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Docosahexaenoic Acid Ingestion Inhibits Natural Killer Cell Activity and Production of Inflammatory Mediators in Young Healthy Men[1]

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ABSTRACT: The purpose of this study was to examine the effects of feeding docosahexaenoic acid (DHA) as triacylglycerol on the fatty acid composition, eicosanoid production, and select activities of human peripheral blood mononuclear cells (PBMNC). A 120-d study with 11 healthy men was conducted at the Metabolic Research Unit of Western Human Nutrition Reach Center. Four subjects (control group) were fed the stabilization diet throughout the study; the remaining seven subjects were fed the basal diet for the first 30 d, followed by 6 g DHA/d for the next 90 d. DHA replaced an equivalent amount of linoleic acid; the two diets were comparable in their total fat and all other nutrients. Both diets were supplemented with 20 mg D α-tocopherol acetate per day. PBMNC fatty acid composition and eicosanoid production were examined on day 30 and 113; immune cell functions were tested on day 22, 30, 78, 85, 106, and 113. DHA feeding increased its concentration from 2.3 to 7.4 wt% in the PBMNC total lipids, and decreased arachidonic acid concentration from 19.8 to 10.7 wt%. It also lowered prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) production, in response to lipopolysaccharide, by 60–75%. Natural killer cell activity and *in vitro* secretion of interleukin-1β and tumor necrosis factor α were significantly reduced by DHA feeding. These parameters remained unchanged in the subjects fed the control diet. B-cell functions as reported here and T-cell functions that we reported previously were not altered by DHA feeding. Our results show that inhibitory effects of DHA on immune cell functions varied with the cell type, and that the inhibitory effects are not mediated through increased production of PGE2 and LTB4.

Paper no. L8089 in Lipids 34, 317–324 (April 1999).

The low incidence of mortality from coronary heart disease among the Eskimos was initially attributed to the relatively large proportion of n-3 fatty acids from fish in their diet (1). Subsequent human and animal experimental studies also demonstrated the beneficial effects of n-3 fatty acids on cardiovascular health. Fish oils may improve cardiovascular health because of their antiinflammatory effects, lowering of serum triglyceride, and inhibition of platelet aggregation.

The n-3 polyunsaturated fatty acids (PUFA) from marine and plant sources inhibit several aspects of human immune response (IR), including the production of inflammatory cytokines tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β), natural killer (NK) cell activity, and lymphocyte proliferation (2–16). Most of the studies with n-3 PUFA neither held total fat intake constant nor provided extra antioxidant nutrients to meet the need for increased oxidative stress. Both these factors may alter human IR (11,17–19). Thus it is not clear whether the inhibition of IR in previous studies was due to n-3 PUFA, increased total fat intake, increased oxidative stress, or a combination of these factors.

Fish oils contain a variable mixture of eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), along with many other fatty acids. It is not known whether the decline in IR caused by the fish oils is due to EPA, DHA, both, or other fatty acids. Purified esters of EPA lowered several aspects human IR (9,10,15). *In vitro* addition of DHA to human (20) and rat lymphocytes (21) inhibited their proliferation. Similar results were also obtained in a DHA feeding study conducted with rats (22). Addition of EPA or DHA to human peripheral blood mononuclear cells (PBMNC) in culture indicates that different mechanisms may mediate the alteration of lymphocyte function by these two fatty acids (20,23).

Dietary fatty acids may alter IR by altering eicosanoid production and the fatty acid composition of the participating cells. Prostaglandin E2 (PGE2) and leukotriene B4 (LTB4), both derived from arachidonic acid (20:4n-6), are considered to be some of the most important eicosanoids that alter immune cell functions. PGE2 inhibited a number of lymphocyte

[1]Parts of data included here were published in abstracts for the Experimental Biology 98, and the International Congress of Immunology 98, meetings.

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Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HPLC, high-performance liquid chromatography; IL, interleukin; IR, immune response; LTB4, leukotriene B4; LPS, lipopolysaccharide; NK, natural killer; PBMNC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; PGE2, prostaglandin E2; PUFA, polyunsaturated fatty acids; TLC, thin-layer chromatography; TNFα, tumor necrosis factor α.

functions such as proliferation and IL-2 secretion in a dose-dependent manner, while the LTB4 was stimulatory at low concentrations and inhibitory at higher concentrations (24). The n-3 fatty acids, EPA and DHA, may reduce the arachidonic acid concentration within the immune cells and thus reduce the production of inflammatory eicosanoids.

