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Critical Review

Modulation of Inflammation and Cytokine Production by Dietary (n-3) Fatty Acids

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ABSTRACT The production of pro-inflammatory cytokines, such as interleukin-1 and tumor necrosis factor, is pivotal in the response to infection. However, overproduction of these cytokines might be detrimental. It has been suggested that (n-3) fatty acids suppress inflammation and ameliorate the course of infection by decreasing the production of pro-inflammatory cytokines. We here, review these effects. Use of (n-3) fatty acids induced moderate clinical improvements in rheumatoid arthritis, psoriasis and colitis, but not in systemic lupus erythematosus. Data on critically ill burn or postoperative cancer patients are still inconclusive. The (n-3) fatty acids markedly inhibited sterile inflammation in animal studies and improved survival in some experimental infections. T cell responses decreased in healthy volunteers but remained unchanged or increased in certain patient groups. The production of pro-inflammatory cytokines decreased in most human studies. The (n-3) fatty acids increased cytokine production capacity in mice. Differences in cytokine-producing cell types studied may account for these paradoxical responses in humans and mice. Although the increased cytokine production in mice is partly mediated by effects on prostaglandins, mechanisms of action in other species remain to be elucidated. The (n-3) fatty acids may be of moderate benefit in some chronic inflammatory diseases. Their therapeutic value and possible hazards in critically ill patients remain to be established. J. Nutr. 126: 1515–1533, 1996.

INDEXING KEY WORDS:

- *(n-3) fatty acids* • *cytokines*
- *inflammation* • *infection*

The (n-3) polyunsaturated fatty acids have been investigated for use in the treatment of inflammatory diseases such as rheumatoid arthritis, psoriasis and ulcerative colitis. Presently, enteral tube feeding preparations containing relatively large amounts of (n-3) fatty acids are advanced for use in critically ill patients because of the presumed anti-inflammatory effects of these fatty acids (Cerra et al. 1991a and 1991b, Daly et al. 1992, Gottschlich et al. 1990). Although the anti-inflammatory effects of (n-3) fatty acids originally were attributed to changes in the production of prostaglandins and leukotrienes (Kinsella et al. 1990, Lee et al. 1985, Needleman et al. 1979), recent studies have emphasized reduced production of cytokines as a possible mechanism (Billiar et al. 1988, Endres et al. 1989). Here, we review studies in humans and animals on the effects of (n-3) fatty acids on inflammation, resistance to infection, and cytokine production.

CYTOKINES AND INFLAMMATION

Cytokines are small proteins that are produced by a variety of cell types. They act on nearly every tissue and organ system. The cytokines interleukin-1α (IL-1α),[2] interleukin-1β (IL-1β), tumor necrosis factor α (TNF) and interleukin-6 (IL-6) are often designated pro-inflammatory cytokines. They are mainly produced by the mononuclear phagocytic cells, including both circulating monocytes and tissue macrophages. These cytokines mediate the response of the host to inflammatory stimuli. They induce fever, activation of B and T lymphocytes and endothelial cells, synthesis of acute phase proteins by the liver, and many other effects that are all part of the acute phase response (**Table 1**) (Dinarello 1991). The term "acute phase response" refers to a set of local and systemic neurologic, endocrine and metabolic changes through which the organism reacts to various stimuli, including infections, forms of sterile

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[2] Abbreviations used: IL-1, interleukin-1; IL-2, interleukin-2; IL-6, interleukin-6; LTB_4, leukotriene B_4; PGE_2, prostaglandin E_2; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor.

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TABLE 1

Biological effects of the pro-inflammatory cytokines interleukin-1 (IL-1), tumor necrosis factor (TNF) and interleukin-6 (IL-6)[1]

Biological effect	IL-1	TNF	IL-6
Induction of fever in experimental animals and humans	++	++	+
Production of acute phase proteins by the liver	+	+	++
Activation of T lymphocytes	++	+	++
Activation of B lymphocytes	++	+	++
Activation of hematopoiesis	+	–	+
Gene expression of cyclooxygenase and phospholipase A_2[2]	+	+	–
Activation of endothelial cells	+	+	–
Activation of synovial cells (e.g., collagenase production)	+	++	–
Osteoclast activation (bone resorption)	+	+	–
Induction of hypotension	+	+	–
Induction of IL-1, TNF, IL-6 and IL-8	+	+	–

[1] – indicates that the effect is absent, + indicates a moderate effect, ++ indicates a strong effect.

[2] The induction of these enzymes is pivotal in the formation of the so-called lipid mediators: prostaglandins, leukotrienes and platelet-activating factor.

inflammation such as auto-immune disease, bleeding, injury (e.g., burn trauma and major surgery) and heavy exercise. The early events in the acute phase response are local and serve to check bleeding, to demarcate damaged tissues and to recruit cells for the subsequent reparative phase, which requires a systemic response. This second, systemic phase involves fever, anorexia, leukocytosis and metabolic changes that include an increased flow of amino acids from muscle to liver and a rearrangement of the pattern of protein synthesis in the liver: albumin production decreases and acute phase proteins such as fibrinogen, C-reactive protein and serum amyloid A are synthesized. Their appearance in the plasma contributes to a well-known phenomenon in inflammation: the elevated erythrocyte sedimentation rate.

Most of these changes are mediated by IL-1, TNF and IL-6. They can trigger cells to secrete other inflammatory mediators, including other cytokines such as the T lymphocyte products interferon gamma and interleukin-2 (IL-2) and lipid mediators such as prostaglandins, leukotrienes and platelet-activating factor. Elevated plasma concentrations of IL-6, TNF or IL-1 have been observed in patients with infections, endotoxemia, trauma or burns, but also in acute bouts of auto-immune disease such as rheumatoid arthritis or systemic lupus erythematosus (SLE) and in patients undergoing organ transplant rejection (Dinarello 1992). Increased IL-1 production by peripheral blood mononuclear cells and by colonic mononuclear cells has been reported in patients with active inflammatory bowel disease (Mahida et al. 1989, Mazlam and Hodgson 1992).

Although production of pro-inflammatory cytokines is believed to benefit the host defense system, high concentrations of circulating pro-inflammatory cytokines have been shown to correlate with poor outcome in severe disease states such as meningococcal septic shock (Waage et al. 1987 and 1989), other forms of septic shock (Calandra et al. 1990, Cannon et al. 1990) and cerebral malaria (Grau et al. 1989). Elevated concentrations of circulating or in vitro stimulated TNF and IL-1 were also reported in AIDS patients by several groups (Berman et al. 1987, Lahdevirta et al. 1988, Lepe-Zuniga et al. 1987, Wright et al. 1988), although not by others (Molina et al. 1989 and 1990). Anti-cytokine strategies such as treatment with antibodies against TNF and treatment with the interleukin-1 receptor antagonist were shown to reduce the severity of inflammation and to protect against mortality in animal models of septic shock, cerebral malaria and colitis (Cominelli et al. 1990, Curfs et al. 1992, Grau et al. 1989, Ohlsson et al. 1990, Tracey et al. 1987). From these observations, the concept of "lethal cytokinemia" has emerged, which implies that exaggerated cytokine production may be harmful or even lethal (van der Meer 1989). It is also felt that the chronically elevated concentrations of cytokines in rheumatoid arthritis contribute to the perpetuating inflammation and are harmful to the patient (Eastgate et al. 1988, Firestein and Zvaifler 1992). These observations have caused researchers to investigate the possible benefits of reducing cytokine production capacity through dietary intervention; of such interventions, the use of very long chain essential fatty acids of the (n-3) family has received considerable attention.

