role in the regulation of the whole-body response to infection and injury. Thus, inflammation and the inflammatory response are part of the innate immune response that is normally protective to the host. However, in some inflammatory conditions and diseases the inflammatory response occurs in an uncontrolled or inappropriate manner and causes excessive damage to the host tissues and disease can ensue. A common characteristic of these conditions and diseases is excessive or inappropriate production of inflammatory mediators, including eicosanoids and cytokines. High concentrations of TNFα, IL-1β and IL-6 are particularly destructive and are implicated in chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel diseases. Chronic overproduction of these cytokines can cause adipose tissue and muscle wasting and loss of bone mass and may account for alterations in body composition and tissue loss seen in inflammatory diseases and in cancer cachexia, which is a syndrome of progressive weight loss, anorexia and persistent erosion of host body cell mass in response to a malignant growth (Morley *et al*. 2006). As well as its clear and obvious association with classic inflammatory

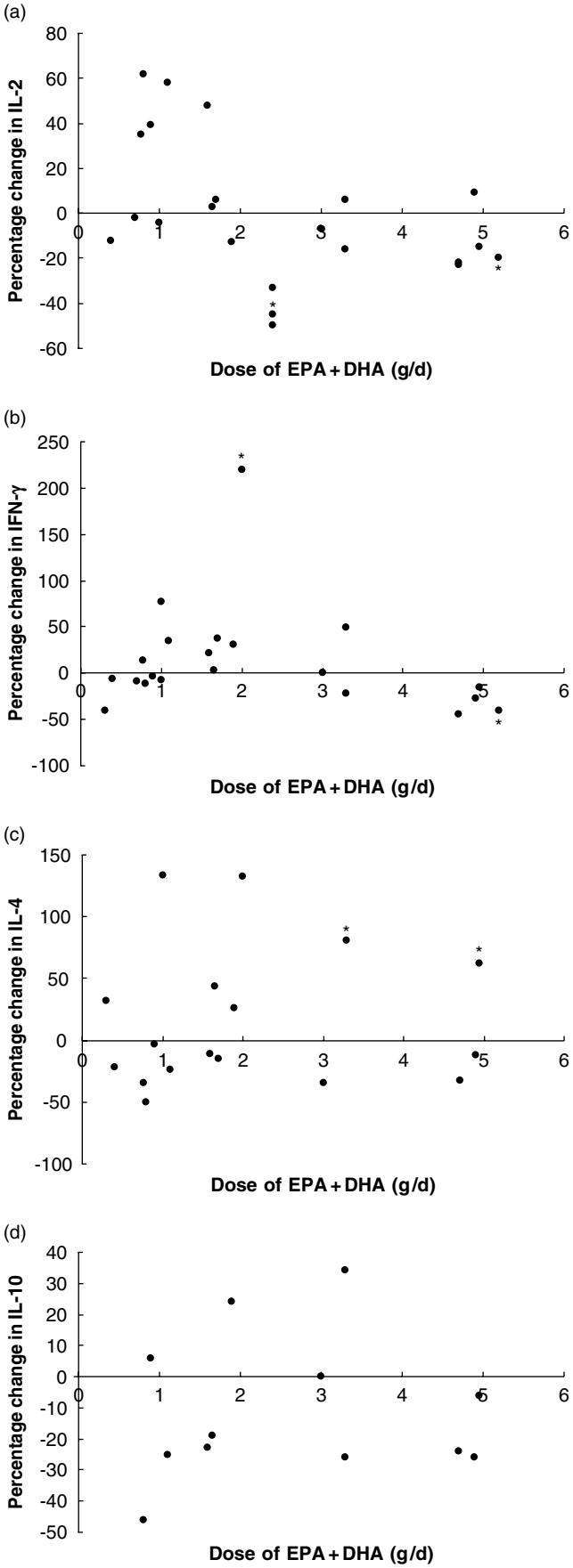

Fig. 2. Relationship between the daily intake of EPA and DHA and *ex vivo* cytokine production by lymphocytes in healthy human subjects. Each data point represents the percentage change in mitogen-induced cytokine production (IL-2 (a), IFN-γ (b), IL-4 (c) and IL-10 (d)) by lymphocytes observed in a cohort in an EPA + DHA supplementation study. Percentage changes were reported to be significantly different from their controls: $^*P<0{\cdot}05$. Data are taken from the studies listed in Table 2.

diseases, inflammation is now recognised to play an important role in the pathology of other diseases such as CVD, HIV progression and neurodegenerative diseases of aging. Additionally, the realisation that adipose tissue is a source of inflammatory cytokines has given rise to the notion that obesity, the metabolic syndrome and type 2 diabetes have an inflammatory component (Calder, 2006).

This section focuses on studies with long-chain *n*-3 PUFA in inflammatory conditions that report some of the immune markers that are most commonly studied in intervention studies in healthy volunteers rather than on disease-specific clinical outcomes (which should be considered as the most important marker of effectiveness). In addition to the immune markers reviewed in the previous section, this section reports the effects of EPA + DHA on circulating cytokines. Plasma levels of inflammatory cytokines are usually below detection level in healthy subjects but are elevated in most inflammatory conditions and are an important target of intervention because of the destructive nature of their presence in the bloodstream in excessive concentrations. The concentrations found in the blood are the net outcome of production by various cells and tissues, including infiltrated monocytes and macrophages at inflamed sites, muscle and fat tissue, and removal and degradation by various cells and tissues. Their levels reflect the *in vivo* pro-inflammatory state (Albers *et al.* 2005) and are partly linked with *ex vivo* production of cytokines as they are derived in part from the same type of cells. Thus, it is expected that if supplemental EPA + DHA reduces the extent to which cells are predisposed to produce excessive levels of cytokines on receiving a stimulus *ex vivo*, this outcome will at least partially correlate with lower *in vivo* concentrations of the same cytokines in conditions in which such stimuli are present.

Complex *n*-3 PUFA-containing nutritional products (e.g. 'immunonutrition') and parenteral supplementation of EPA + DHA-enriched emulsions have been used in patients with various inflammatory conditions. However, similar to the overview in the previous section, this section is limited to studies of oral intake of pure sources of EPA + DHA. Table 4 gives an overview of the studies addressed in this section.

Cancer

Cancer cachexia is a major factor contributing to the weakening of the already compromised immune system of patients with cancer. Cancer cachexia affects approximately 30% of patients with cancer, thus accounting for >400 000 patients in the USA alone (Morley *et al.* 2006). A current belief is that the mechanism underlying cancer

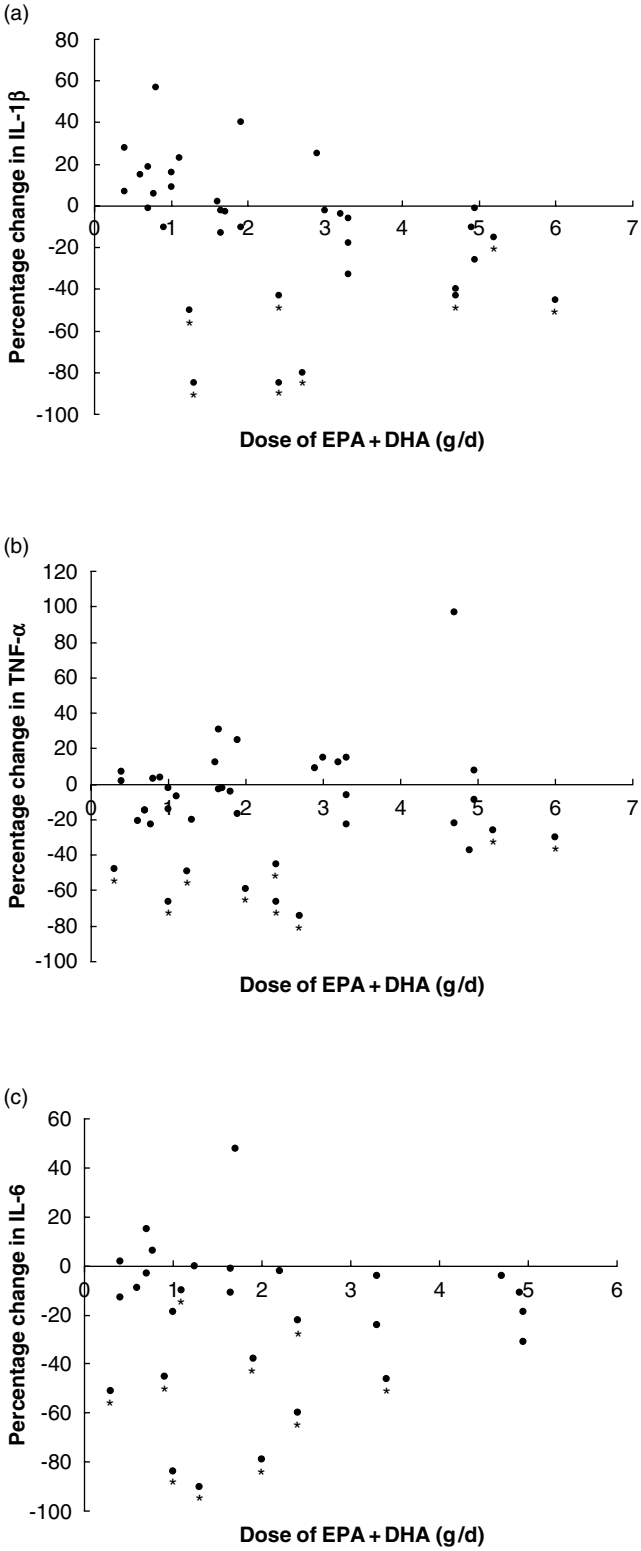

Fig. 3. Relationship between the daily intake of EPA and DHA and *ex vivo* cytokine production (IL-1β (a), TNFα (b) and IL-6 (c)) by monocytes in healthy human subjects. Each data point represents the percentage change in lipopolysaccharide-induced cytokine production by monocytes observed in a cohort in an EPA + DHA supplementation study. Percentage changes were reported to be significantly different from their controls: *$P<0{\cdot}05$. Data are taken from the studies listed in Table 3.

cachexia involves the host's production of certain cytokines, such as IL-1β, IL-6 and TNFα (Todorov *et al*. 1996). As increased production of these cytokines may play a major role in weight loss, an important clinical question with fish oil intervention is therefore whether treatment strategies based on anti-cachexia treatment might reduce cachexia and thereby improve immune function, life expectancy and life quality. Indeed, the findings of a recent large study (Fearon *et al*. 2006) suggest that supplementation with EPA in cancer cachexia results in weight gain compared with placebo, particularly in gastrointestinal cancer.