The effect of dietary DHA on production of inflammatory mediators or other aspects of human IR has not been published. This is because until recently, purified DHA was not available for human consumption. Such studies are now possible with the availability of DHASCO™ oil, which contains 40% DHA as natural triacylglycerol. Studies with DHA are important, because it is the major n-3 fatty acid in tissues, and the body tends to conserve it more than EPA. The purpose of this study was to examine the effect of a moderately high intake (6 g/d) of DHA, in the absence of EPA, on select parameters of human IR, including inflammatory cytokine production, NK cell activity, and *in vivo* antibody production. Possible mechanisms by which DHA may alter immune cell functions were determined by examining PBMNC fatty acid composition, *in vitro* eicosanoid production, and the serum concentrations of apoprotein E and antioxidant vitamins. We held total fat intake constant and supplemented diets with D α-tocopherol acetate (20 mg/d) to avoid increased oxidative stress from DHA consumption.

MATERIALS AND METHODS

Subjects and study design. Twelve healthy men between the ages of 20–40 yr were selected from the San Francisco Bay area through local advertisements. They passed a physical examination given by a licensed physician, and had body weights within 20% of ideal body weights based on 1983 Metropolitan Life Insurance tables. They were all nonsmokers and nondrug users. Mean body weights, age, and body mass index (BMI) for the study subjects are given in Table 1. Body weights of the subjects were maintained within 2% of their initial weights throughout the study by adjusting their energy intake when necessary.

The study protocol was approved by the Human use committee of the University of California at Davis and the United States Department of Agriculture committee at Houston, Texas. The study lasted for 120 d (April 1 to July 29, 1996) although no immunological tests were conducted after day 113, because of other scheduled procedures. All subjects were immunized on day 85 with a trivalent 1995–1996 influenza vaccine (Connaught Laboratories Inc., Swiftwater, PA). Subjects lived at the Metabolic Research Unit (MRU) of the Western Human Nutrition Research Center for the duration of the study, except when going for daily walks (2 × 2 miles) or other scheduled outings. They consumed only those foods prepared by the staff of the MRU and were under constant supervision. Subjects were divided into two groups. Four subjects (control group) were fed the control or stabilization diet throughout the study. The remaining eight subjects (DHA group) were fed the control diet for the first 30 d of the study and a DHA-supplemented diet for the last 90 d. One subject from the DHA group did not complete the study, so data from only seven subjects were evaluated. More subjects were included in the DHA group than in the control group, because most of the DHA effects were evaluated by comparing the pre- and post-DHA indices in the DHA group.

Diets. Diets were made up of natural foods, except DHA and vitamin E, and were fed in a 5-d rotating menu, comprising three meals and a postdinner snack. Dietary composites from each of the 5-d menus for both diets were analyzed for macronutrients. In both diets, protein, fat, and carbohydrates provided approximately 15, 30, and 55% energy, respectively (Table 2). The micronutrient contents of the diets were calculated by using *USDA Handbook 8* (25); all nutrients were at or above the recommended dietary allowances (RDA) and were identical in the two diets. Diets contained about one RDA of vitamin E from natural foods (calculated using values from *USDA Handbook 8*) and were supplemented with an additional 20 mg/d of D α-tocopherol acetate (Bronson Pharmaceutical, St. Louis, MO).

DHA diet contained 15 g of DHASCO™ oil (a gift from Martek Corporation, Columbia, MD), which was incorporated by replacing an isocaloric amount of safflower oil; all other foods were identical in the two diets. The main effect of incorporating DHASCO™ oil into diet was the replacement of 6 g linoleic acid (18:2n-6) with an equivalent amount of DHA. Fatty acid composition of the two diets is given in Table 3. The DHASCO™ oil was kept in sealed containers at –20°C, and all open bottles were flushed with nitrogen before

TABLE 1
Physical Characteristics of Study Subjects[a]

Index	DHA group (n = 7)	Control group (n = 4)
Age, yr	33.1 ± 1.8	33.3 ± 3.1
Weight, kg	78.6 ± 4.2	74.7 ± 4.2
BMI, kg/m^2	23.7 ± 0.8	23.1 ± 1.1

[a]DHA, docosahexaenoic acid; BMI, body mass index. Mean ± SEM.

TABLE 2
Nutrient Composition of Experimental Diets[a]

	Energy %	
Nutrient	DHA diet	Control diet
Protein	15.2 ± 0.6	14.6 ± 0.5
Carbohydrate	53.9 ± 3.0	56.4 ± 3.1
Fat, total	30.9 ± 2.1	29.0 ± 1.8
Saturated	8.9 ± 0.4	8.1 ± 0.4
Monounsaturated	9.2 ± 0.3	9.2 ± 0.3
n-6 Polyunsaturated	6.5 ± 0.3	8.5 ± 0.3
n-3 Polyunsaturated	2.8 ± 0.1	1.1 ± 0.1
trans	1.9 ± 0.1	2.1 ± 0.1
P/S ratio	1.2	1.1
Cholesterol (mg/d)	360	360

[a]Mean ± SEM (n = 5). Dietary composites were prepared for each 5-d rotating menu. Both diets were supplemented with D α-tocopherol acetate, 20 mg/d. P/S, polyunsaturated fatty acid/saturated fatty acid. See Table 1 for other abbreviation.