(n-3) FATTY ACIDS AND INFLAMMATION IN HUMANS

Table 2 summarizes studies on the effects of dietary (n-3) fatty acids in inflammatory disease states in humans. Several investigators have developed tube feeding formulas with, among other modifications, (n-3) fatty acids as the major lipid source. These modified formulas have been investigated in burn patients (Gottschlich et al. 1990) and patients operated on for upper gastrointestinal malignancies (Daly et al. 1992). Both studies claimed reduction in infectious complications and in the length of hospital stay. However, both studies were *not double blind*, and disease severity or age were not evenly distributed between the study groups. In a better controlled study by Moore et al. (1994), trauma patients receiving a modified formula high in (n-3) fatty acids experienced fewer intra-abdominal abscesses and less multiple organ failure.

In placebo-controlled studies, patients with rheumatoid arthritis experienced small improvements of symptoms after ingestion of a fish oil concentrate for

TABLE 2

Effects of dietary (n-3) fatty acids on inflammatory disease in humans

Disease state	Number of studies: Total	Number of studies: Double-blind, placebo-controlled	Reference	No. of patients studied	Duration of diet	(n-3) Fatty acids dose	Effects (no. of studies/total)	Comment
						g/d		
Postoperative cancer patients	1	0	Daly et al. 1992	85	±7 d	3.3–5.2	Infectious complications ↓ (not significantly) Length of hospital stay ↓	Placebo-controlled but not double blind; heterogeneous study groups; confounded by additional presence of arginine and RNA in treatment group enteral product.
Burn trauma	1	0	Gottschlich et al. 1990	50	1–9 wk	±3–6	Wound infections ↓ Length of hospital stay ↓	Placebo-controlled but not double blind; heterogeneous study groups.
Trauma patients	1	0	Moore et al. 1994	98	7 d	2.1	Intra-abdominal abscesses ↓ Multiple organ failure ↓	Placebo-controlled but not double blind; confounded by additional presence of arginine and RNA in treatment group enteral product.
Rheumatoid arthritis	8	8	Kremer et al. 1985, Kremer et al. 1987, Sperling et al. 1987, Belch et al. 1988, Cleland et al. 1988, van der Tempel et al. 1990, Kremer et al. 1990, Espersen et al. 1992	263	6 wk–12 mo	0.24–6.7	No. of tender joints ↓ (5/7) No. of swollen joints ↓ (2/7) Morning stiffness ↓ (3/7) Grip strength ↑ (2/7) Subjective improvement (6/7)	Biochemical markers of inflammation not influenced.
Psoriasis	5	2	Ziboh et al. 1986, Maurice et al. 1987, Bittiner et al. 1988, Bjorneboe et al. 1988, Lassus et al. 1990	161	6–8 wk	1.8–20	Scaling ↓ (4/5) Erythema ↓ (4/5) Pruritus ↓ (2/5) Subjective improvement for joint pain (1/5)	Only moderate improvements.
Ulcerative colitis	5	3	McCall et al. 1989, Salomon et al. 1990, Lorenz et al. 1989, Stenson et al. 1992, Aslan and Triadafilopoulos 1992	90	8 wk–4 mo	3.2–6.0	Clinical symptoms ↓ (4/5) Histological parameters ↓ (3/5) Prednisone dosage ↓ (4/5)	Placebo-controlled studies had crossover designs
Systemic lupus erythematosus	3	3	Moore et al. 1987, Clark et al. 1989, Westberg and Tarkowski 1990	68	5 wk–12 mo	0.3–6.0	No effect on clinical or biochemical parameters of inflammation	
Bronchial asthma	2	2	Arm et al. 1988, Arm et al. 1989	42	10 wk	5.4	Allergen-induced late asthmatic response ↓ (1/2)	

periods ranging from 8 to 24 wk. However, hard endpoints such as erythrocyte sedimentation rate, concentration of circulating acute phase proteins, and rheumatoid factor titer were not influenced by dietary fish oil supplementation. Similarly, minimal to moderate clinical improvements were reported in patients with psoriasis, bronchial asthma and ulcerative colitis after dietary supplementation with fish oil.

Even less convincing are the results on fish oil feeding in patients with SLE (Moore et al. 1987). In one double-blind, crossover study using olive oil as the control, patients with moderately active SLE showed significant clinical and serological benefits after 3 mo of fish oil consumption, but after 6 mo there was no difference between the treatment groups (Westberg and Tarkowski 1990). Dietary fish oil supplementation had no effect on renal outcome in patients with SLE-associated nephritis (Clark et al. 1989).

Taken together, these studies suggest some attenuation of inflammation and thus of the acute phase response by (n-3) fatty acids in humans. However, the rise in concentration of circulating acute phase proteins induced by heavy exercise was increased in healthy volunteers after 3 wk of dietary supplementation with (n-3) fatty acids (Ernst et al. 1991), suggesting increased rather than decreased formation of pro-inflammatory mediators after fish oil.

Thus, (n-3) fatty acids seem to have some beneficial effects in rheumatoid arthritis, psoriasis and ulcerative colitis, but only after prolonged ingestion, and laboratory indices of inflammation were not affected. The effects in bronchial asthma and SLE were minimal to absent. Data on short-term treatment with (n-3) fatty acids in burn patients and in postoperative cancer patients are still inconclusive, although the results of one study in trauma patients are promising (Moore et al. 1994).

(n-3) FATTY ACIDS AND INFLAMMATION IN ANIMALS

Studies on the effects of dietary (n-3) fatty acids in experimental inflammations in animals are summarized in **Table 3.** Several studies have shown favorable effects of dietary fish oil supplementation in mouse models of SLE. These genetically susceptible mice spontaneously develop a SLE-like syndrome including glomerulonephritis, and the animals die before reaching 1 y of age. Administration of fish oil convincingly retarded both the development of glomerulonephritis and premature death (Kelley et al. 1985, Prickett et al. 1981). In a model of autoimmune arthritis in mice, dietary fish oil supplementation reduced the severity of joint inflammation (Leslie et al. 1985). However, in similarly afflicted rats this effect was absent (Prickett et al. 1984). In mice immunized with collagen, plasma concentrations of amyloid P component, an acute phase protein, were significantly reduced following a fish oil diet (Cathcart et al. 1987). Inflammatory responses have been implicated in the etiology of noninsulin-dependent diabetes mellitus. In BHE rats, an animal model of noninsulin-dependent diabetes mellitus, longevity was shortened after a life-long fish oil diet (Berdanier et al. 1992). However, the development of glucose intolerance and lipemia was delayed in these animals. Rats with immune complex–induced enteropathy developed significantly fewer severe inflammatory lesions after a fish oil diet than after a beef tallow diet (Bloch et al. 1989). Thus, most animal studies demonstrated a modulating effect of (n-3) fatty acids on inflammation.