The first study to report effects of fish oil supplementation on immune markers in cancer cachexia (Wigmore *et al*. 1997) has examined the effect of an intake level increasing from 1 g EPA/d to 6 g EPA/d during a 4-week intervention in patients with pancreatic cancer. After 4 weeks of supplementation with EPA the production of IL-6 by blood mononuclear cells was found to be significantly ($P<0{\cdot}05$) decreased compared with levels seen for healthy controls, but no significant effect on serum IL-6 concentration was found. The supernatant fraction of the stimulated mononuclear cells following EPA supplementation was shown to reduce the potential of isolated human hepatocytes to produce C-reactive protein. Indeed, serum levels of C-reactive protein were found to be decreased after EPA supplementation, indicating that EPA can down regulate the acute-phase response in cancer cachexia, most likely via suppression of IL-6 production. Gogos *et al*. (1998) have failed to find effects of supplementation of 5·1 g EPA + DHA/d for 6 weeks on serum TNFα, IL-6 and IL-1 levels and on *ex vivo* IL-1 and IL-6 production by blood mononuclear cells. However, in the malnourished subpopulation with decreased TNFα production, *ex vivo* TNFα production by mononuclear cells was shown to be restored to a normal level following *n*-3 PUFA supplementation. In this study survival was prolonged in the patients receiving the *n*-3 PUFA supplement. Furukawa *et al*. (1999) have studied the effect of an oral supplement providing 1·8 g EPA/d to patients with oesophageal cancer post surgery who were also receiving parenteral soyabean oil. The EPA intervention was started on day 7 before surgery and was continued until post-operative day 21. The authors have reported that serum IL-6 is significantly lower ($P<0{\cdot}05$) on post-operative day 7 and lymphocyte proliferation and NK cell activity are significantly higher ($P<0{\cdot}05$) on post-operative day 21. Similar data have been presented by the same authors in a research letter (Takagi *et al*. 2001) and a symposium report (Tashiro *et al*. 1998), so these data are likely to derive from the same study. Finally, a very high intake of fish oil (8·1 g EPA + DHA/d) for 4 weeks has been studied in gastrointestinal cancer cachexia (Persson *et al*. 2005). Although fish oil supplementation was shown to result in weight stabilisation, particularly when combined with melatonin treatment, no effects on circulating TNFα, IL-1β, IL-6 or IL-8 concentrations were observed.

Thus, these studies indicate that the production of IL-6 in these populations, either measured after *ex vivo* stimulation of mononuclear cells or in plasma, is decreased or not affected by EPA (and DHA) supplementation. Similar

results have been reported in studies with EPA-enriched enteral nutrition (Barber *et al*. 2001). The reported increase in *ex vivo* TNFα production by mononuclear cells, lymphocyte proliferation and NK activity in some studies may reflect a (partial) immune restoration by *n*-3 PUFA supplementation.

Inflammatory bowel diseases

Ulcerative colitis and Crohn's disease are chronic inflammatory diseases of the alimentary tract. In ulcerative colitis the mucosa of the colon is mainly affected, while in Crohn's disease any part of the alimentary tract from the mouth to the anus can be affected, although it is usually the ileum and colon. In both diseases the intestinal mucosa contains elevated levels of inflammatory eicosanoids such as LTB_4 (Sharon & Stenson, 1984) and cytokines. In particular, the activation of IL-2- and IFN-γ-producing Th1 cells in the lamina propria of the Crohn's disease-affected gut plays a pivotal role in the pathogenesis (Hommes & van Deventer, 2000).

At least twelve placebo-controlled studies using long-chain *n*-3 PUFA in patients with inflammatory bowel diseases are now available and are reviewed elsewhere (Calder, 2006). Only two of these studies have reported effects on cytokine production. The earliest study (Almallah *et al*. 1998, 2000*a*,*b*) has reported a significant reduction ($P<0{\cdot}05$) in serum IL-2 and soluble IL-2 receptor levels following 26 weeks of supplementation with 5·6 g EPA + DHA/d in patients with ulcerative colitis. This change was found to be accompanied by significant reductions ($P<0{\cdot}05$) in serum LTB_4 concentration, NK cell activity and sigmoidoscopic and histological scores, and decreased disease activity. More recently, Trebble *et al*. (2004) have shown that 2·7 g EPA + DHA/d for 24 weeks reduces the *ex vivo* production of IFN-γ and PGE_2, but not TNFα, by stimulated mononuclear cells from patients with Crohn's disease. These data indicate that *n*-3 PUFA may decrease disease-related inflammatory markers in inflammatory bowel diseases.

Rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory disease characterised by joint inflammation that manifests as swelling, pain, functional impairment, morning stiffness, osteoporosis and muscle wasting. Joint lesions are characterised by infiltration of activated macrophages, T lymphocytes and plasma cells into the synovium (the tissue lining the joints) and by proliferation of synovial cells termed synoviocytes. Synovial biopsies from patients with rheumatoid arthritis contain high levels of TNFα, IL-1β, IL-6, IL-8 and granulocyte–macrophage-colony-stimulating factor, and synovial cells cultured *ex vivo* produce TNFα, IL-1β, IL-6, IL-8 and granulocyte–macrophage-colony-stimulating factor for extended periods of time without additional stimulus (Feldmann & Maini, 1999). Cyclooxygenase-2 expression is increased in the synovium of patients with rheumatoid arthritis, and in the joint tissues in rat models of arthritis (Sano *et al*. 1992). PGE_2, LTB_4, 5-hydroxyeicosatetraenoic acid and also platelet-activating factor are found in the synovial fluid of patients with active rheumatoid arthritis (Sperling, 1995). The efficacy of non-steroidal anti-inflammatory drugs in rheumatoid arthritis indicates the importance of pro-inflammatory cyclooxygenase pathway products in the pathophysiology of the disease. Thus, these data provide a mechanistic basis for benefits of fish oil supplementation in rheumatoid arthritis.

At least seventeen placebo-controlled studies have investigated the effect of *n*-3 PUFA in patients with rheumatoid arthritis (for review, see Calder, 2006). Five of these studies have reported effects on inflammatory cytokines (Table 4). Kremer *et al*. (1990) have found no effects of either 2·9 g EPA + DHA/d or 5·9 g EPA + DHA/d on Con A-induced IL-2 production and Con A- or PHA-induced lymphocyte proliferation. LPS-induced IL-1 production by monocytes was reported to be significantly decreased ($P<0{\cdot}05$) by 5·9 g EPA + DHA/d but not by 2·9 g EPA + DHA/d in the same study. Espersen *et al*. (1992) and Kremer *et al*. (1995) have reported decreased serum IL-1β following 3·2 and 7·1 g EPA + DHA/d respectively. No effect of fish oil supplementation on serum TNFα was found following 3·2 (Espersen *et al*. 1992), 3·4 (Sundrarjun *et al*. 2004), 4·2 (Adam *et al*. 2003) or 7·1 (Kremer *et al*. 1995) g EPA + DHA/d. However, decreased soluble TNFα receptor levels were found (Sundrarjun *et al*. 2004) after 12 weeks of supplementation with 3·4 g EPA + DHA/d. Serum IL-6 levels were unchanged in the same study (Sundrarjun *et al*. 2004) and following 7·1 g EPA + DHA/d (Kremer *et al*. 1995). In addition, no effects on serum IL-8 and IL-2 concentrations were found following fish oil supplementation in the latter study.

These studies indicate that fish oil may decrease IL-1 production in rheumatoid arthritis and that it has little effect on other cytokines. However, in four of these studies fish oil was found to improve clinical outcomes (Calder, 2006). Indeed, in almost all published trials of fish oil intervention in rheumatoid arthritis clinical benefits have been reported (Calder, 2006). These data therefore suggest that disease-specific clinical outcomes might be a more sensitive marker of anti-inflammatory effects of EPA + DHA than inflammatory cytokines.