TABLE 3
Fatty Acid (wt%) Composition of Experimental Diets[a]

Fatty acid	DHA diet	Control diet
12:0	1.3 ± 0.1	0.7 ± 0.1[b]
14:0	4.3 ± 0.3	2.2 ± 0.4[b]
16:0	16.6 ± 0.7	16.3 ± 0.7
16:1n-9	0.7 ± 0.2	0.9 ± 0.2
18:0	7.1 ± 0.5	7.5 ± 0.4
18:1*t*, all isomers	6.2 ± 0.5	7.0 ± 0.5
18:1n-9	26.6 ± 0.8	26.0 ± 0.7
18:1n-7	1.7 ± 0.1	2.0 ± 0.1
18:1n-5	1.5 ± 0.2	2.1 ± 0.4
18:2*tt* and 19:0	0.5 ± 0.1	0.6 ± 0.1
18:2n-6	21.6 ± 1.2	28.3 ± 1.0[b]
18:3n-3	2.6 ± 0.2	3.2 ± 0.1
22:0	0.2 ± 0.0	0.2 ± 0.0
20:5n-3	0.4 ± 0.1	0.3 ± 0.1
22:6n-3	6.5 ± 0.22	<0.1 ± 0.1[b]
Total	98.0 ± 0.4	97.2 ± 0.3
Unknowns	2.0 ± 0.2	2.8 ± 0.1

[a]Mean ± SEM (n = 5). See Table 1 for abbreviation.
[b]Significantly different between two diets ($P < 0.05$).

being returned to the refrigerator. It was served only in cold foods such as yogurt, dips, or salads. Thus the chances of DHA oxidation were minimized.

Laboratory procedures. Blood samples were collected between 7: 00 and 8:00 A.M. after an overnight fast on study days 23, 30, 78, 85, 106, and 113. Samples were collected by antecubital venipuncture into evacuated tubes without anticoagulants (for sera preparation) or containing heparin (for cell culture experiments). For both dietary groups two determinations of cytokine production, NK cell activity, and serum antioxidant vitamins were made at the end of stabilization, middle, and end of the intervention periods (days 23, 30, 78, 85, 106, and 113). Means of the two measurements are shown in the results section. PBMNC fatty acid composition and eicosanoid production were determined only on study days 30 and 113. Influenza antibody titers were determined in the sera prepared from blood samples drawn on days 30, 85, 106, and 113.

Isolation and culture of PBMNC for cytokine and eicosanoid secretion. PBMNC were isolated using Histopaque-1077 as previously reported (26) and cultured with or without lipopolysaccharide (LPS, 1.0 mg/L) in 24-well flat-bottom culture plates (5×10^5 PBMNC/mL/well). The culture medium used was RPMI-1640 (Gibco, Grand Island, NY), containing 10% autologous serum and L-glutamine (2 mmol/L), penicillin (100 KU/L), streptomycin (100 mg/L) and gentamicin (20 mg/L). The tissue culture media were collected by centrifugation 24 h after stimulation with LPS and stored frozen at −70°C until the cytokine and eicosanoid concentrations were determined. Enzyme-linked immunosorbent assay (ELISA) kits for cytokine assays were purchased from Immunotech (Miami, FL) and those for LTB4 from Cayman Chemical Company (Ann Arbor, MI).

PGE2 concentration in the media was determined by gas chromatography–mass spectrometry (GC–MS) as the methoxime-pentafluorobenzyl ester-trimethylsilyl ether derivative. Analyte and internal standard were extracted from 200 μL culture media with C_{18} Sep-Pak cartridges after dilution with 10 mL acidified (pH 3.0) water, and addition of 1 ng of (3,3,4,4-$^2H_4$) PGE2 internal standard. They were eluted with methyl formate/petroleum ether (1:1) after sequential rinsing first with acidified water, then with methyl formate/petroleum ether (5:95). The prostaglandins were esterified with pentafluorobenzyl bromide in CH_3CN, then were treated with methoxylamine hydrochloride in pyridine. The PGE2-pentafluorobenzyl ester-methoxime derivative was purified on silica gel thin-layer chromatography plates and finally converted to trimethylsilyl ether derivative with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) in pyridine (1:1). Gas chromatography–mass spectrometry in the electron capture chemical ionization (ECCI) mode was carried out with a Finnigan-MAT TSQ-70B instrument operated at 70 eV with methane as the moderating gas. The ions monitored were *m/z* 524 (M − 181) for the endogenous analyte and *m/z* 528 for the tetradeuterated internal standard.

