Modulation of Cytokine Production by Dietary Polyunsaturated Fatty Acids (43415)

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The existence of a bidirectional interaction between nutrition, host defense, and infectious, inflammatory and neoplastic diseases is well established. The nutritional status of a host has a profound effect on the immune response and its ability to defend against invading pathogens. On the other hand, the metabolic changes associated with these diseases can adversely affect the nutritional status of the host. Cytokines play an important role in both aspects of this interrelationship. Both the production and biologic function of cytokines are regulated by eicosanoids (1). Thus, it is imperative to study the effect of dietary fatty acid, precursors of eicosanoids, on the production and biologic function of cytokines. The production of several cytokines, such as interleukin (IL)-1, IL-2 and tumor necrosis factor (TNF), has been shown to be decreased by prostaglandin (PG) E_2 (2–6). Lipoxygenase products have been shown either to decrease or increase cytokine production (7). Some of the biologic effects of cytokines, i.e., fever induction, appetite loss, and inflammation, are believed to be mediated by PGE_2 (2).

Cytokines

Cytokines are protein mediators that serve as communication signals among different cells. The cells of the immune system are the main source of cytokines, although other cell types such as fibroblasts, keratinocytes, and endothelial cells are also capable of synthesizing these substances. Similarly, cytokines act on targets that can include both immune and nonimmune cell types, further expanding their physiologic and pathologic scope beyond the immune system. A notable feature of cytokines is that they act as both "mediators of defense" and "mediators of disease." As mediators of defense, these compounds are essential in the T cell response, antibody formation, tumor killing, hematopoiesis, nonspecific resistance, radio protection, and anti-inflammation. As mediators of disease, they induce fever, inflammation, pain, metabolic dysfunction, tissue degradation, anorexia, cachexia, shock, and death (1). Cytokines have been implicated in the pathogenesis of several diseases, including cardiovascular, bone, joint, and skin diseases. More than a dozen cytokines have been characterized and several are available in recombinant form.

Interleukin 1. IL-1 is synthesized as a 33-kDa precursor and then processed to forms with molecular mass in range of 13–17 kDa. IL-1 exists as two polypeptides, IL-1α and IL-1β, with limited amino acid homology. IL-1 is one of the key mediators in the immune response to microbial invasion, immunologic reactions, inflammatory responses, and tissue injury (2). It is produced by a variety of cell types, such as macrophages, fibroblasts, endothelial cells, keratinocytes, and smooth muscle cells. However, macrophages are the major souce of IL-1. IL-1 has a broad spectrum of biologic effects, including the regulation of T and B lymphocytes, development and activation, induction of other cytokines and cytokine receptor synthesis, central nervous system effect, metabolic effects, and proinflammatory effects. The production of IL-1 is negatively controlled by PGE_2 (3). On the other hand, IL-1 increases PGE_2 production, which, in turn, mediates the biologic effect of IL-1, such as fever and decreased appetite (2).

Tumor Necrosis Factor. The term TNF designates two different polypeptides, TNF-α (produced by monocyte/macrophages) and TNF-β (derived from lymphocytes). TNF shares several biologic properties of IL-1 and, in some cases, acts in synergy with IL-1. In addition, TNF is cytostatic and cytotoxic for several tumor cell lines *in vitro* and for some tumors *in vivo* (8, 9). Similar to IL-1, PGE_2 has been shown to decrease TNF production (6).

Interleukin 2. IL-2 is produced by T cells and is needed for antigen- and mitogen-stimulated T cell proliferation. It is also needed for antibody production by B cells, cytotoxic activity, and proliferation of natural

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killer cells (1). IL-2 production decreases with aging and in several pathologic conditions, such as cancer, autoimmune diseases, and congenital and acquired immunodeficiency states (10). IL-2 production is also under negative control by PGE_2 (4, 5).

Interleukin 6. IL-6 is produced by activated lymphocytes and stimulates growth and differentiation of T and B lymphocytes. IL-6 has also been indicated as a component of the acute phase response (1).

Granulocyte-Macrophage Colony-Stimulating Factor. Granulocyte-macrophage colony-stimulating factor is a polypeptide produced by activated lymphocytes. Its primary function, as indicated by its name, is to stimulate growth and differentiation of the precursor of granulocytes and macrophages. In addition, it enhances bactericidal and tumoricidal activity of these cells.