Lung inflammation

COPD is characterised by reduced airflow on expiration as a result of airway obstruction. COPD is currently the fifth leading cause of death worldwide, and in the next decades its prevalence and mortality rates are expected to increase (Murray & Lopez, 1997). COPD is characterised by chronic inflammation in the small airways and lung parenchyma accompanied by infiltration of neutrophils and macrophages (Barnes *et al*. 2003). This inflammation is considered to mediate excess mucous production, fibrosis and proteolysis via neutrophil recruitment. The most important neutrophil chemotactic factors implicated in COPD are IL-8, TNFα and LTB_4 (Barnes, 2000). Airflow obstruction and a chronic persistent inflammatory process also characterise asthma, but the nature of the inflammation differs markedly from that in COPD. The

Table 4. Effects of EPA + DHA supplementation on circulating cytokine levels, *ex vivo* cytokine production or lymphocyte proliferation following mitogen stimulation in intervention studies in patients with inflammatory disorders

Reference	Type of study	Dose of EPA + DHA (g/d)	Duration (weeks)	Subjects				Markers studied	Effect seen*
				n	Gender	Age (years)	Condition		
Cancer									
Tashiro *et al.* (1998); Furukawa *et al.* (1999); Takagi *et al.* (2001)	DB, Non-PC, R	1·8 EPA	4	7	M + F	mean 61	Post surgery oesophageal cancer	Serum IL-6 Con A- and PHA-induced LP	IL-6 ↓ (after 1 week) Con A, ↑ 143%; PHA, ↑ 153%
Wigmore *et al.* (1997)	Open	EPA, 1 g wk 1, 2 g wk 2, 4 g wk 3, 6 g wk 4	4	6	M + F	18–80, mean 58	Pancreatic cancer cachexia	Serum IL-6 LPS-induced IL-6	None IL-6, ↓ 65%
Gogos *et al.* (1998)	PC, R	5·1 (3·1 + 2·1)	6	30	M + F	mean 57	Cancer, half of patients malnourished	Serum TNFα, IL-6, IL-1 LPS-induced TNFα, IL-6, IL-1	None TNFα, ↑ malnourished; IL-6, IL-1, none
Persson *et al.* (2005)	Open, non-PC, R	8·1 (4·9 + 3·2)	4	13	M + F	52–78, mean 66	Gastrointestinal cancer cachexia	Serum TNFα, IL-1β, sIL-2R, IL-6, IL-8	None
Inflammatory bowel diseases									
Trebble *et al.* (2004)	DB, PC, R	2·7 (1·6 + 1·1)	24	31	M + F	mean 45	Crohn's disease, IFN-γ increased in pathology	Con A-induced IFN-γ LPS-induced TNFα	↓ 50% None
Almallah *et al.* (2000*a*)	PC, R	5·6 (3·2 + 2·4)	26	9	M + F		Procto-colitis	Serum IL-2, IL-2R	IL-2, ↓; IL-2R, ↓
Rheumatoid arthritis (RA)									
Kremer *et al.* (1990)	DB, PC, R parallel	2·9 (1·7 + 1·2)	24	20	M + F	32–81, mean 59	RA	LPS-induced IL-1 Con A-induced IL-2 Con A- and PHA-induced LP	None None None
Espersen *et al.* (1992)	DB, PC, R	3·2 (2·0 + 1·2)	12	18	M + F		RA	Serum IL-1β, TNFα	IL-1β, ↓; TNFα, none
Sundrarjun *et al.* (2004)	PC, R	3·4 (1·9 + 1·5)	12	23	M + F	mean 46	RA	Serum IL-6, TNFα, sTNFR	IL-6, TNFα, none; sTNFR ↓
Adam *et al.* (2003)	PC, R	4·2 (2·4 + 1·8)	12	30	M + F	mean 58	RA	Serum IL-1β, TNFα	None
Kremer *et al.* (1990)	DB, PC, R, parallel	5·9 (3·5 + 2·4)	24	17	M + F	30–80, mean 58	RA	LPS-induced IL-1 Con A-induced IL-2 Con A- and PHA-induced LP	↓ 55% None None
Kremer *et al.* (1995)	DB, PC	7·1 (4·6 + 2·5)	26 to 30	23	M + F	mean 58	RA	Serum IL-1β, IL-6, TNFα, IL-8, IL-2	IL-1β, ↓; IL-6, TNFα, IL-8, IL-2, none
Lung inflammation									
Matsuyama *et al.* (2005)	DB, PC, R	0·6	104	32	M + F	mean 66	COPD	Sputum IL-8, TNFα Serum IL-8 TNFα	IL-8, ↓ 55%; TNFα, ↓ 48% None
Broekhuizen *et al.* (2005)	DB, PC, R	1·0 (0·7 + 0·3) + 0·7 GLA + 1·2 ALA + 0·4 STA	8	51	M + F	mean 64	COPD	Serum IL-6, TNFα	None
Mickleborough *et al.* (2006)	DB, PC, R	5·2 (3·2 + 2·0)	3	8	M + F	mean 23	Exercise-induced broncho-constriction in asthma	Sputum IL-1β, TNFα	IL-1β, ↓; TNFα, ↓

HIV									
Virgili *et al.* (1997)	Open, parallel	1·8 (1·1 + 0·7)	6	9	M	30–52, mean 36	HIV	LPS-induced IL-1β	IL-1β, ↓ 33%
Bell *et al.* (1996)	DB, PC, R	2 (1·0 + 1·0)	6	10	M	30–43, mean 38	HIV	LPS-induced TNFα, IL-6	IL-6 ↑, TNFα, none
Hellerstein *et al.* (1996)	Open	4·5 (2·7 + 1·8)	10	16	M	mean 41	HIV wasting	Serum TNFα, IL-1β,IFN-α LPS- and PHA-induced TNFα, IL-1β	None None
Obesity									
Jellema *et al.* (2004)	DB, cross-over	1·1 (0·6 + 0·5)	6	11	M	mean 59	BMI 30–35 kg/m^2	Serum sTNFαR, IL-6, TNFα	None
Chan *et al.* (2002)	DB, PC, R	3·4 (1·8 + 1·6)	6	12	M	mean 54	Central obesity, BMI >29 kg/m^2	Serum TNFα, IL-6	None
Diabetes									
Molvig *et al.* (1991)	Open	3·2 (2 + 1·2)	7	8	M	mean 24	Type 1 diabetes	PHA-induced LP LPS-induced TNFα, IL-1β	↓ 50% None
Mori *et al.* (2003)	DB, PC, R, parallel	3·7 DHA	6	18	M + F	40–75, mean 61	Type 2 diabetes	Serum TNFα, IL-6	None
Mori *et al.* (2003)	DB, P,C R, parallel	3·8 EPA	6	17	M + F	40–75, mean 61	Type 2 diabetes	Serum TNFα, IL-6	None
Other inflammatory conditions									
Gallai *et al.* (1995)	Open	5·2 (3·1 + 2·1)	26	15	M + F	20–50	Multiple sclerosis	Serum sIL-2R PHA-induced IL-2 Con A-induced IFN-γ LPS-induced TNFα, IL-1β	↓ 30% ↓ 25% ↓ 45% IL-1β, ↓ 24%; TNFα, ↓ 25%
Soyland *et al.* (1994)	DB, PC, R	5·0 (3·1 + 1·9)	16	21	M + F		Psoriasis (*n* 11), atopic dermatitis (*n 10*)	PHA-induced LP, IL-2, IL-6, TNFα	None
Cappelli *et al.* (1997)	Open non-PC	2·9	52	10	M + F	mean 52	Chronic renal disease	PHA-induced IL-2, IL-1β, TNFα	TNFα ↓ 34%, IL-2, IL-1β: None

DB, double-blind; PC, placebo-controlled; R, randomised; ALA, α-linolenic acid (18: 3*n*-3); STA, stearidonic acid (18: 4*n*-3); GLA, γ-linolenic acid (18: 3*n*-6); RA, rheumatoid arthritis; COPD, chronic obstructive pulmonary disease; LP, lymphocyte proliferation; Con A, concanavalin A; PHA, phytohaemaglutinin; LPS, lipopolysaccharide; s, soluble; IL-2R, IL-2 receptor; IFN-γ, interferon-γ; TNFR, TNF receptor; ↓, reduction; ↑, increase.

*The effect is shown if it was significant ($P < 0·05$).

inflammation in asthma is predominantly eosinophilic and the most important cytokines involved are IL-4, IL-5 and IL-13 (Barnes, 2000). Control of inflammatory mediators is an important aspect in the treatment strategy of both inflammatory lung diseases.

Two recent studies (Broekhuizen *et al*. 2005; Matsuyama *et al*. 2005) have investigated the effects of relatively-low supplemental intake of EPA + DHA on inflammatory markers, as well as exercise capacity, in COPD. Broekhuizen *et al*. (2005) have reported improved exercise capacity in a cycling test but no effect on systemic levels of IL-6 and TNFα following intake of a supplement containing 1·0 g EPA + DHA/d. Matsuyama *et al*. (2005) have measured both systemic (serum) and sputum TNFα and IL-8 levels in a 2-year intervention with a supplemental intake of 0·6 g EPA + DHA/d. Notably, although no differences in serum cytokine concentrations were found in this study, decreased sputum TNFα and IL-8 levels were observed in the group receiving *n*-3 PUFA, accompanied by improved exercise capacity in a walk test. The only comparable study of asthma has reported the effects of a high level of fish oil (5·2 g EPA + DHA/d) on sputum inflammatory cytokines and pulmonary function in exercise-induced broncho-constriction (Mickleborough *et al*. 2006). Concentrations of TNFα and IL-1β in the sputum supernatant fraction were found to be significantly lower ($P<0·05$) in the fish oil group and this change was accompanied by improved pulmonary function.

In conclusion, these studies indicate that EPA + DHA supplementation in inflammatory lung diseases results in a local rather than a systemic decrease in inflammatory cytokines, even at relatively low intake levels. As these local effects are associated with improved clinical outcomes, cytokine concentrations in inflamed tissue might be a more sensitive marker and predictor of anti-inflammatory efficacy of EPA + DHA supplementation than systemic cytokines.