Exogenous administration of IL-1 or TNF to experimental animals induces an acute phase response, including anorexia and fever (McCarthy et al. 1985, Moldawer et al. 1988, Tracey et al. 1988). Apart from interference with cytokine production, dietary fish oil supplementation has been associated with attenuation of some of the responses to exogenously administered cytokines. In guinea pigs, the febrile response to an injection of recombinant murine IL-1 was attenuated after 6 wk of a fish oil–enriched diet compared with control diet (Pomposelli et al. 1989). In rats, feeding fish oil for 6 wk attenuated the catabolic response to a combined infusion of IL-1 and TNF, as reflected by reduced whole-body leucine oxidation and increased net hepatic protein anabolism (Hirschberg et al. 1990). Similarly, the anorexia induced by IL-1 administration was markedly decreased in rats fed fish oil for 6 wk (Hellerstein et al. 1989), and thermogenic and pyrogenic responses to IL-1 were inhibited in rats after 4 to 9 wk of (n-3) fatty acid supplementation (Cooper and Rothwell 1993). These fish oil effects may be mediated by a reduced formation of prostaglandins, because both IL-1–induced fever and anorexia are attenuated by treatment with cyclooxygenase inhibitors (Hellerstein et al. 1989, Okusawa et al. 1988).

Thus, in several animal models of auto-immune disease and in experimentally induced acute phase response, severity of inflammation seems to be favorably influenced by fish oils. In these animal studies, the effects of (n-3) fatty acids seem much stronger than in the clinical studies mentioned above. This difference could relate to the fact that most of these studies involve feeding experimental diets to the animals before the appearance of any disease symptoms, whereas studies in humans have exclusively examined the activity of (n-3) fatty acids against a background of an existing clinical disease. Moreover, the relative amount of (n-3) fatty acids ingested in the animal studies is substantially higher than in the human studies.

(n-3) FATTY ACIDS AND IMMUNE RESPONSE

Does such a suppression of sterile inflammation coincide with a general downregulation of the immune

TABLE 3

Effects of dietary (n-3) fatty acids on experimental inflammation in animals[1]

Experimental disease state	Reference	Species	Number of animals studied[2]	Duration of (n-3) fatty acids intake	Source of (n-3) fatty acids	Effects in the animals receiving (n-3) fatty acids	Comments
Systemic lupus erythematosus	Kelley et al. 1985, Prickett et al. 1981, Prickett et al. 1983, Robinson et al. 1986	Genetically prone mice	>60 per study	Lifelong (except in Robinson's study)	FO	Survival ↑ Proteinuria ↓ Lymphadenopathy ↓ Anti-ds DNA antibodies ↓ (Prickett et al. 1983)	Controls: beef tallow, except in Kelley's study (safflower oil). Robinson: significant improvement even after establishment of overt renal disease.
Type II collagen–induced autoimmune arthritis	Leslie et al. 1985	Mice	189	26 d	FO	Joint inflammation ↓	Controls: corn oil.
Type II collagen–induced autoimmune arthritis	Prickett et al. 1984	Rats	113	5–6 wk	FO	Incidence of arthritis ↑ Severity of joint inflammation =	Controls: beef tallow
Immune complex–induced enteropathy	Bloch et al. 1989	Rats	50	6–8 wk	FO	Number of inflammatory lesions ↓	Controls: beef tallow.
Hapten-induced colitis	Guarner et al. 1992	Rats	60	4 wk	FO	Number of inflammatory lesions ↓ Histology score ↓	Controls: sunflower oil.
Acetic acid–induced colitis	Empey et al. 1991	Rats	45	6 wk	FO	Histologic injury ↓ Fluid absorption ↑	Controls: cottonseed oil or safflower oil. Effects only apparent after pretreatment with PGE_1-analog misoprostol.
IL-1–induced fever	Pomposelli et al. 1989	Guinea pigs	24	6 wk	FO	Fever ↓	Controls: safflower oil. No effect after 5 wk of diet.
Thermogenic and pyrogenic responses to IL-1 administration	Cooper and Rothwell 1993	Rats	60	4–9 wk	FO	Fever ↓ Oxygen consumption ↓	Controls: olive oil. Low-dose (n-3) fatty acids in micro-gelatin encapsulated FO formula.
IL-1– and TNF-induced protein synthesis	Hirschberg et al. 1990	Rats	65	6 wk	FO	Protein anabolism in the liver ↑	Controls: safflower oil.
IL-1–induced anorexia	Hellerstein et al. 1989	Rats	120	6 wk	FO	Anorexia ↓	Controls: corn oil or nonpurified diet.

[1] Abbreviations used: FO, fish oil; IL-1, interleukin-1; PG, prostaglandin; TNF, tumor necrosis factor.
[2] In most studies, results are given of repeated experiments. This number refers to the total number of animals studied.

response, thereby decreasing host resistance to infection? This question has been addressed in various ways. In several studies, human peripheral blood mononuclear cells from healthy volunteers were cultured in vitro. In these studies, T cell proliferation was inhibited by the addition of (n-3) fatty acids to the culture medium (Calder et al. 1994, Soyland et al. 1993). In vivo studies on peripheral blood mononuclear cells after dietary supplementation with (n-3) fatty acids are summarized in **Table 4.** Most studies in healthy volunteers show a decreased proliferative response of T lymphocytes to specific stimulants such as phytohemagglutinin or concanavalin A. The production capacity of T cell–derived cytokines and the T4/T8 ratio were found to be unchanged or depressed. On the other hand, studies in certain patient groups such as asthma patients and intensive care unit patients found a positive effect of dietary (n-3) fatty acids on T cell responses. One recent study even reported increased production of interferon-γ following consumption of (n-3) fatty acids (Kemen et al. 1995). At present it is unclear why T cell responses to dietary (n-3) fatty acids differ between healthy subjects and certain patient groups. Depressed T cell responses at baseline may play a role here. Well-controlled studies comparing different patient groups with healthy volunteers at baseline and after consumption of dietary (n-3) fatty acids are certainly needed to clarify this issue.

Because decreased T cell functions may affect resistance to cancer, the possible impact of dietary (n-3) fatty acids on carcinogenesis has been of concern. Epidemiological studies show that total fat intake is related to breast and colon cancer mortality (Kromhout 1990, Wynder et al. 1986). Animal studies suggest that (n-6) fatty acids may have a tumor-promoting effect and that (n-3) fatty acids may exert an inhibitory effect on chemically induced mammary and colon tumorigenesis (Carroll and Braden 1984). In a recent human study, low tissue levels of α-linolenic acid [18:3(n-3)] were found to be associated with early metastasis in breast cancer, suggesting a protective effect of (n-3) fatty acids (Bougnoux et al. 1994). At present it is not clear whether the effects of dietary (n-3) fatty acids on carcinogenesis are related to their influence on T cell function.

The incidence of certain infectious diseases such as tuberculosis and diarrheal diseases has reportedly been increased in Eskimo populations. Because many socioeconomic and genetic factors contribute to the incidence of infectious diseases, it is difficult to establish the contribution of dietary (n-3) fatty acids to this phenomenon (Grzybowski and Dorken 1983).

