Immunologic effects of marine- and plant-derived n-3 polyunsaturated fatty acids in nonhuman primates\textsuperscript{1,3}

Dayong Wu, Simin Nikbin Meydani, Mohsen Meydani, Michael G Hayek, Peter Huth, and Robert J Nicolosi

**ABSTRACT** The effect of marine- and plant-derived n-3 polyunsaturated fatty acids (PUFAs) on T cell-mediated immune response was studied in cynomolagus monkeys. Animals were first fed a 14-wk baseline diet; 10 animals were then fed diets containing 1.3% or 3.3% of energy as eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) while the other 10 were fed diets containing 3.5% or 5.3% of energy as \(\alpha\)-linolenic acid (ALA) for two consecutive 14-wk periods. Both diets significantly decreased the percentage of T cells (except 1.3% EPA + DHA), T helper cells (except 1.3% EPA + DHA and 3.5% ALA), and T suppressor cells. Proliferative response of lymphocytes to T cell mitogens significantly increased after the diet containing 3.3% EPA + DHA. Interleukin 2 production significantly increased after the diets containing 1.3% and 3.3% EPA + DHA. No significant changes in mitogenic response or interleukin 2 production were found after ALA diets. Feeding 1.3% or 3.3% EPA + DHA or 5.3% ALA significantly suppressed prostaglandin \(E_2\) production in response to T cell mitogens. Plasma tocopherol concentrations were decreased significantly only in monkeys fed ALA diets. We conclude that after adjustment for the tocopherol concentration, marine-derived n-3 PUFAs but not plant-derived n-3 PUFAs increased T cell-mediated mitogenic response and interleukin 2 production. This is most likely due to diet-induced quantitative differences in cellular fatty acid composition and, thus, in prostaglandin \(E_2\) production and tocopherol status. *Am J Clin Nutr* 1996;63:273–80.

**KEY WORDS** Nonhuman primates, n-3 polyunsaturated fatty acids, eicosapentaenoic acid, docosahexaenoic acid, \(\alpha\)-linolenic acid, immune response

**INTRODUCTION**

Marine-derived n-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), have been shown to have beneficial effects in reducing the pathogenesis of inflammatory and cardiovascular diseases (1–3). It has, however, also been observed that they suppress (4, 5), have no effect on (6), or increase (7, 8) T cell-mediated immune function when fed in moderate to high amounts. To some extent, this discrepancy reflects varied experimental conditions, including species of animals, age, dietary composition (in particular, the concentration of tocopherol and other fatty acids), supplementation dose, feeding period, and health status. Although it is known that an increase in PUFA intake increases the requirement for tocopherol, most of the previous studies did not correct tocopherol concentrations on the basis of degree of unsaturation. Furthermore, in several previous studies, concentrations of other fatty acids were not kept constant.

The effect of plant-derived n-3 PUFAs, particularly \(\alpha\)-linolenic acid (ALA, 18:3n-3), on the immune response has not been well documented. It has been proposed that this precursor of long-chain n-3 PUFAs may provide the same benefit as supplementation with fish oil but with fewer adverse effects (9, 10).

The present study was undertaken to examine the effects of diets enriched with different amounts of either marine-derived n-3 PUFAs (mainly EPA and DHA) or plant-derived n-3 PUFAs (mainly ALA) in the presence of adequate concentrations of tocopherol (calculated with existing formulas) and controlled concentrations of other fatty acids on plasma fatty acid composition, \(\alpha\)-tocopherol, phosphatidylcholine hydroperoxide (PCOOH), eicosanoid production, and T cell–mediated immune responses of cynomolagus monkeys.

**MATERIALS AND METHODS**

**Animals and diets**

Twenty male cynomolagus monkeys (age, 9.7 ± 0.5 y; weight, 8.1 ± 0.5 kg) were used in this study. The animals were maintained in accordance with the guidelines of the Animal Care and Use Committee of the University of Massachusetts at Lowell Research Foundation and with the guidelines prepared by the Committee on Care and Use of Labora-

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\textsuperscript{3} Address reprint requests to SN Meydani, Nutritional Immunology Laboratory, JM USDA HNRCA at Tufts University, 711 Washington Street, Boston, MA 02111.

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tory Animals of the Institute of Laboratory Animal Resources, National Research Council.

All animals were fed a baseline diet containing 30% of energy as fat for 14 wk. They were then randomly divided into two groups (n = 10 in each group). One group was fed a diet containing 3.5% of energy as ALA for 14 wk followed by a diet containing 5.3% of energy as ALA for another 14 wk. The second group was fed a diet containing 1.3% of energy as EPA plus DHA for 14 wk followed by a diet containing 3.3% of energy as EPA plus DHA for another 14 wk. Compositions of the experimental diets are shown in Table 1. Tocopherol concentrations for each diet were calculated based on the percentage of different PUFAs in each diet according to the mathematical formula described by Muggli (11) and are shown in Table 1. Food and water were available ad libitum.