HIV infection and AIDS

Generally, HIV infection and disease progression is not considered to be predominantly an inflammatory disease. Gradual depletion of T lymphocytes is considered to be the most distinct and important immunological feature of HIV disease progression. However, there is substantial evidence to indicate that HIV disease progression is also associated with persistent presence of (subclinical) inflammation, particularly in the intestinal mucosa. It is uncertain whether a mucosal inflammatory response in the intestine is a result of either HIV infection or altered enterocyte function and activity. It is clear, however, that during the progression of the disease there are distinct kinetics of production of local pro-inflammatory cytokines (McGowan *et al*. 1994, 2004; Reka *et al*. 1994; Sharpstone *et al*. 1996). There is elevation of TNFα, IL-1β and IL-6 in particular and to a lesser extent IFN-γ in intestinal biopsies from patients infected with HIV. The kinetics of the production of these cytokines and the increased local tissue levels strongly depend on the stage of disease, i.e. TNFα and IL-1β increase during the progression of disease. It has been shown in models of intestinal barrier function that pro-inflammatory cytokines can have strong detrimental effects on intestinal barrier disruption by increasing paracellular permeability (McKay & Baird, 1999; Nusrat *et al*. 2000). Disruption of intestinal barrier integrity, as determined by increased epithelial permeability, has also been reported in patients infected with HIV. This barrier disruption seems to worsen during the course of disease (Lima *et al*. 1997). It has therefore been hypothesised that the underlying mechanism most likely involves pro-inflammatory cytokines such as TNFα, IFN-γ and IL-1β (Stockmann *et al*. 1998, 2000; Schmitz *et al*. 2002). A more-widely-acknowledged aspect of the pro-inflammatory response during HIV infection is the AIDS-related wasting syndrome. It has been estimated that in the USA 35 % of patients with AIDS are cachectic (Morley *et al*. 2006). Elaboration of pro-inflammatory cytokines is probably the major factor responsible for AIDS wasting (Morley *et al*. 2006). Studies investigating the effects of fish oil supplementation on cytokine concentrations and responses in patients who were HIV positive were conducted against the background of potential benefits on lean body mass.

Three relatively-small studies (*n* 9–16; Bell *et al*. 1996; Hellerstein *et al*. 1996; Virgili *et al*. 1997) have investigated the effects of fish oil supplementation on cytokine concentrations and responses in patients who were HIV positive. Virgili *et al*. (1997) have reported decreased LPS-induced IL-1β production following 1·8 g EPA + DHA/d for 6 weeks. On the other hand, Bell *et al*. (1996) have reported increased LPS-induced IL-6 production and no effect on TNFα production after 2·0 g EPA + DHA/d for 6 weeks. Finally, Hellerstein *et al*. (1996) have reported no effects of 4·5 g EPA + DHA/d on serum TNFα, IL-1β and IFN-γ concentrations, LPS- and PHA-induced production of TNFα and IL-1β or weight loss in a study of patients with AIDS-associated weight loss. None of these studies have reported significant effects of *n*-3 PUFA on T-cell counts.

In conclusion, as a fish oil-rich supplement has no adverse effect on T-cell counts in these or other studies of individuals infected with HIV or positive for AIDS (Pichard *et al*. 1998; de Luis Roman *et al*. 2001; Keithley *et al*. 2002), this intervention appears to be safe in HIV infection and AIDS. Although there appears to be no consistent effect of fish oil supplementation on systemic cytokine production in patients infected with HIV or positive for AIDS, to date there are no data available on the effect of EPA + DHA enrichment on local markers of inflammation in the intestinal mucosa. It would therefore be useful to investigate the effects of EPA + DHA on mucosal inflammation and intestinal barrier integrity in patients infected with HIV, as such an approach might be more likely to reveal potential clinically-relevant benefits of EPA + DHA supplementation to these patients.

Obesity

Obese individuals are at increased risk for a range of metabolic diseases, including insulin resistance, dyslipidaemia and hypertension. Adipose tissue is an important endocrine organ, secreting a range of inflammatory mediators, including TNFα and IL-6. Circulating concentrations of these cytokines are increased in obesity and may contribute

to the pathogenesis of metabolic diseases (Browning, 2003). Hence, obesity is considered as a low-grade chronic inflammatory state.

Two studies (Chan *et al*. 2002; Jellema *et al*. 2004) have investigated the effects of supplementation with fish oil (providing either 1·1 (Jellema *et al*. 2004) or 3·4 (Chan *et al*. 2002) g EPA + DHA/d) for 6 weeks on serum TNFα and IL-6 concentrations in obese men. Fish oil supplementation was not found to significantly affect the concentrations of circulating cytokines in either study. Hence, these studies do not provide evidence that EPA + DHA supplementation has favourable effects on markers for the low-grade inflammatory state in obesity.

Diabetes

Inflammatory cytokines have been implicated in the inflammatory processes leading to the destruction of the Islets of Langerhans in type 1 diabetes (Mandrup-Poulsen *et al*. 1989). In addition, prospective epidemiological studies have found that patients with type 2 diabetes have increased levels of inflammatory markers such as ARA-derived F2-isoprostanes (Gopaul *et al*. 1995), IL-6 (Pradhan *et al*. 2001), TNFα (Nilsson *et al*. 1998) and C-reactive protein (Pradhan *et al*. 2001). It has been suggested that elevated levels of these markers are associated with increased oxidative stress in patients with type 2 diabetes (Mori *et al*. 2003). Hence, both insulin-dependent and non-insulin-dependent diabetes are considered to be disorders with an inflammatory component.

Molvig *et al*. (1991) have studied the effects of 3·2 g EPA + DHA/d in a small cohort of men with type 1 diabetes parallel to an age-matched cohort of healthy men. As in the healthy subjects, no effect on LPS-induced TNFα and IL-1β production was found but PHA-induced lymphocyte proliferation was found to be decreased by 50 % in the patients with diabetes. Mori *et al*. (2003) have studied the effects of a supplemental intake of either 3·8 g purified EPA/d or 3·7 g purified DHA/d on serum IL-6 and TNFα concentrations in patients with type 2 diabetes who were hypertensive. Although no statistically significant effects on serum IL-6 and TNFα were found, TNFα concentrations tended to be lower after 6 weeks of treatment with either EPA or DHA compared with baseline levels. Post-intervention TNFα concentrations were 20 % lower in the group receiving EPA and 33 % lower in the group receiving DHA.

Other inflammatory conditions

In addition to the inflammatory disorders addressed previously, the effects of EPA + DHA on the selected immune markers in psoriasis, chronic renal disease and multiple sclerosis have been investigated in only one study for each of these disorders. Soyland *et al*. (1994) have found no effects on PHA-induced lymphocyte proliferation or IL-2, IL-6 and TNFα production following treatment with 5·0 g EPA + DHA/d for 16 weeks in patients with psoriasis. Cappelli *et al*. (1997) have reported decreased PHA-induced TNFα production but no effects on PHA-induced IL-1β and IL-2 production in patients with chronic progressive renal disease following treatment with 2·9 g EPA + DHA/d for 1 year. Gallai *et al*. (1995) have studied the effects of 5·2 g EPA + DHA/d for 26 weeks in healthy subjects (for details, see Tables 2 and 3) and patients with multiple sclerosis and have reported decreased PHA-induced IL-2 production, decreased Con A-induced IFN-γ production and decreased LPS-induced TNFα and IL-1β production in the patients with multiple sclerosis. These effects were similar to those for the healthy subjects in this study.

Discussion and conclusions

Dietary supplementation with long-chain *n*-3 PUFA from fish oil (EPA and DHA) increases the proportion of these fatty acids in immune cells and changes the production of important mediators and regulators of inflammation and immune responses, such as PG, LT and resolvins, towards a more anti-inflammatory profile. The incorporation of EPA and DHA into human inflammatory cells (Healy *et al*. 2000) and the decreased production of pro-inflammatory PGE_2 occur in a dose-dependent fashion (Rees *et al*. 2006). Furthermore, an inverse relationship between mononuclear cell EPA content and the production of TNFα and IL-1β by these cells has been reported (Caughey *et al*. 1996). These observations suggest that the effects of EPA and DHA on inflammation and markers of immune function might be dose-dependent. The study by Rees *et al*. (2006) suggests that the threshold value of such an effect may be between 1·65 g EPA + DHA/d and 3·3 g EPA + DHA/d, at least in healthy volunteers, as decreased PGE_2 production was found at 3·3 g/d, with a larger decrease at 4·95 g/d, but no significant effect at 1·65 g/d.

Overall, the current data from supplementation studies in healthy subjects fail to reveal a threshold value for, and dose–response effects on, immunomodulation with EPA + DHA. First, there is no clear trend that intervention cohorts with decreased inflammatory cytokine and lymphocyte proliferation responses are found more often in the upper range of intake. Second, there are no clear indications that the percentage decrease in cytokine production and lymphocyte proliferation following EPA + DHA supplementation is higher in the higher dose range. Third, most studies in healthy subjects, particularly those with the best-quality design (double-blind, placebo-controlled, randomised and parallel), have not found effects of EPA and DHA on cytokine production and lymphocyte proliferation. The latter observation particularly indicates that healthy subjects are relatively insensitive to modulation of the cell and cytokine response with long-chain *n*-3 PUFA, even at intake levels that raise EPA concentration in mononuclear cell phospholipids from approximately 0·6 % total fatty acids to 4·1 % total fatty acids (Rees *et al*. 2006). Rees *et al*. (2006) have observed that older subjects incorporate EPA more readily than younger subjects, and that older subjects are more sensitive to the immunological effects of EPA. This observation is consistent with differences in lymphocyte proliferation responsiveness between older and young subjects (see p. 240). In addition, based on direct comparison of young and older subjects

there is some evidence to suggest that older women are more sensitive to the ability of long-chain *n*-3 PUFA to decrease production of inflammatory cytokines (Meydani *et al*. 1991), but this finding is not confirmed in a study comparing young and older males (Rees *et al*. 2006), and there is no evidence to suggest that older subjects are more sensitive to modulation of T-cell cytokine responses than younger subjects. Taken together, there is no conclusive evidence that the immunomodulatory effects of EPA + DHA in healthy subjects are dose dependent, which may be related to the apparent low sensitivity of healthy subjects to such modulation.

