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Lack of effect of foods enriched with plant- or marine-derived n-3 fatty acids on human immune function¹⁻³

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ABSTRACT

Background: Greatly increasing dietary flaxseed oil [rich in the n-3 polyunsaturated fatty acid (PUFA) α -linolenic acid (ALA)] or fish oil [rich in the long-chain n-3 PUFAs eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids] can reduce markers of immune cell function. The effects of more modest doses are unclear, and it is not known whether ALA has the same effects as its long-chain derivatives.

Objective: The objective was to determine the effects of enriching the diet with ALA or EPA+DHA on immune outcomes representing key functions of human neutrophils, monocytes, and lymphocytes.

Design: In a placebo-controlled, double-blind, parallel study, 150 healthy men and women aged 25-72 y were randomly assigned to 1 of 5 interventions: placebo (no additional n-3 PUFAs), 4.5 or 9.5 g ALA/d, and 0.77 or 1.7 g EPA+DHA/d for 6 mo. The n-3 PUFAs were provided in 25 g fat spread plus 3 oil capsules. Blood samples were taken at 0, 3, and 6 mo.

Results: The fatty acid composition of peripheral blood mononuclear cell phospholipids was significantly different in the groups with higher intakes of ALA or EPA+DHA. The interventions did not alter the percentages of neutrophils or monocytes engaged in phagocytosis of *Escherichia coli* or in phagocytic activity, the percentages of neutrophils or monocytes undergoing oxidative burst in response to *E. coli* or phorbol ester, the proliferation of lymphocytes in response to a T cell mitogen, the production of numerous cytokines by monocytes and lymphocytes, or the in vivo delayed-type hypersensitivity response.

Conclusion: An intake of ≤ 9.5 g ALA/d or ≤ 1.7 g EPA+DHA/d does not alter the functional activity of neutrophils, monocytes, or lymphocytes, but it changes the fatty acid composition of mononuclear cells. *Am J Clin Nutr* 2003;77:1287-95.

KEY WORDS Fish oil, α -linolenic acid, n-3 polyunsaturated fatty acids, immunity, lymphocyte, monocyte, neutrophil, cytokine, phagocytosis, oxidative burst

INTRODUCTION

Inflammatory cells such as neutrophils, monocytes, and macrophages form part of the innate immune response that is responsible for early host defense against invading bacteria (1). Inflammatory cells recognize bacteria in a nonspecific way and act to destroy them by phagocytosis, by the production of superoxide and related reactive oxygen species in the respiratory burst, or

both (1). Bacterial cell wall components such as lipopolysaccharide (LPS) stimulate the production of cytokines such as tumor necrosis factor (TNF) and interleukin 1 (IL-1) and IL-6 by monocytes and macrophages. The inflammatory cytokines provide one link between inflammatory cells and specific immunity because they can stimulate T and B lymphocytes (1). Monocytes and macrophages can also act as antigen-presenting cells, which provides an additional link between the innate and specific immune systems. When T lymphocytes are presented with antigen, they become activated, secrete cytokines, and ultimately enter the cell cycle and divide (1). In cell culture, the stimulation and subsequent proliferation of T lymphocytes can be achieved by mitogens such as concanavalin A (Con A) (2). T lymphocytes are classified into helper T cells, distinguished by the presence of the molecule CD4 on their surface, and suppressor (cytotoxic) T cells, distinguished by the presence of CD8 on their surface. T lymphocytes can also be subdivided functionally according to the pattern of cytokines they produce. Type 1 helper T lymphocytes produce IL-2 and interferon γ (IFN- γ), while type 2 helper T lymphocytes produce IL-4, IL-5, and IL-10 (1).

There is continuing interest in the effects of n-3 polyunsaturated fatty acids (PUFAs) on human immune function (3, 4). Studies have generally been of short duration and have focused on the effects of the long-chain n-3 PUFAs eicosapentaenoic acid (EPA;

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TABLE 1
Characteristics of the treatment groups at baseline (0 mo)¹

	Group				
	Placebo (n = 30)	Low-ALA (n = 30)	High-ALA (n = 31)	Low-EPA+DHA (n = 30)	High-EPA+DHA (n = 29)
Age (y)	55 ± 2 ²	53 ± 2	54 ± 2	52 ± 2	54 ± 2
Sex, M:F	18:12	17:13	18:13	17:13	17:12
BMI (kg/m ²)	25.8 ± 0.6	27.2 ± 0.6	26.1 ± 0.6	26.3 ± 0.6	26.5 ± 0.6

¹ALA, α -linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. There were no significant differences among treatment groups.