Blood collection

At the end of each dietary period, animals were sedated with Ketamine (Bristol Laboratories, Veterinary Products, Syracuse, NY). Ten milliliters of heparin-treated blood was collected from the femoral vein after animals were deprived of food overnight. Two blood samples were collected from each animal at each dietary phase to account for day-to-day variability. Two milliliters of EDTA-treated blood was also collected for a complete blood count, white blood cell differentials, and flow cytometric analysis of lymphocyte subpopulations.

Peripheral blood mononuclear cell preparation

Peripheral blood mononuclear cells (PBMCs) were separated from heparin-treated blood with Percoll (1.075; Pharmacia, Piscataway, NJ). Cells were washed twice in endotoxin-free RPMI 1640 medium supplemented with 1 × 10^5 U penicillin/L, 100 mg streptomycin/L, 2 mmol l-glutamine/L, and 25 mmol HEPES/L (Gibco, Grand Island, NY). The cells were counted under a light microscope, and viability was assessed by the trypan blue dye exclusion method. Cells were then suspended at the appropriate concentration in RPMI 1640 with 10% heat-inactivated autologous plasma for measurement of mitogenic lymphocyte proliferation, interleukin 2 (IL-2), and prostaglandin E2 (PGE2) production.

Lymphocyte proliferation

PBMCs (at a concentration of 5 × 10^6 cells/L) were cultured in the presence or absence of the T cell mitogens concanavalin A (ConA; Sigma, St Louis) or phytohemagglutinin P (PHA; Difco Laboratories, Detroit) in concentrations of 1, 10, and 100 mg/mL. Cells were cultured for 72 h at 37 °C in an atmosphere of 5% carbon dioxide and 95% humidity. Cultures were pulsed with 18.5 μBq [3H]thymidine (specific radioactivity, 247.9 GBq; DuPont NEN Products, Boston) during the final 4 h of incubation. The cells were harvested onto glass filter paper and [3H]thymidine incorporation was determined by liquid-scintillation counting in a liquid-scintillation counter (Beckman Instruments, Palo Alto, CA). The counter had an efficiency of 50% for [3H].

PGE2 and IL-2 measurements

For PGE2 and IL-2 measurements, PBMCs (at a concentration of 1 × 10^6 cells/L) were cultured in the presence of 10 mg ConA or PHA/L for 48 h. Cell-free supernates were collected and stored at −70 °C for later analysis. The concentration of PGE2 was measured by radioimmunoassay as described by McCosk et al (12). PGE2 antibody was a gift from J Du Pont of Iowa State University and M Mathias of Colorado State University. The antibody cross-reactivity and specificity were described previously (13). The PGE2 antibody used in this study had a cross-reactivity of 5.6% with PGE3. IL-2 was measured

### Table 1

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Baseline diet</th>
<th>EPA plus DHA diet</th>
<th>ALA diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (% of energy)</td>
<td>1.3%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.3%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.5%&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>0.8</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>20:1n-9</td>
<td>3.5</td>
<td>3.7</td>
</tr>
<tr>
<td>conducts for each diet were calculated based on the percentage of different PUFAs in each diet according to the mathematical formula described by Muggli (11) and are shown in Table 1. Food and water were available ad libitum.</td>
<td>12.1</td>
<td>11.3</td>
<td>10.0</td>
</tr>
<tr>
<td>Other fatty acids</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Tocopherol (mg/kg)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>29.5</td>
<td>35.1</td>
<td>46.1</td>
</tr>
</tbody>
</table>

<sup>1</sup> All diets contained equal amounts of the following nutrients: protein (18.8% of energy), casein (9.4% of energy), soy protein isolate (9.4% of energy), carbohydrate (50.4% of energy), dextrin (28.6% of energy), sucrose (21.8% of energy), fiber (cellulose; 50 g/kg), vitamin and mineral mix (30 g/kg), and cholesterol (300 mg/dL). Protein, carbohydrate, and fiber were from Bioserv Inc (Frenchtown, NJ). Two vitamin and mineral mixes were used. The Newbreme and Hayes salt mix (catalog #F8590, Bioserv Inc) contained the following (g/kg): magnesium oxide, 32.0; calcium carbonate, 290.5; potassium sulfate dibasic, 314.2; calcium phosphate dibasic, 72.6; magnesium sulfate, 98.7; sodium chloride, 162.4; ferric citrate, 27.0; potassium iodide, 0.077; manganese sulfate, 1.22; zinc chloride, 0.91; cupric sulfate, 0.29; chromium acetate, 0.044; sodium selenite, 0.004; and sodium fluoride, 0.02. The Amsan and Hayes vitamin E-free mix (catalog #F8280, Bioserv Inc) contained the following (g/kg): thiamine HCl, 0.80; riboflavin, 1.60; pyridoxine HCl, 0.80; calcium pantothenate, 5.00; nicotinamide, 8.00; folic acid, 1.20; biotin (1.0%), 4.00; cynocobalamin (0.1%), 40; menadione, 0.1; retinyl palmitate (500 000 IU/g), 5.00; cholecalciferol (100 000 IU/g), 2.50; ascorbic acid, 107; inositol, 100; and taurine, 50; made to 1 kg with 664 g sucrose. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, α-linolenic acid.

