Dietary supplementation with eicosapentaenoic acid, but not with other long-chain n−3 or n−6 polyunsaturated fatty acids, decreases natural killer cell activity in healthy subjects aged >55 y[1–3]

Frank Thies, Gerhard Nebe-von-Caron, Jonathan R Powell, Parveen Yaqoob, Eric A Newsholme, and Philip C Calder

ABSTRACT

Background: Animal studies showed that dietary flaxseed oil [rich in the n−3 polyunsaturated fatty acid α-linolenic acid (ALA)], evening primrose oil [rich in the n−6 polyunsaturated fatty acid γ-linolenic acid (GLA)], and fish oil [rich in the long-chain n−3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] can decrease natural killer (NK) cell activity. There have been no studies of the effect on NK cell activity of adding these oils to the diet of humans.

Objective: Our objective was to determine the effect of dietary supplementation with oil blends rich in ALA, GLA, arachidonic acid (AA), DHA, or EPA plus DHA (fish oil) on the NK cell activity of human peripheral blood mononuclear cells.

Design: A randomized, placebo-controlled, double-blind, parallel study was conducted. Healthy subjects aged 55–75 y consumed 9 capsules/d for 12 wk; the capsules contained placebo oil (an 80:20 mix of palm and sunflower seed oils) or blends of placebo oil and oils rich in ALA, GLA, AA, DHA, or EPA plus DHA. Subjects in these groups consumed 2 g ALA, 770 mg GLA, 680 mg AA, 720 mg DHA, or 1 g EPA plus DHA (720 mg EPA + 280 mg DHA) daily, respectively. Total fat intake from the capsules was 4 g/d.

Results: The fatty acid composition of plasma phospholipids changed significantly in the GLA, AA, DHA, and fish oil groups. NK cell activity was not significantly affected by the placebo, ALA, GLA, AA, or DHA treatment. Fish oil caused a significant reduction (mean decline: 48%) in NK cell activity that was fully reversed by 4 wk after supplementation had ceased.

Conclusion: A moderate amount of EPA but not of other n−6 or n−3 polyunsaturated fatty acids can decrease NK cell activity in healthy subjects. *Am J Clin Nutr* 2001;73:539–48.

KEY WORDS Fish oil, immunity, natural killer cell, n−6 fatty acid, n−3 fatty acid, polyunsaturated fatty acids, α-linolenic acid, γ-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, arachidonic acid

INTRODUCTION

Natural killer (NK) cells are a class of lymphocytes found mainly in the blood and spleen. They are derived from the bone barrow but are not T or B lymphocytes and do not undergo thymic maturation. They are involved in the natural immune response to virus-infected and tumor cells and play a role in graft rejection (1). NK cell activity declines with age in both humans and laboratory animals (2, 3).

A reduction in fat intake (to <30% or 22% of total energy) is associated with a significant increase in the NK cell activity of human peripheral blood mononuclear cells (PBMCs; a mixture of lymphocytes including NK cells and monocytes) (4, 5), suggesting that high fat consumption suppresses NK cell activity. This is supported by some studies in laboratory rodents (6) but not by others (7). Animal feeding studies show that the type of fatty acid in the diet affects NK cell activity; n−3 polyunsaturated fatty acids (PUFAs) appear to have particularly potent effects. Increasing the amount of α-linolenic acid (ALA; 18:3n−3) in a rat diet lowers spleen NK cell activity compared with that measured after feeding diets rich in linoleic acid (8, 9). Feeding rats or mice diets containing large amounts of fish oil [FO; which is rich in eicosapentaenoic acid (EPA; 20:5n−3) and docosahexaenoic acid (DHA; 22:6n−3)] results in suppressed spleen NK cell activity compared with that measured after feeding low-fat diets or high-fat diets rich in saturated fat or n−6 PUFAs (10–15). Feeding rats diets rich in γ-linolenic acid (GLA; 18:3n−6) also lowers spleen NK cell activity compared with that measured after feeding some other diets (13, 14). These studies are supported by cell culture studies in which human NK cell activity was suppressed by culture of the cells with GLA (16, 17), EPA (17–19), and DHA (17–19).

Despite these cell culture and animal feeding studies, few investigations have been conducted on the effect of dietary fatty

[1] From the Department of Biochemistry, University of Oxford, Oxford, United Kingdom; Unilever Research Colworth Laboratory, Sharnbrook, United Kingdom; The Hugh Sinclair Unit of Human Nutrition, Department of Food Science and Technology, University of Reading, Reading, United Kingdom; and The Institute of Human Nutrition, University of Southampton, Southampton, United Kingdom.

[2] Supported by a grant to EAN and PCC from the Ministry of Agriculture, Fisheries and Food; by Unilever Research Colworth Laboratory; and by Nutricia Research under the Agro-Food LINK Programme (AFQ51). K562 cells were a gift from the Sir William Dunn School of Pathology, University of Oxford.

[3] Address reprint requests to PC Calder, The Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, United Kingdom. E-mail: pcc@soton.ac.uk.

Received March 31, 2000.

Accepted for publication July 28, 2000.

TABLE 1
Characteristics of the treatment groups[1]

	Placebo (n = 8)	ALA (n = 8)	GLA (n = 7)[2]	AA (n = 8)	DHA (n = 8)	FO (n = 7)[2]
Male:female	5:3	4:4	3:4	4:4	5:3	3:4
No. of smokers	1	0	0	0	0	0
Age (y)	62 (56–69)[3]	66 (60–74)	64 (55–71)	61 (56–70)	65 (58–71)	62 (60–68)
BMI (kg/m^2)	25.1 (21.3–29.7)	25.5 (22.2–28.0)	23.3 (18.6–27.1)	24.1 (21.3–27.3)	23.5 (19.6–28.4)	26.7 (22.8–31.1)

[1] ALA, α-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; FO, fish oil.
[2] Excludes data for one woman who dropped out of the study.
[3] $\bar{x}$; range in parentheses.

acids on human NK cell activity. If effects such as those identified in cell culture and animal feeding experiments occur in humans, there could be implications for the safety and clinical applications of the fatty acids concerned. Thus, it seems important to ascertain the effects of fatty acid supplementation on human NK cell activity. We therefore studied the effect of moderate supplementation with encapsulated oil blends rich in ALA, GLA, arachidonic acid (AA; 20:4n−6), DHA, or EPA plus DHA on NK cell activity in healthy, free-living subjects aged >55 y.

SUBJECTS AND METHODS

Materials

Phosphate-buffered saline (PBS) tablets were obtained from Unipath Ltd (Basingstoke, United Kingdom). Histopaque, bovine serum albumin (fatty acid free), HEPES-buffered RPMI medium, glutamine, antibiotics (penicillin and streptomycin), and propidium iodide were obtained from Sigma Chemical Co Ltd (Poole, United Kingdom). Fluorescent-labeled monoclonal antibodies (anti-CD45/CD14 and anti-CD3/CD16/CD56) were purchased from Coulter Corp (Hialeah, FL). FACS-lysing solution was purchased from Becton Dickinson (Mountain View, CA). Cytotoxicity kits were purchased from Proteins International Inc (Rochester, MI). Solvents were purchased from Fisher Scientific (Loughborough, United Kingdom).

Subjects and study design

Ethical permission for all procedures involving human volunteers was obtained from the Central Oxford Research Ethics Committee (COREC no. 96.182). All volunteers completed a health and lifestyle questionnaire before entering the study and a doctor's consent for inclusion in the study was obtained. Volunteers were excluded if they were taking any prescribed medication; had diagnosed hypercholesterolemia, hypertriglyceridemia, coronary artery disease, or diabetes; had a chronic inflammatory disease; took aspirin regularly; were vegetarian; or consumed FO, evening primrose oil, or vitamin capsules. The characteristics of the 46 subjects who completed the study are given in **Table 1**; the subjects' mean age and body mass index did not differ significantly among the groups. All subjects were white and lived in their own homes; none were disabled or immobile in any way. Twenty-seven subjects were employed full-time and 19 were retired. All female subjects were postmenopausal.