In animal studies, the effects of dietary (n-3) fatty acids on infections have been investigated with live pathogenic microorganisms or bacterial endotoxins, because Gram-negative bacteria such as *Escherichia coli* and meningococci are pathogenic largely because of the endotoxins that they release. In controlled studies, guinea pigs were protected against metabolic acidosis and death from endotoxin administration when they had been fed a fish oil–supplemented diet for 6 wk (Mascioli et al. 1989, Teo et al. 1991), and the physiological response to endotoxin infusion was favorably influenced when guinea pigs had been on total parenteral nutrition containing (n-3) fatty acids for only 3.5 d prior to administration of endotoxin (Pomposelli et al. 1990) (**Table 5**). Two studies reported a negative effect of (n-3) fatty acids on outcome of experimental infection with live microorganisms. Chang et al. (1992) observed a decreased survival rate of fish oil–fed Swiss mice infected perorally with *Salmonella typhimurium*, and D'Ambola et al. (1991) reported diminished lung clearance of inspired *Staphylococcus aureus* in neonatal rabbits supplemented with high dose of fish oil or safflower oil. However, most studies indicate that the anti-inflammatory effect of dietary fish oil supplementation in animals in general does not lead to an increased susceptibility to infection, and in several models of infection, outcome was even improved (**Fig. 1**) (Table 5). This suggests that in certain cases (n-3) fatty acids will prevent the excessive production of cytokines that, if unchecked, leads to death in septic animals.

(n-3) FATTY ACIDS AND CYTOKINE PRODUCTION

Methodological problems

Cytokine assays and their validity. Cytokines can exert their effects at extremely low concentrations (ng/L), and under normal physiological conditions (e.g., in the absence of disease or trauma) circulating cytokines are often below the detection limits for most assay systems. Therefore, in many clinical studies with healthy human subjects cytokine-producing cells are isolated, cultured and then stimulated in vitro to obtain detectable cytokine concentrations. Such studies tell us more about the cytokine production *capacity* of a given cell type in vitro than about changes in actual cytokine production occurring in vivo.

In most human studies, peripheral blood mononuclear cells are isolated using a density gradient. Subsequently, these cells are cultured for a short period (e.g., overnight) and stimulated to produce cytokines. In many animal studies, resident macrophages are isolated from the peritoneal cavity or from organs such as the liver (Kupffer cells). Although culture principles for human and animal cells are basically the same, it cannot be excluded that the differences in origin of these cells have some bearing on their cytokine production capacity in vitro.

Cytokines can be quantified by bioassay or by immunochemical methods. In bioassays, cell lines are used

TABLE 4

Effects of (n-3) fatty acids (FA) on T and B lymphocytes

Reference	Subjects	Number	(n-3) Fatty acid dose	Duration of diet	Control group	T cell proliferation	T4/T8 ratio	T cell–derived cytokines	B cell response
Virella et al. 1991	Healthy men	6	2.4	6 wk	Olive oil	=	=	IL-2 ↓	Inconclusive
Kelley et al. 1991	Healthy men	10	±20[1]	56 d	Iso-energetic basal diet	↓	=	IL-2 =	=
Meydani et al. 1991	Healthy older and younger women	12	2.4	3 mo	None	↓	ND[2]	IL-2 ↓	ND
Meydani et al. 1993	Healthy men and women over age 40 y	22	1.23	24 wk	Low fat, 0.27 g/d (n-3) FA[3]	↓	↓	IL-2 ↓	=
Payan et al. 1986	Patients with persistent asthma	6	4	8 wk	0.1 g/d eicosapentoenoic acid	↑	=	ND	ND
Soyland et al. 1994	Psoriasis and atopic dermatitis patients	40	5	4 mo	Corn oil	=	ND	ND[4]	ND
Kremer et al. 1990	Rheumatoid arthritis patient	49	3.3/6.7	24 wk	Olive oil	↑	ND	IL-2 ↓	=
Cerra et al. 1991b	Intensive care unit patients with enteral tube feeding	20	±45[5,6]	±7 d	Equivalent in fat and energy	↑	=	ND	ND
Moore et al. 1994	Trauma patients with enteral tube feeding	98	2.1[6]	7 d	Vivonex (low fat)	=	↑	L-2 = IFN-γ =	ND
Kemen et al. 1995	Post-operative cancer patients with enteral tube feeding	42	192[6]	16 d	Isoenergetic, isonitrogenous placebo	↑	↑	IFN-γ ↑	↑

[1] Dietary supplementation was given as flax seed oil, abundant in linolenic acid (18:3, n-3). The study had a cross-over design.
[2] ND = not determined.
[3] The (n-3) fatty acids in the control group were plant derived, whereas the (n-3) fatty acids in the experimental group were fish derived.
[4] The expression of interleukin-2 (IL-2) receptor was decreased in the (n-3) fatty acids–supplemented groups.
[5] Dose of the feeding formula was increased during the study period.
[6] The experimental enteral tube feeding formula contained (n-3) fatty acids, nucleotides and arginine.

TABLE 5

Effects of dietary (n-3) fatty acids on experimental infections in animals

Type of infection	Reference	Species	Number of animals studied[1]	Duration of (n-3) fatty acids intake	Source of (n-3) fatty acids	Effects in the animals receiving (n-3) fatty acids	Comments
Endotoxic shock	Mascioli et al. 1989	Guinea pigs	90	6 wk	FO[2]	Survival ↑	Controls: safflower oil ad libitum or restricted. Differences in survival significant only when compared with ad libitum safflower oil.
Endotoxic shock	Pomposelli et al. 1990	Guinea pigs	?	3.5 d	FO	Lactic acidosis ↓	Controls: soybean oil. Fat administered parenterally.
Listeria monocytogenes, Pseudomonas aeruginosa, Candida albicans, murine CMV	Rubin et al. 1989	Autoimmune disease–prone (NZB × NZW) F1 mice	275	4 wk	FO	No differences in survival	Controls: beef tallow.
Salmonella typhimurium	Chang et al. 1992	Mice	98	4 wk	FO	Mortality ↑	Controls: low-fat diet, coconut oil, or corn oil.
Salmonella typhimurium, Pseudomonas aeruginosa	Clouva-Molyvdas et al. 1992	Mice	320	2–3 wk	FO	No differences in survival	Controls: no fat, or various concentrations of coconut, safflower, or oleic oil.
Klebsiella pneumoniae, cerebral malaria induced by *Plasmodium berghei*	Blok et al. 1992	Mice	180	6 wk	FO	Survival ↑ in both infections	Controls: corn oil, palm oil, or normal lab chow. Indomethacin treatment no effect on survival. No reduction in bacterial counts.
Murine AIDS	Fernandes et al. 1992	Mice	160	8 wk	FO	Survival ↑	Controls: corn oil. Differences significant between 20% fish oil and 20% corn oil.
Abscess (*Staphylococcus aureus, Bacteroides fragilis*)	Barton et al. 1991	Rats	40	12 d	FO	Survival ↑ (not significantly)	Controls: safflower oil. All deaths occured within 48 h after abscess.
Plasmodium berghei	Kuraratilake et al. 1992	Mice	?	4 d	DHA-micelles i.p.	Parasitemia ↓	Controls: vehicle or saline Treatment with i.p. DHA started after infection
Staphylococcus aureus (inspired)	D'Ambola et al. 1991)	Neonatal rabbits	156	7 d	FO	Clearance of bacteria ↓	Controls: safflower oil or saline Bacterial clearance reduced in both fish-oil and safflower-oil treated rats compared to saline

[1] In most studies, results are given of repeated experiments. This number refers to the total number of animals studied.
[2] Abbreviation used: DHA, docosahexaenoic acid; FO, fish oil.