<sup>2</sup> Percentage of energy as EPA plus DHA or ALA.

<sup>3</sup> Fatty acids were produced from an oil blend containing different amounts of palm, palm kernel, TriSun 80, soy stearene, and safflower oil (Kraft Food Inc, Memphis, TN); sardine oil (Ota Oil Mill, Okazaki City, Japan); and perilla oil (Nippon Suisan Kaisha Ltd, Tokyo).

<sup>4</sup> Tocopherol concentration in oils was adjusted to the targeted amount for each diet by addition of 2-ambo-a-tocopherol (Hoffmann-La Roche, Nutley, NJ) to the vitamin E-free vitamin mix.
via a bioassay in which cytotoxic T lymphocyte line 2 (CTLL-2) cells were used (14). Recombinant human IL-2 (Genzyme Corporation, Boston) was used as a standard. The amount of recombinant IL-2 that caused half-maximal incorporation of [³H]thymidine in 5 × 10⁵ CTLL-2 cells was defined as 1 U/mL. IL-2 activity was calculated by probit analysis (14).

Measurements in blood and plasma

Complete blood counts were obtained with a Baker 9000 Hematology Analyzer (Serono-Baker Instrument Inc, Allenstown, PA) and white blood cell differentials were assessed by microscopic examination of blood smears after Wright-Giemsa staining. For flow cytometric analysis, 100 μL whole blood was added to 200 μL phosphate-buffered saline containing 1 μg fluorescein isothiocyanate–labeled monoclonal antibodies for T cells (T11, mouse immunoglobulin G1 anti-CD2; Coulter, Hialeah, FL), B cells (B1, mouse immunoglobulin G2a anti-CD20; Coulter), T helper cells (anti-4, mouse immunoglobulin G2a anti-CD4; Olympus, Lake Success, NY), T suppressor cells (DAKO-CD8, mouse immunoglobulin G1 anti-CD8, Dako Corporation, Carpenteria, CA), or appropriate controls. Cells were incubated on ice for 45 min, after which time red blood cells were lysed with a Whole Blood Lysing Reagent Kit (Coulter), washed several times in phosphate-buffered saline containing 2% bovine serum albumin, fixed in 0.5% paraformaldehyde, and stored in the dark at 4°C. Cells were analyzed for fluorescein isothiocyanate staining on a FACSCAN (Becton Dickinson Instruments, Cambridge, MA) at an excitation wavelength of 488 nm and an emission wavelength of 530 nm.

Plasma fatty acids were analyzed as described previously (15). Plasma tocopherol was analyzed by a modified HPLC method of Bieri et al (16) as described previously (17). Plasma PCOOH₁ was analyzed by an HPLC method as described previously (18).

Statistical analysis

Data were analyzed for overall treatment effects by repeated-measures analysis of variance (ANOVA). Individual mean differences were determined by Fisher’s protected test. All analyses were conducted with the SYSTAT statistical package (SYSTAT Inc, Evanston, IL). Differences at P < 0.05 were considered to be significant.

RESULTS

Plasma fatty acids

Plasma fatty acid profiles for the most part reflected the fatty acid compositions of the respective dietary lipid sources (Table 2 and Table 3). Plasma from animals fed either diet had the same concentrations of ALA, EPA, DHA, and other fatty acids at baseline. Repeated-measures ANOVA indicated an overall diet effect. Both the ALA and the EPA plus DHA diets resulted in reduced concentrations of plasma n-6 PUFAs. Despite equivalent or higher amounts of dietary linoleate in the ALA diets, the decrease in plasma n-6 PUFAs was more dramatic after consumption of the EPA plus DHA diets (22.4% and 52.6% in 18:2n-6, 84.7% and 92.5% in 20:3n-6, and 20.3% and 25.0% in 20:4n-6, after the 1.3% and 3.3% EPA plus