Determination of NK cell activity. NK cell activity was determined by using the nonadherent PBMNC and the ^{51}Cr labeled K-562 cells at effector/target cell ratios of 50:1, 25:1, 12.5:1, 6.2:1, and 3.1:1 as previously reported (26). Six wells were used for each effector cell concentration and for the spontaneous and maximal release (caused by 3% centrimide) of ^{51}Cr. After 4 h incubation at 37°C in 5% CO_2, the plates were centrifuged and aliquots of supernatant collected to determine the amount ^{51}Cr released.

Percent lysis was calculated as follows:

$$\% \text{ lysis} = \frac{(\text{experimental CPM} - \text{spontaneous CPM})}{(\text{maximum CPM} - \text{spontaneous CPM})} \times 100 \quad [1]$$

Serum antibody titers. The antibody titers for the viral strains A/TEXAS/36/91, A/Johannesburg/33/94, and B/Harbin/7/94 (strains included in the vaccine) were determined by using the hemagglutination inhibition assay (27). Results for antibody titers are expressed as the geometric mean (GM) of the antibody titers and 95% confidence intervals.

PBMNC fatty acid composition. PBMNC for fatty acid analysis were isolated using Histopaque-1077 as discussed above. Cells were further washed twice with Dulbecco's phosphate buffered saline (PBS) resuspended in 1 mL PBS and layered over 1.5 mL Histopaque-1077. The tubes were centrifuged for 15 min at 1000 × *g* to remove contaminating erythrocytes. PBMNC were washed with PBS, mixed with 0.8 mL LYMPHO-KWIK (Canoga Park, CA) and incubated for 15 min at 37°C. The PBMNC were then overlaid with 0.2 mL PBS and centrifuged for 2 min at 2000 × *g*. The cell pellet was washed one more time with PBS and stored frozen at −20°C until fatty acid analysis. This isolation procedure removed most of contaminating platelets and erythrocytes, and yielded cells containing 90–95% mononuclear cells as determined by differential cell counting.

PBMNC lipids were extracted by the procedures previously described by Nelson (28,29) using chloroform/

methanol (2:1, vol/vol). The total lipid extract was transmethylated with methanolic HCl (7%, w/w) by the procedures described previously (30,31). The impurities extracted into the hexane phase after termination of the reaction were removed by thin-layer chromatography (29). We have described the conditions of the capillary gas-liquid chromatography (GLC) previously (30). Briefly, the column was a 30-m × 0.025 mm fused-quartz column coated with SP-2340 (Supelco, Inc., Bellefonte, PA). The GLC data were processed with a Hewlett-Packard ChemStation software running on an IBM compatible desktop computer.

Serum antioxidant vitamins. Fasting blood samples were collected from all subjects and sera were separated by centrifugation at 1000 × *g* for 15 min at 4°C. Aliquots of sera were stored under liquid nitrogen until antioxidant analysis. For the analysis of α-tocopherol and retinol in sera, 100 µL of each of the internal standards retinyl acetate and α-tocopherol acetate were transferred into disposable glass tubes and mixed vigorously with 200 µL of sera. For extraction of the antioxidants 400 µL of hexane was added and the contents were mixed vigorously for 1 min. The tubes were centrifuged and the solvent layer was transferred to a centrifuge tube and the contents were evaporated under a stream of nitrogen. The antioxidants in the tube were dissolved in 25 µL of diethyl ether followed by 75 µL of methanol, and 20 µL of the sample solution was injected for high-performance liquid chromatography (HPLC) analysis. The HPLC conditions for α-tocopherol and retinol were as follows: A Waters LC with a stainless steel column packed with microBondapak C18 (10 µm, 300 mm × 3.9 i.d.) as the solid phase was used. The mobile phase was methanol/water (97:3, vol/vol) at a flow rate of 1.0 mL/min. The eluate was monitored with an ultraviolet (UV) detector at 280 nm.