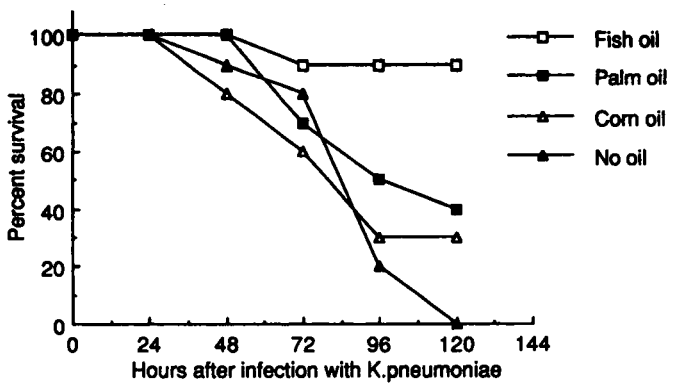

FIGURE 1 Survival of Swiss mice after *Klebsiella pneumoniae* infection. Fish oil–fed animals survived significantly longer than control animals fed palm oil, corn oil or nonpurified diet ($P < 0.05$). Reproduced with permission from Blok et al. (1992).

that are dependent on specific cytokines for growth. Such bioassays can detect cytokines at low concentrations, but their specificity is poor. More recently, the availability of specific monoclonal and polyclonal antibodies against various cytokines has enabled the development of immunological detection methods such as ELISA and RIA. In body fluids, cytokines may be bound to specific proteins such as soluble receptors for TNF or to nonspecific ligands such as $\alpha 2$ macroglobulin and uromodulin. Such molecules may interfere with both bioassays and immunoassays. Receptor blocking molecules such as the naturally occurring protein designated IL-1 receptor antagonist interfere with bioassays only (Dinarello 1991). On the other hand, with ELISA and RIA one might be measuring biologically non-active proteins. In general, the reproducibility and validity of immunoassays are superior to those of bioassays.

Design of dietary trials. Several human studies evaluating the influence of (n-3) fatty acids on cytokine production have compared baseline values with post-treatment values, without a concurrent control group. The contribution of aspecific fluctuations in cytokine production such as reduced stress through adaptation to the study protocol cannot be appreciated in such a before-and-after design. It *also cannot* be deduced from such studies whether the observed effects are specific for the type of polyunsaturated fatty acids employed.

On the other hand, in placebo-controlled studies the type and amount of placebo fatty acids may be problematic. In human studies, the dietary fat supplementation constitutes only a small percentage of the total energy intake, but in animal studies the fat supplement may account for 30% of total energy intake. When control groups receive such an amount of corn oil or safflower oil, both very rich in (n-6) fatty acids, this may well influence the production of prostaglandins and leukotrienes and thus may have some bearing on the resultant cytokine production measured in these studies (Kinsella et al. 1990). Ideally, in both human and animal supplementation studies, control groups should receive an isoenergetic quantity of fatty acids without disturbing the ratio of (n-3) to (n-6) fatty acids that was present in their diets before the start of the study.

The effects of (n-3) fatty acids on cytokine production in humans

Table 6 presents an overview of human studies concerning the effects of (n-3) fatty acids on cytokine production. In several studies peripheral blood mononuclear cells were isolated and profound decreases in ex vivo production of IL-1, TNF and IL-6 were noted (Endres et al. 1989, Meydani et al. 1991). Unfortunately, these studies did not employ a placebo group taking an isoenergetic amount of a fatty acid supplement low in polyunsaturated fatty acids and a (n-6) to (n-3) ratio similar to that in the subjects' normal diet. Meydani et al. (1993) reported on cytokine production capacity in 22 subjects before and after 24 wk of a low fat diet. The diets were either low or high in (n-3) fatty acids, which were provided as natural food components. Ex vivo production of IL-1β, TNF and IL-6 by isolated peripheral blood mononuclear cells fell significantly in the high (n-3) fatty acids group. In contrast, IL-1β and TNF production increased in the low (n-3) fatty acids group. The two groups were not studied simultaneously, as the authors state, to avoid seasonal differences. Molvig et al. (1991) studied ex vivo cytokine production after low and high dose dietary fish oil supplementations in healthy young men and in subjects with diabetes. Control subjects took an isoenergetic amount of a triglyceride blend. After 7 wk of oil supplementation, concentrations of secreted IL-1β and of secreted and cell-associated TNF were not different between groups. Only cell-associated IL-1β was decreased in the fish oil group. This finding is surprising, because it is IL-1α that remains cell associated whereas IL-1β is mainly released into the culture medium (Lonnemann et al. 1989). In contrast with the findings of Endres et al. (1989) no differences in ex vivo cytokine production were noted between the groups 10 wk after cessation of the diet. Even though the authors had an appropriate control group, the conclusions from this study should be viewed with caution. This is because the authors do not present the values from the start of the study. Such a design (i.e., repeated measures) would have greatly strengthened this study, because it can help control for inherent intrasubject variation. In other words, it is the *changes* in cytokine production that (n-3) fatty acids cause that is really most important and not the absolute amount of cytokine made by each subject. In a study of rheumatoid arthritis patients, IL-1 fell over time in all groups, including the control group taking olive oil (Kremer et al. 1990). Cooper et al. (1993) studied young volunteers before and after dietary supplementation with fish oil during 6 to 8 wk. A concurrent control group did not alter their usual diets.

TABLE 6

Effects of (n-3) fatty acids on cytokine production in humans[1]

Reference	Subjects	Number	(n-3) FA dose	Duration of diet	Control group	Source of cytokines	Cytokine assay	Effect of (n-3) FA (Maximum change in %)
			g/d					
Endres et al. 1989	Healthy men	13	4.6	6 wk	Normal diet	PBMC[2]	RIA	IL-1β ↓ (−61%)[3] IL-1α ↓ (−39%) TNF ↓ (−40%)
Meydani et al. 1991)	Healthy older and younger women	12	2.4	1, 2 and 3 mo	None	PBMC	RIA	IL-1β ↓ (−90%)[4] TNF ↓ (−70%) IL-6 ↓ (−60%)
Meydani et al. 1993	Healthy men and women over age 40	22	1.23	24 wk	Low-fat, 0.27 g/d (n-3) FA[5]	PBMC	RIA	IL-1β ↓ (−40%)[6] TNF ↓ (−35%) IL-6 ↓ (−34%)
Kremer et al. 1990	Rheumatoid arthritis patients	49	3.3/6.7	24 wk	Olive oil	PBMC	Bioassay[7]	IL-1 ↓ in treated (−54.7%) and in controls (−38.5%)
Molvig et al. 1991	Healthy men; diabetics	33	1.5/3.0	7 wk	Triglyceride blend	PBMC[8]	ELISA	IL-1β and TNF secretion not different between groups[7]
Virella et al. 1991	Healthy men	6	3.0	6 wk	Olive oil	PBMC	EIA	IL-2 ↓
Espersen et al. 1992	Rheumatoid arthritis patients	32	3.6	12 wk	Mixture of fatty acids	Plasma	RIA	IL-1β ↓[9] TNFα =
Cooper et al. 1993	Healthy men	29	1.15–1.56	6–8 wk	Normal diet	Whole blood culture	Bioassay	IL-1 ↓[10] IL-6 ↓ TNF =
Cannon et al. 1995	Older healthy subjects	12	3.0	4 mo	Isoenergetic neutral fat	PBMC	RIA	IL-1β =[11]

[1] Abbreviation used: ELISA, enzyme linked immunosorbent assay; FA, fatty acids; IL-1, interleukin-1; IL-2, interleukin-2; IL-6, interleukin-6; PBMC, peripheral blood mononuclear cells; RIA, radiolabeled immunoassay; TNF, tumor necrosis factor.

