Oral (n-3) Fatty Acid Supplementation Suppresses Cytokine Production and Lymphocyte Proliferation: Comparison Between Young and Older Women1,2,3

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ABSTRACT The effect of (n-3) fatty acid supplementation on cytokine production and lymphocyte proliferation was investigated in young (23–33 y) and older (51–68 y) women. Subjects supplemented their diets with 2.4 g of (n-3) fatty acid/d for 3 mo. Blood was collected before and after 1, 2 and 3 mo of supplementation. The (n-3) fatty acid supplementation reduced total interleukin (IL)-1β synthesis by 48% in young women but by 90% in older women; tumor necrosis factor was reduced by 58% in young and 70% in older women. Interleukin-6 was reduced in young women by 30% but by 60% in older women. Older women produced less IL-2 and had lower mitogenic responses to phytohemagglutinin (PHA) than young women prior to (n-3) fatty acid supplementation. The (n-3) fatty acid supplementation reduced IL-2 production in both groups; however, this reduction was significant only in older women. The PHA-stimulated mitogenic response was significantly reduced by (n-3) fatty acid in older women (36%). Thus, long-term (n-3) fatty acid supplementation reduced cytokine production in young women and cytokine production and T cell mitogenesis in older women. The reduction was more dramatic in older women than in young women. Although (n-3) fatty acid-induced reduction in cytokine production may have beneficial anti-inflammatory effects, its suppression of IL-2 production and lymphocyte proliferation in older women may not be desirable. J. Nutr. 121: 547–555, 1991.

INDEXING KEY WORDS:
- cytokines • immune response • humans • age • fish oil

The lower incidence of coronary heart disease in Greenland Eskimos [1] has been attributed to their high consumption of diets rich in (n-3) fatty acids, eicosapentaenoic acid (EPA)5 and docosahexaenoic acid [DHA]. Eicosapentaenoic acid is a precursor of the 3-series of prostanoids and the 5-series of leukotrienes, which have been shown to exhibit less potent aggregatory and inflammatory properties than the corresponding arachidonic acid (AA) metabolites [2, 3]. These findings have renewed the interest in fish oil and its potential use in the prevention and/or therapy of certain chronic cardiovascular and inflammatory diseases. Animal experiments and clinical trials have indicated a potentially beneficial effect of (n-3) fatty acid supplementation on atherosclerosis and atherothrombotic disorders [4] and autoimmune and inflammatory diseases [5]. The prevalence of all these disorders increases with age.

Aging is associated with an altered regulation of the immune system [6]. Age-related functional changes have been well characterized for both humoral and cell-mediated immune responses. However, the major alterations occur in T cell-mediated...
function. In vitro the proliferative response of human and rodent lymphocytes to phytohemagglutinin (PHA) and concanavalin A (Con A) becomes depressed with age [6]. Several groups have shown that antigen and mitogen-stimulated interleukin (IL)-2 production declines with age and contributes to T cell-mediated defects observed with aging (7–9); changes in B cell response and IL-1 production are equivocal (8, 10, 11).

Short-term supplementation with 4.6 g of [n-3] fatty acid/d in young men has been shown to decrease the inducible production of IL-1 and tumor necrosis factor (12). The effect of lower levels of supplementation with [n-3] fatty acid for longer periods of time on IL-1, tumor necrosis factor, other cytokines and lymphocyte proliferation of healthy subjects has not been studied. Furthermore, the question of age difference in [n-3]-induced changes has not been addressed. The latter point is especially important because of the age-associated changes of cell-mediated immune function.

In this study, we investigated the effect of supplementation with 2.4 g of [n-3] fatty acid/d for up to 3 mo on cytokine production and lymphocyte proliferation of young and older females.

**MATERIALS AND METHODS**

**Subjects and experimental design.** The study was approved by the Human Investigation Review Committee of the New England Medical Center Hospitals. Six healthy young (23–33 y), premenopausal women and six healthy older (51–68 y), postmenopausal women were recruited from the Boston area. Women with unusual dietary habits, high alcohol consumption (> 150 mL of alcohol per week) as well as those with a history of cancer, heart disease, arthritis, hypertension, diabetes, renal or liver disease or irregular menstrual cycles were excluded. Also excluded from participation in the study were smokers, women using prescribed medication such as oral contraceptives, corticosteroids, antibiotics, aspirin and nonsteroidal anti-inflammatory drugs, and those whose body weights were >110% or <90% of their ideal body weights. All postmenopausal women had gone through natural menopause and had been postmenopausal for at least 2 y prior to the study. After initial screening, blood samples were analyzed for standard clinical chemistry, complete blood count and white blood cell differential.