The data summarised in Table 4 show that inflammatory cytokine concentrations or production are influenced by fish oil in a relatively large number of studies conducted in patients with inflammatory conditions. This observation suggests that patients with an inflammatory condition might be more sensitive to the immunomodulatory effects of long-chain *n*-3 PUFA than healthy subjects. This difference could potentially be related to depletion of the buffering capacity present in healthy subjects, e.g. as a result of a higher turnover rate of immune cells in disease and of the fatty acids in immune cell phospholipids for use as substrate for eicosanoid synthesis or as ligands for transcription factors. In addition, some studies, particularly those in patients with COPD, indicate that local effects at the site of inflammation might be more pronounced than systemic effects (for references, see Table 4). In addition, the Trebble *et al*. (2004) study of Crohn's disease indicates that disease-related T-cell markers might be more sensitive to immunomodulation by fish oil than the same T-cell markers in healthy subjects. The presence of inflamed tissue or 'sensitised' immune cells in inflammatory disorders and the absence of these factors in healthy subjects might (partially) explain the differential immunomodulation seen. As the designs and experimental conditions used differ between studies it is not possible to thoroughly investigate the potential presence of dose–response effects. The limited data that allow direct comparison within the same inflammatory disorder provide no clear indication of a dose–response effect of EPA + DHA supplementation on immune markers. Importantly, in a substantial number of these studies clinical benefits related to the inflammatory state of the condition were observed in the absence of significant effects on immune markers of inflammation. This observation indicates that EPA + DHA might exert anti-inflammatory effects without revealing these effects if only certain immune markers are considered. This possibility implies that condition-specific clinical end points such as joint tenderness and morning stiffness in rheumatoid arthritis or exercise performance in COPD might be more sensitive markers of modulation by EPA + DHA than cytokines. Taken together, the observations indicate that studies in healthy subjects are a useful tool to describe the general principles of immunomodulation by *n*-3 PUFA, as in general the direction of immunomodulation in healthy subjects (if any) and in inflammatory conditions is the same. However, the extent of the effect might be very different in inflammatory conditions, indicating that studies in healthy subjects are not very appropriate for establishing dose levels for specific applications in inflammatory conditions. In some specific situations, such as in immune suppression induced by malnourishment (Gogos *et al*. 1998) or surgery (stress; Furukawa *et al*. 1999) in patients with cancer the direction of the modulation might be opposite to that seen in healthy subjects and in patients with a chronic inflammatory disorder. Also, it is possible that in such conditions EPA + DHA contribute to a normalisation of the immune response.

Traditionally, EPA rather than DHA has been considered as the most important and potent immunomodulatory *n*-3 PUFA, as its mechanistic basis of being an alternative substrate for eicosanoid synthesis is well described. More recently, DHA-derived mediators D-series resolvins, docosatrienes and neuroprotectins, also produced by cyclooxygenase-2 and lipoxygenase under some conditions, have been identified that also appear to be anti-inflammatory and inflammation resolving (Hong *et al*. 2003; Marcheselli *et al*. 2003; Mukherjee *et al*. 2004). Only two studies in the current overview provide direct comparison of the effects of EPA and DHA. The first study (Kew *et al*. 2004) has shown that a similarly high intake of either EPA or DHA has no effect on cytokine production but that only DHA reduces CD69 expression, a marker of T-cell activation, in healthy subjects. The second study (Mori *et al*. 2003) has shown that serum TNFα concentrations are at least equally affected by DHA intake compared with EPA intake in subjects with type 2 diabetes. Conclusive evidence of the relative contribution of EPA and DHA is still lacking but these observations, together with the mechanistic understanding of DHA-based effects that is now available, may change the traditional view of the relative contributions of EPA and DHA.

The importance of the dampening effect of EPA + DHA on some markers of immune function in relation to the immune response in general and disease resistance is not well described. The observations in some studies that EPA + DHA decrease cytokine and lymphocyte proliferation responses may lead to the conclusion that EPA + DHA are immunosuppressive and therefore disadvantageous to the host's immune function and disease resistance. However, there are no data to suggest that either supplementation with fish oil or high background intakes of EPA + DHA (e.g. in the Japanese, Greenland Inuit, Norwegian or Icelandic populations) increase susceptibility to infectious diseases. The immunomodulatory effects observed in some studies with healthy subjects might equally well reflect a correction towards normalised, less exaggerated, responses, indicating a more balanced and effective immune response. The later hypothesis is supported by a few observations. The findings of a large study on the risk of community-acquired pneumonia and fatty acid intake (Merchant *et al*. 2005) indicate that pneumonia risk is reduced by 31 % for every 1 g/d increase in intake of α-linolenic acid (18 : 3*n*-3) and by 4 % for every 1 g/d increase in linoleic acid intake. In this study linoleic and α-linolenic acids were derived from common food sources, so their effects could not be separated. α-Linolenic acid has a much larger effect than linoleic acid and increased α-linolenic acid intake increases EPA levels in immune cells (Burdge & Calder, 2005). Thus, increased EPA levels in immune cells might explain this observation. Moreover,

among subjects with low *n*-6 and *n*-3 fatty acid intakes from plant sources, high fish intake is associated with reduced pneumonia risk (Merchant *et al*. 2005). Second, observations associated with an epidemic of measles in Greenland in 1951, triggered by an infected Danish sailor, support this view. The epidemic in this naïve population shows the same characteristics (e.g. expected numbers of cases, complications) as previous epidemics recorded in other naïve populations elsewhere in the world (Kronborg *et al*. 1992). As this naïve Inuit population in Greenland traditionally consume a long-chain *n*-3 PUFA-rich diet, these observations suggests that these fatty acids do not worsen the response to the virus (Calder, 2001*b*). Third, evidence from clinical trials of patients with trauma and cancer who were hospitalised (Heyland *et al*. 2001) suggests that EPA + DHA-enriched 'immunonutrition' may decrease, not increase, infectious complication rates. However, because of the combination of nutrients such as arginine, nucleotides and long-chain *n*-3 PUFA in the enteral formulas used in these studies, it is not possible to discern how much of the reported effects are attributable to *n*-3 PUFA. Thus, these limited data support the view that high EPA + DHA consumption does not impair immune function and may be beneficial for infectious disease resistance. For conclusive statements on this issue, well-powered supplementation studies designed to identify effects of EPA + DHA on infection rates are required.

In conclusion, the current review provides no evidence for strong dose-dependent immunomodulatory effects of EPA + DHA in healthy subjects. The apparent absence of dose-dependent effects might be a result of the relative insensitivity of healthy subjects to such modulation. The presence of an inflammatory condition might increase the sensitivity to the immunomodulatory effects of EPA + DHA. In addition, there is substantial evidence to suggest that some condition-specific clinical end points are more sensitive markers to these effects than immune markers. Studies in healthy subject are a useful tool for investigating the general principles of EPA + DHA modulation, rather than for determining the dose required in specific inflammatory conditions. The concern that the potential immunosuppressive effects of EPA + DHA might impair immune function or infectious disease resistance is not supported by the studies considered here. Indeed, in some conditions the immunomodulatory effects of EPA + DHA might improve immune function and disease resistance.

References

Abbate R, Gori AM, Martini F, Brunelli T, Filippini M, Francalanci I, Paniccia R, Prisco D, Gensini GF & Neri Serneri GG (1996) n-3 PUFA supplementation, monocyte PCA expression and interleukin-6 production. *Prostaglandins, Leukotrienes, and Essential Fatty Acids* **54**, 439–444.

Adam O, Beringer C, Kless T, Lemmen C, Adam A, Wiseman M, Adam P, Klimmek R & Forth W (2003) Anti-inflammatory effects of a low arachidonic acid diet and fish oil in patients with rheumatoid arthritis. *Rheumatology International* **23**, 27–36.

Albers R, Antoine JM, Bourdet-Sicard R, Calder PC, Gleeson M, Lesourd B, Samartin S, Sanderson IR, Van Loo J, Vas Dias FW & Watzl B (2005) Markers to measure immunomodulation in human nutrition intervention studies. *British Journal of Nutrition* **94**, 452–481.

Almallah YZ, El-Tahir A, Heys SD, Richardson S & Eremin O (2000*a*) Distal procto-colitis and n-3 polyunsaturated fatty acids: the mechanism(s) of natural cytotoxicity inhibition. *European Journal of Clinical Investigation* **30**, 58–65.

Almallah YZ, Ewen SW, El-Tahir A, Mowat NA, Brunt PW, Sinclair TS, Heys SD & Eremin O (2000*b*) Distal proctocolitis and n-3 polyunsaturated fatty acids (n-3 PUFAs): the mucosal effect in situ. *Journal of Clinical Immunology* **20**, 68–76.