For ascorbic acid, serum proteins were precipitated with 60% methanol in water and 1 mM EDTA. Serum (50 µL) was mixed with 200 µL of 60% methanol/water/ EDTA, incubated for 10 min on ice, centrifuged at 12,000 × *g* at 4°C for 8 min, and then filtered. A 20 µL sample of filtrate was immediately analyzed for L-ascorbic acid. A stainless steel column packed with Inersil-ODS (5 mm, 150 mm × 4.6 mm i.d., Gaskurokogyo Ltd., Japan) was used. The mobile phase was methanol/phosphate buffer containing 0.005 M tetra-*n*-butylammonium bromide (20:80, vol/vol) at a flow rate of 0.5 mL/min. The eluate was monitored at 265 nm with a UV detector.

For serum β-carotene, serum was twice deproteinized with ethanol and then extracted with hexane. The extract was centrifuged, the hexane layer removed, and then evaporated with nitrogen. The dried extracts were dissolved in isopropanol for HPLC with a Nova-pak C_{18}. The mobile phase was acetonitrile/methanol/acetone (40:40:20, by vol). The eluate was monitored with a UV detector at 450 nm.

Data analysis. A repeated measure of analysis of variance model was used to determine the effects of DHA on the indices of IR tested. A univariate, split-plot approach was taken using SAS PROC Mixed (32). Contrasts were constructed for within-group comparisons among the stabilization and intervention periods. Paired *t*-test was used to compare PBMNC fatty acid composition and eicosanoid secretions. Changes in the parameters examined are considered significant for $P < 0.05$, unless otherwise stated.

RESULTS

Effect of DHA feeding on NK cell activity. Figure 1 contains the data for NK cell activity for the DHA group determined at the effector/target cell ratio of 50:1 on study days 30, 85, and 113. NK cell activity was not altered by dietary DHA within the first 55 d (study day 85) of its feeding ($P = 0.82$). However, DHA feeding for 83 d (study day 113) caused a 20% decrease in NK cell activity as compared to the activity found prior to the start of DHA feeding (day 30). This decrease in NK cell activity associated with DHA feeding was statistically significant ($P = 0.004$). NK cell activity for the control group did not change throughout the study. Similar results were obtained at the effector/target cell ratios of 25:1, 12.5:1, 6.2:1, and 3.1:1 (data not shown).

Effect of DHA feeding on the secretion of proinflammatory cytokines. The concentrations of IL-1β and TNFα secreted by PBMNC from the subjects in the DHA group are shown in Figures 2 and 3, respectively. DHA feeding caused a reduction in the secretion of both IL-1β and TNFα within 55 d (study day 85), although it was not statistically significant at that time ($P = 0.093$ for IL-1β and 0.31 for TNFα). With the continued feeding of DHA, the secretion of these cytokines continued to decrease. By the end of the study, their concentrations were reduced by approximately 40–45% as compared to the concentrations before DHA was fed ($P = 0.0004$ for IL-1β and 0.0002 for TNFα). In the control group, the secretion of these two cytokines did not change during the study.

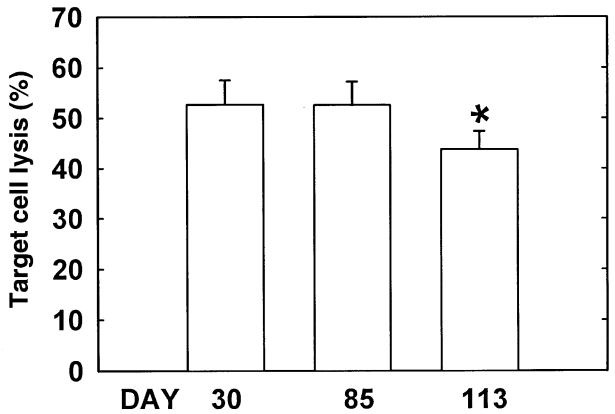

FIG. 1. Docosahexaenoic acid (DHA) feeding decreased natural killer (NK) cell activity. NK cell activity was determined with an effector/target cell ratio of 50:1 and data shown are the mean ± SEM ($n = 7$). NK cell activity was significantly reduced on day 113, compared to the value at day 30 ($P = 0.004$), but not on day 85. The corresponding values for percentage target cell lysis in the control group on days 30, 85, and 113 were 38.5 ± 14.0, 39.0 ± 14.0, and 41.0 ± 18.0, respectively.