Prior to entering the study, prospective subjects were interviewed by a nutritionist to ensure that they consumed diets considered typical for persons living in the United States (35–40% of energy from fat; 300–400 mg cholesterol/d) and had normal eating habits (i.e., no frequent reducing diets, food binges or vegetarian diets). Each subject’s usual diet was supplemented with [n-3] fatty acid contained in six capsules of Pro-Mega (Parke Davis, Warner Lambert Co., Morris Plains, NJ) daily for 12 wk. Each 500 mg Pro-Mega capsule contained 280 mg of EPA, 120 mg of DHA, 100 mg of other fatty acids and 1 IU of d-alpha tocopherol (vitamin E). Therefore, each subject received 1680 mg of EPA, 720 mg of DHA, 600 mg of other fatty acid and 6 IU of vitamin E per day. The total amount of fat contributed by the fish oil capsules was 3 g, or 1.5% of the energy intake.

The volunteers reported no side effects from ingestion of the fish oil capsules. The mean age for the young women was 26.7 ± 1.7 y; for the older women it was 60.7 ± 2.9 y. The mean weight and height for young and older women were 67.8 ± 3.7 kg, 168.7 ± 3.8 cm and 70.5 ± 3.2 kg, 164.3 ± 3.0 cm, respectively. The subjects maintained their weight throughout the study.

Compliance was monitored by measurement of total plasma fatty acid as described in the Methods section, and the subjects were asked to return the unused capsules. Blood was collected on two consecutive days from young women during the follicular phase of their menstrual cycle (d 4–7) and on two consecutive days from older women. The means of these two determinations were used for further analysis. Samples were collected at baseline and at the end of 1, 2 and 3 mo of supplementation with fish oil. Heparinized blood (40 mL) was collected for in vitro immunologic tests and 6 mL of blood was collected in EDTA for white blood cell count differential, fatty acid and vitamin E analysis. Subjects were weighed weekly. All samples were coded, and the codes were not revealed until all analyses were completed.

**Isolation of mononuclear cells.** Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood according to the procedures of Boyum [13]. Peripheral blood mononuclear cells were removed from the interface and washed twice in RPMI 1640 supplemented with 100 µg of penicillin/mL, 100 µg of streptomycin/mL, 2 mmol of L-glutamine/L and 25 mmol of HEPES/L (Gibco, Grand Island, NY). RPMI was subjected to ultrafiltration to remove endotoxin as previously described [12]. Cells were resuspended in medium and counted under a light microscope. Cell viability was assessed using trypan blue exclusion. Cells then were suspended at appropriate concentrations for measurement of mitogenic lymphocyte proliferation, induction of cytokines and prostaglandin E₂ formation. A sample of heparinized plasma was heat-inactivated at 56°C for 30 min to be used as autologous plasma in the cell cultures.

**Lymphocyte proliferation.** Lymphocyte proliferation was measured by [3H]thymidine incorporation following stimulation with T cell mitogens. Dilutions of mitogens from 1 µg/mL to 100 µg/mL for PHA (PHA-P; Difco, Detroit, MI) and Con A (Sigma, St. Louis, MO) were prepared in RPMI 1640 with 10%
autologous plasma, and optimal dilution for each mitogen was determined. One hundred microliters of each mitogen was plated in triplicate into 96-well, flat-bottomed microtiter plates [Becton Dickinson, Oxnard, CA]. Peripheral blood mononuclear cells were suspended at 1 x 10^6 cells/mL in RPMI 1640. Then, 100 µL of the cell suspension was plated with and without mitogens and incubated for 72 h at 37°C in an atmosphere of 5% CO_2 and 95% humidity. Four hours before termination of incubation, 18.5 KBq [0.5 µCi] of [³H]thymidine [specific activity 247.9 GBq/mmoll] (6.7 Ci/mmoll), New England Nuclear, Boston, MA) in 20 µL was added to each well. Cells were harvested onto glass microtiter filter paper using a cell harvester [PHD, Cambridge, MA]. Filter disks were placed in minivials and counted in a liquid scintillation counter [Beckman Instruments, Palo Alto, CA]. The results are reported as corrected counts per minute, the average counts per minute of mitogen-stimulated cultures minus the average counts per minute of cultures without mitogens. The scintillation counter had an efficiency of 45% for tritium.