Almallah YZ, Richardson S, O'Hanrahan T, Mowat NA, Brunt PW, Sinclair TS, Ewen S, Heys SD & Eremin O (1998) Distal procto-colitis, natural cytotoxicity, and essential fatty acids. *American Journal of Gastroenterology* **93**, 804–809.

Bach JF (2002) The effect of infections on susceptibility to autoimmune and allergic diseases. *New England Journal of Medicine* **347**, 911–920.

Bagga D, Wang L, Farias-Eisner R, Glaspy JA & Reddy ST (2003) Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. *Proceedings of the National Academy of Sciences USA* **100**, 1751–1756.

Barber MD, Fearon KC, Tisdale MJ, McMillan DC & Ross JA (2001) Effect of a fish oil-enriched nutritional supplement on metabolic mediators in patients with pancreatic cancer cachexia. *Nutrition and Cancer* **40**, 118–124.

Barnes PJ (2000) Mechanisms in COPD: differences from asthma. *Chest* **117**, 10S–14S.

Barnes PJ, Shapiro SD & Pauwels RA (2003) Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *European Respiratory Journal* **22**, 672–688.

Bechoua S, Dubois M, Vericel E, Chapuy P, Lagarde M & Prigent AF (2003) Influence of very low dietary intake of marine oil on some functional aspects of immune cells in healthy elderly people. *British Journal of Nutrition* **89**, 523–531.

Bell SJ, Chavali S, Bistrian BR, Connolly CA, Utsunomiya T & Forse RA (1996) Dietary fish oil and cytokine and eicosanoid production during human immunodeficiency virus infection. *Journal of Parenteral and Enteral Nutrition* **20**, 43–49.

Blok WL, Deslypere JP, Demacker PN, van der Ven-Jongekrijg J, Hectors MP, van der Meer JW & Katan MB (1997) Pro- and anti-inflammatory cytokines in healthy volunteers fed various doses of fish oil for 1 year. *European Journal of Clinical Investigation* **27**, 1003–1008.

Broekhuizen R, Wouters EF, Creutzberg EC, Weling-Scheepers CA & Schols AM (2005) Polyunsaturated fatty acids improve exercise capacity in chronic obstructive pulmonary disease. *Thorax* **60**, 376–382.

Browning LM (2003) n-3 Polyunsaturated fatty acids, inflammation and obesity-related disease. *Proceedings of the Nutrition Society* **62**, 447–453.

Burdge GC & Calder PC (2005) Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reproduction, Nutrition, Development* **45**, 581–597.

Calder PC (2001*a*) N-3 polyunsaturated fatty acids, inflammation and immunity: pouring oil on troubled waters or another fishy tale? *Nutrition Research* **21**, 309–341.

Calder PC (2001*b*) Polyunsaturated fatty acids, inflammation, and immunity. *Lipids* **36**, 1007–1024.

Calder PC (2003) N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. *Lipids* **38**, 343–352.

Calder PC (2006) n-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. *American Journal of Clinical Nutrition* **83**, S1505–S1519.

Calder PC, Yaqoob P, Thies F, Wallace FA & Miles EA (2002) Fatty acids and lymphocyte functions. *British Journal of Nutrition* **87**, S31–S48.

Cannon JG, Fiatarone MA, Meydani M, Gong J, Scott L, Blumberg JB & Evans WJ (1995) Aging and dietary modulation of elastase and interleukin-1 beta secretion. *American Journal of Physiology* **268**, R208–R213.

Cappelli P, Di Liberato L, Stuard S, Ballone E & Albertazzi A (1997) N-3 polyunsaturated fatty acid supplementation in chronic progressive renal disease. *Journal of Nephrology* **10**, 157–162.

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

Chan DC, Watts GF, Barrett PHR, Beilin LJ & Mori TA (2002) Effect of atorvastatin and fish oil on plasma high-sensitivity C-reactive protein concentrations in individuals with visceral obesity. *Clinical Chemistry* **48**, 877–883.

Ciubotaru I, Lee YS & Wander RC (2003) Dietary fish oil decreases C-reactive protein, interleukin-6, and triacylglycerol to HDL-cholesterol ratio in postmenopausal women on HRT. *Journal of Nutritional Biochemistry* **14**, 513–521.

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

Daviglus ML, Stamler J, Orencia AJ, Dyer AR, Liu K, Greenland P, Walsh MK, Morris D & Shekelle RB (1997) Fish consumption and the 30-year risk of fatal myocardial infarction. *New England Journal of Medicine* **336**, 1046–1053.

de Luis Roman DA, Bachiller P, Izaola O, Romero E, Martin J, Arranz M, Eiros Bouza JM & Aller R (2001) Nutritional treatment for acquired immunodeficiency virus infection using an enterotropic peptide-based formula enriched with n-3 fatty acids: a randomized prospective trial. *European Journal of Clinical Nutrition* **55**, 1048–1052.

Dooper MM, Wassink L, M'Rabet L & Graus YM (2002) The modulatory effects of prostaglandin-E on cytokine production by human peripheral blood mononuclear cells are independent of the prostaglandin subtype. *Immunology* **107**, 152–159.

Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JW *et al.* (1989) The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *New England Journal of Medicine* **320**, 265–271.

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

Espersen GT, Grunnet N, Lervang HH, Nielsen GL, Thomsen BS, Faarvang KL, Dyerberg J & Ernst E (1992) Decreased interleukin-1 beta levels in plasma from rheumatoid arthritis patients after dietary supplementation with n-3 polyunsaturated fatty acids. *Clinical Rheumatology* **11**, 393–395.

Fearon KC, Barber MD, Moses AG, Ahmedzai SH, Taylor GS, Tisdale MJ & Murray GD (2006) Double-blind, placebo-controlled, randomized study of eicosapentaenoic acid diester in patients with cancer cachexia. *Journal of Clinical Oncology* **24**, 3401–3407.

Feldmann M & Maini RN (1999) The role of cytokines in the pathogenesis of rheumatoid arthritis. *Rheumatology* **38**, Suppl. 2, 3–7.

Furukawa K, Tashiro T, Yamamori H, Takagi K, Morishima Y, Sugiura T *et al.* (1999) Effects of soybean oil emulsion and eicosapentaenoic acid on stress response and immune function after a severely stressful operation. *Annals of Surgery* **229**, 255–261.

Gallai V, Sarchielli P, Trequattrini A, Franceschini M, Floridi A, Firenze C, Alberti A, Di Benedetto D & Stragliotto E (1995) Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. *Journal of Neuroimmunology* **56**, 143–153.

Gibney MJ & Hunter B (1993) The effects of short- and long-term supplementation with fish oil on the incorporation of n-3 polyunsaturated fatty acids into cells of the immune system in healthy volunteers. *European Journal of Clinical Nutrition* **47**, 255–259.

Gogos CA, Ginopoulos P, Salsa B, Apostolidou E, Zoumbos NC & Kalfarentzos F (1998) Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy: a randomized control trial. *Cancer* **82**, 395–402.

Goldman DW, Pickett WC & Goetzl EJ (1983) Human neutrophil chemotactic and degranulating activities of leukotriene B5 (LTB5) derived from eicosapentaenoic acid. *Biochemical and Biophysical Research Communications* **117**, 282–288.

Gopaul NK, Anggard EE, Mallet AI, Betteridge DJ, Wolff SP & Nourooz-Zadeh J (1995) Plasma 8-epi-PGF2 alpha levels are elevated in individuals with non-insulin dependent diabetes mellitus. *FEBS Letters* **368**, 225–229.

Grimble RF, Howell WM, O'Reilly G, Turner SJ, Markovic O, Hirrell S, East JM & Calder PC (2002) The ability of fish oil to suppress tumor necrosis factor alpha production by peripheral blood mononuclear cells in healthy men is associated with polymorphisms in genes that influence tumor necrosis factor alpha production. *American Journal of Clinical Nutrition* **76**, 454–459.

Hallgren CG, Hallmans G, Jansson JH, Marklund SL, Huhtasaari F, Schutz A, Stromberg U, Vessby B & Skerfving S (2001) Markers of high fish intake are associated with decreased risk of a first myocardial infarction. *British Journal of Nutrition* **86**, 397–404.

Hawkes JS, Bryan DL, Makrides M, Neumann MA & Gibson RA (2002) A randomized trial of supplementation with docosahexaenoic acid-rich tuna oil and its effects on the human milk cytokines interleukin 1 beta, interleukin 6, and tumor necrosis factor alpha. *American Journal of Clinical Nutrition* **75**, 754–760.

Healy DA, Wallace FA, Miles EA, Calder PC & Newsholm P (2000) Effect of low-to-moderate amounts of dietary fish oil on neutrophil lipid composition and function. *Lipids* **35**, 763–768.

Hellerstein MK, Wu K, McGrath M, Faix D, George D, Shackleton CH, Horn W, Hoh R & Neese RA (1996) Effects of dietary n-3 fatty acid supplementation in men with weight loss associated with the acquired immune deficiency syndrome: Relation to indices of cytokine production. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* **11**, 258–270.

Heyland DK, Novak F, Drover JW, Jain M, Su X & Suchner U (2001) Should immunonutrition become routine in critically ill patients? A systematic review of the evidence. *Journal of the American Medical Association* **286**, 944–953.

Hibbeln JR (1998) Fish consumption and major depression. *Lancet* **351**, 1213.

Hibbeln JR (2002) Seafood consumption, the DHA content of mothers' milk and prevalence rates of postpartum depression: a cross-national, ecological analysis. *Journal of Affective Disorders* **69**, 15–29.