Forty-eight subjects were randomly allocated to receive in a double-blind fashion 6 types of encapsulated oil blends (n = 8 per treatment group): placebo, ALA, GLA, AA, DHA, and FO. The types and amounts of oils used in the blends are shown in **Table 2**. The fatty acid composition of the oil blends (as determined by gas chromatography of total lipid extracts) is shown in **Table 3**. The capsules were gelatin-coated, nontransparent, and green. Each capsule contained 445 mg of the oil blend and subjects consumed 9 capsules/d (ie, 4 g encapsulated oil/d) for 12 wk. Therefore, subjects in the ALA group consumed an extra 2.0 g ALA/d; subjects in the GLA, AA, and DHA groups consumed an extra 700 mg (approximately) GLA, AA, or DHA/d, respectively; and subjects in the FO group consumed an extra 1 g EPA plus DHA/d (720 mg EPA + 280 mg DHA). All capsules contained 300 μg α-tocopherol equivalents plus 180 μg ascorbyl-palmitate/g oil. Thus, all subjects consumed an extra 1.2 mg α-tocopherol/d. Two female subjects dropped out of the study, one from the GLA group because of illness

TABLE 2
Types and amounts of oils used to make the blends used in the study[1]

	Placebo	ALA	GLA	AA	DHA	FO
			% by wt of final oil blend			
Palm oil[2]	80	50	21	43	43	—
Sunflower seed oil[2]	20	13	5	11	11	—
Super refined flaxseed oil[2]	—	37	—	—	—	—
GLA-rich triacylglycerol[3]	—	—	74	—	—	—
AA-rich fungal oil[4]	—	—	—	46	—	—
DHA-rich fungal oil[5]	—	—	—	—	46	—
Chilean FO	—	—	—	—	—	100

[1] ALA, α-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; FO, fish oil.
[2] Supplied by Loders Croklaan Bv, Wormeveer, Netherlands.
[3] Supplied by Scotia Pharmaceuticals Ltd, Stirling, United Kingdom.
[4] ARASCO; supplied by Martek Corp, Columbia, MD.
[5] DHASCO; supplied by Martek Corp.

TABLE 3
Fatty acid composition of the oil blends used in the study[1]

	Placebo	ALA	GLA	AA	DHA	FO
			% by wt of total fatty acids			
Myristic acid	0	0	0	0	8.0	10.7
Palmitic acid	37.4	9.9	9.6	25.1	24.5	19.1
Palmitoleic acid	0	0	0	0	1.1	9.8
Stearic acid	4.0	2.8	1.1	4.9	2.4	3.2
Oleic acid	36.0	18.0	10.4	34.2	29.8	11.9
Linoleic acid	22.7	15.8	58.6	16.3	13.4	1.2
GLA	0	0	20.3	1.5	0	0.5
ALA	0.2	53.5	0.3	0.2	0.3	0.9
AA	0	0	0	17.9	0	1.4
EPA	0	0	0	0	0	18.8
DHA	0	0	0	0	19.1	7.4

[1] ALA, α-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; FO, fish oil.

and one from the FO group because of an inability to comply as a result of stomach upset.

Blood was sampled 4 wk before and immediately before the start of supplementation, every 4 wk during supplementation, and after a 4-wk washout period. Plasma phospholipid fatty acid composition, plasma concentrations of thiobarbituric acid–reactive substances (TBARS), and blood leukocyte numbers and subsets were not measured in the first baseline sample. NK cell activities were measured in both baseline samples; there were no significant differences between the 2 baseline samples and the average of the 2 measurements is reported here. Throughout this article, week 0 represents the baseline measurements; weeks 4, 8, and 12 the supplementation period; and week 16 the end of the washout period. All treatment groups completed the study in parallel. The study ran from June 1997 (early summer) to December 1997 (early winter).

The capsules were provided to the subjects in blister packs (9 capsules per pack; 7 blister packs per box) along with clear instructions on how they should be administered (3 capsules 3 times daily immediately before breakfast, lunch, and dinner). During the supplementation period subjects received fresh blister packs of capsules every 4 wk. Compliance was assessed by questionnaire and biochemically by determining the plasma phospholipid fatty acid composition.

Subjects completed a 7-d food diary. Habitual nutrient intakes were determined by using FOODBASE (V1.3; Institute of Brain Chemistry, London).

Preparation of PBMCs

Blood samples were collected into heparin-treated tubes and diluted 1:1 with PBS. The diluted blood was layered onto histopaque (density: 1.077 g/L; ratio of diluted blood to histopaque: 4:3) and centrifuged for 15 min at 800 × *g* and 20°C. The cells were washed once with PBS, resuspended in 2.5 mL PBS, and layered onto 5 mL histopaque. They were then centrifuged once more to achieve a lower degree of erythrocyte contamination, washed with PBS, and finally resuspended in the appropriate medium.

Measurement of NK cell activity

The activity of NK cells, in preparations of PBMCs, against the K562 target cell line was determined by measuring the release of cellular lactate dehydrogenase (LDH) on target cell lysis. PBMCs were prepared as described above and resuspended in HEPES-buffered RPMI medium supplemented with 2 mmol glutamine/L and antibiotics. K562 cells were washed 3 times and resuspended in HEPES-buffered RPMI medium supplemented with 10 g bovine serum albumin/L and antibiotics. PBMCs (1×10^6, 5×10^5, or 2.5×10^5/100 μL) and K562 cells (1×10^4/50 μL) were added to each well of a 96-well microtiter plate to give ratios of PBMCs to K562 cells of 100:1, 50:1, or 25:1 in a final volume of 150 μL. Maximal target cell lysis was assessed by incubating K562 cells with the lysing reagent provided in the cytotoxicity kit. Spontaneous release of LDH by PBMCs was assessed by incubating the PBMCs in the absence of target cells; spontaneous release of LDH by K562 cells was assessed by incubating the K562 cells in the absence of effector cells. Plates were centrifuged for 3 min at 100 × *g* and room temperature to bring effector and target cells into contact and were then incubated at 37°C in air:carbon dioxide (19:1) for 4 h. After incubation, 50 μL ice-cold RPMI medium was added to each well and the plates were centrifuged for 5 min at 400 × *g* and room temperature. A portion (100 μL) of the medium was removed from each well and transferred to the wells of a flat-bottomed 96-well plate. The activity of LDH in the medium was assayed according to the instructions provided by the manufacturer of the kit. Percentage cytolysis was calculated as follows:

$$\begin{aligned}\text{Percentage cytolysis} = (&\text{LDH release}\\ &- \text{PBMC spontaneous LDH release}\\ &- \text{K562 spontaneous LDH release})\\ &/(\text{maximum K562 LDH release}\\ &- \text{K562 spontaneous LDH release})\\ &\times 100 \qquad (1)\end{aligned}$$

Analysis of leukocyte numbers

Leukocyte numbers and subsets were analyzed only in the blood samples collected at weeks 0, 12, and 16. For the determination of lymphocyte subsets, whole blood (100 μL) was incubated with fluorescent-labeled monoclonal antibodies (20 μL) for 40 min at 12°C; the monoclonal antibodies used were anti-CD45/CD14 and anti-CD3/CD16/CD56. Erythrocytes were then lysed and the leukocytes fixed with 3 mL FACS-lysing solution for 10 min. Leukocytes were collected by centrifugation (250 × *g*, 5 min, 12°C), resuspended in 3 mL PBS, and centrifuged again. Finally, the leukocytes were resuspended in 1 mL PBS and analyzed in an XL/MCL flow cytometer (Coulter Corp). Fluorescence data were collected for 1×10^4 cells and were analyzed by using SYSTEM II software (Coulter Corp).