**Interleukin-2 and interleukin-6.** To determine interleukin 2 and 6 activity, 1 x 10^6 cells/mL in RPMI with 10% autologous plasma were cultured in 24-well, flat-bottomed plates [Becton Dickinson] with Con A or PHA (10 µg/mL) for 48 h. Cell-free supernatant was stored at −70°C for later analysis of IL-2 and IL-6. Activity of IL-2 was measured using a microassay method described by Gillis et al. [14]. Recombinant human IL-2 [Genzyme Corp., Boston, MA] was used as standard, 1 U/mL was defined as the amount of recombinant IL-2 that causes a half-maximal incorporation of [³H]thymidine in 5 x 10^4 cytotoxic T cell line cells in culture. Activity of IL-2 was calculated using probit analysis [14]. Cytotoxic T cell line cells were a gift from S. Gillis of Immunex [Seattle, WA]. Interleukin-6 was measured by radioimmunoassay as previously described [15].

**Interleukin-1 and tumor necrosis factor.** Peripheral blood mononuclear cells were suspended at 5 x 10^6 cells/mL in RPMI with 2% autologous plasma. This cell suspension [0.5 mL] was added to 0.5 mL RPMI or RPMI containing heat-killed *Staphylococcus epidermidis* [at 20 organisms per PMBC] or endotoxin [lipopolysaccharide (LPS) *Escherichia coli* 1335, Sigma, 1 ng/mL] for 24 h. The plates were frozen until the end of the study, at which time all plates from each donor were thawed and exposed to two more freeze-thaw cycles simultaneously to complete cell lysis. The contents of the wells, consisting of cell lysates and supernatants, were analyzed by radioimmunoassay for IL-1β and tumor necrosis factor as previously described [16, 17]. Each radioimmunoassay is specific for the respective cytokine and does not cross-react with IL-1α, granulocyte-monocyte colony stimulating factor or interferon-α, -β or -γ.

**Prostaglandin E_2 production.** Peripheral blood mononuclear cells [1 x 10^6 cells/mL] were cultured in 24-well, flat-bottomed plates [Becton Dickinson] in the presence or absence of endotoxin [LPS *E. coli*, 1 µg/mL] and 10% autologous plasma for 48 h in a 37°C, 5% CO_2 humidified incubator. Cell-free supernatant was saved at −70°C for prostaglandin E_2 analysis by radioimmunoassay as described by McCosh et al. [18]. Prostaglandin E_2 antibody was a gift from J. Dupont of Iowa State University and M. Mathias of Colorado State University. The antibody cross-reactivity and specificity have already been described [19].

**Complete blood count and white cell differential.** Complete blood count was obtained using a Baker 9000 Hematology Analyzer [Serono-Baker Instrument Inc., Allentown, PA] and white cell differential was assessed by microscopic examination of blood smears following Wright-Giemsa staining.

**Plasma tocopherol.** Plasma samples were saved under nitrogen at −70°C for tocopherol analysis. Plasma was analyzed by a modified high performance liquid chromatography method of Bieri et al. [21] as previously described [22].

**Fatty acid analysis.** The analysis and transesterification of plasma fatty acid was performed as follows: 100 µL of a solution containing 40 mg of heptadecanoic acid in 100 mL of isooctane was added to each tube in which a plasma sample was analyzed. The isooctane was evaporated under a stream of nitrogen. Then, 100 µL of plasma was added to the tube, followed by the addition of 2 mL of methanol-benzene 4:1 (v/v). Then, 200 µL of acetyl chloride was added while the solution was stirred. The tubes were capped and heated at 100°C for 1 h. The tubes were cooled to room temperature and 5 mL of 6% (wt/v) K_2CO_3 solution was mixed thoroughly. The tubes were then centrifuged for 20 min [950 x g at 4°C]. The benzene layer was removed and added to a gas chromatography vial ready for analysis. The extract was injected into a Hewlett Packard 5890 gas liquid chromatograph fitted with a 105 m silica capillary column [RTX 2330, Restek Corp., Port Matilda, PA] and detected with a flame ionization detector. The fatty acid methyl esters of over 40 fatty acids [13:0 to 28:0, including cis- and trans-configurations] are separated by this method. Peaks were identified, and the procedure was validated by chromatography of mixtures of authenticated fatty acid methyl esters. A computer/integrator was used to store the data, to integrate and identify peaks and to calculate the percentage of fatty acid. Data were normalized by comparing the areas of the fatty acid peaks with the area of the internal standard peak, heptadecanoic acid, after correction for the various response factors. The values presented are calculated as percentages of the total area of the identified fatty acid peaks.