Hommes DW & van Deventer SJ (2000) Anti- and proinflammatory cytokines in the pathogenesis of tissue damage in

Crohn's disease. *Current Opinion in Clinical Nutrition and Metabolic Care* **3**, 191–195.
Hong S, Gronert K, Devchand PR, Moussignac RL & Serhan CN (2003) Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. *Journal of Biological Chemistry* **278**, 14677–14687.
Jellema A, Plat J & Mensink RP (2004) Weight reduction, but not a moderate intake of fish oil, lowers concentrations of inflammatory markers and PAI-1 antigen in obese men during the fasting and postprandial state. *European Journal of Clinical Investigation* **34**, 766–773.
Keithley JK, Swanson B, Zeller JM, Sha BE, Cohen M, Hershow R & Novak R (2002) Comparison of standard and immune-enhancing oral formulas in asymptomatic HIV-infected persons: a multicenter randomized controlled clinical trial. *Journal of Parenteral and Enteral Nutrition* **26**, 6–14.
Kelley DS, Taylor PC, Nelson GJ & Mackey BE (1998*a*) Arachidonic acid supplementation enhances synthesis of eicosanoids without suppressing immune functions in young healthy men. *Lipids* **33**, 125–130.
Kelley DS, Taylor PC, Nelson GJ & Mackey BE (1998*b*) Dietary docosahexaenoic acid and immunocompetence in young healthy men. *Lipids* **33**, 559–566.
Kelley DS, Taylor PC, Nelson GJ, Schmidt PC, Ferretti A, Erickson KL, Yu R, Chandra RK & Mackey BE (1999) Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. *Lipids* **34**, 317–324.
Kelley DS, Taylor PC, Nelson GJ, Schmidt PC, Mackey BE & Kyle D (1997) Effects of dietary arachidonic acid on human immune response. *Lipids* **32**, 449–456.
Kew S, Banerjee T, Minihane AM, Finnegan YE, Muggli R, Albers R, Williams CM & Calder PC (2003) Lack of effect of foods enriched with plant- or marine-derived n-3 fatty acids on human immune function. *American Journal of Clinical Nutrition* **77**, 1287–1295.
Kew S, Mesa MD, Tricon S, Buckley R, Minihane AM & Yaqoob P (2004) Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell composition and function in healthy humans. *American Journal of Clinical Nutrition* **79**, 674–681.
Kramer TR, Schoene N, Douglass LW, Judd JT, Ballard-Barbash R, Taylor PR, Bhagavan HN & Nair PP (1991) Increased vitamin E intake restores fish-oil-induced suppressed blastogenesis of mitogen-stimulated T lymphocytes. *American Journal of Clinical Nutrition* **54**, 896–902.
Kremer JM, Lawrence DA, Jubiz W, DiGiacomo R, Rynes R, Bartholomew LE & Sherman M (1990) Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Clinical and immunologic effects. *Arthritis and Rheumatism* **33**, 810–820.
Kremer JM, Lawrence DA, Petrillo GF, Litts LL, Mullaly PM, Rynes RI *et al.* (1995) Effects of high-dose fish oil on rheumatoid arthritis after stopping nonsteroidal antiinflammatory drugs. Clinical and immune correlates. *Arthritis and Rheumatism* **38**, 1107–1114.
Kronborg D, Hansen B & Aaby P (1992) Analysis of the incubation period for measles in the epidemic in Greenland in 1951 using a variance components model. *Statistics in Medicine* **11**, 579–590.
Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese J 3rd, Spur BW, Robinson DR, Corey EJ, Lewis RA & Austen KF (1985) Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *New England Journal of Medicine* **312**, 1217–1224.
Lee TH, Menica-Huerta JM, Shih C, Corey EJ, Lewis RA & Austen KF (1984) Characterization and biologic properties of 5,12-dihydroxy derivatives of eicosapentaenoic acid, including leukotriene B5 and the double lipoxygenase product. *Journal of Biological Chemistry* **259**, 2383–2389.
Lewis RA, Austen KF & Soberman RJ (1990) Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. *New England Journal of Medicine* **323**, 645–655.
Lima AA, Silva TM, Gifoni AM, Barrett LJ, McAuliffe IT, Bao Y, Fox JW, Fedorko DP & Guerrant RL (1997) Mucosal injury and disruption of intestinal barrier function in HIV-infected individuals with and without diarrhea and cryptosporidiosis in northeast Brazil. *American Journal of Gastroenterology* **92**, 1861–1866.
McGowan I, Elliott J, Fuerst M, Taing P, Boscardin J, Poles M & Anton P (2004) Increased HIV-1 mucosal replication is associated with generalized mucosal cytokine activation. *Journal of Acquired Immune Deficiency Syndromes* **37**, 1228–1236.
McGowan I, Radford-Smith G & Jewell DP (1994) Cytokine gene expression in HIV-infected intestinal mucosa. *AIDS* **8**, 1569–1575.
McKay DM & Baird AW (1999) Cytokine regulation of epithelial permeability and ion transport. *Gut* **44**, 283–289.
Mandrup-Poulsen T, Helqvist S, Molvig J, Wogensen LD & Nerup J (1989) Cytokines as immune effector molecules in autoimmune endocrine diseases with special reference to insulin-dependent diabetes mellitus. *Autoimmunity* **4**, 191–218.
Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, Serhan CN & Bazan NG (2003) Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. *Journal of Biological Chemistry* **278**, 43807–43817.
Matsuyama W, Mitsuyama H, Watanabe M, Oonakahara K, Higashimoto I, Osame M & Arimura K (2005) Effects of omega-3 polyunsaturated fatty acids on inflammatory markers in COPD. *Chest* **128**, 3817–3827.
Merchant AT, Curhan GC, Rimm EB, Willett WC & Fawzi WW (2005) Intake of n-6 and n-3 fatty acids and fish and risk of community-acquired pneumonia in US men. *American Journal of Clinical Nutrition* **82**, 668–674.
Meydani SN, Endres S, Woods MM, Goldin BR, Soo C, Morrill-Labrode A, Dinarello CA & Gorbach SL (1991) Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *Journal of Nutrition* **121**, 547–555.
Meydani SN, Lichtenstein AH, Cornwall S, Meydani M, Goldin BR, Rasmussen H, Dinarello CA & Schaefer EJ (1993) Immunologic effects of national cholesterol education panel step-2 diets with and without fish-derived N-3 fatty acid enrichment. *Journal of Clinical Investigation* **92**, 105–113.
Mickleborough TD, Lindley MR, Ionescu AA & Fly AD (2006) Protective effect of fish oil supplementation on exercise-induced bronchoconstriction in asthma. *Chest* **129**, 39–49.
Miles EA, Allen E & Calder PC (2002) In vitro effects of eicosanoids derived from different 20-carbon fatty acids on production of monocyte-derived cytokines in human whole blood cultures. *Cytokine* **20**, 215–223.
Miles EA, Aston L & Calder PC (2003) In vitro effects of eicosanoids derived from different 20-carbon fatty acids on T helper type 1 and T helper type 2 cytokine production in human whole-blood cultures. *Clinical and Experimental Allergy* **33**, 624–632.
Miles EA, Banerjee T & Calder PC (2004*a*) The influence of different combinations of gamma-linolenic, stearidonic and

eicosapentaenoic acids on the fatty acid composition of blood lipids and mononuclear cells in human volunteers. *Prostaglandins, Leukotrienes, and Essential Fatty Acids* **70**, 529–538.

Miles EA, Banerjee T, Dooper MM, M'Rabet L, Graus YM & Calder PC (2004*b*) The influence of different combinations of gamma-linolenic acid, stearidonic acid and EPA on immune function in healthy young male subjects. *British Journal of Nutrition* **91**, 893–903.

Miles EA, Banerjee T, Wells SJ & Calder PC (2006) Limited effect of eicosapentaenoic acid on T-lymphocyte and natural killer cell numbers and functions in healthy young males. *Nutrition* **22**, 512–519.

Molvig J, Pociot F, Worsaae H, Wogensen LD, Baek L, Christensen P *et al.* (1991) Dietary supplementation with omega-3-polyunsaturated fatty acids decreases mononuclear cell proliferation and interleukin-1 beta content but not monokine secretion in healthy and insulin-dependent diabetic individuals. *Scandinavian Journal of Immunology* **34**, 399–410.

Mori TA, Woodman RJ, Burke V, Puddey IB, Croft KD & Beilin LJ (2003) Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects. *Free Radical Biology and Medicine* **35**, 772–781.

Morley JE, Thomas DR & Wilson MM (2006) Cachexia: pathophysiology and clinical relevance. *American Journal of Clinical Nutrition* **83**, 735–743.

Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Wilson RS, Aggarwal N & Schneider J (2003) Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease. *Archives of Neurology* **60**, 940–946.

Mosmann TR & Sad S (1996) The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunology Today* **17**, 138–146.

Mukherjee PK, Marcheselli VL, Serhan CN & Bazan NG (2004) Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. *Proceedings of the National Academy of Sciences USA* **101**, 8491–8496.

Murray CJ & Lopez AD (1997) Alternative projections of mortality and disability by cause 1990–2020: global Burden of Disease Study. *Lancet* **349**, 1498–1504.

Nilsson J, Jovinge S, Niemann A, Reneland R & Lithell H (1998) Relation between plasma tumor necrosis factor-alpha and insulin sensitivity in elderly men with non-insulin-dependent diabetes mellitus. *Arteriosclerosis, Thrombosis, and Vascular Biology* **18**, 1199–1202.

Nusrat A, Turner JR & Madara JL (2000) Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: nutrients, cytokines, and immune cells. *American Journal of Physiology* **279**, G851–G857.