[2] Peripheral blood mononuclear cells were isolated after 6 wk of supplementation and again at 10 and 20 wk after cessation of the diet. Cytokine production by the cultured cells was stimulated with endotoxin, phytohemagglutinin or heat-killed *Staphylococcus epidermidis*. Cytokines were determined by specific RIA of the supernatants after lysis of the cells, and thus consisted of both secreted and cell-associated fractions.

[3] Changes in cytokine production capacity were maximal at 10 wk after cessation of the supplementation.

[4] The reductions were more pronounced in older women than in younger women. The authors did not report on cytokine production after cessation of the dietary intervention.

[5] The (n-3) fatty acids in the control group were plant derived.

[6] In a non-concurrent control group receiving a low fat, low (n-3) fatty acids diet, IL-1β increased with 62% TNF increased with 47%.

[7] Twenty patients received low dose and 17 patients received high dose fish oil concentrate per day, and control patients received olive oil. Peripheral blood mononuclear cells were cultured for 24 h with or without endotoxin. It should be noted that the bioassay employed in this study does not discriminate between IL-1 and IL-6. Phytohemagglutinin-stimulated IL-2 production also tended to decrease over time in both age groups during fish oil supplementation, but this reduction did not reach statistical significance.

[8] Peripheral blood mononuclear cells were isolated at baseline, after 7 wk of oil supplementation, and again 10 wk after cessation of the diet. The cells were cultured for 20 h with and without endotoxin, and secreted and cell-associated cytokines were determined separately. From this paper the percentage of change from baseline cannot be deduced. Only cell-associated IL-1β was decreased in the (n-3) fatty acid group.

[9] Initial plasma IL-1β concentrations were not elevated; however, IL-1β was still reduced after fish oil supplementation.

[10] Local and systemic inflammatory responses to typhoid vaccine injection were attenuated in the subjects taking fish oil supplement.

[11] From this paper the percent change from baseline cannot be deduced.

Using a somewhat different method of cytokine measurement (whole blood culture), they found that ex vivo production of IL-1 and IL-6 was suppressed after fish oil supplementation. However, production of TNF was not significantly altered. Interestingly, these investigators found an attenuation of the inflammatory response to an intramuscular injection of typhoid vaccine in the fish oil group. Recently, Cannon et al. (1995) found no differences in ex vivo stimulated IL-1β production in older subjects after 4 mo of dietary fish oil supplementation compared with controls taking a placebo of neutral fat composition. Again, in this study no conclusions can be drawn about changes in cytokine concentrations induced by fish oil, and possible effects may be masked by intersubject variations. Espersen et al. (1992) reported a reduction in plasma IL-1β concentrations after fish oil supplementation in rheumatoid arthritis patients that did not occur in the concurrent control group.

In the Endres-Meydani (Endres et al. 1989, Meydani et al. 1991 and 1993) peripheral blood mononuclear cells were cultured in the presence of autologous serum or plasma, whereas in Cannon's and Molvig's studies (Cannon et al. 1995, Molvig et al. 1991), pooled normal human serum was used. Theoretically, the fatty acids present in the autologous serum could have influenced the in vitro cytokine production. However, preincubation of human peripheral blood mononuclear cells with eicosapentanoic acid resulted only in a marginal suppression of IL-1β synthesis, whereas TNF synthesis was not affected at all (Sinha et al. 1991).

Whether (n-3) fatty acids reduce inflammation in certain human disease states through inhibition of cytokine production remains speculative, but most studies, controlled or not, report a decrease in one or more pro-inflammatory cytokines after dietary (n-3) fatty acids.

(n-3) fatty acids and cytokine production in animals

Animal studies concerning the production of cytokines after dietary (n-3) polyunsaturated fatty acid supplementation are summarized in **Table 7.**

Rats. Billiar et al. (1988) reported decreased bioactivity of IL-1 and TNF produced in vitro by liver macrophages of rats after 6 wk of dietary supplementation with fish oil. Control animals were fed an equal amount of corn oil. However, animals receiving safflower oil, which is rich in (n-6) polyunsaturated fatty acids, showed a decrease in IL-1 and TNF production similar to that in animals fed fish oil. This latter finding is difficult to interpret, because in both corn oil and safflower oil the predominant fatty acid is linoleic acid. Grimm et al. (1994) also found decreased production of IL-6 and TNF in peripheral blood mononuclear cells of rats fed (n-3) fatty acids for 4 d.

In contrast to these findings, Turek et al. (1991) reported increased TNF production capacity of resident peritoneal macrophages in rats fed linseed oil for 30 d. Linseed oil is rich in α-linolenic acid, an (n-3) fatty acid and precursor of eicosapentanoic acid. Control animals received corn oil. Interestingly, when the peritoneal cells were elicited by the in vivo administration of complete Freund's adjuvant, the differences in TNF production in the two dietary groups were no longer apparent.

Mice. There seems to be a contrast between the effects of (n-3) fatty acids on cytokine production in mice and in the other species studied. Watanabe et al. (1991) studied the effects of feeding α-linolenic acid on TNF production by murine peritoneal macrophages. Control animals received safflower oil, rich in linoleic acid and low in (n-3) fatty acids. Diets were given for 4 to 5 wk. Endotoxin-stimulated TNF production by peritoneal macrophages was significantly higher in the α-linolenic acid group than in the control group. The authors also reported a considerably higher serum TNF activity in the α-linolenic acid group after in vivo injection of endotoxin.

Lokesh et al. (1990) studied the synthesis of IL-1 and TNF by mouse peritoneal macrophages after 4 wk of dietary supplementation with fish oil, corn oil or olive oil. An IL-1 bioassay was performed on the supernatants of cultured peritoneal macrophages stimulated with endotoxin after lysis of the cells, and thus probably reflected mainly IL-1α. The TNF bioactivity was measured in the supernatants of these macrophages. Both IL-1 and TNF were markedly increased in the mice fed fish oil compared with control animals. Similarly, Hardardottir and Kinsella (1992) found enhanced in vitro stimulated TNF production by resident peritoneal macrophages in mice that had been fed an (n-3) fatty acid–supplemented diet for 5 wk. Enlarging on these observations, the same group studied the kinetics of TNF release by in vitro–stimulated murine resident peritoneal macrophages after 4 wk of fish oil or control diet (Hardardottir et al. 1992). Although total TNF production was again increased, the kinetics of maximum TNF production appeared not to be affected by the diets. These findings were basically confirmed by Somers and Erickson (1994).

Our group has studied ex vivo production of IL-1 and TNF by peritoneal cells from mice fed fish oil, corn oil or palm oil for 6 wk (Blok et al. 1992). Interleukin-1α, IL-1β and TNF were measured by specific immunoassays. Interleukin-1α and TNF production by peritoneal cells were significantly higher in the fish oil–fed animals than in controls (**Fig. 2**). Ertel et al. (1993) found increased IL-1β release by peritoneal macrophages of mice following experimental hemorrhagic shock.