TABLE 4
Habitual fatty acid intakes of all subjects and of subjects in the different treatment groups during supplementation[1]

	All subjects	Placebo	ALA	GLA	AA	DHA	FO
				g/d			
Myristic acid	3.49 ± 0.19	3.62 ± 0.33	3.94 ± 0.40	3.53 ± 0.64	2.80 ± 0.30	4.44 ± 0.64	3.21 ± 0.43
Palmitic acid	14.90 ± 0.53	16.75 ± 0.34	16.72 ± 1.13	14.84 ± 1.52	13.75 ± 0.81	18.17 ± 1.53	13.74 ± 1.57
Palmitoleic acid	1.27 ± 0.05	1.29 ± 0.08	1.35 ± 0.07	1.22 ± 0.13	1.16 ± 0.07	1.48 ± 0.15	1.49 ± 0.13
Stearic acid	7.21 ± 0.27	7.26 ± 0.32	8.17 ± 0.60	7.17 ± 0.74	6.41 ± 0.60	8.19 ± 0.82	6.67 ± 0.71
Oleic acid	19.82 ± 0.67	21.23 ± 1.18	21.75 ± 1.53	19.86 ± 1.10	19.06 ± 1.54	22.82 ± 1.67	19.40 ± 2.79
Linoleic acid	9.79 ± 0.62	10.17 ± 0.80	10.22 ± 1.68	12.36 ± 1.79	10.34 ± 1.25	10.79 ± 2.10	9.70 ± 1.69
GLA	0.005 ± 0.001	0.005 ± 0.002[a]	0.005 ± 0.001[a]	0.775 ± 0.001[d]	0.064 ± 0.002[c]	0.005 ± 0.001[a]	0.024 ± 0.002[b]
ALA	0.89 ± 0.05	0.73 ± 0.05[a]	2.94 ± 0.17[b]	0.98 ± 0.14[a]	0.84 ± 0.07[a]	0.94 ± 0.08[a]	0.99 ± 0.19[a]
AA	0.15 ± 0.01	0.17 ± 0.03[a]	0.17 ± 0.02[a]	0.13 ± 0.02[a]	0.82 ± 0.02[b]	0.15 ± 0.02[a]	0.19 ± 0.02[a]
EPA	0.09 ± 0.01	0.11 ± 0.03[a]	0.05 ± 0.01[a]	0.09 ± 0.03[a]	0.11 ± 0.02[a]	0.10 ± 0.02[a]	0.80 ± 0.02[b]
DHA	0.15 ± 0.01	0.18 ± 0.05[a]	0.09 ± 0.02[a]	0.14 ± 0.04[a]	0.19 ± 0.03[a]	0.85 ± 0.02[c]	0.40 ± 0.03[b]

[1] $\bar{x}$ ± SEM; n = 46 (all subjects) or 7 or 8 (per treatment group). ALA, α-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; FO, fish oil. Means within a row with different superscript letters are significantly different (one-factor ANOVA): $P < 0.0001$ (GLA, ALA, AA, and EPA) and $P = 0.0003$ (DHA).

To determine leukocyte and lymphocyte numbers, whole blood (40 μL) was incubated with 2 mL FACS-lysing solution for 30 min to lyse the erythrocytes and fix the leukocytes. The leukocytes were then stained with propidium iodide (10 μL of a 1-g/L solution) and counted in an XL/MCL flow cytometer with a 60-μL volume stop setting. Absolute lymphocyte numbers were calculated by multiplying the total leukocyte number by the proportion of leukocytes staining $CD45^+/CD14^-$.

Analysis of fatty acid composition

Lipid was extracted from plasma with chloroform:methanol (2:1, by vol) and phospholipids were isolated by thin-layer chromatography by using a mixture of hexane:diethyl ether:acetic acid (90:30:1, by vol) as the elution phase. Fatty acid methyl esters were prepared by incubation with 14% boron trifluoride at 80°C for 60 min. Fatty acid methyl esters were isolated by solvent extraction, dried, and separated in a gas chromatograph (model 6890; Hewlett-Packard, Avondale, PA) fitted with a 30 m × 0.32 mm BPX70 capillary column with a film thickness of 0.25 μm. Helium at 1.0 mL/min was used as the carrier gas and the split-splitless injector was used with a split-splitless ratio of 20:1. Injector and detector temperatures were 275°C. The column oven temperature remained at 170°C for 12 min after sample injection and was programmed to then increase to 210°C in increments of 5°C/min and then remain at 210°C for 15 min. The separation was recorded with HP GC CHEM STATION software (Hewlett Packard). Fatty acid methyl esters were identified by comparison with standards run previously.

Measurement of plasma TBARS concentrations

Plasma TBARS were measured by incubating 100 μL plasma with 1.2 mL of 3.35 g thiobarbituric acid/L in 100 g trichloroacetic acid/L for 15 min at 95°C and recording the absorbance at 535 nm after cooling. TBARS were calculated by using an extinction coefficient of 1.56×10^5 $(mmol/L)^{-1} \cdot cm^{-1}$.

Statistical analysis

The sample size (ie, the number of subjects per treatment group) was calculated on the basis of measurements of NK cell activity and plasma TBARS concentrations made previously in our laboratory by use of the same methods as in this study. We determined that a sample size of 8 would detect a difference in NK cell activity (determined at a PBMC-to-K562 target cell ratio of 100:1) of = 25% at $P = 0.05$ with 80% power and a difference in plasma TBARS concentration of = 25% at $P = 0.05$ with 90% power.

Results are expressed as means ± SEMs for 7 or 8 subjects per treatment group. The statistical significances of treatment, of time, and of their interaction were determined by using two-factor repeated-measures analysis of variance (ANOVA). If the interaction between treatment and time was significant, the effects of treatment and of time were analyzed further by one-factor ANOVA with Bonferroni's correction for multiple comparisons. All statistical tests were performed by using SPSS (version 6.0; SPSS Inc, Chicago) and a P value < 0.05 was taken to indicate statistical significance.

RESULTS

Habitual nutrient intakes

There were no significant differences between men and women with respect to habitual intakes of total energy; of fat, carbohydrate, and protein as a percentage of dietary energy; of total fat and individual fatty acids (g/d); or of cholesterol, α-tocopherol, β-carotene, and vitamin C (data not shown). Men consumed significantly more alcohol than did women (14.1 ± 2.4 compared with 7.0 ± 1.7 g/d).

There were no significant differences among treatment groups with respect to habitual intakes of total energy (11.6 ± 0.6 MJ/d for the 46 subjects who completed the study); of fat, carbohydrate, and protein as a percentage of energy (32.8 ± 0.8%, 45.0 ± 0.8%, and 19.5 ± 0.9%, respectively); or of cholesterol (284.5 ± 13.5 mg/d), α-tocopherol (7.6 ± 0.5 mg/d), β-carotene (2.7 ± 0.5 mg/d), and vitamin C (140 ± 18 mg/d). Habitual intakes of individual fatty acids did not differ significantly among the treatment groups; data for all subjects combined are shown in **Table 4**. Likewise, habitual intakes of total saturated fatty acids, total monounsaturated fatty acids, total PUFAs, total n−6 PUFAs, and total n−3 PUFAs did not differ significantly among treatment groups (data not shown); the ratio of n−6 to n−3 PUFAs in the habitual diet also did not differ significantly among groups (9.0 ± 0.7).

Fatty acid intakes during treatment

Intakes of individual fatty acids during the treatment period were calculated by adding habitual intakes to intakes from the supplements (Table 4). Intakes of myristic (14:0), palmitic (16:0), palmitoleic (16:1n−7), stearic (18:0), oleic (18:1n−9), and linoleic (18:2n−6) acids were higher than habitual intakes in at least some treatment groups during supplementation, but not significantly so. However, intakes of these fatty acids were not significantly different among the treatment groups. In contrast, there were significant differences in intakes of ALA, GLA, AA, EPA, and DHA among the different treatment groups.

Fatty acid composition of plasma phospholipids

The fatty acid composition of plasma phospholipids was not significantly affected by the placebo or ALA treatment. ALA and GLA did not appear in plasma phospholipids, even in those subjects in the ALA and GLA groups. Two-factor ANOVA did not detect any significant effects of time or treatment on the proportions of palmitic acid (≈30% by wt of total fatty acids) or stearic acid (≈13% by wt of total fatty acids). There were effects of time and treatment on the proportions of oleic acid ($P = 0.015$ for effect of time and $P < 0.001$ for effect of treatment) and linoleic acid ($P < 0.001$ for effects of both time and treatment) in plasma phospholipids, although there were no significant time × treatment interactions. In contrast, there were significant time-dependent effects of treatment on the proportions of dihomo-γ-linolenic acid (DGLA; 20:3n−6), AA, EPA, and DHA in plasma phospholipids (two-factor ANOVA effects of both time and treatment, $P < 0.001$ in all cases; time × treatment interaction, $P < 0.004$ for DGLA and $P < 0.001$ for AA, EPA, and DHA). These effects were investigated further by one-factor ANOVA.