Dietary Lipid Modulation of Cytokine Production and Biologic Function

Since the production and several biologic functions of cytokines are under the control of products of arachidonic acid metabolism (2–7), it has been proposed that dietary effects on eicosanoid production could modify cytokine production. Billar *et al.* (11) demonstrated that IL-1 production by Kupffer cells from rats fed fish oil (15 %en) for 6 weeks was significantly lower than IL-1 production by cells from rats fed 15 %en corn oil. Endres *et al.* (12) gave supplements of 18 g/day of MaxEPA fish oil containing 2.7 g of eicosapentaenoic acid (EPA) and 1.85 g of docosahexaenoic acid (DHA) to nine volunteers. A significant decrease (43%) was observed in the production of IL-1β by peripheral blood mononuclear cells after 6 weeks of supplementation. The reduced production of IL-1β continued for 10 weeks following cessation of fish oil supplements (61% decrease), but returned to normal 20 weeks after cessation of the supplement (12).

Virella *et al.* (13) and Santoli and Zurier (14) showed that *in vitro* addition of EPA to human peripheral blood mononuclear cells inhibits production of IL-2 and expression of the IL-2 receptor. In the study by Santoli and Zurier (14), dihomogammalinoleic acid also reduced IL-2 production. Supplementation *in vivo* with (n-3) polyunsaturated fatty acids (PUFA) has been shown to decrease T cell and B cell proliferation and delayed type hypersensitivity skin response, an *in vivo* measure of T cell-mediated immune responses (13, 15, 16).

We studied the effect of dietary (n-3) PUFA on cytokine production in the context of the aging immune response (17). Aging is associated with an altered regulation of the immune system, with T cell-mediated immune response exhibiting the major changes (10). *In vitro*, the proliferative response of human and rodent lymphocytes to phytohemagglutinin and concanavalin A becomes depressed with age. Several groups have shown that antigen and mitogen-stimulated IL-2 production declines with age and contributes to T cell-mediated defects observed with aging, while decreases in B cell response and IL-1 production are equivocal. The decrease in cytokine production with aging has been attributed to increased production of PGE_2 (18, 19).

Six healthy young (23–33 y) and six healthy older (51–68 y) women supplemented their typical American diet with (n-3) PUFA contained in six capsules of Pro-Mega daily for 12 weeks. Each subject, therefore, received 1680 mg of EPA, 720 mg of DHA, 600 mg of other fatty acids, and 6 IU of vitamin E per day (17). Plasma EPA significantly increased both in younger women ($0.64 \pm 0.04\%$ before vs $3.6 \pm 0.91\%$ after supplementation, $P < 0.04$) and in older women ($0.73 \pm 0.05\%$ before vs $7.30 \pm 0.28\%$ after supplementation, $P < 0.001$). Similarly DHA increased both in younger women ($1.81 \pm 0.12\%$ before vs $2.96 \pm 0.44\%$ after supplementation, $P < 0.06$) and older women ($1.76 \pm 0.17\%$ before vs $4.30 \pm 0.36\%$ after supplementation, $P < 0.001$). The arachidonic acid (AA) to EPA ratio also decreased in younger and older women. These changes were more dramatic in older women than young women, so that the AA to EPA ratio decreased 12-fold in older women and only 4-fold in young women. Similarly, the decrease in PGE_2 production was more dramatic in older women. Dietary treatment or age did not have an effect on plasma tocopherol level, total number of white blood cells, or the percentage of mononuclear cells.

IL-1β, TNF, and IL-6 production was not significantly different between young and older women prior to (n-3) PUFA supplementation (Fig. 1). (n-3) PUFA supplementation significantly decreased production of these cytokines in both young and older women. The decrease was more dramatic in older women than young women, so that the older women had significantly lower production of IL-1β, TNF, and IL-6 than young women after 3 months of (n-3) PUFA supplementation (Fig. 1). IL-1β and TNF production after 8 weeks of supplementation was <50% of baseline values. Further reductions were observed after 12 weeks of supplementation. Although the presupplementation production of IL-1β and TNF was not different between young and older women, the (n-3) PUFA supplementation induced a greater reduction in older women compared with young women. This was associated with a larger increase in plasma EPA and DHA and a greater decrease in the AA to EPA ratio seen in older women compared with young women following (n-3) PUFA supplementation. Granulocyte-macrophage colony-stimulated factor production was similarly decreased by fish oil supplementation (data not shown).