<table>
<thead>
<tr>
<th>Table 2</th>
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<tr>
<td>Effect of dietary eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) supplementation on plasma fatty acid composition&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fatty acid</td>
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<tr>
<td>---</td>
</tr>
<tr>
<td>% by wt</td>
</tr>
<tr>
<td>16:0</td>
</tr>
<tr>
<td>16:1n-7</td>
</tr>
<tr>
<td>18:0</td>
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<tr>
<td>18:1n-9</td>
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<tr>
<td>18:3n-3</td>
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<tr>
<td>20:3n-6</td>
</tr>
<tr>
<td>20:4n-6</td>
</tr>
<tr>
<td>20:5n-3</td>
</tr>
<tr>
<td>22:6n-3</td>
</tr>
<tr>
<td>Ratio of n-6 to n-3</td>
</tr>
</tbody>
</table>

<sup>1</sup>x ± SEM; n = 10. NC, not calculated.
<sup>2</sup>Percentage of energy as EPA plus DHA.
<sup>3-5</sup>Significantly different from baseline diet: <sup>3</sup>P < 0.05, <sup>4</sup>P < 0.01, <sup>5</sup>P < 0.001.
<sup>6</sup>Significantly different from 1.3% EPA plus DHA diet: <sup>6</sup>P < 0.05, <sup>7</sup>P < 0.001, <sup>8</sup>P < 0.01.

DHA diets, respectively) than after consumption of the ALA diets (17.8% and 19.1% in 18:2n-6, 58.9% and 67.6% in 20:3n-6, and 15.9% and 16.6% in 20:4n-6, after consumption of the 3.5% and 5.3% ALA diets, respectively). Significant increases in plasma ALA, EPA, and DHA were observed after consumption of both ALA diets (Table 3). Consumption of both EPA plus DHA diets significantly increased plasma EPA and DHA concentrations (Table 2). The concentration of n-3 PUFAs found in plasma after the baseline diet was < 0.2%. Thus, the ratio of n-6 to n-3 PUFAs could not be calculated for this diet period. This ratio was 2.34 and 0.76 after the 1.3%
and 3.3% EPA plus DHA diets, and 5.01 and 3.21 after the 3.5% and 5.3% ALA diets, respectively.

**Lymphocyte proliferation**

Mitogenic responses of PBMCs to different concentrations of ConA and PHA were examined. No significant difference was observed in the proliferative response of PBMCs between different dietary groups in the absence of mitogen (data not shown). Repeated-measures ANOVA indicated a significant difference in overall diet effect on PBMC proliferative response to optimal concentrations of mitogens. As shown in Figure 1, the EPA plus DHA diet, but not the ALA diet, enhanced the mitogenic response of monkey PBMCs (P = 0.047 and 0.012 for ConA and PHA, respectively, for diet effect). The mitogenic response to ConA and PHA increased by 51% and 47%, respectively, after consumption of the diet containing 3.3% EPA plus DHA. Seventy percent and 100% of monkeys consuming the 3.3% EPA plus DHA diet showed an increase in mitogenic response to ConA and PHA, respectively. Neither diet caused a statistically significant change in proliferative response to sub- or supraoptimal doses of mitogens (data not shown).

**PGE\(_2\) and IL-2 production**

Repeated-measures ANOVA indicated an overall dose-dependent diet effect on PGE\(_2\) production by PBMCs from subjects in both diet groups. This effect tended to be different between the two diet groups (P = 0.05 and 0.08 for ConA- and PHA-induced PGE\(_2\) production, respectively). More specifically, both the ALA and the EPA plus DHA diets decreased PGE\(_2\) production (Table 4), although this inhibition was not significant after the diet containing 1.3% EPA plus DHA (for ConA- and PHA-induced PGE\(_2\) production). Consumption of the diet containing 3.3% EPA plus DHA significantly decreased PGE\(_2\) production by PBMCs in response to ConA by 95% and in response to PHA by 91%. Consumption of the diet containing 3.5% ALA significantly decreased ConA-stimulated PGE\(_2\) production by 46%, whereas consumption of a higher concentration of ALA (ie, 5.3% of energy) decreased PGE\(_2\) production in response to ConA by 74% and in response to PHA by 63%.

Figure 2 shows ConA- and PHA-stimulated IL-2 production by PBMCs from animals consuming the EPA plus DHA or ALA diet. Repeated-measures ANOVA indicated a significant difference in diet effect. A significant (P = 0.05) diet \(\times\) fat concentration interaction was also observed in the EPA plus DHA diet group. ConA-stimulated IL-2 production was elevated 111% and 128% after consumption of the 1.3% and 3.3% EPA plus DHA diets, respectively. The percentage of monkeys showing an increase in ConA-stimulated IL-2 production after consumption of the 1.3% and 3.3% EPA plus DHA diets was 75% and 100%, respectively. A significant increase (140%) in PHA-stimulated IL-2 production was seen only after consumption of the diet containing 3.3% EPA plus DHA (Figure 2A). The percentage of monkeys showing an increase in PHA-stimulated IL-2 production after consumption of the diet containing 3.3% EPA plus DHA was 87%. In contrast, feeding animals diets containing ALA did not have any significant effect on ConA- or PHA-stimulated IL-2 production (Figure 2B).