Thus, the effects of dietary (n-3) fatty acids on cytokine production in mice are opposite to those in humans: in mice, several controlled studies have independently shown increased cytokine production after (n-3) fatty acid supplementation, whereas in humans decreased cytokine production was found. As can be seen in Tables 6 and 7, human studies have exclusively used

TABLE 7

Effects of (n-3) fatty acids on cytokine production in animals[1]

Reference	Species	Number of animals studied	% of energy intake as fat	Type of (n-3) FA	Duration of diet	Control group	Source of cytokines	Cytokine assay	Effects of (n-3) FA
Billiar et al. 1988	Rat	9	15	FO	6 wk	Corn oil or safflower oil	Kupffer cells	Bioassay	IL-1 ↓[2] TNF ↓
Turek et al. 1991	Rat	12	20	FO	30 d	Corn oil	Peritoneal macrophages	Bioassay	TNF ↓
Grimm et al. 1994	Rat	50	30	FO	4 d	Safflower oil or saline	PBMC	ELISA	IL-6 ↓ TNFα ↓
Lokesh et al. 1990	Mouse	21	30	FO	4 wk	Corn oil or olive oil	Peritoneal macrophages	Bioassay	IL-1 ↑ TNF ↑
Hardardottir and Kinsella 1991	Mouse	60	20	FO	5 wk	Different ratios of (n-3):n-6 fatty acids	Peritoneal macrophages	ELISA	TNF ↓[3]
Hardardottir et al. 1992	Mouse	30	20	FO	4 wk	Different ratios of (n-3):(n-6) fatty acids	Peritoneal macrophages	ELISA	TNF ↓[4]
Blok et al. 1992	Mouse	30	30	FO	6 wk	Corn oil or palm oil	Peritoneal cells; plasma	RIA ELISA	IL-1 ↑ TNF ↑
Watanabe et al. 1991	Mouse	30	20	α-LA	4 to 5 wk	Safflower oil	Peritoneal macrophages; plasma	Bioassay	TNF ↑
Ertel et al. 1993	Mouse	?	12	FO	3 wk	Corn oil or safflower oil	Peritoneal macrophages	Bioassay	IL-1 ↑
Somers and Erickson 1994	Mouse	20	20	FO	4 wk	Safflower oil	Thioglycollate-elicited peritoneal macrophages	Bioassay	TNF ↑
Morris et al. 1991	Horse	6	16	α-LA	8 wk	None	Peritoneal macrophages	Bioassay	TNF ↓[5]

[1] Abbreviations used: α-LA, α-linolenic acid [18:3 (n-3)]; FO, fish oil; FA, fatty acids; IL-1, interleukin-1; IL-6, interleukin-6; PBMC, peripheral blood mononuclear cells; RIA, radiolabeled immunoassay; TNF, tumor necrosis factor.

[2] Cytokine production decreased in (n-3) FA–fed as well as in safflower oil–fed animals.

[3] Tumor necrosis factor was increased only in the high (n-3) FA group, receiving 1.5% (n-3) FA and 1.5 (n-6) FA (wt/wt).

[4] Kinetics of maximum in vitro TNF production was not affected by the diets.

[5] Unstimulated peritoneal macrophages showed increased TNF production capacity after (n-3) FA supplementation, whereas endotoxin-stimulated cells showed a decrease.

peripheral blood cells, whereas most animal studies (rats and mice) used isolated tissue macrophages. Thus, as pointed out before, differences in origin of the cell types used to study cytokine production may account for the paradoxical responses to (n-3) fatty acids in humans and animals. This hypothesis is supported by the only animal study using peripheral blood mononuclear cells, which found decreased IL-6 and TNF production (Grimm et al. 1994). To clarify this issue, studies are needed to evaluate the influence of dietary (n-3) fatty acids on cytokine production in isolated peripheral blood mononuclear cells and tissue macrophages simultaneously.

As in the human studies, there is no evidence that the alleviation of inflammation by (n-3) fatty acids in animal studies is mediated by alterations in cytokine production. Studies employing (n-3) fatty acids simultaneously with control measures such as agonists or antagonists of cytokines have not been performed.

POSSIBLE MECHANISMS OF THE MODULATION OF CYTOKINE PRODUCTION BY DIETARY (n-3) FATTY ACID INTAKE

At present, the mechanisms by which dietary (n-3) fatty acids modulate cytokine production have not been elucidated. Until recently, the alleviation of inflammation after the ingestion of (n-3) fatty acids has mainly been attributed to a reduced production by leukocytes of the eicosanoids prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4). Inflammatory agonists stimulate eicosanoid synthesis by enhancing the release of arachidonic acid from the intracellular phospholipid pool through activation of phospholipases. Subsequently, the free arachidonic acid is metabolized into prostaglandins and leukotrienes by the enzymes cyclooxygenase and lipoxygenase, respectively. Eicosapentanoic acid, one of the (n-3) polyunsaturated fatty acids found in high concentrations in fish oil, is rapidly incorporated into cell membrane phospholipids, where it replaces arachidonic acid as a substrate and is converted into the biologically less active prostaglandin E_3 (PGE_3) and leukotriene B_5 (LTB_5) (Lee et al. 1985). Moreover, (n-3) fatty acids have been shown to be poorly metabolized by cyclooxygenase, thereby reducing the total production of eicosanoids (Lee et al. 1985).

Prostaglandins. Changes in the production of the eicosanoids PGE_2 and LTB_4 have been postulated as mechanisms of altered cytokine production after dietary fatty acid supplementation. In vitro addition of PGE_2 to human peripheral blood mononuclear cells inhibits TNF production, probably by increasing intracellular cyclic AMP (Endres et al. 1991, Renz et al. 1988). Initially, the in vitro production of IL-1 has also been reported to be under inhibitory control of PGE_2, but

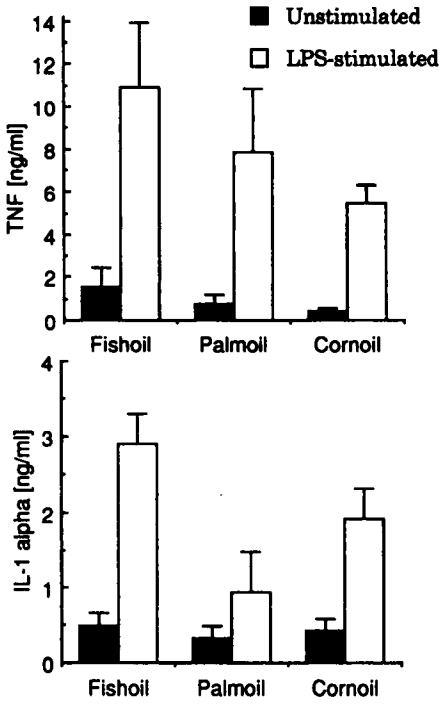

FIGURE 2 Cytokine production capacity of murine peritoneal cells. Swiss mice were fed fish oil, palm oil or corn oil for 6 wk. Peritoneal cells were isolated and cultured with (dark bars) or without endotoxin (light bars). Tumor necrosis factor (TNF) was measured in the culture medium; interleukin-1α (IL-1α) was measured after lysis of the cells. Both TNF and IL-1α were significantly higher in the fish oil–fed mice than in those fed corn oil or palm oil ($P < 0.05$). Reproduced with permission from Blok et al. (1992). LPS = lipopolysaccharide.

since the availability of specific IL-1 assays it has become clear that in vitro IL-1 secretion is not inhibited by PGE_2 (Endres et al. 1991, Scales et al. 1989). In vivo inhibition of PGE_2 production by administration of a cyclooxygenase inhibitor has been reported to increase circulating concentrations of IL-6 and TNF during acute endotoxinemia in humans (Spinas et al. 1991). Because PGE_2 concentrations are decreased following consumption of an (n-3) fatty acid–rich diet, it is unlikely that prostaglandins play an important role in the decreased TNF production observed in humans and some other species. In mice, however, decreased PGE_2 production could account for the observed increases in TNF after (n-3) fatty acids. Two groups reported that the increase in TNF production by peritoneal macrophages from mice fed fish oil or control diets could be substantially reduced by treating these cells with PGE_2 (Hardardottir and Kinsella 1991, Hubbard et al. 1991). However, TNF production capacity was still elevated in the mice fed (n-3) fatty acids compared with controls. Hardardottir and Kinsella 1991) also showed that after the abolition of PGE_2 production in all dietary groups by indomethacin, peritoneal macrophages of fish oil–fed mice still produced significantly more TNF. This indicates that in mice, reduced PGE_2 production is not

the only mechanism responsible for the increased TNF production.