Older women had significantly lower production

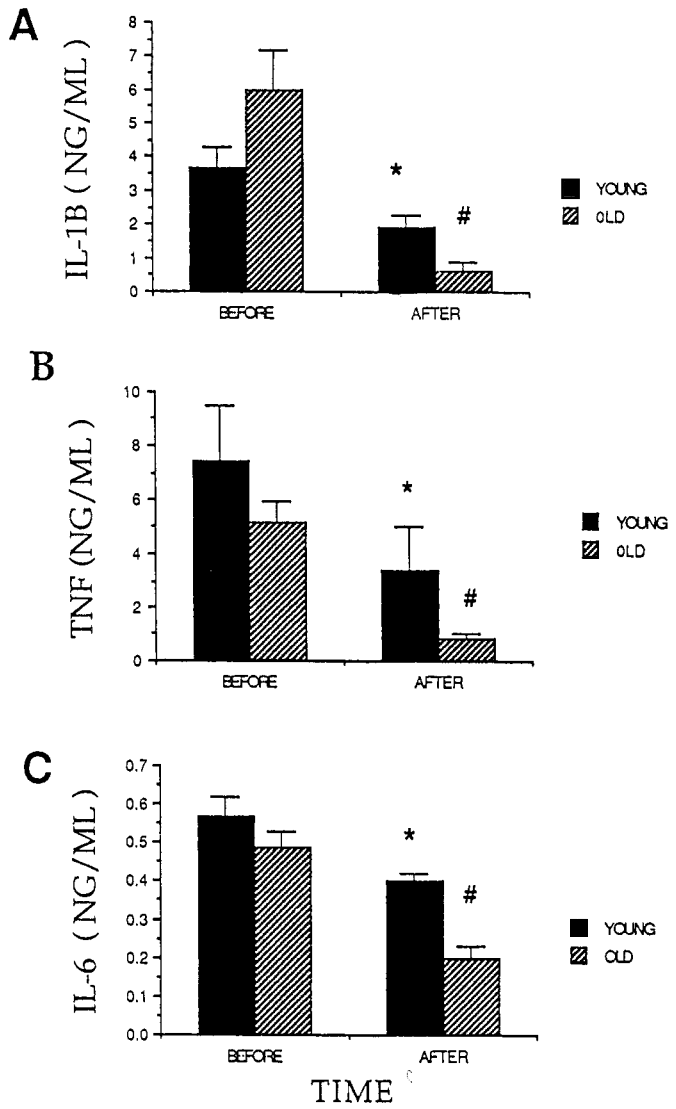

Figure 1. Effect of (n-3) PUFA supplementation (2.4 g/day for 3 months) on (A) IL-1β, (B) TNF, and (C) IL-6 production in young and older women (mean ± SE, $n = 6$). Peripheral blood mononuclear cells (1×10^6 cells/ml for IL-6 and 5×10^6 cells/ml for TNF and IL-1β) were stimulated with 1 μg/ml of endotoxin (for IL-1β) and with 40 organisms of *Staphyloccus aureus* per peripheral blood mononuclear cell (for TNF) for 24 hr and 10 μg/ml of concanavalin A (for IL-6) for 48 hr. Cytokines were measured as described in Ref. 13. Asterisk indicates significantly lower than before values at $P < 0.05$. Crosshatch indicates significantly lower than young at $P < 0.05$.

of IL-2 and mitogenic response to phytohemagglutinin and concanavalin A than young women (Table I). Fish oil supplementation resulted in a significant reduction in IL-2 production and mitogenic response of lymphocytes to phytohemagglutinin in older women only. The decrease in cytokine production and lymphocyte proliferation when dietary (n-3) fats are increased cannot be readily explained by a decrease in PGE_2 production, because PGE_2 has been shown to suppress IL-1, IL-2 production, and lymphocyte proliferation. The effect of (n-3) PUFA supplementation, therefore, appears to be independent of PGE_2 changes. On the other hand, a

Table I. Effect of (n-3) PUFA Supplementation on IL-2 Production and Mitogenic Response of Peripheral Blood Mononuclear Cells from Young and Older Women (Mean ± SE, $n = 6$)[a]