**FIGURE 1.** Effect of diets enriched in eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) or \(\alpha\)-linolenic acid (ALA) on mitogenic responses of peripheral blood mononuclear cells (PBMCs) from cynomolgus monkeys. PBMCs were cultured with optimal concentrations (10 mg/L) of concanavalin A (ConA) or phytohemagglutinin (PHA) for 72 h and pulsed with \(^{3}H\)thymidine during the last 4 h to calculate DNA incorporation. Data were obtained from monkeys fed EPA plus DHA (A) and ALA (B) diets, respectively. \(\bar{x}\) ± SEM, \(n = 10\) in EPA plus DHA group, \(n = 9\) in ALA group. Cpm, corrected count per minute, which is the cpm of stimulated cultures minus the cpm of nonstimulated cultures. Repeated-measures ANOVA indicated a significant difference in overall diet effect on the proliferative response of PBMCs to optimal amounts of mitogens: \(P = 0.047\) and 0.012 for ConA- and PHA-stimulated proliferation, respectively.

**Complete blood count, white blood cell differential, and flow cytometric analysis**

The total number of white blood cells or percentages of lymphocytes, monocytes, neutrophils, eosinophils, and basophils was not significantly changed after consumption of the EPA plus DHA or ALA diet (data not shown). The lymphocyte subpopulation shifted significantly from baseline after consumption of the EPA plus DHA and ALA diets. As shown in Table 5, the percentage of T cells was reduced in a dose-dependent manner by consumption of both diets, except that the reduction after the diet containing 1.3% EPA plus DHA
was not significant. The percentage of T cells decreased by 12% and 46% after the diets containing 1.3% and 3.3% EPA plus DHA, and by 9% and 37% after the diets containing 3.5% and 5.3% ALA, respectively. This decrease reflects decreases in both T helper cells and T suppressor cells. The percentage of T helper cells decreased by 35% and 31% after the diets containing 3.3% EPA plus DHA and 5.3% ALA, respectively. T suppressor cells decreased in a dose-dependent manner after the diets containing 1.3% and 3.3% EPA plus DHA (25% and 48%, respectively) and after the diets containing 3.5% and 5.3% ALA (16% and 41%, respectively). The changes in absolute numbers of T cells, T helper cells, and T suppressor cells were generally consistent with the changes in percentage with the exception of the diet containing 3.5% ALA. Although a decrease was observed in both T helper and T suppressor cells, a higher percentage decrease was observed in the percentage of T suppressor cells, resulting in an insignificant increase in the ratio of T helper to T suppressor cells in both the EPA plus DHA and ALA diet groups. No significant change in either percentage or absolute number of B cells was observed, with the exception of a moderate increase after consumption of the diet containing 3.5% ALA ($P < 0.05$).

**DISCUSSION**

We investigated the effect of two amounts of n-3 PUFAs from marine and plant sources on T cell–mediated immune responses. The diets were designed so that the amounts of total fat and fatty acids were held constant. The primary differences in fatty acid composition were in the amount of n-6 and n-3 PUFAs. The diets containing 3.5% of energy as ALA and 3.3% of energy as EPA plus DHA differed only in percentage of ALA and EPA plus DHA. Furthermore, the amount of toco-pherol added to the diets varied according to the degree of unsaturation. The results show that consumption of marine-derived or plant-derived n-3 PUFAs alters fatty acid composition of monkey plasma accordingly. However, the changes in immunologic indexes varied with the source and amount of the n-3 PUFA supplement.

High dietary intake of ALA was reported to not have a significant effect on plasma concentrations of arachidonic acid (AA) or EPA (19, 20). In this study, however, we observed a moderate yet significant decrease in AA after consumption of either of the diets containing 3.5% or 5.3% of energy as ALA. Decreases after each of the two ALA diets were not significantly different. Furthermore, ALA consumption increased

**TABLE 4**

Effect of diets containing eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) or α-linolenic acid (ALA) on mitogen-stimulated prostaglandin E<sub>2</sub> production by peripheral blood mononuclear cells of cynomolgus monkeys

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>EPA plus DHA diet</th>
<th>ALA diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1.3%&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>ConA</td>
<td>379 ± 78</td>
<td>300 ± 203</td>
</tr>
<tr>
<td>PHA</td>
<td>561 ± 144</td>
<td>335 ± 197</td>
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<sup>1</sup> ± SEM; n = 9 in each diet group. Peripheral blood mononuclear cells (1 × 10<sup>6</sup> cells) were cultured with 10 mg concanavalin A (ConA) or phytohemagglutinin (PHA)/L for 48 h. The cell-free supernates were used to examine prostaglandin E<sub>2</sub> content by radioimmunoassay.