**Statistical analysis.** Data were analyzed by two-way ANOVA for the effect of age, duration of [n-3]
TABLE 1

Plasma fatty acid composition at baseline and after 3 months of (n-3) fatty acid supplementation in young and older women

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Young women (n = 5)</th>
<th>Older women (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 3 mo</td>
</tr>
<tr>
<td>EPA * 20:5(n-3)</td>
<td>0.64 ± 0.04</td>
<td>3.6 ± 0.91</td>
</tr>
<tr>
<td>DHA * 22:6(n-3)</td>
<td>1.81 ± 0.12</td>
<td>2.96 ± 0.44</td>
</tr>
<tr>
<td>AA * 20:4(n-6)</td>
<td>6.70 ± 0.20</td>
<td>6.90 ± 0.50</td>
</tr>
<tr>
<td>AA:EPA ratio</td>
<td>10.60 ± 0.40</td>
<td>2.50 ± 0.70</td>
</tr>
</tbody>
</table>

1Values are means ± SEM.
2One sample was lost during sample handling.
3Results of ANOVA.
4Significantly different from young women (after supplementation) at p < 0.03, ANOVA. c = Significantly different from young women (after supplementation) at p < 0.05, ANOVA.
5Percentage of identified fatty acids (see Methods).
6Abbreviations used: AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Results

Plasma fatty acids. Compliance was confirmed by a significant increase in both plasma EPA and DHA (Table 1). All young and older subjects showed the increase in EPA and DHA; however, the increase in EPA and DHA was more dramatic in older women than in young women (10-fold increase for EPA and 2.5-fold increase for DHA in older women vs. fivefold increase for EPA and 1.6-fold increase for DHA in young women). Arachidonic acid (AA) was significantly decreased in older women, whereas it did not change in young women. The AA:EPA ratio was significantly decreased in both young and older women (91% decrease in older women vs. 76% decrease in young women). At baseline there was no difference in percentage of EPA, DHA and AA or the ratio of AA:EPA between young and older women. However, after 3 mo of (n-3) fatty acid supplementation, older women had a significantly higher percentage of EPA, DHA and a lower ratio of AA:EPA in their plasma fatty acid than young women.

The total number of white blood cells and the percentage of mononuclear and polymorphonuclear cells in peripheral blood did not change as a result of fish oil consumption (data not shown) and was not affected by the age of the subjects. Plasma tocopherol level was not changed by (n-3) supplementation (data not shown).

Interleukin-1, tumor necrosis factor and interleukin-6. Figure 1 shows endotoxin-stimulated IL-1β production in young and older women. Production of IL-1β was not significantly different between young and older women prior to fish oil supplementation. Production of IL-1β was significantly reduced in both groups following consumption of fish oil. The decrease in IL-1β was more dramatic in older (90% decrease) than in young women (48% decrease); after 3 mo of (n-3) fatty acid supplementation, older women produced significantly less IL-1β than young women.

![Figure 1](https://example.com/figure1.png)

**FIGURE 1** Effect of (n-3) fatty acid supplementation on endotoxin-stimulated interleukin (IL)-1β production in young and older women. Peripheral blood mononuclear cells from women taking (n-3) fatty acid supplements for 3 mo were stimulated with 1 ng/mL lipopolysaccharide for 24 h at baseline and 1, 2 and 3 mo after (n-3) fatty acid supplementation (two measurements at each time point). Total (cell-associated plus secreted) IL-1β was determined by RIA. Mean ± SEM, n = 6. Significant reduction over time at P = 0.01 for young and P = 0.004 for older women by contrast analysis. *Significantly lower than young women at P = 0.043 by ANOVA.
Endotoxin-stimulated tumor necrosis factor production is shown in Figure 2, and staphylococcus-stimulated tumor necrosis factor production is shown in Figure 3. In young women endotoxin-stimulated tumor necrosis factor synthesis was significantly reduced over time of fish oil supplementation (58% decrease) and staphylococcus-stimulated tumor necrosis factor production decreased (54% decrease) but did not reach statistical significance. In older women both endotoxin- and staphylococcus-stimulated tumor necrosis factor production was reduced significantly (70% decrease for endotoxin- and 84% decrease for staphylococcus-stimulated production). Baseline tumor necrosis factor production was not different between young and older women; however, older women tended to have lower staphylococcus-stimulated tumor necrosis factor production after 3 mo of [n-3] fatty acid supplementation than young women.