Parameter	Young		Older	
	Before	After	Before	After
IL-2 (units/ml)	88 ± 28	38 ± 20	60 ± 30[b]	22 ± 9[c]
Mitogenic response to PHA (cpm × 10^3)	84 ± 16	78 ± 9	54 ± 6[b]	34 ± 8[c]

[a] Women supplemented their typical American diet with 2.4 g/day of (n-3) PUFA for 3 months (Ref. 13). Con A-stimulated (10 μg/ml) IL-2 production and PHA-stimulated (5 μg/ml) mitogenic response were measured as described in Ref. 13.
[b] Significantly lower than young at $P < 0.05$.
[c] Significantly lower than before values at $P < 0.05$.

reduction in leukotriene (LT) B_4 by (n-3) PUFA can suppress IL-1, IL-2 production, and the subsequent lymphocyte proliferation, since LTB_4 (in some but not all studies) has been shown to increase IL-1 as well as IL-2 production and lymphocyte proliferation (20). This explanation, however, is unlikely, since we recently showed that *in vitro* addition of both PGE_2 and LTB_4 decreases lymphocyte proliferation (21). Thus, we now consider the immunosuppressive effect of (n-3) PUFA to be due to the formation of EPA-derived eicosanoids, because PGE_3 and LTB_5 were more effective than PGE_2 and LTB_4 in reducing lymphocyte proliferation (21). Santoli and Zurier (14) showed that (n-3) PUFA can reduce IL-2 production directly, independent of changes in cyclooxygenase products. An increased production of lipid peroxides following (n-3) PUFA supplementation (22) might also contribute to the immunosuppressive effect of these fatty acids, since products of lipid peroxidation such as H_2O_2 have been shown to decrease lymphocyte proliferation *in vitro* (23). Furthermore, antioxidant nutrient deficiency decreases immune responsiveness, whereas supplementation enhances the immune response (24). A statistically nonsignificant reduction was also observed in young women. The decrease in cytokine production and lymphocyte proliferation by fish oil is also observed with long-term feeding of diets enriched with (n-3) PUFA in the form of fish (25).

This decrease in cytokine production and lymphocyte proliferation may compromise cell-mediated immunity, especially in the older women, since T cell-mediated functions have been shown to decrease with aging. A decline in T cell-mediated functions has been implicated as a contributory factor in the increased incidence of infectious diseases and tumors in the elderly. For example, D'Ambola *et al.* (26) showed that feeding high doses of either fish oil or safflower oil (5 g/kg/day) for 7 days decreased lung clearance of *Staphylococcus aureus* by 50% in neonatal rabbits. On the other hand, IL-1β, TNF, and IL-6 have been implicated

in the pathogenesis of autoimmune inflammatory diseases, and a reduction in the synthesis of these cytokines by (n-3) PUFA of fish oil may contribute to the reported beneficial effect of (n-3) PUFA in rheumatoid arthritis (27) and amyloidosis (28). Furthermore, IL-1 is implicated in the pathogenesis of osteoporosis by virtue of its ability to induce bone resorption (29), the incidence of which increases in older women. Therefore, (n-3) PUFA supplementation may prove to be beneficial in retarding the development of arthritis and osteoporosis.

Fatty acids of the (n-3) class can also influence the metabolic effect of cytokines. In this regard, Hellerstein *et al.* (30) showed that rats fed chow or semipurified diets containing 10% (wt/wt) corn oil had a significant decrease in food intake following injection of IL-1 (18.9 ± 11.0% decrease for chow-fed rats and 31 ± 15.4% decrease for corn oil-fed rats). The IL-1-induced decrease in food intake was not observed in rats fed fish oil.

Conclusion

Cytokines play a key role in the interrelationships among nutrition, host defense, and diseases. Several investigations indicate that the synthesis as well as biologic function of the cytokines can be modified by (n-3) PUFA. The (n-3) PUFA-induced changes in cytokine production appear to be partially mediated via changes in the formation of AA- and EPA-derived metabolites. The relative contribution of the AA-derived and EPA-derived eicosanoids to the changes in cytokine production needs to be determined. Because of the wide spectrum of biologic and pathologic events that can be affected by cytokines, dietary manipulation by (n-3) PUFA for a specific biologic function or disease state affected by cytokines cannot be done in isolation. Rather, the implications of such changes for other systems and disease states should be carefully considered.

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