² $\bar{x} \pm \text{SEM}$.

20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) found in fish oil rather than on those of their precursor, α -linolenic acid (ALA; 18:3n-3). Fish oil providing >2.4 g EPA+DHA/d decreased the production of superoxide by neutrophils (5-7) and monocytes (8); decreased the production of TNF- α , IL-1 β , and IL-6 by LPS-stimulated mononuclear cells (9-13); decreased the proliferation of lymphocytes (11, 12, 14-18); and decreased the production of IL-2 (11, 12, 14, 18) and IFN- γ (12). Only a few studies have investigated the immunologic effects of ALA in humans. Increasing the intake of ALA to \approx 14 g/d for 4 wk reduced TNF- α and IL-1 β production by LPS-stimulated mononuclear cells (9), whereas an intake of 18 g ALA/d for 8 wk reduced Con A-stimulated lymphocyte proliferation and the delayed-type hypersensitivity (DTH) response (19). Most often, the possibility that n-3 PUFAs diminish inflammatory and immune cell functions is interpreted in a favorable way—that their effects are anti-inflammatory and so will be beneficial to health (3, 4). However, a reduction in the activity of immune cells could compromise host defenses. The amounts of n-3 PUFAs provided in most studies performed to date greatly exceed habitual intakes (20, 21) and intakes that are recommended (20-22) or that could be attained by most persons through dietary change. It is important to ensure that there is no adverse immunologic impact of a more modest increase in the consumption of these PUFAs and that their effects are examined over a longer time than most studies span to date. Therefore, the current study investigated the immunologic effects of increases in the intake of ALA or EPA+DHA provided largely through food over a 6-mo period; 2 intakes of ALA or EPA+DHA were compared.

SUBJECTS AND METHODS

Materials

Tablets of phosphate-buffered saline were obtained from Unipath Ltd (Basingstoke, United Kingdom). Histopaque, HEPES-buffered RPMI medium, glutamine, antibiotics (penicillin and streptomycin), Con A, *E. coli* 0111:B4 LPS, boron trifluoride, butylated hydroxytoluene, formaldehyde, solvents, and standard chemicals were purchased from Sigma Chemical Co Ltd (Poole, United Kingdom). Fluorescein isothiocyanate-labeled mouse anti-human CD3, CD14, and CD19 and R-phycoerythrin-labeled mouse anti-human CD4, CD8, CD16, and CD54 were purchased from Serotec Ltd (Kidlington, United Kingdom). Kits were used for measurement of phagocytosis and oxidative burst in whole blood (PHAGOTEST and BURSTTEST, respectively; Becton Dickinson, Oxford, United Kingdom). [³H]Thymidine was purchased from Amersham International Ltd (Amersham, United Kingdom). Cytokine EASIA enzyme-linked immunosorbent assay

kits were obtained from BioSource International (Nivelles, Belgium). Cell-mediated immunity test kits (MultiTest-CMI) were purchased from Merieux Institute Inc (Paris).

Subjects and study design

This study was part of a larger investigation of the effects of ALA and EPA+DHA on human health-related outcomes [known as the Ministry of Agriculture, Fisheries, and Food (MAFF)-LINK AFQ111]. Ethical permission for all procedures involving human volunteers was obtained from the University of Reading Ethics and Research Committee and the West Berkshire Health Authority Ethics Committee. Moderately hyperlipidemic but otherwise healthy adults aged 25-72 y were invited to participate in the study. Moderate hyperlipidemia was defined as a fasting total cholesterol concentration between 4.6 and 8.0 mmol/L and a fasting triacylglycerol concentration between 0.8 and 3.2 mmol/L. All volunteers completed a questionnaire on health and lifestyle before entering the study. Volunteers were excluded if they were taking any prescribed hypolipidemic or anti-inflammatory medication; had been diagnosed as having cardiovascular disease, diabetes, liver or endocrine dysfunction, or chronic inflammatory disease; were pregnant or lactating; were vegetarian; consumed fish oil, evening primrose oil, or vitamin supplements; smoked >15 cigarettes/d; exercised strenuously >3 times/wk; had a body mass index (BMI; in kg/m²) <20 or >32; consumed >2 portions of oily fish/wk; or were nonconsumers of margarine. Two hundred subjects were recruited to the study and were randomly allocated to 1 of 5 interventions, with randomization stratified for age, BMI, and fasting plasma triacylglycerol concentration. One hundred fifty subjects completed the study; reasons for dropping out included lack of time, inability to consume the required portion of fat spread, a desire to lose weight, or unspecified. The characteristics of the 150 subjects who completed the study are given in **Table 1**; mean age and BMI did not differ significantly among the treatment groups.