Effects of GLA supplementation

The proportion of DGLA in plasma phospholipids was significantly higher after 12 wk of GLA supplementation than at baseline (**Table 5**), and at this time point GLA was significantly higher in subjects in the GLA group than in each of the other groups, including the placebo group. The proportion of DGLA was significantly higher at weeks 4 and 8 in the GLA group than in the ALA, AA, DHA, and FO groups. GLA supplementation also increased the proportion of AA in plasma phospholipids, which was significantly higher after 12 wk supplementation than at baseline.

Effects of AA supplementation

The proportion of AA in plasma phospholipids was significantly higher after 4, 8, and 12 wk of AA supplementation than at baseline and after the washout period (Table 5). After 4 wk of washout the proportion of AA in plasma phospholipids was not significantly different from that observed at baseline. The proportion of AA was significantly higher at weeks 4, 8, and 12 in the AA group than in each of the other groups, including the placebo group. There was also a nonsignificant trend toward a lower proportion of EPA in the plasma phospholipids of subjects in the AA group after 12 wk of supplementation than at baseline.

Effects of DHA supplementation

The proportion of DHA in plasma phospholipids tended to increase after 4 wk of DHA supplementation (NS) and was significantly higher after 8 and 12 wk of DHA supplementation than at baseline and after washout (Table 5). After 4 wk of washout the proportion of DHA in plasma phospholipids was not significantly different from that at baseline. The proportion of DHA was significantly higher at weeks 8 and 12 in the DHA group than in each of the other groups, including the placebo group. The proportion of AA in plasma phospholipids after 8 wk was significantly lower in subjects in the DHA group than in each of the other groups.

Effects of FO supplementation

There was a significant increase in the proportion of EPA in the plasma phospholipids of subjects taking the FO supplement, such that the proportion of EPA was significantly higher after 4, 8, or 12 wk of supplementation than at baseline and was significantly higher in the FO group than in each of other groups, including the placebo group, after 4, 8, and 12 wk of supplementation (Table 5). This increase in the proportion of EPA in plasma phospholipids was near maximal after 4 wk of FO supplementation. The maximal increase in the proportion of EPA was ≈2-fold above baseline. There were trends toward an increase in the proportion of DHA and a decrease in the proportion of AA in plasma phospholipids but these were not significant.

Effect on plasma TBARS concentrations and on leukocyte numbers and subsets

There was no significant effect of any of the treatments on plasma TBARS concentrations (**Table 6**). There was also no significant effect of any of the treatments on the total number of leukocytes, lymphocytes, T lymphocytes, or NK cells/L blood (**Table 7**). Likewise, there was no significant effect of any of the treatments on the proportion of leukocytes as total lymphocytes or on the proportion of lymphocytes as T lymphocytes or NK cells.

Effects on NK cell activity

NK cell activity measured at each PBMC-to-K562 target cell ratio did not differ significantly among the treatment groups at baseline; data for the PBMC:K562 of 100:1 are shown in **Table 8**. There were no significant effects of time or treatment nor was there a significant time × treatment interaction for NK cell activity measured at a PBMC:K562 of 25:1 or 50:1. However at the PBMC:K562 of 100:1 there were significant effects of time and treatment ($P < 0.001$ and $P = 0.001$, respectively) and a significant time × treatment interaction ($P = 0.024$). One-factor ANOVA indicated that at this ratio NK cell activity was not significantly affected by treatment with placebo, ALA, GLA, AA, or DHA (Table 8). However, treatment with FO resulted in a significant decrease in NK cell activity, such that activity was significantly lower after 12 wk of supplementation than after 0 and 4 wk (Table 8 and **Figures 1** and **2**). After 4 wk of washout (ie, week 16), NK cell activity in the FO group had returned to baseline values; activity at 16 wk was significantly higher than at the end of supplementation (12 wk; Table 8 and Figure 1).

NK cell activity determined at a PBMC:K562 of 100:1 declined in all subjects in the FO group. The mean decline was 20% after 8 wk and 48% (range: 10–75%) after 12 wk of FO supplementation (Figure 1). The absolute change in NK cell activity determined at a PBMC:K562 of 100:1 after 12 wk of treatment with FO was significantly different from the absolute change in the placebo group (Table 8). Although NK cell activity determined at a PBMC:K562 of 50:1 and 25:1 was not significantly affected by treatment, there was a nonsignificant trend toward a decrease

TABLE 5
Effect of supplementation on the fatty acid composition of plasma phospholipids[1]

Treatment and week	Oleic acid	Linoleic acid	DGLA	ARA	EPA	DHA
			% by wt of total fatty acids			
Placebo						
0 (Baseline)	10.8 ± 0.6	24.3 ± 1.4	2.66 ± 0.25	8.56 ± 0.33	0.90 ± 0.10	3.37 ± 0.37
4	11.1 ± 0.7	21.8 ± 1.2	3.09 ± 0.14	8.54 ± 0.36	1.07 ± 0.11	3.26 ± 0.40
8	10.9 ± 0.5	21.3 ± 1.3	3.08 ± 0.17	9.20 ± 0.44	1.45 ± 0.34	3.03 ± 0.40
12	10.5 ± 0.6	22.0 ± 1.7	2.63 ± 0.27	9.01 ± 0.86	0.83 ± 0.08	3.28 ± 0.36
16 (Washout)	11.1 ± 0.4	21.3 ± 1.0	3.25 ± 0.21	8.85 ± 0.51	1.17 ± 0.20	3.27 ± 0.41
ALA						
0 (Baseline)	10.0 ± 0.8	24.9 ± 1.4	2.85 ± 0.12	8.21 ± 0.50	1.44 ± 0.24	3.59 ± 0.40
4	10.4 ± 0.6	22.1 ± 1.0	2.86 ± 0.13	9.38 ± 0.50	1.64 ± 0.30	3.53 ± 0.50
8	10.5 ± 0.4	22.6 ± 0.7	2.58 ± 0.10	9.60 ± 0.64	1.85 ± 0.38	3.60 ± 0.50
12	10.2 ± 0.5	22.8 ± 1.1	2.88 ± 0.21	9.26 ± 0.45	1.24 ± 0.16	3.28 ± 0.30
16 (Washout)	10.8 ± 0.5	22.3 ± 1.0	2.96 ± 0.17	9.44 ± 0.46	1.25 ± 0.07	2.94 ± 0.14
GLA						
0 (Baseline)	12.2 ± 1.0	23.6 ± 1.0	2.84 ± 0.25	8.44 ± 0.39	1.10 ± 0.11	3.84 ± 0.36
4	10.1 ± 0.5	20.4 ± 0.7	3.80 ± 0.24	9.49 ± 0.47	1.63 ± 0.33	4.60 ± 0.70
8	9.9 ± 0.5	20.7 ± 0.8	4.10 ± 0.33	9.44 ± 0.37	1.43 ± 0.30	4.09 ± 0.26
12	9.9 ± 0.7	22.2 ± 0.6	4.63 ± 0.52[2,3]	10.69 ± 2.14[2]	0.80 ± 0.11	3.16 ± 0.35
16 (Washout)	10.7 ± 0.8	21.4 ± 0.9	3.18 ± 0.31	9.08 ± 0.33	1.47 ± 0.17	3.60 ± 0.45
AA						
0 (Baseline)	10.5 ± 0.8	22.6 ± 1.2	2.43 ± 0.32	9.29 ± 0.41	1.45 ± 0.19	3.67 ± 0.33
4	10.1 ± 0.5	17.5 ± 0.9	2.53 ± 0.20	15.91 ± 0.25[2–4]	1.26 ± 0.24	3.26 ± 0.26
8	10.2 ± 0.6	18.9 ± 1.0	2.40 ± 0.12	16.15 ± 0.76[2–4]	1.20 ± 0.22	3.08 ± 0.30
12	9.4 ± 0.3	18.6 ± 1.4	2.30 ± 0.24	17.20 ± 0.49[2–4]	0.86 ± 0.17	3.71 ± 0.38
16 (Washout)	10.5 ± 0.4	20.8 ± 1.2	2.70 ± 0.24	10.23 ± 0.48	1.57 ± 0.39	3.84 ± 0.35
DHA						
0 (Baseline)	12.2 ± 0.7	23.4 ± 2.0	2.70 ± 0.28	7.94 ± 0.35	1.21 ± 0.24	3.48 ± 0.38
4	11.9 ± 0.4	21.4 ± 1.3	2.63 ± 0.17	7.72 ± 0.46	1.50 ± 0.22	4.79 ± 0.29
8	11.8 ± 0.6	21.5 ± 1.4	2.49 ± 0.27	6.71 ± 0.39[3]	1.50 ± 0.25	6.04 ± 0.21[2–4]
12	11.6 ± 0.6	22.2 ± 1.2	2.23 ± 0.19	7.40 ± 0.34	0.91 ± 0.15	6.66 ± 0.37[2–5]
16 (Washout)	11.6 ± 0.3	22.1 ± 1.2	2.66 ± 0.16	8.26 ± 0.36	1.18 ± 0.19	4.05 ± 0.43
FO						
0 (Baseline)	10.7 ± 0.3	21.4 ± 0.4	3.10 ± 0.20	9.50 ± 0.31	1.07 ± 0.09	3.32 ± 0.20
4	9.9 ± 0.5	20.1 ± 0.7	2.83 ± 0.22	9.36 ± 0.24	2.79 ± 0.31[2,3]	4.16 ± 0.20
8	9.9 ± 0.3	19.1 ± 0.7	2.99 ± 0.26	9.70 ± 0.29	3.20 ± 0.44[2,3]	4.70 ± 0.70
12	9.6 ± 0.3	20.0 ± 1.5	2.59 ± 0.29	9.27 ± 0.60	3.53 ± 0.47[2,3]	4.40 ± 0.20
16 (Washout)	10.6 ± 0.4	19.2 ± 1.1	3.18 ± 0.19	10.12 ± 0.45	2.40 ± 0.61	3.98 ± 0.50