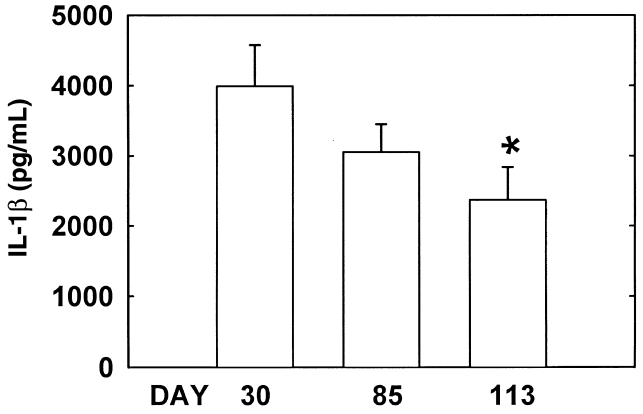

FIG. 2. DHA feeding reduced *in vitro* secretion of interleukin-1β (IL-1β). Peripheral blood mononuclear cells (PBMNC) were cultured with lipopolysaccharide (1.0 mg/L) for 24 h, and the IL-1β secreted was quantified with an enzyme-linked immunosorbent assay (ELISA). Data shown are the mean ± SEM (n = 7). DHA feeding reduced IL-1β secretion, when compared to the value at day 30 (P = 0.093 for day 85 and P = 0.0004 for day 113). The corresponding values for IL-1β secretion in the control group on days 30, 85, 113 were 2912 ± 294, 3104 ± 201 and 2981 ± 678, respectively. See Figure 1 for abbreviation.

DHA feeding and serum influenza antibody titers. The pre- (day 30) and postimmunization (day 113) antibody titers against the three strains of influenza virus for both groups of subjects are shown in Table 4. The preimmunization (day 30) mean titers for A/Johannesburg and B/Harbin strains between the two groups were not different, while the titer for A/Texas strain was about fourfold higher in the DHA group than in the control group. Immunization caused a significant increase (three- to tenfold) in the mean titers for all three strains in both the groups. The postimmunization titers for all three strains were not different between the two dietary groups. However, the differential increase between the two dietary groups after immunization by the A/Texas strain was almost significant (P = 0.059). This difference was most likely due to the higher initial antibody titer for this strain in the DHA group. Antibody titers for all three strains in both dietary groups on day 106 were not significantly different from titers on day 113 (data not shown).

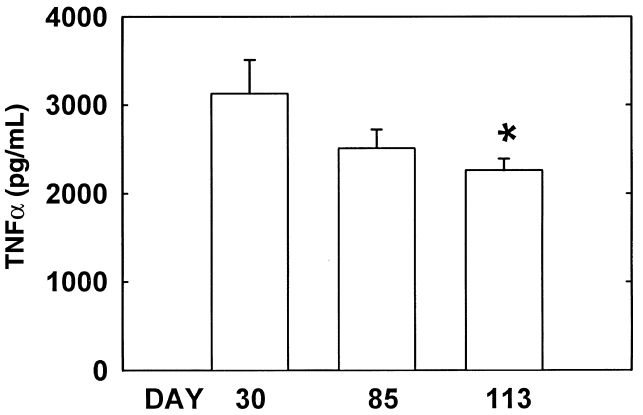

FIG. 3. DHA feeding reduced tumor necrosis factor α (TNFα) secretion. PBMNC culture and TNFα analysis procedures were similar to those given in Figure 2. DHA feeding reduced the secretion of TNFα, with P values of 0.31 and 0.0002, for days 85 and 113, respectively. The corresponding values for TNFα secretion in the control group on days 30, 85, and 113 were 2611 ± 526, 2834 ± 480, and 2648 ± 348, respectively. See Figures 1 and 2 for abbreviations.

TABLE 4
Effect of DHA Feeding on Serum Influenza Antibody Titers[a]

		DHA group (n = 7)		Control group (n = 4)	
Strain	Day	GM	CI	GM	CI
A/Texas	85	195	98–389	57	23–141
	113	585	278–1234	640	257–1596
A/Johannesburg	85	27	13–56	34	13–88
	113	109	51–234	135	51–354
B/Harbin	85	54	27–109	40	16–101
	113	148	70–311	160	63–405

[a]GM, geometric mean; CI, 95% confidence interval. For other abbreviation see Table 1. All subjects were immunized on day 85. Immunization significantly (P < 0.05) increased the antibody titers for all three strains in both groups. The difference in the increase between the DHA and control group was almost significant (P = 0.059) for A/Texas, but not for the other two strains.

Influence of DHA feeding on the fatty acid composition of PBMNC. Table 5 contains data regarding the major fatty acids of the PBMNC isolated on study days 30 and 113 for both dietary groups. PBMNC fatty acid composition on day 30 was not different between the two dietary groups. Dietary DHA increased the concentration of DHA in the PBMNC from 2.3 wt% on day 30 to 7.4 wt% on day 113. The arachidonic acid content of the PBMNC decreased from 19.8 to 10.7 wt% with DHA feeding. The EPA concentration in PBMNC lipids was 1.3 wt% and it did not change with DHA feeding (not shown). The concentration of major fatty acids in the PBMNC from control group (Table 5), as well as that of other trace fatty acids in both groups (data not shown), did not change during the study.