The intervention was a combination of fat spreads (25 g spread/d) and oil capsules (three 1-g capsules/d). Subjects in the placebo intervention arm consumed a standard linoleic acid-rich margarine plus capsules containing a mixture of oils with the fatty acid composition of the average diet in the United Kingdom (placebo capsules). Subjects in the ALA intervention arm consumed a spread containing 3.7 or 8.7 g ALA/25 g body weight and placebo capsules; these groups are referred to as the low- and high-ALA groups, respectively. The aim of the low- and high-ALA interventions was to increase total ALA intake to 5 and 10 g/d, respectively, with an estimated contribution from the background diet of 1.5 g ALA/d taken into account (21). Subjects in the low-EPA+DHA intervention arm consumed a spread containing 0.18 g EPA + 0.28 g DHA/25 g body weight and placebo capsules. Subjects in the



TABLE 2

Dietary intakes of the major polyunsaturated fatty acids in the treatment groups during intervention¹

	Group				
	Placebo	Low-ALA	High-ALA	Low-EPA+DHA	High-EPA+DHA
	<i>g/d</i>				
LA	22.9 ± 0.9 ^a	16.2 ± 0.7 ^b	13.1 ± 0.7 ^b	20.8 ± 0.8 ^a	21.1 ± 1.0 ^a
AA	0.23 ± 0.02	0.14 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.20 ± 0.02
ALA	1.5 ± 0.1 ^a	4.5 ± 0.6 ^b	9.5 ± 0.1 ^c	1.3 ± 0.5 ^a	1.4 ± 0.1 ^a
EPA	0.22 ± 0.02 ^{a,b}	0.16 ± 0.03 ^a	0.15 ± 0.03 ^a	0.30 ± 0.02 ^b	0.66 ± 0.02 ^c
DHA	0.30 ± 0.03 ^a	0.21 ± 0.02 ^b	0.19 ± 0.02 ^b	0.47 ± 0.02 ^c	1.02 ± 0.03 ^d

¹ $\bar{x} \pm \text{SEM}$; $n = 29-31$ per treatment group. ALA, α -linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; AA, arachidonic acid. Values in a row with different superscript letters are significantly different, $P < 0.05$ (one-factor ANOVA with post hoc Tukey test).

high-EPA+DHA intervention arm consumed a spread containing 0.18 g EPA + 0.28 g DHA/25 g body weight and fish oil capsules providing 0.31 g EPA + 0.48 g DHA. The aim of the low- and high-EPA+DHA interventions was to increase total EPA+DHA intake to 0.7 and 1.5 g/d, respectively, with an estimated contribution from the background diet of 0.2 g EPA+DHA/d taken into account (21). The target intakes of EPA+DHA were based on recommended intakes of those fatty acids (21), whereas the target intakes of ALA were based on existing literature suggesting that the effect of 7 g ALA in increasing tissue long-chain n-3 PUFA concentrations is approximately equivalent to that of 1 g EPA+DHA (23).

The amount of vitamin E in the spreads was standardized on the basis of the amount and type of PUFAs present (24). According to an analysis of the spreads and capsules, the content of vitamin E (expressed as α -tocopherol equivalents/25 g spread plus 3 oil capsules) was 11.4 for the placebo intervention, 13.7 for the low-ALA intervention, 14.0 for the high-ALA intervention, 14.0 for the low-EPA+DHA intervention, and 15.2 for the high-EPA+DHA intervention.