[1] $\bar{x}$ ± SEM; n = 7 or 8 per treatment group. ALA, α-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; FO, fish oil. Treatment × time interaction (two-factor ANOVA): $P < 0.004$ (DGLA) and $P < 0.001$ (ARA, EPA, and DHA).

[2] Significantly different from baseline, $P < 0.003$.

[3] Significantly different from all other groups, including placebo, at the same time point, $P < 0.045$.

[4] Significantly different from the end of the washout period, $P < 0.0001$.

[5] Significantly different from week 4, $P < 0.0001$.

after 12 wk of FO supplementation (mean decrease of 25% at both ratios) (Figure 2).

DISCUSSION

Daily supplementation of the diet of healthy elderly subjects with ≈700 mg GLA, AA, or DHA or 1 g EPA plus DHA significantly altered plasma phospholipid fatty acid composition, with changes apparent after 4 wk of supplementation. Despite the marked changes in fatty acid composition caused by the GLA, AA, and DHA treatments, these treatments did not significantly alter the NK cell activity of PBMCs. In contrast, supplementation with FO for 12 wk significantly decreased NK cell activity measured at a PBMC-to-K562 target cell ratio of 100:1 and tended to decrease NK cell activity measured at the ratios of 50:1 and 25:1.

The habitual intake of ALA by the subjects in the current study was 0.7–1 g/d, which is consistent with other reports for the UK adult population (20, 21). The amount of ALA included in the supplement (2 g/d) increased total ALA intake by 2- to 2.8-fold to 3 g/d. ALA was absent from plasma phospholipids in most subjects at baseline and did not increase significantly in the subjects in the ALA group. The products of ALA elongation and desaturation (EPA and DHA) were also not significantly elevated in plasma phospholipids in the ALA group. These observations are consistent with those of a recent study in which an increase in ALA intake from 0.9 to 3.7 g/d for 28 d did not significantly alter the proportions of ALA, EPA, or DHA in plasma phospholipids (22). Thus, it appears that when ALA is included in the diet at moderate amounts it is not incorporated into plasma phospholipids in significant amounts. Furthermore, if it is elongated

TABLE 6
Plasma concentrations of thiobarbituric acid–reactive substances (TBARS)[1]

Week	Placebo	ALA	GLA	AA	DHA	FO
			μmol/L			
0 (Baseline)	4.9 ± 0.5	4.2 ± 0.4	4.5 ± 0.6	5.4 ± 0.6	4.1 ± 0.2	4.8 ± 0.7
4	5.2 ± 0.5	3.9 ± 0.3	4.1 ± 0.3	4.5 ± 0.7	4.2 ± 0.3	3.9 ± 0.6
8	4.9 ± 0.4	3.7 ± 0.3	4.3 ± 0.5	4.5 ± 0.3	4.0 ± 0.5	4.4 ± 0.3
12	4.0 ± 0.4	3.9 ± 0.3	4.3 ± 0.5	4.0 ± 0.4	3.8 ± 0.3	3.7 ± 0.5
16 (Washout)	4.4 ± 0.5	3.8 ± 0.4	4.3 ± 0.5	4.3 ± 0.4	3.9 ± 0.2	4.3 ± 0.5

[1] $\bar{x}$ ± SEM; n = 7 or 8 per treatment group. ALA, α-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; FO, fish oil.

and desaturated, the products of this are not preferentially incorporated into plasma phospholipids.

Habitual intakes of GLA have not been reported and GLA is not mentioned in the UK Department of Health dietary reference values (21). It seems likely that GLA intake is normally negligible; habitual intake of GLA among subjects in the current study was ≈5 mg/d. Thus, the GLA supplement used in the current study (770 mg/d) represented a significant increase (>150-fold) in GLA intake. Nevertheless, GLA did not appear in plasma phospholipids. Amounts of the elongation product of GLA, DGLA, were increased during GLA supplementation, however, suggesting that some of the GLA is elongated before incorporation into phospholipids. These observations are consistent with those of Johnson et al (23), who found no increase in serum GLA concentrations after 3 wk of supplementation with 1.5 g GLA/d but an increase in serum DGLA. Wu et al (24) recently reported a small increase in the proportion of GLA in total plasma lipids (from 0.5% to 0.8% by wt of total fatty acids) in healthy subjects given ≈700 mg GLA/d for 8 wk. The reason for the difference between the results of Wu et al (24) and those of the current study may relate to the nature of the lipid fraction studied: Wu et al reported the fatty acid composition of total plasma lipids, whereas we report the composition of the plasma phospholipid fraction. It is possible that GLA is found in higher proportions in the triacylglycerol and cholesteryl ester fractions than in the phospholipid fraction, in which case it would be more readily detected in total lipids than in purified phospholipids. Observations of Johnson et al (23) support this explanation: in that study, the amount of GLA in neutral lipids (tri- and diacylglycerols and cholesteryl esters) was higher than the amount in the phospholipid fraction.