DHA feeding and eicosanoid secretion. The concentrations of PGE2 and LTB4 secreted into the tissue culture media by the PBMNC stimulated with LPS from both dietary groups are shown in Table 6. DHA feeding caused a 60–75% reduction in the secretion of both these eicosanoids when compared to the concentrations on day 30. Eicosanoids secreted by the PBMNC from control group did not change during the study.

TABLE 5
Major Fatty Acids (wt%) of Peripheral Blood Mononuclear Cells[a]

	DHA group (n = 7)		Control group (n = 4)	
Fatty acid	Day 30	Day 113	Day 30	Day 113
16:0	19.2 ± 1.1	18.4 ± 1.2	20.9 ± 1.6	18.9 ± 2.3
18:0	21.2 ± 1.1	18.0 ± 1.1	21.5 ± 0.3	19.1 ± 0.6
18:1n-9	8.0 ± 0.7	8.8 ± 0.8	7.5 ± 0.8	7.5 ± 2.2
18:2n-6	10.2 ± 0.4	12.1 ± 2.7	9.9 ± 0.3	8.7 ± 2.8
20:4n-6	19.8 ± 1.3	10.7 ± 0.8*	20.2 ± 1.4	15.4 ± 0.5
22:6n-3	2.3 ± 0.1	7.4 ± 1.0*	1.5 ± 0.2	1.4 ± 0.1

[a]Mean ± SEM. *P < 0.05. See Table 1 for abbreviation.

TABLE 6
Effect of Dietary DHA on *in vitro* Secretion of Eicosanoids by PBMNC[a]

	DHA group (n = 7)		Control group (n = 4)	
Eicosanoid	Day 30	Day 113	Day 30	Day 113
PGE2, ng/10^6 PBMNC	13.1 ± 2.0	5.0 ± 1.0*	15.1 ± 2.8	17.1 ± 3.0
LTB4, pg/10^6 PBMNC	140 ± 30	34 ± 10*	118 ± 41	92 ± 28

[a]Eicosanoids secreted into the medium (mean ± SEM) in 24 h after LPS (1 mg/L) addition. Abbreviations: PGE2, prostaglandin E2; LTB4, leukotriene, B4; PBMNC, peripheral blood mononuclear cells. For other abbreviation see Table 1. PGE2 was quantified by gas chromatography–mass spectrometry and LTB4 by enzyme-linked immunosorbent assay (ELISA). For each eicosanoid, comparisons were made between day 30 and 113 within each group, and those with the asterisk (*) are significantly different ($P < 0.01$).

TABLE 7
DHA Feeding and Serum Antioxidant Vitamins[a]

	DHA group (n = 7)		Control group (n = 4)	
Vitamin	Day 30	Day 113	Day 30	Day 113
Retinol, μg/mL	0.52 ± 0.06	0.48 ± 0.19	0.40 ± 0.08	0.44 ± 0.06
β-Carotene, μg/mL	78.2 ± 17.1	84.4 ± 17.3	95.3 ± 28.6	113.7 ± 19.9
α-Tocopherol, μg/mL	2.20 ± 0.12	1.82 ± 0.19*	2.37 ± 0.42	1.92 ± 0.44*
Ascorbate, mg/dL	0.48 ± 0.20	0.61 ± 0.28	0.36 ± 0.20	0.52 ± 0.32

[a]Mean ± SEM. *α-Tocopherol concentration on day 113 was lower in both groups than the corresponding values on day 30 ($P < 0.04$); the concentrations of other three vitamins did not change. For abbreviation see Table 1.

In both groups, the concentrations of PGE2 secreted by 1×10^6 PBMNC was about 100-fold higher than that of LTB4.

Effect of DHA feeding on the serum concentration of antioxidant vitamins. The concentrations of retinol, β-carotene, α-tocopherol, and ascorbate in the sera of the subjects in both dietary groups on study days 30 and 113 are shown in Table 7. None of these antioxidant vitamins except α-tocopherol changed during the study in either group. The concentration of α-tocopherol on day 113 was significantly decreased ($P < 0.04$) in both dietary groups when compared to the corresponding values on day 30.

DISCUSSION

This study was conducted to determine whether DHA feeding would inhibit select indices of human IR that have previously been reported to be inhibited with dietary EPA, fish, or flax seed oils. In contrast to most of the previous studies with fish oils, we maintained total fat intake constant, supplemented diets with D α-tocopherol acetate at 20 mg/d, and used diets devoid of EPA. All these factors are important in evaluating the effects of n-3 PUFA on human IR. In an attempt to determine the possible mechanisms underlying the effects of DHA feeding, we examined the fatty acid composition of PBMNC, the production of eicosanoids, the serum concentrations of apoprotein E and antioxidant vitamins.