Subjects completed a previously validated 180-question food-frequency questionnaire before the start of the study and again near the end of the study (at ≈ 5 mo), and nutrient intakes were determined with the use of FOODBASE software, version 1.3 (Institute of Brain Chemistry, London). Daily intakes of n-6 and n-3 PUFAs in the different groups during the intervention are shown in **Table 2**. The ratio of n-6 to n-3 PUFAs in the diet of subjects in the placebo intervention group was ≈ 111 with a ratio of linoleic acid to ALA of $\approx 15:1$. In contrast, the ratio of n-6 to n-3 PUFAs in the diets of the low- and high-ALA intervention groups was $\approx 3.3:1$ and $1.4:1$, respectively, with this ratio being achieved by the replacement of linoleic acid with ALA. The ratio of n-6 to n-3 PUFAs in the diets of the low- and high-EPA+DHA intervention groups was $\approx 10:1$ and $7:1$, respectively, although in these diets the ratio of linoleic acid to ALA was maintained at $\approx 15:1$.

All subjects consumed the placebo spread and capsules for 4 wk before beginning the interventions. Subsequently the interventions were consumed for 6 mo. Subjects were studied in 3 cohorts that did not overlap; approximately one-third of subjects were recruited to each cohort. Mean compliance with the interventions, as measured from the return of empty fat-spread containers and capsule packs, was $>92\%$ and $>88\%$, respectively, across all intervention groups, and it did not differ significantly among intervention groups. Fasting blood was sampled immediately before the interventions and at 3 and 6 mo.

Preparation of PBMCs

Blood samples were collected into heparinized evacuated tubes between 0800 and 1000 after a fast of ≥ 10 h. The blood was layered onto Histopaque (density: 1.077 g/L; ratio of blood to Histopaque: 1:1) and centrifuged for 15 min at $800 \times g$ at 20°C . The cells, called peripheral blood mononuclear cells (PBMCs), were collected from the interphase and washed once with RPMI medium containing 0.75 mmol/L glutamine and antibiotics (penicillin and streptomycin; culture medium). After resuspension in 4 mL culture medium, the cells were layered onto 4 mL Histopaque. They were centrifuged once more (15 min at $800 \times g$ and 20°C) to achieve a lower degree of erythrocyte contamination, washed with culture medium, and finally resuspended and counted on a Z1 Cell Counter (Coulter Electronics, Luton, United Kingdom).

Analysis of PBMC subsets

For the determination of PBMC subsets, whole blood (100 μL) was incubated with various combinations of fluorescence-labeled monoclonal antibodies (10 μL of each antibody) for 30 min at 4°C . Monoclonal antibody combinations used were anti-CD3 and anti-CD4 (to distinguish T lymphocytes as CD3⁺ and helper T lymphocytes as CD3⁺CD4⁺), anti-CD3 and anti-CD8 (to distinguish cytotoxic T lymphocytes as CD3⁺CD8⁺), anti-CD3 and anti-CD16 (to distinguish natural killer cells as CD3⁻CD16⁺), anti-CD19 and anti-CD54 [to distinguish B lymphocytes as CD19⁺ and to determine the expression of intercellular adhesion molecule-1 (CD54) on B lymphocytes], and anti-CD14 and anti-CD54 (to distinguish monocytes as CD14⁺ and to determine the expression of intercellular adhesion molecule-1 on monocytes). Erythrocytes were then lysed with 2 mL lysing solution (3.75 mL formaldehyde, 4.5 mL diethylene glycol, 1.75 mL Tris at 0.2 mol/L made up to 1 L with distilled water), and the leukocytes were washed and fixed with 0.2 mL fixing solution (phosphate-buffered saline containing 2 mL formaldehyde/100 mL). Fixed leukocytes were analyzed in a flow cytometer (FACSCalibur; Becton Dickinson, Oxford, United Kingdom). Fluorescence data were collected on 2×10^4 cells and analyzed with the use of CELLQUEST software (Becton Dickinson).

Analysis of PBMC phospholipid fatty acid composition

Lipid was extracted from PBMCs with chloroform:methanol (2:1, vol:vol), and phospholipids were isolated with the use of thin-layer chromatography using a mixture of hexane:diethyl

ether:acetic acid (90:30:1, vol:vol:vol) as the elution phase. Fatty acid methyl esters were prepared by incubation with 140 g boron trifluoride/L in methanol at 80 °C for 60 min and analyzed by gas chromatography as described elsewhere (25).