The habitual AA intake of subjects in the current study was in the range of 100–200 mg/d, which is consistent with earlier reports for other Western populations (25–27). The amount of AA provided in the supplement in the current study (680 mg/d) increased AA intake almost 5-fold. This resulted in significant enrichment of plasma phospholipids with AA, which was

TABLE 7
Circulating total lymphocyte and lymphocyte subpopulations[1]

Cell and week	Placebo	ALA	GLA	AA	DHA	FO
			× 10^9/*L blood*			
Leukocytes						
0 (Baseline)	5.225 ± 0.457	4.614 ± 0.491	4.271 ± 0.507	3.329 ± 0.233	3.943 ± 0.329	4.260 ± 0.360
12	4.763 ± 0.439	4.100 ± 0.464	4.171 ± 0.509	3.471 ± 0.210	4.229 ± 0.460	4.300 ± 0.685
16 (Washout)	5.613 ± 0.687	4.457 ± 0.203	4.600 ± 0.567	3.743 ± 0.339	4.171 ± 0.362	4.840 ± 0.496
Lymphocytes[2]						
0 (Baseline)	1.664 ± 0.235	1.434 ± 0.133	1.372 ± 0.183	1.130 ± 0.158	1.375 ± 0.233	1.236 ± 0.090
12	1.393 ± 0.195	1.293 ± 0.231	1.181 ± 0.151	1.111 ± 0.128	1.350 ± 0.235	1.398 ± 0.101
16 (Washout)	1.342 ± 0.144	1.210 ± 0.148	1.192 ± 0.160	1.088 ± 0.076	1.527 ± 0.309	1.094 ± 0.091
T lymphocytes[3]						
0 (Baseline)	1.191 ± 0.181 (72.1)[4]	1.039 ± 0.114 (72.5)	1.060 ± 0.178 (77.3)	0.865 ± 0.143 (76.5)	1.056 ± 0.182 (76.8)	0.877 ± 0.047 (71.0)
12	1.061 ± 0.172 (76.2)	0.933 ± 0.154 (72.2)	0.930 ± 0.137 (78.7)	0.855 ± 0.116 (77.0)	0.972 ± 0.194 (72.0)	0.991 ± 0.084 (70.9)
16 (Washout)	0.951 ± 0.129 (70.9)	0.873 ± 0.114 (72.1)	0.902 ± 0.142 (75.7)	0.843 ± 0.060 (77.5)	1.139 ± 0.258 (74.6)	0.773 ± 0.050 (70.7)
NK cells[5]						
0 (Baseline)	0.226 ± 0.049 (13.6)	0.192 ± 0.042 (13.4)	0.183 ± 0.049 (13.3)	0.139 ± 0.020 (12.3)	0.180 ± 0.050 (13.0)	0.189 ± 0.057 (15.3)
12	0.169 ± 0.029 (12.1)	0.158 ± 0.032 (12.2)	0.151 ± 0.021 (12.8)	0.129 ± 0.018 (11.6)	0.245 ± 0.067 (18.1)	0.223 ± 0.035 (16.0)
16 (Washout)	0.222 ± 0.054 (16.5)	0.167 ± 0.035 (13.8)	0.179 ± 0.026 (15.0)	0.132 ± 0.022 (12.1)	0.242 ± 0.058 (15.8)	0.176 ± 0.040 (16.0)

[1] $\bar{x}$ ± SEM; n = 7 or 8 per treatment group. ALA, α-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; FO, fish oil.
[2] Calculated from the percentage of total leukocytes staining $CD45^+/CD14^-$.
[3] Determined as lymphocytes staining $CD3^+$.
[4] Values in parentheses are the percentage of total lymphocytes.
[5] Determined as lymphocytes staining $CD3^-/CD56^+/CD16^+$.

TABLE 8
Natural killer cell activity of peripheral blood mononuclear cells (PBMCs)[1]

Week	Placebo	ALA	GLA	AA	DHA	FO
			% cytolysis			
0 (Baseline)	30.2 ± 5.4	37.9 ± 3.0	35.9 ± 4.2	27.4 ± 4.1	34.6 ± 4.8	35.1 ± 1.5
4	29.2 ± 5.6	35.9 ± 5.1	32.4 ± 5.8	26.9 ± 4.2	36.1 ± 5.4	34.7 ± 1.4
8	32.4 ± 5.5	33.0 ± 2.7	37.2 ± 5.1	27.6 ± 6.4	36.8 ± 6.4	27.4 ± 3.4
12	30.4 ± 5.8	32.7 ± 4.9	28.8 ± 5.3	27.0 ± 5.0	34.2 ± 5.2	19.8 ± 3.2[2]
16 (Washout)	30.8 ± 6.4	35.6 ± 6.0	36.2 ± 4.6	28.8 ± 5.3	41.4 ± 7.4	34.5 ± 3.9
12 − 0	0.8 ± 3.1	−4.8 ± 6.2	−6.9 ± 3.4	−2.8 ± 1.2	1.6 ± 4.2	−14.3 ± 2.1[3]

[1] $\bar{x}$ ± SEM; n = 7 or 8 per treatment group. Values are percentage cytolysis at a PBMC:K562 of 100:1. ALA, α-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; FO, fish oil. Treatment × time interaction, $P = 0.024$ (two-factor ANOVA).
[2] Significantly different from baseline, week 4, and the end of the washout period, $P < 0.05$.
[3] Significantly different from placebo, $P = 0.008$ (one-factor ANOVA).

increased from ≈9% to ≈17% by wt of total fatty acids. This change is consistent with a recent study that reported that providing healthy young men with 1.5 g AA/d for 50 d increased the proportion of AA in plasma phospholipids from ≈10% to ≈18% by wt of total fatty acids (28).

Habitual intake of EPA plus DHA by subjects in the current study was 250–300 mg/d, which is in accordance with other reports for UK adults (20, 21). The amount of EPA plus DHA provided in the FO supplement (1 g/d) increased total EPA plus DHA intake by 4-fold. Intake of both EPA and DHA was increased in the FO group (by 8- and 1.7-fold, respectively) and there was a 2-fold increase in the proportion of EPA in plasma phospholipids and a nonsignificant trend toward increased DHA in this group. In the subjects in the DHA group, the intake of DHA increased ≈5.5-fold, whereas that of EPA did not change. Supplementation with this amount of DHA increased the proportion of DHA in plasma phospholipids without affecting that of EPA. Although it is often thought that FO supplementation decreases the proportion of AA in phospholipids, this was not observed in this study. This agrees with the observations of Allard et al (29), who found that supplementation of the diet with 5.3 g EPA plus DHA/d (ie, 5 times more EPA plus DHA than used in the current study) for 6 wk did not significantly decrease the proportion of AA in plasma phospholipids.

There was no significant effect of ALA, GLA, AA, or DHA on human NK cell activity. The lack of effect of AA is consistent with the observations of Kelley et al (30), who reported no significant effect on NK cell activity after 55 d of supplementation of the diet of young men with 1.5 g AA/d. Also, 6 g DHA/d as part of a low-fat diet for 55 d did not alter human NK cell activity, although NK cell activity decreased (by 20%) after 83 d (31). In the current study, we observed no significant effect on NK cell activity of 720 mg DHA/d for 12 wk. Another study found no significant effect of GLA, AA, or DHA in amounts of 4.4% by wt of total dietary fatty acids on rat spleen NK cell activity after 6 wk (32). The results of that animal study (32), the earlier human studies involving AA and DHA (30, 31), and the current study indicate that significantly increasing the amounts of ALA, GLA, AA, and DHA in the human diet will not compromise NK cell activity. Nevertheless, higher amounts of these fatty acids in the diet might impair NK cell activity, as shown by Kelley et al (31) for DHA.

The current study is the first to report decreased NK cell activity after FO supplementation of the human diet, although Yamashita et al (19) reported a significant decrease in NK cell activity 24 h after infusion of an EPA-rich triacylglycerol into human volunteers. The reduction we observed in NK cell activity after FO supplementation (providing 720 mg EPA plus 280 mg DHA/d) but not after supplementation with 720 mg DHA/d strongly suggests that EPA but not DHA was responsible for the effect. Alternatively, the effect of FO on NK cell activity may have been due to a component found in FO other than the long chain n−3 PUFAs. However, in support of an effect of EPA, rather than of DHA or a contaminant, are data showing that a diet containing EPA as 4.4% by wt of total fatty acids inhibited rat spleen NK cell activity, whereas a diet containing the same amount of DHA did not (32).