Leukotrienes. As suggested by Endres et al. (1989), a fish oil–induced decrease in the synthesis of lipoxygenation products of arachidonic acid may account for inhibition of cytokine production. However, studies on the effect of leukotrienes on IL-1 production have shown divergent results. Leukotriene B_4 was reported to enhance lipopolysaccharide-induced IL-1 production by human monocytes in vitro (Rola-Pleszczynski and Lemaire 1985), but specific leukotriene inhibitors failed to reduce IL-1 production by human and mouse mononuclear cells at concentrations that completely inhibited leukotriene synthesis (Hoffman et al. 1991, Parkar et al. 1990). In mice, treatment with lipoxygenase inhibitors suppressed the circulating TNF concentrations after endotoxin injection as well as the ex vivo TNF production by peritoneal macrophages (Schade et al. 1989). Endres et al. (1989) suggested that the profound decrease in IL-1 and TNF production at 10 wk after cessation of the fish oil diet might have been due to reduced production of LTB_4. However, others reported complete restoration of LTB_4 formation by neutrophils and peripheral blood mononuclear cells within 8 wk after cessation of a similar diet (Tulleken et al. 1989). These data suggest that reduced synthesis of leukotrienes is unlikely to cause the reduced cytokine production after (n-3) fatty acid administration.

Dual inhibition. Studies with agents that inhibit both the lipoxygenase- and cyclooxygenase-mediated metabolism of arachidonic acid are of interest, because dietary (n-3) fatty acid supplementation results in a similar reduction of both prostaglandins and leukotrienes. One of these agents, tebufelone, enhanced IL-1 and TNF synthesis by human peripheral blood mononuclear cells at concentrations that suppressed leukotriene formation (Sirko et al. 1991). Another dual cyclooxygenase/lipoxygenase inhibitor, SK&F 86002, protected mice from endotoxin-induced mortality and reduced circulating TNF concentrations (Badger et al. 1989). Differences in species studied may account for these divergent findings. However, because dual cyclooxygenas/lipoxygenase inhibitors seem to increase cytokine production in human cells and decrease cytokine production in mice, these observations are opposite to the observed decrease in cytokine production in humans and the increased cytokine production in mice induced by (n-3) fatty acids. Thus, these observations again argue against reduced eicosanoid synthesis as an explanation for these effects.

Vitamin E and cytokine production. Fish oil concentrates used in clinical and experimental studies are enriched in vitamin E to prevent lipid peroxidation. The effects of dietary fish oil supplementation on cytokine production could thus theoretically be due to the vitamin E rather than to the (n-3) fatty acids.

Meydani et al. (1989) studied this issue in a group of elderly human volunteers who consumed 800 IU of all-*rac*-α-tocopheryl acetate daily for 30 d. Endotoxin-stimulated IL-1 bioactivity was not affected by this vitamin E supplementation. Cannon et al. (1991) studied male volunteers who supplemented their diets with 800 IU of all-*rac*-α-tocopherol daily for 48 d. After a session of heavy exercise, IL-1β production capacity rose in subjects receiving placebo but not in vitamin E–supplemented subjects. The TNF production capacity was not affected by vitamin E; IL-6 production capacity was, however, significantly reduced in the vitamin E group. These studies show that vitamin E supplementation in itself may exert some influence on cytokine production. However, the amount of vitamin E employed in these studies is much higher than that ingested in the fish oil studies (800 IU vs. approximately 20 IU/d). Therefore it remains questionable whether vitamin E may account for the changes observed after dietary fish oil supplementation.

Modulation of signal transduction. Some studies have addressed the question whether (n-3) fatty acids alter intracellular signal transduction pathways involved in the synthesis of cytokines. In mice, 10 d of dietary supplementation with (n-3) fatty acids significantly increased the diacylglycerol concentration in concanavalin A–stimulated splenocytes (Fowler et al. 1993). The (n-3) fatty acids seemed to modulate the activities of protein kinase C and cAMP-dependent protein kinases in a lymphoma cell line (Speizer et al. 1991). It is thus conceivable that (n-3) fatty acids induce changes in the concentrations or activities of intracellular factors involved in cytokine production. Clearly, these data need to be confirmed and related to changes in cytokine production.

CONCLUSIONS

Anti-inflammatory effects of dietary fish oil supplementation have been observed in clinical studies of rheumatoid arthritis, psoriasis and ulcerative colitis. The effects of (n-3) fatty acids seem to be stronger in animal models of chronic inflammation than in the clinical studies. This may relate to the early institution of supplementation and the very high intake of (n-3) fatty acids in the animal studies. In humans, the anti-inflammatory potential of fish oil seems to be limited. Studies in critically ill burn or postoperative cancer patients yielded inconclusive results, but one study in trauma patients is promising. It is unclear whether the anti-inflammatory effects of fish oil in humans are mediated through a reduction in cytokine production or through other mechanisms such as reduced production of lipid mediators; studies in humans have reported impressive reductions in cytokine production capacity, although not all studies were properly controlled. The data from animal studies show the opposite: increased IL-1 and TNF production capacity after (n-3) fatty acid

supplementation. Differences in origin of cytokine-producing cells may account for this paradoxical response to (n-3) fatty acids in humans and mice.

Interference with the negative feedback of prostaglandins on TNF production partly accounts for the increased TNF production after (n-3) fatty acid supplementation in mice. The mechanism of action of (n-3) fatty acids on IL-1 production in mice and on cytokine production in other species remains to be elucidated.

The issue whether dietary (n-3) fatty acids decrease host resistance to infections is still unresolved. T cell responses increased in certain patient groups after (n-3) fatty acid consumption but decreased in healthy subjects. Baseline T cell activation may play a role here. Some animal studies suggest a beneficial effect of (n-3) fatty acids on outcome of infections, whereas others report an adverse or no effect. Thus, at present there is no firm scientific evidence that enteral feedings enriched in (n-3) fatty acids are of benefit in the treatment of critically ill patients. Future research in this field may be directed in several ways. First, studies are needed to relate the contributions of the various lipid mediators and the intracellular signal transduction systems to the observed effects of (n-3) fatty acids on inflammation and on cytokine production. Second, different cytokine-producing cell types should be studied simultaneously in one species to unravel the paradoxical responses to dietary (n-3) fatty acids in humans and mice. Third, in view of the preliminary studies already performed and given the increasing number of immunocompromised patients in our hospitals and intensive care units, well-designed studies are needed to assess the potential benefit of dietary (n-3) fatty acids in this group of patients.

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