<sup>2</sup> Percentage of energy as EPA plus DHA or ALA.

<sup>1,4</sup> Significantly different from respective baseline diet: <sup>1</sup>$P < 0.01$, <sup>4</sup>$P < 0.05$.

**FIGURE 2.** Effect of diets enriched in eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) or α-linolenic acid (ALA) on interleukin 2 (IL-2) production by peripheral blood mononuclear cells (PBMCs) from cynomolgus monkeys. PBMCs were cultured with optimal concentrations (10 mg/L) of ConA or PHA for 48 h. Cell-free supernates were used for the IL-2 bioassay as described in the text. Results are from monkeys fed EPA plus DHA (A) and ALA (B) diets, respectively. ± SEM, n = 8 in EPA plus DHA group, n = 9 in ALA group. Repeated-measures ANOVA indicated a significant difference in diet effect between the EPA plus DHA and ALA groups. A diet × fat interaction was also observed in the EPA plus DHA group ($P = 0.05$).
TABLE 5
Effect of diets enriched in eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) or α-linolenic acid (ALA) on lymphocyte subpopulation of cynomolgus monkeys

<table>
<thead>
<tr>
<th></th>
<th>B cells (%)</th>
<th>T cells (%)</th>
<th>T helper (%)</th>
<th>T suppressor (%)</th>
<th>Total lymphocytes (%)</th>
<th>Th:Ts6</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1.3%2</td>
<td>3.3%2</td>
<td>ALA diet</td>
<td>ALA diet</td>
<td>ALA diet</td>
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<tr>
<td>Lymphocyte</td>
<td>Baseline</td>
<td></td>
<td>Baseline</td>
<td>ALA diet</td>
<td>3.5%2</td>
<td>5.3%2</td>
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<td></td>
<td></td>
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<td>Baseline</td>
<td>ALA diet</td>
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<td></td>
<td>12.6 ± 3.3</td>
<td>21.7 ± 4.2</td>
<td>21.7 ± 4.2</td>
<td>21.7 ± 4.2</td>
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<tr>
<td>(cells/μL whole blood)</td>
<td>408 ± 69</td>
<td>455 ± 53</td>
<td>455 ± 53</td>
<td>455 ± 53</td>
<td>455 ± 53</td>
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<tr>
<td></td>
<td>74.7 ± 4.2</td>
<td>65.7 ± 3.7</td>
<td>40.0 ± 3.96</td>
<td>76.2 ± 3.7</td>
<td>69.6 ± 3.56</td>
<td>47.9 ± 4.36</td>
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<td>(cells/μL whole blood)</td>
<td>3678 ± 1246</td>
<td>2320 ± 538</td>
<td>1485 ± 2534</td>
<td>3649 ± 448</td>
<td>3915 ± 596</td>
<td>2677 ± 5136</td>
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<tr>
<td>T helper (%)</td>
<td>31.9 ± 4.8</td>
<td>30.4 ± 2.4</td>
<td>20.5 ± 2.06</td>
<td>32.7 ± 2.2</td>
<td>32.1 ± 2.06</td>
<td>22.3 ± 2.0</td>
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<tr>
<td>(cells/μL whole blood)</td>
<td>1676 ± 645</td>
<td>1059 ± 237</td>
<td>810 ± 187</td>
<td>1626 ± 254</td>
<td>1840 ± 305</td>
<td>1265 ± 2526</td>
</tr>
<tr>
<td>T suppressor (%)</td>
<td>50.3 ± 5.2</td>
<td>37.9 ± 4.5</td>
<td>26.1 ± 2.76</td>
<td>60.2 ± 3.7</td>
<td>50.5 ± 1.96</td>
<td>35.3 ± 3.6</td>
</tr>
<tr>
<td>(cells/μL whole blood)</td>
<td>2428 ± 776</td>
<td>1264 ± 237</td>
<td>983 ± 1654</td>
<td>2970 ± 457</td>
<td>2905 ± 472</td>
<td>2020 ± 419</td>
</tr>
<tr>
<td>Total lymphocytes/μL whole blood</td>
<td>4509 ± 977</td>
<td>4097 ± 932</td>
<td>3537 ± 536</td>
<td>4798 ± 519</td>
<td>5602 ± 746</td>
<td>5284 ± 673</td>
</tr>
</tbody>
</table>

1 x ± SEM; n = 8 for EPA plus DHA and n = 10 for ALA groups. 5 × 10^6 cells were used for flow cytometric analysis of each specific lymphocyte subset.
2 Percentage of energy from EPA plus DHA or ALA.
3–5 Significantly different from respective baseline diet: 3 P < 0.05, 4 P < 0.001, 5 P < 0.01.
6 Ratio of T helper to T suppressor cells.

plasma EPA and DHA concentrations. This indicates that in monkeys, 18:3 n−3 can be converted to EPA or DHA, or both, although with low efficiency. Similar results were reported in humans (21). Significant decreases in plasma AA and dose-dependent increases in plasma EPA and DHA were also observed after the diets containing 1.3% and 3.3% of energy as EPA plus DHA were fed. It is interesting that both the ALA and the EPA plus DHA diets caused a significant reduction in 20:3n−6, a precursor to AA. The decrease in 20:3n−6 was more dramatic than that observed in AA or in 18:2n−6 after consumption of ALA. This is likely due to competitive inhibition by ALA of specific steps of desaturation or elongation, or both.