One concern with recommendations to increase intakes of certain long-chain PUFAs (either in the general population or in specific groups such as pregnant women and newborns) and with the widespread availability of products rich in some of these PUFAs is the potential for adverse immunologic effects resulting from excessive intakes. However, the current study indicates that significantly increased intakes of ALA, GLA, AA, and DHA may not adversely affect immune function. However, this study

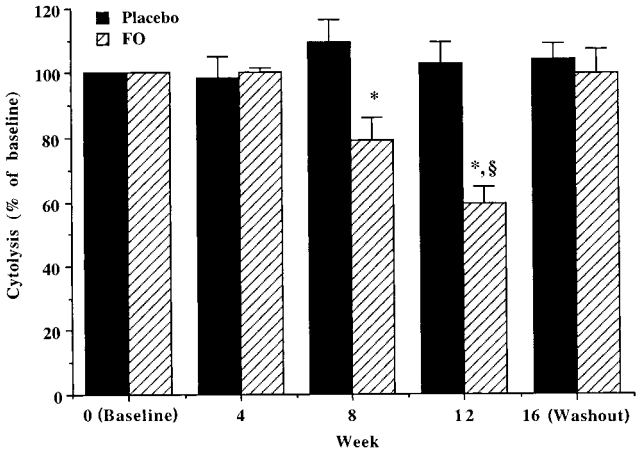

FIGURE 1. Time-dependent changes in natural killer (NK) cell activity in the placebo and fish oil (FO) groups. Data are expressed as mean (±SEM) NK cell activity (determined at a ratio of peripheral blood mononuclear cells to K562 target cells of 100:1) as a percentage of activity at baseline (n = 7 or 8). *Significantly different from placebo, $P = 0.045$ at week 8 and $P = 0.028$ at week 12. §Significantly different from baseline, week 4, and the end of the washout period, $P = 0.031$.

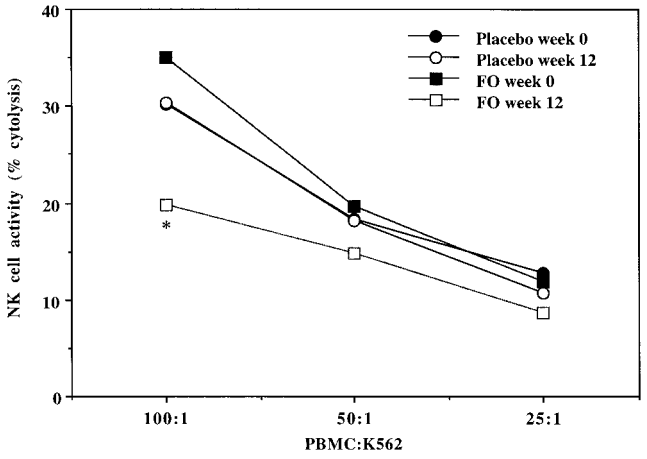

FIGURE 2. Mean natural killer (NK) cell activity in the placebo and fish oil (FO) groups before supplementation (week 0) and at the end of supplementation (week 12). NK cell activity was determined at a ratio of peripheral blood mononuclear cells to K562 target cells (PBMC:K562) of 25:1, 50:1, and 100:1 (n = 7 or 8); error bars were omitted for clarity. *Significantly different from baseline (week 0), P = 0.034.

was of subjects aged >55 y and extrapolation of our results to other groups such as pregnant women and newborn infants may not be appropriate.

Although the inhibition of NK cell activity by EPA (in FO) was fully reversible, this effect suggests that increased EPA intake could have some adverse immunologic effects. NK cells play an important role in host defense against virus infection (33) and in immunosurveillance against tumor cells (34). Thus, it might be inappropriate for groups at risk of viral infection and some cancers to increase their intake of EPA. Other studies showed that increased intakes of EPA can decrease T lymphocyte functions such as proliferation and interleukin 2 production in certain populations (35, 36). It will be important to conduct studies to identify whether increased intakes of FO alter rates of infection in humans. The results of some animal studies indicated that a high intake of FO results in decreased resistance to infectious agents (37–41), although some other animal studies reported that FO does not alter (42), or even increases (43), resistance to some pathogens.

There may be some clinical situations in which an EPA-induced decrease in NK cell activity might be beneficial. Rejection of transplanted bone marrow and organs is in part mediated by NK cells (44) and so a transient decrease in NK cell activity might be useful before and soon after transplantation, although this might provide an opportunity for viral infection and tumor growth. FO prolongs the survival of cardiac transplants in rats (45–47) and renal transplants survive or function better in patients who receive FO (48–51). It is tempting to suggest that a part of this improvement is due to EPA-induced inhibition of NK cell activity, but none of these studies measured NK cell activity in the patients.

The plasma TBARS concentrations observed in this study compare well with the value of 4.0 ± 0.4 μmol/L reported by Meydani et al (52) for 10 women aged 51–71 y. In the current study, none of the treatments, including FO, significantly changed plasma TBARS concentrations. Therefore, it appears that oxidative stress resulting in lipid peroxidation was not induced by any of the treatments in the current study and that lipid peroxidation did not play a role in the alteration in NK cell activity observed after treatment with FO. However, some studies reported that the suppressive effects of FO on T lymphocyte functions can be prevented by adding suitable amounts of α-tocopherol to the diet (53, 54). Therefore, a role of oxidant stress in eliciting the observed decrease in NK cell activity cannot be totally excluded and warrants further investigation.

We thank Paul Quinlan (Unilever Research Colworth Laboratory), Gert Meijer (Unilever Vlaardingen), and Jacques Bindels and Monique Al [Nutricia (now Numico) Research] for their contributions to the study and Ria Banerjee for technical assistance.

REFERENCES

1. Lewis CE, McGee JO'D. The natural killer cell. Oxford, United Kingdom: Oxford University Press, 1992.
2. Penschow J, Mackay IR. NK and K cell activity of human blood: differences according to sex, age, and disease. Ann Rheum Dis 1980;39:82–6.
3. Kiessling RB, Klein E, Wigzell H. Natural killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to phenotype. Eur J Immunol 1975;5:112–7.
4. Barone J, Hebert JR, Reddy MM. Dietary fat and natural killer cell activity. Am J Clin Nutr 1989;50:861–7.
5. Hebert JR, Barone J, Reddy MM, Backlund JY. Natural killer cell activity in a longitudinal dietary fat intervention trial. Clin Immunol Immunopathol 1990;54:103–16.
6. Morrow WJW, Ohashi Y, Hall J, et al. Dietary fat and immune function. I. Antibody responses, lymphocyte and accessory cell function in (NZB × NZW)F1 mice. J Immunol 1985;135:3857–63.
7. Leung KH, Ip MM. Effect of dietary polyunsaturated fat and 7,12-dimethyl(*a*)-anthracene on rat splenic natural killer cells and prostaglandin E synthesis. Cancer Immunol Immunother 1986;21: 161–3.
8. Jeffery NM, Sanderson P, Sherrington EJ, Newsholme EA, Calder PC. The ratio of n−6 to n−3 polyunsaturated fatty acids in the rat diet alters serum lipid levels and lymphocyte functions. Lipids 1996;31:737–45.
9. Jeffery NM, Newsholme EA, Calder PC. The level of polyunsaturated fatty acids and the n−6 to n−3 polyunsaturated fatty acid ratio in the rat diet both affect serum lipid levels and lymphocyte functions. Prostaglandins Leukot Essent Fatty Acids 1997;57: 149–60.
10. Meydani SN, Yogeeswaran G, Liu S, Baskar S, Meydani M. Fish oil and tocopherol-induced changes in natural killer cell-mediated cytotoxicity and PGE_2 synthesis in young and old mice. J Nutr 1988;118:1245–52.
11. Berger A, German JB, Chiang BL, et al. Influence of feeding unsaturated fats on growth and immune status of mice. J Nutr 1993;123: 225–33.
12. Lumpkin EA, McGlone JJ, Sells JL, Hellman JM. Modulation of murine natural killer cell cytotoxicity by dietary fish oil, corn oil or beef tallow. J Nutr Immunol 1993;2:43–53.
13. Yaqoob P, Newsholme EA, Calder PC. Inhibition of natural killer cell activity by dietary lipids. Immunol Lett 1994;41:241–7.
14. Sanderson P, Yaqoob P, Calder PC. Effects of dietary lipid manipulation upon rat spleen lymphocyte functions and the expression of lymphocyte surface molecules. J Nutr Environ Med 1995;5:119–32.
15. Peterson LD, Thies F, Sanderson P, Newsholme EA, Calder PC. Low levels of eicosapentaenoic and docosahexaenoic acids mimic the effects of fish oil upon rat lymphocytes. Life Sci 1998;62:2209–17.
16. Rice C, Hudig D, Newton RS, Mendelsohn J. Effect of unsaturated fatty acids on human lymphocytes. Disparate influences of oleic and linoleic acids on natural cytotoxicity. Clin Immunol Immunopathol 1981;20:389–401.