DHA feeding caused a significant decrease in NK cell activity and the secretion of proinflammatory cytokines, IL-1β, and TNFα by monocytes stimulated with LPS. The time required for the decrease in these functions after DHA feeding was different. After 55 d of DHA feeding, cytokine production was inhibited, but not NK cell activity. However, the NK cell activity was decreased within 83 d of DHA feeding. Our results showing reduction in NK cell activity and the secretion of proinflammatory cytokines by dietary DHA are consistent with the results from earlier human studies involving EPA, fish, or flax seed oils (2–16). Reduction in NK cell activity may lower resistance to cancer and viral infections.

DHA feeding did not alter B-cell functions (Table 4). Nor did it change T-cell functions in this study, as previously reported by us (33). The amount of DHA fed was equivalent to 15–20 g fish oils, which has been found to inhibit T- and B-cell responses within 90 d of its feeding (8–14). Our dietary protocol differed from those used in previous investigations, because we held total fat constant and provided extra vitamin E. These factors may be responsible for our lack of an observed change in T- and B-cell functions.

Dietary DHA increased DHA concentration in PBMNC lipids from 2.3 to 7.4 wt%. It was incorporated into PBMNC lipids primarily by replacing arachidonic acid. Although DHA can be converted back to EPA by humans (34,35), and the serum concentration of EPA in the DHA group of our study subjects increased from 0.38 to 3.39 wt% (36), the concentration of EPA in PBMNC lipids was not altered by DHA feeding (data not shown).

Reduction in PBMNC arachidonic acid concentration with DHA feeding was associated with a concomitant decrease in the production of PGE2 and LTB4. Since PGE2 inhibits NK and macrophage cell functions (24), the decrease in NK cell activity and proinflammatory cytokine production could not be due to reduction in PGE2 production. These results are consistent with the results from other studies using inhibitors of eicosanoid synthesis (37–41). The decrease in immune cell functions was probably not due to increased oxidative stress either, because the serum concentration of retinol, β-carotene, and ascorbic acid were not altered by DHA feeding. The serum concentration of tocopherol decreased in both groups; therefore, it could not be attributed to DHA feeding.

Serum concentration of apoprotein E was not different between the two groups on study day 30 and did not change throughout the study in the control group (not shown). However, its concentration increased from 7.7 ± 1.6 (mean ± SEM) on day 30 to 12.0 ± 1.7 mg/dL on day 113 in the DHA group. Because apoprotein E has been reported to inhibit lymphocyte and monocyte functions (45), it is possible that it contributed to the reduction in NK cell activity and cytokine production in the DHA group. These alterations in immune cell functions may also result from a reduction in LTB4 production, which has been shown to enhance these functions *in vitro* (42,43).

It is possible that some of the effects observed in this study were due to a reduction in linoleic acid intake rather than the increase in DHA intake. The intake of linoleic acid was de-

creased by about 7 g/d in the high-DHA diet compared to the basal diet. The subjects on the high-DHA diet still received more than 20 g/d linoleic acid, an amount of substrate in excess of that required for the fatty acid metabolic acid pathways. It was well above any known requirements for linoleic acid. The plasma linoleic acid content in the basal and high-DHA diets fed subjects was 39 and 36% of total fatty acids, respectively, and was not significantly different (36). The basal diet provided less than 50 mg/d of DHA , and the high-DHA diet provided 6 g/d. Thus it is unlikely that any of the change in immune cell function or eicosanoid production was due to a 20% reduction in linoleic acid intake. These were most likely the result of an increase in DHA intake.

Results from this study show that feeding DHA to humans inhibited select immune cell functions, but it seems to be a less potent and selective inhibitor than that predicted by previous results obtained with fish oils. Although the subjects in the DHA group in our study did not show increased incidence of infections, they may be potentially at a higher risk. The risk for infections may further increase with the increased duration of DHA feeding, increased total fat intake, or increased oxidative stress. The antiinflammatory effects of DHA may be useful in the management of autoimmune disorders; however, such benefits need to be balanced with the increased risk of infections.

ACKNOWLEDGMENTS

The authors are appreciative of the support provided by the dietary and nursing staff of the Metabolic Research Unit in conducting this study. We thank the volunteers who participated in the study, the bioanalytical laboratory for sample processing, Health Services Department of California in Berkeley for determining influenza antibody titers, and the Martek Corporation for donating the DHASCO oil.

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[Received December 18, 1998, and in revised form and accepted February 18, 1999]