Production of IL-6 in young and older women is shown in Figure 4. Production of IL-6 was not significantly different between young and older women prior to fish oil supplementation. A significant reduction over time was observed in both groups; however, in young women there was only a 30% reduction, whereas in older women a 60% reduction occurred after 3 mo of supplementation. This resulted in a significantly lower IL-6 production after 3 mo of supplementation by older women than by young women.

Interleukin-2 and lymphocyte proliferation. Figure 5 shows Con A-stimulated IL-2 production by PBMC in young and older women. The PBMC of older women produced significantly less IL-2 in response to Con A than those of young women at baseline and after 1 mo of fish oil supplementation. The amount of Con A-stimulated IL-2 production in older women was 33% of that by young women. Consumption of fish oil reduced IL-2 production in both young and older women, and no age difference in IL-2 production was observed after 2 and 3 mo of fish oil supplementation. A fish oil-induced reduction in IL-2 production over time approached statistical significance in older women only. Similar results were obtained with PHA-stimulated IL-2 production (data not shown).
Figure 6 shows PHA-stimulated lymphocyte proliferation. Older women had lower PHA-stimulated lymphocyte proliferation than young women at all time points. Con A-stimulated mitogenic responses in older women were also lower than those in young women, reaching statistical significance after 2 and 3 mo of fish oil supplementation (data not shown). The fish oil supplementation reduced mitogenic responses in young and older women. However, statistical significance was observed only in the PHA-stimulated mitogenic response of older women (36% reduction after 3 mo).

Prostaglandin E₂ production. After 3 mo of supplementation, endotoxin-stimulated prostaglandin E₂ production decreased by 57% (2.78 ± 0.42 before supplementation vs. 1.18 ± 0.47 ng/1 x 10⁶ cells after supplementation, \( P = 0.03 \)) in older women. Prostaglandin E₂ production in young women decreased by 40% (2.23 ± 0.31 before supplementation vs 1.34 ± 0.34 ng/1 x 10⁶ cells after supplementation). However, this decrease was not statistically significant.

DISCUSSION

Our results show that supplementation with low levels of (n-3) fatty acid for 3 mo suppresses inducible production of IL-1β, tumor necrosis factor, IL-6 and IL-2. The suppression is more dramatic in older women, especially for IL-1β, tumor necrosis factor and IL-2 production. Furthermore, mitogenic responses to PHA were significantly reduced in older women by (n-3) fatty acid supplementation.

Several investigators have reported a suppressive effect of large doses of (n-3) fatty acid on neutrophil and monocyte function; however, few studies have focused on the effect of (n-3) fatty acid on lymphocyte function (24–26). Furthermore, the effect of (n-3) fatty acid on cytokine production and lymphocyte proliferation has not been studied in women or older adults.

Endres et al. (12) demonstrated that (n-3) fatty acid supplementation (4.69 g/d) in healthy men for 6 wk reduces IL-1 and tumor necrosis factor total synthesis. The amount of IL-1β and tumor necrosis factor produced after 6 wk of supplementation was 60 and 80% of baseline values, respectively. In the present study, which used less (n-3) fatty acid (2.4 mg/d), the synthesis of IL-1β and tumor necrosis factor produced after 8 wk of supplementation was less than 50% of baseline values. Further reductions were observed after 12 wk of supplementation. Although the supplementation production of IL-1β and tumor necrosis factor was not different between young and older women, the (n-3) fatty acid supplement induced a greater reduction in older women than in young women. This is associated with the larger increase in plasma EPA and DHA and a greater decrease in AA in older women compared with young women after (n-3) fatty acid supplementation. The reason for higher incorporation of EPA and DHA into plasma lipids of older women is not clear. The analysis of food frequency questionnaires indicate that the diet of young...
and older women had a similar amount of total fat (41% in young and 40% in older women), saturated fat (16% in young and 15% in older women), monounsaturated fat (15%), polyunsaturated fat (6% in young and 7% in older women) and polyunsaturated fatty acid:saturated fatty acid ratio (0.38 in young and 0.44 in older women). Furthermore, plasma EPA of older male rats than in that of young rats was produced significantly less 11-2, and PHA-stimulated lymphocyte proliferation was similar in young and older groups (Table 1). We have no reason to think that the two age groups had any difference in compliance because they all had increases in EPA and DHA, and our counting of the returned capsules did not indicate any difference between the two age groups. That this is a true age difference and not a compliance problem is further supported by animal studies in which old rats showed a higher percentage of increase in EPA and DHA than young rats after fish oil supplementation [27]. One possible reason for higher incorporation of EPA and DHA into plasma lipids of older women is a more efficient absorption of [n-3] fatty acid by older women compared with young women. Hollander et al. [28] reported a more efficient absorption of linoleic acid by intestinal segments of old rats compared with young rats. The differences observed also may be the result of hormonal differences between the young and old women, because the older women were all postmenopausal. However, this is unlikely because Suzuki et al. [27] also reported a larger increase in plasma EPA of older male rats than in that of young rats following fish oil consumption.