17. Purasiri P, McKechnie A, Heys SD, Eremin O. Modulation in vitro of human natural cytotoxicity, lymphocyte proliferative response to mitogens and cytokine production by essential fatty acids. Immunology 1997;92:166–72.
18. Yamashita N, Yokoyama A, Hamazaki T, Yano S. Inhibition of natural killer cell activity of human lymphocytes by eicosapentaenoic acid. Biochem Biophys Res Commun 1986;138:1058–67.
19. Yamashita N, Maruyama M, Yamazaki K, Hamazaki T, Yano S. Effect of eicosapentaenoic and docosahexaenoic acid on natural killer cell activity in human peripheral blood lymphocytes. Clin Immunol Immunopathol 1991;59:335–45.
20. British Nutrition Foundation's Task Force report. Unsaturated fatty acids: nutritional and physiological significance. London: Chapman and Hall, 1992.
21. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. Dietary reference values for food energy and nutrients for the United Kingdom. London: Her Majesty's Stationery Office, 1994.
22. Li D, Sinclair A, Wilson A, et al. Effect of dietary α-linolenic acid on thrombotic risk factors in vegetarian men. Am J Clin Nutr 1999; 69:872–82.
23. Johnson MM, Swan DD, Surette ME, et al. Dietary supplementation with γ-linolenic acid alters fatty acid content and eicosanoid production in healthy humans. J Nutr 1997;127:1435–44.
24. Wu D, Meydani M, Leka LS, et al. Effect of dietary supplementation with black currant seed oil on the immune response of healthy elderly subjects. Am J Clin Nutr 1999;70:536–43.
25. Mann NJ, Johnson LG, Warrick GE, Sinclair AJ. The arachidonic acid content of the Australian diet is lower than previously estimated. J Nutr 1995;125:2528–35.
26. Jonnalagadda SS, Egan SK, Heimbach JT, Harris SS, Kris-Etherton PM. Fatty acid consumption pattern of Americans: 1987–1988 USDA Nationwide Food Consumption Survey. Nutr Res 1995;15:1767–81.
27. Sinclair AJ, O'Dea K. The significance of arachidonic acid in hunter-gatherer diets: implications for the contemporary Western diet. J Food Lipids 1993;1:143–57.
28. Nelson GJ, Schmidt PC, Bartolini G, et al. The effect of dietary arachidonic acid on plasma lipoprotein distributions, apoproteins, blood lipid levels, and tissue fatty acid compositions in humans. Lipids 1997;32:427–33.
29. Allard JP, Kurian R, Aghdassi E, Muggli R, Royall D. Lipid peroxidation during n−3 fatty acid and vitamin E supplementation in humans. Lipids 1997;32:535–41.
30. Kelley DS, Taylor PC, Nelson GJ, Schmidt PC, Mackey BE, Kyle D. Effects of dietary arachidonic acid on human immune response. Lipids 1997;32:449–56.
31. Kelley DS, Taylor PC, Nelson GJ, et al. Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. Lipids 1999;34: 317–24.
32. Peterson LD, Jeffery NM, Thies F, Sanderson P, Newsholme EA, Calder PC. Eicosapentaenoic and docosahexaenoic acids alter rat spleen leukocyte fatty acid composition and prostaglandin E_2 production but have different effects on lymphocyte functions and cell-mediated immunity. Lipids 1998;33:171–80.
33. Welsh RM, Vargas-Cortes M. Natural killer cells in viral infection. In: Lewis CE, McGee JO'D, eds. The natural killer cell. Oxford, United Kingdom: Oxford University Press, 1992:107–50.
34. Lewis CE, McGee JO'D. Natural killer cells in tumour biology. In: Lewis CE, McGee JO'D, eds. The natural killer cell. Oxford, United Kingdom: Oxford University Press, 1992:175–203.
35. Meydani SN, Endres S, Woods MM, et al. Oral (n−3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. J Nutr 1991;121:547–55.
36. Meydani SN, Lichtenstein AH, Cornwall S, et al. Immunologic effects of National Cholesterol Education Panel Step-2 diets with and without fish-derived n−3 fatty acid enrichment. J Clin Invest 1993;92:105–13.
37. Mayatepek E, Paul K, Leichsenring M, et al. Influence of dietary (n−3) polyunsaturated fatty acids on leukotriene B_4 and prostaglandin E_2 synthesis and the time course of experimental tuberculosis in guinea pigs. Infection 1994;22:106–12.
38. Fritsche KL, Shahbazian LM, Feng C, Berg JN. Dietary fish oil reduces survival and impairs bacterial clearance in C3H/Hen mice challenged with *Listeria monocytogenes*. Clin Sci (Colch) 1997;92: 95–101.
39. Chang HR, Dulloo AG, Vladoianu IR, et al. Fish oil decreases natural resistance of mice to infection with *Salmonella typhimurium*. Metabolism 1992;41:1–2.
40. D'Ambola JB, Aeberhard EE, Trang N, Gaffar S, Barrett CT, Sherman MP. Effect of dietary (n−3) and (n−6) fatty acids on in vivo pulmonary bacterial clearance by neonatal rabbits. J Nutr 1991;121: 1262–9.
41. Byleveld PM, Pang GT, Clancy RL, Roberts DCK. Fish oil feeding delays influenza virus clearance and impairs production of interferon-γ and virus-specific immunoglobulin A in the lungs of mice. J Nutr 1999;129:328–35.
42. Rubin RH, Wilkinson RA, Xu L, Robinson DR. Dietary marine lipid does not alter susceptibility of (NZB × NZW)F1 mice to pathogenic microorganisms. Prostaglandins 1989;38:251–62.
43. Blok WL, Vogels MTE, Curfs JHAJ, Eling WMC, Buurmann WA, van der Meer JMW. Dietary fish oil supplementation in experimental gram-negative infection and in cerebral malaria in mice. J Infect Dis 1992;165:898–903.
44. Hogan PG. Natural killer cells in transplantation. In: Lewis CE, McGee JO'D, eds. The natural killer cell. Oxford, United Kingdom: Oxford University Press, 1992:151–74.
45. Otto DA, Kahn DR, Hamm MW, Forrest DE, Wooten JT. Improved survival of heterotopic cardiac allografts in rats with dietary n−3 polyunsaturated fatty acids. Transplantation 1990;50:193–8.
46. Grimm H, Tibell A, Norrlind B, Schott J, Bohle RA. Nutrition and allorejection impact of lipids. Transplant Immunol 1995;3:62–7.
47. Grimminger F, Grimm H, Fuhrer D, et al. Omega 3 lipid infusion in a heart allotransplant model: shift in fatty acid and lipid mediator profiles and prolongation of transplant survival. Circulation 1996; 93:365–71.
48. Berthoux FC, Guerin C, Berthoux P, Burgard G, Alamartine E. One-year randomized controlled trial with omega-3 fatty acid-rich fish oil in clinical renal transplant. Transplant Proc 1992;24:2578–82.
49. van der Heide HJJ, Bilo HJG, Donker JM, Wilmink JM, Tegzess AM. Effect of dietary fish oil on renal function and rejection in cyclosporine-treated recipients of renal transplants. N Engl J Med 1993;329:769–73.
50. Bennett WM, Carpenter CB, Shapiro ME, et al. Delayed omega-3 fatty acid supplements in renal transplantation. Transplantation 1995;59:352–6.
51. Maachi K, Berthoux P, Burgard G, Alamartine E, Berthoux F. Results of a 1-year randomized controlled trial with omega-3 fatty acid fish oil in renal transplantation under triple immunosuppressive therapy. Transplant Proc 1995;27:846–9.
52. Meydani M, Natiello F, Goldin B, et al. Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. J Nutr 1991;121:484–91.
53. Kramer TR, Schoene N, Douglass LW, et al. Increased vitamin E intake restores fish-oil-induced suppressed blastogenesis of mitogen-stimulated T lymphocytes. Am J Clin Nutr 1991;54:896–902.
54. Wu D, Meydani SN, Meydani M, Hayek MG, Huth P, Nicolosi RJ. Immunologic effects of marine- and plant-derived n−3 polyunsaturated fatty acids in nonhuman primates. Am J Clin Nutr 1996; 63:273–80.