Several studies have focused on the effects of n−3 PUFAs on AA incorporation into membrane lipids and changes in its metabolism by cyclooxygenase and lipoxygenase. Dietary supplementation with fish oil or purified EPA plus DHA lowered the concentrations of PGE2 in mouse plasma (22), rat macrophages (23), splenocytes (24), platelets (19), and human PBMCs (21). Studies with plant-derived n−3 PUFAs have shown either a less-pronounced decrease in PGE2 production (19) or a lack of an effect (23). Our data, however, indicate a significant decrease in PGE2 production after consumption of diets containing 5.3% ALA (74% and 63% decrease, in response to ConA and PHA, respectively). PGE2 production by PBMCs in response to ConA and PHA stimulation decreased by 95% and 91%, respectively, after consumption of a diet containing 3.3% EPA plus DHA. Thus, decreases in PGE2 production can be achieved after consumption of ALA.

Similar to the effect on fatty acid composition, the decrease in PGE2 production induced by the ALA diet was less efficient than that induced by the EPA plus DHA diet. This difference can be related to the different mechanisms and efficacy of the two diets for interfering with the metabolism of AA and synthesis of 2-series prostaglandins. Lower concentrations of AA in the phospholipid pool (as reflected in plasma fatty acid composition) reduce the availability of substrate for prostaglandin synthesis. The extent of the decrease in AA concentrations was much less in monkeys fed the ALA diet compared with those fed the EPA plus DHA diet (5.3% ALA and 1.3% EPA plus DHA caused similar percentage changes in AA content). Furthermore, EPA can compete with AA for cyclooxygenase and consequently decrease the syntheses of those eicosanoids derived from AA. ALA (18:3n−3) on the other hand, may competitively inhibit the desaturation and elongation of linoleic acid (18:2n−6) for the conversion to longer-chain PUFAs.

The reported effects of n−3 PUFA intake on cell-mediated immunity have been contradictory. Both positive and negative effects on T cell–mediated function have been reported in the literature. However, it appears that the majority report an unfavorable effect, especially with marine-derived, long-chain n−3 PUFAs. An impaired early thymocyte response to ConA was observed by Valette et al (25) after rats were fed a diet containing 8% fish oil for only 3 wk. Dietary enrichment with 2% EPA inhibited the ability of mouse splenocytes to present antigen to murine T helper cell clones after 4–5 wk of the diet (26). We previously reported that human subjects receiving n−3 PUFAs (EPA and DHA) showed decreases in IL-2 production, delayed-type hypersensitivity skin response, and lymphocyte proliferation (5, 21). This is surprising because diets enriched in n−3 PUFAs reduce the production of PGE2, a substance that inhibits T cell–mediated function. From those studies, we speculated that the suppressive effect of marine-derived n−3 PUFAs on T cell–mediated function is due to compromised tocopherol status (17, 21).

Along these lines, we observed in another study (27) that mice fed n−3 PUFAs had lower splenocyte mitogenic responses and liver vitamin E concentrations than did those fed n−6 PUFAs. Addition of vitamin E enhanced mitogenic re-
response more dramatically in mice fed n-3 PUFAs (281%) than in mice fed n-6 PUFAs (109%). Therefore, in the present study we adjusted the tocopherol concentration on the basis of the PUF content of the diets. Consequently, a significant enhancement of both mitogenic response and IL-2 production was observed after monkeys were fed diets containing 3.3% EPA plus DHA. Considering that a smaller and statistically insignificant increase in PBMC proliferation and IL-2 production was observed after consumption of 1.3% EPA plus DHA, we suggest that the effect of EPA plus DHA on these indices might be dose-dependent. ALA, a shorter-chain n-3 PUFAs, did not have any significant effect on mitogenic response of PBMCs at the same or a higher percentage. Production of IL-2 from these PBMCs was also not altered by ALA diets.