Oomen CM, Feskens EJ, Rasanen L, Fidanza F, Nissinen AM, Menotti A, Kok FJ & Kromhout D (2000) Fish consumption and coronary heart disease mortality in Finland, Italy, and The Netherlands. *American Journal of Epidemiology* **151**, 999–1006.

Persson C, Glimelius B, Ronnelid J & Nygren P (2005) Impact of fish oil and melatonin on cachexia in patients with advanced gastrointestinal cancer: a randomized pilot study. *Nutrition* **21**, 170–178.

Peterson LD, Jeffery NM, Thies F, Sanderson P, Newsholme EA & Calder PC (1998) Eicosapentaenoic and docosahexaenoic acids alter rat spleen leukocyte fatty acid composition and prostaglandin E2 production but have different effects on lymphocyte functions and cell-mediated immunity. *Lipids* **33**, 171–180.

Pichard C, Sudre P, Karsegard V, Yerly S, Slosman DO, Delley V, Perrin L & Hirschel B (1998) A randomized double-blind controlled study of 6 months of oral nutritional supplementation with arginine and omega-3 fatty acids in HIV-infected patients. Swiss HIV Cohort Study. *AIDS* **12**, 53–63.

Pradhan AD, Manson JE, Rifai N, Buring JE & Ridker PM (2001) C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal of the American Medical Association* **286**, 327–334.

Rees D, Miles EA, Banerjee T, Wells SJ, Roynette CE, Wahle KW & Calder PC (2006) Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men. *American Journal of Clinical Nutrition* **83**, 331–342.

Reka S, Garro ML & Kotler DP (1994) Variation in the expression of human immunodeficiency virus RNA and cytokine mRNA in rectal mucosa during the progression of infection. *Lymphokine and Cytokine Research* **13**, 391–398.

Sano H, Hla T, Maier JA, Crofford LJ, Case JP, Maciag T & Wilder RL (1992) In vivo cyclooxygenase expression in synovial tissues of patients with rheumatoid arthritis and osteoarthritis and rats with adjuvant and streptococcal cell wall arthritis. *Journal of Clinical Investigation* **89**, 97–108.

Schmidt EB, Varming K, Moller JM, Bulow Pedersen I, Madsen P & Dyerberg J (1996) No effect of a very low dose of n-3 fatty acids on monocyte function in healthy humans. *Scandinavian Journal of Clinical and Laboratory Investigation* **56**, 87–92.

Schmitz H, Rokos K, Florian P, Gitter AH, Fromm M, Scholz P, Ullrich R, Zeitz M, Pauli G & Schulzke JD (2002) Supernatants of HIV-infected immune cells affect the barrier function of human HT-29/B6 intestinal epithelial cells. *AIDS* **16**, 983–991.

Serhan CN (2005) Novel eicosanoid and docosanoid mediators: resolvins, docosatrienes, and neuroprotectins. *Current Opinion in Clinical Nutrition and Metabolic Care* **8**, 115–121.

Serhan CN, Arita M, Hong S & Gotlinger K (2004) Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. *Lipids* **39**, 1125–1132.

Sharon P & Stenson WF (1984) Enhanced synthesis of leukotriene B4 by colonic mucosa in inflammatory bowel disease. *Gastroenterology* **86**, 453–460.

Sharpstone DR, Rowbottom AW, Nelson MR, Lepper MW & Gazzard BG (1996) Faecal tumour necrosis factor-alpha in individuals with HIV-related diarrhoea. *AIDS* **10**, 989–994.

Simopoulos AP (1998) Overview of evolutionary aspects of omega 3 fatty acids in the diet. *World Review of Nutrition and Dietetics* **83**, 1–11.

Soyland E, Lea T, Sandstad B & Drevon A (1994) Dietary supplementation with very long-chain n-3 fatty acids in man decreases expression of the interleukin-2 receptor (CD25) on mitogen-stimulated lymphocytes from patients with inflammatory skin diseases. *European Journal of Clinical Investigation* **24**, 236–242.

Sperling RI (1995) Eicosanoids in rheumatoid arthritis. *Rheumatic Diseases Clinics of North America* **21**, 741–758.

Sperling RI, Benincaso AI, Knoell CT, Larkin JK, Austen KF & Robinson DR (1993) Dietary omega-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. *Journal of Clinical Investigation* **91**, 651–660.

Stockmann M, Schmitz H, Fromm M, Schmidt W, Pauli G, Scholz P, Riecken EO & Schulzke JD (2000) Mechanisms of epithelial barrier impairment in HIV infection. *Annals of the New York Academy of Sciences* **915**, 293–303.

Stockmann M, Schmitz H, Fromm M, Schmidt W, Rokos K, Pauli G, Scholz P, Riecken EO & Schulzke JD (1998) The

mechanism of diarrhea in HIV is based on an impaired epithelial barrier function that could be induced by a specific cytokine pattern. *Annals of the New York Academy of Sciences* **859**, 267–270.

Sundrarjun T, Komindr S, Archararit N, Dahlan W, Puchaiwatananon O, Angthararak S, Udomsuppayakul U & Chuncharunee S (2004) Effects of n-3 fatty acids on serum interleukin-6, tumour necrosis factor-alpha and soluble tumour necrosis factor receptor p55 in active rheumatoid arthritis. *Journal of International Medical Research* **32**, 443–454.

Takagi K, Yamamori H, Furukawa K, Miyazaki M & Tashiro T (2001) Perioperative supplementation of EPA reduces immunosuppression induced by postoperative chemoradiation therapy in patients with esophageal cancer. *Nutrition* **17**, 478–479.

Tashiro T, Yamamori H, Takagi K, Hayashi N, Furukawa K & Nakajima N (1998) n-3 versus n-6 polyunsaturated fatty acids in critical illness. *Nutrition* **14**, 551–553.

Thies F, Miles EA, Nebe-von-Caron G, Powell JR, Hurst TL, Newsholme EA & Calder PC (2001*a*) Influence of dietary supplementation with long-chain n-3 or n-6 polyunsaturated fatty acids on blood inflammatory cell populations and functions and on plasma soluble adhesion molecules in healthy adults. *Lipids* **36**, 1183–1193.

Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA & Calder PC (2001*b*) Dietary supplementation with eicosapentaenoic acid, but not with other long-chain n-3 or n-6 polyunsaturated fatty acids, decreases natural killer cell activity in healthy subjects aged >55 y. *American Journal of Clinical Nutrition* **73**, 539–548.

Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA & Calder PC (2001*c*) Dietary supplementation with gamma-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. *Journal of Nutrition* **131**, 1918–1927.

Tilley SL, Coffman TM & Koller BH (2001) Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *Journal of Clinical Investigation* **108**, 15–23.

Todorov P, Cariuk P, McDevitt T, Coles B, Fearon K & Tisdale M (1996) Characterization of a cancer cachectic factor. *Nature* **379**, 739–742.

Trebble T, Arden NK, Stroud MA, Wootton SA, Burdge GC, Miles EA, Ballinger AB, Thompson RL & Calder PC (2003*a*) Inhibition of tumour necrosis factor-alpha and interleukin 6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. *British Journal of Nutrition* **90**, 405–412.

Trebble TM, Arden NK, Wootton SA, Calder PC, Mullee MA, Fine DR & Stroud MA (2004) Fish oil and antioxidants alter the composition and function of circulating mononuclear cells in Crohn disease. *American Journal of Clinical Nutrition* **80**, 1137–1144.

Trebble TM, Wootton SA, Miles EA, Mullee M, Arden NK, Ballinger AB, Stroud MA, Burdge GC & Calder PC (2003*b*) Prostaglandin E2 production and T cell function after fish-oil supplementation: response to antioxidant cosupplementation. *American Journal of Clinical Nutrition* **78**, 376–382.

Vega-Lopez S, Kaul N, Devaraj S, Cai RY, German B & Jialal I (2004) Supplementation with omega3 polyunsaturated fatty acids and all-rac alpha-tocopherol alone and in combination failed to exert an anti-inflammatory effect in human volunteers. *Metabolism: Clinical and Experimental* **53**, 236–240.

Virella G, Fourspring K, Hyman B, Haskill-Stroud R, Long L, Virella I, La Via M, Gross AJ & Lopes-Virella M (1991) Immunosuppressive effects of fish oil in normal human volunteers: correlation with the in vitro effects of eicosapentanoic acid on human lymphocytes. *Clinical Immunology and Immunopathology* **61**, 161–176.

Virgili N, Farriol M, Castellanos JM, Giro M, Podzamczer D & Pita AM (1997) Evaluation of immune markers in asymptomatic AIDS patients receiving fish oil supplementation. *Clinical Nutrition* **16**, 257–261.

Wallace FA, Miles EA & Calder PC (2003) Comparison of the effects of linseed oil and different doses of fish oil on mononuclear cell function in healthy human subjects. *British Journal of Nutrition* **89**, 679–689.

Wigmore SJ, Fearon KC, Maingay JP & Ross JA (1997) Down-regulation of the acute-phase response in patients with pancreatic cancer cachexia receiving oral eicosapentaenoic acid is mediated via suppression of interleukin-6. *Clinical Science London* **92**, 215–221.

Wu D, Han SN, Meydani M & Meydani SN (2004) Effect of concomitant consumption of fish oil and vitamin E on production of inflammatory cytokines in healthy elderly humans. *Annals of the New York Academy of Sciences* **1031**, 422–424.

Yaqoob P, Pala HS, Cortina-Borja M, Newsholme EA & Calder PC (2000) Encapsulated fish oil enriched in alpha-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. *European Journal of Clinical Investigation* **30**, 260–274.