Both IL-1 and tumor necrosis factor are involved in the pathogenesis of inflammatory diseases, and a reduction in the synthesis of these cytokines by [n-3] supplementation may contribute to the beneficial effect of [n-3] fatty acid in rheumatoid arthritis [5]. Furthermore, IL-1 is implicated in the pathogenesis of osteoporosis by virtue of its ability to induce bone resorption [29]. The incidence of both arthritis and osteoporosis increases in older women; therefore, [n-3] fatty acid supplementation may prove beneficial in retarding the development of these disease states.

A suppressive effect of oral [n-3] supplementation on IL-6 and IL-2 production and mitogenic lymphocyte proliferation was found. Older women produced significantly less IL-2, and PHA-stimulated mitogenic responses were similarly lower. This is in agreement with a previous report [9]. The reduction in IL-6 during [n-3] supplementation seemed slower than reduction in IL-1β and tumor necrosis factor. For example, a substantial reduction in IL-1β and tumor necrosis factor production took place in older women after 1 mo of supplementation, but a significant reduction in IL-6 was not observed until after 3 mo of supplementation. The reduction by [n-3] supplement in IL-2 production and PHA mitogenesis in older women is of interest because T cell function decreases with aging. The decline in T cell-mediated function has been implicated as a contributory factor in the increased incidence of infectious diseases and tumors in the elderly.

In our study significant reduction in PHA-stimulated mitogenesis was observed only in older adults. This decrease in cytokine production and lymphocyte proliferation may compromise cell-mediated immunity, and it is supported by the study of Yoshino and Ellis [30], who showed that Sprague-Dawley rats fed fish oil concentrate had a lower delayed-type hypersensitivity response than those fed water, oleic acid or safflower oil.

We observed a decrease in prostaglandin E2 production following [n-3] supplementation. Prostaglandin E2 has been shown to suppress IL-1, IL-2 and lymphocyte proliferation [31, 32]. We previously showed that a decrease in prostaglandin E2 production by tocopherol supplementation enhances IL-2 production and lymphocyte proliferation in old mice [33] and elderly human subjects [34]. Therefore, the effect of [n-3] fatty acid supplementation seems to be independent of prostaglandin E2 changes. Conversely, a reduction in LTB4 by [n-3] fatty acid can suppress IL-1, IL-2 production and the subsequent lymphocyte proliferation because LTB4 in some, but not all, studies has been shown to increase IL-1 as well as IL-2 production and lymphocyte proliferation [35]. Santoli and Zurier [36] showed that polyunsaturated fatty acid can reduce IL-2 production directly and independently of changes in cyclooxygenase products. Products of lipid peroxidation such as H2O2 have been shown to have a suppressive effect on lymphocyte proliferation [37]. Meydani et al. [38] showed that supplementation of young and older women with [n-3] fatty acid increases plasma malonaldehyde level. This increase was greater in older women than in young women. Therefore, the decrease in cytokine production and lymphocyte proliferation observed in this study could be the result of an increase in the formation of products of lipid peroxidation following [n-3] fatty acid supplementation. Furthermore, it is not clear whether the decrease in IL-2 production and lymphocyte proliferation is the result of a direct effect of [n-3] fatty acid on IL-2 production or is mediated by the decrease in IL-1 and IL-6.

The clinical implications of our findings need to be determined. Whereas [n-3] fatty acid supplementation may be beneficial as an anti-inflammatory regimen or in decreasing the severity of autoimmune diseases, its suppressive effect on cell-mediated immunity in older adults may not be desirable. Future clinical trials are needed to define the level of [n-3] fatty acid supplementation that provides an anti-inflammatory effect and minimizes the reduction of cell-mediated immunity.

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