Our results indicate that the effects of 18-carbon and 20-22-carbon PUFAs on T cell–mediated functions differ. Diets containing EPA plus DHA, in the presence of an adequate tocopherol concentration, enhanced mitogenic proliferative responses and IL-2 production, whereas no such effect was observed with ALA diets. Some of the contributing factors to this phenomenon are that longer-chain (more unsaturated) PUFAs are believed to be more susceptible to autoxidation, which, in turn, may contribute to their suppression of the immune response. Therefore, in the current study we calculated the amount of α-tocopherol needed for each diet on the basis of existing formulas (11) to prevent increased oxidation and free radical production and to maintain tocopherol concentrations. As predicted, the plasma α-tocopherol concentration was maintained in animals fed EPA plus DHA. Accordingly, a significant enhancement of both mitogenic response and IL-2 production of PBMCs were observed after monkeys were fed the diets containing 3.3% EPA plus DHA. Surprisingly, however, plasma tocopherol concentrations decreased after consumption of ALA diets: 19% and 27% after the 3.5% and 5.3% ALA diets, respectively (from 10.52 ± 1.91 mg/L at baseline to 8.74 ± 1.60 mg/L after 3.5% ALA and to 7.67 ± 1.40 mg/L after 5.3% ALA; P < 0.01).

This inadequate supply of vitamin E was further reflected in the PCOOH concentration adjusted for the unsaturation index. ALA-fed animals tended to have higher PCOOH production per unsaturation index (ie, per double bond) than did those fed EPA plus DHA (0.54 ± 0.19 μmol/L in monkeys fed 5.3% ALA compared with 0.39 ± 0.14 μmol/L in monkeys fed 3.3% EPA plus DHA). As indicated above, no significant increase in mitogenic response or IL-2 production was observed in monkeys fed ALA, even though a significant decrease in PGE₂ production was noted. The change in tocopherol status thus appears to contribute to a lack of a stimulatory effect of ALA in this experiment. Considering the well-confirmed enhancing effect on immune function of α-tocopherol, we suggest that in the ALA-fed animals the beneficial effect of reduced PGE₂ production on immune response might have been offset by the adverse effect of reduced α-tocopherol concentrations. The concentration of α-tocopherol for both diets was calculated with the same formula (11). The formula correctly predicted tocopherol concentrations needed for EPA plus DHA diets but not for the ALA diets. Thus, it appears that the calculated α-tocopherol concentration based on the amount of unsaturation of oils in the diets may not adequately reflect the actual concentration needed in vivo when fatty acids have the potential to be further elongated and desaturated in the body, which appears to be the case with ALA. The further elongation and desaturation of ALA to more unsaturated PUFAs, as evidenced by the existence of EPA and DHA in ALA-fed animals, were not considered when the required concentration of α-tocopherol in the diet was calculated.

In addition, the ALA-induced decrease in PGE₂ production might not have been adequate to enhance T cell–mediated functions significantly. On the other hand, the dramatic decrease in PGE₂ production in animals fed EPA plus DHA in the presence of adequate concentrations of α-tocopherol resulted in a significant improvement of T cell–mediated functions. Further support for a contribution of a decrease in PGE₂ production to the observed effect is provided by our previous studies in which in vitro addition of indomethacin, a cyclooxygenase inhibitor, significantly improved mitogenic response of mice fed fish oil.

The change in IL-2 concentration shown in this study is consistent with the change observed in mitogenic response of PBMCs monkeys fed the two types of diets. The enhancements in mitogenic response and IL-2 production induced by EPA and DHA do not appear related to the changes in the lymphocyte subpopulation because all of the shifts in lymphocyte subpopulations in this study were comparable between the two dietary groups. n-3 PUFAs can also affect T cell–mediated function directly or indirectly (through changes in PGE₂) by influencing membrane-related signal transduction events.

In this study, we observed reduced numbers of total T cells, T helper cells, and T suppressor cells after consumption of either of the n-3 PUFA diets. This is consistent with our previous finding in mice (27). It is interesting that as a result of supplementation with n-3 PUFAs, T cells were significantly decreased whereas B cells remained relatively constant. This indicates that an unidentified (in this study) population of cells is increased after supplementation with n-3 PUFAs. This compartment may represent null cells, natural killer cells, or both.

In summary, we showed that consumption of diets enriched in EPA plus DHA or ALA reduced PGE₂ production by cynomolgus monkey PBMCs, albeit to a lesser degree with ALA enrichment. However, enhanced mitogenic response and IL-2 production were only observed in PBMCs from monkeys fed EPA plus DHA. When the required concentration of tocopherol was calculated on the basis of the fatty acid composition of diet, the formula accurately predicted the α-tocopherol concentration needed for EPA plus DHA diets, whereas it did not accurately predict the α-tocopherol requirement when ALA was fed. Thus, the differential effect of marine- and plant-derived n-3 PUFAs in this study appears to be due to differences in the degree of PGE₂ reduction induced by these diets as well as to differences in tocopherol status between the animals fed these diets.

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REFERENCES

3. Phillipson BE, Rothrock DW, Connor WE, Harris WS, Illingworth