Measurement of phagocytic activity and oxidative burst

Phagocytosis and oxidative burst by neutrophils and monocytes were determined with the use of PHAGOTEST and BURSTTEST kits, respectively. Before use, blood was cooled on ice for 10 min and then spun in a vortex for 5 s. For measurement of phagocytosis, aliquots (100 µL) of blood were then incubated on ice (control) or in a heated water bath at 37 °C for 10 min with opsonized fluorescein isothiocyanate-labeled *E. coli* (20 µL). The reaction was stopped by the addition of ice-cold quenching solution (100 µL). For measurement of oxidative burst, aliquots (100 µL) of blood were incubated in a heated water bath at 37 °C for 10 min with opsonized *E. coli*, phorbol ester, or washing solution as control (20 µL in each case). After incubation, a solution (20 µL) containing the fluorogenic substrate dihydrorhodamine 123 was added, and the samples were incubated for 10 min at 37 °C.

At the completion of phagocytosis and respiratory burst incubations, erythrocytes were lysed, leukocytes were fixed, and the DNA was stained according to the manufacturer's instructions. Cell preparations were then analyzed by flow cytometry in a FACSCalibur flow cytometer. Fluorescence data were collected on 2×10^4 cells and analyzed with the use of CELLQUEST software. Neutrophils and monocytes were identified by forward and side scatter. Both the percentage of neutrophils or monocytes engaging in phagocytosis or oxidative burst (% positive) and the median fluorescence intensity (MFI; a measure of the extent of phagocytosis or oxidative burst per leukocyte) were determined.

Measurement of lymphocyte proliferation in PBMC cultures

PBMCs (2×10^5) were cultured in culture medium supplemented with 50 mL autologous plasma/L and Con A at final concentrations of 5, 15, 25, 50, and 75 mg/L; the final volume of the culture was 200 µL, and all cultures were performed in triplicate. Proliferation was measured as the incorporation of [³H]thymidine over the final 18 h of a 66-h culture period. Thymidine-incorporation values for the triplicate cultures were averaged (CV was always < 10% and usually < 5%). Data are expressed as thymidine incorporation in cpm/well.

Measurement of the production of cytokines by PBMC cultures

PBMCs (2×10^6) were cultured for 24 h in culture medium and supplemented with 50 mL autologous plasma/L and either 25 mg Con A/L or 15 mg LPS/L; the final culture volume was 2 mL. These concentrations of stimulants and the incubation period of 24 h were selected on the basis of the results of preliminary experiments in which these conditions were found to result in maximal production of each of the cytokines being studied here (data not shown). At the end of the incubation, the plates were centrifuged and the culture medium was collected and frozen in aliquots. The concentrations of cytokines were measured by enzyme-linked immunosorbent assays. TNF-α, IL-1β, IL-6, and IL-10 were measured in the supernatant fluids of cells stimulated with LPS, and IL-2, IFN-γ, and IL-4 were measured in the supernatant fluids

of cells stimulated with Con A. Limits of detection for these assays were 3 ng/L for TNF-α; 2 ng/L for IL-1β, IL-6, and IL-4; 1 ng/L for IL-10; 100 U/L for IL-2; and 30 IU/L for IFN-γ (data supplied by the manufacturer of each kit). The interassay and intraassay CVs were < 10% for all cytokine enzyme-linked immunosorbent assays.

Delayed-type hypersensitivity response test

The DTH skin response was assessed with the use of the MultiTest-CMI kit, rather than the conventional skin test, to avoid the boosting effect observed with the repeated administration required by the latter test (26). The test apparatus consisted of a single-use disposable applicator of acrylic resin with 8 heads loaded with either glycerine control or with 1 of 7 recall antigens (tetanus, diphtheria, streptococcus, tuberculin, *Candida albicans*, *Tricophyton mentagrophytes*, or *Proteus mirabilis*). The test consisted of holding the applicator to the skin for a period of 10 s and then removing, which left at each site a droplet of liquid that was blotted from the skin after 2 min. The number and area of positive reactions were assessed 48 h after administration of the test. An imprint of the test area was taken and transferred to an analysis grid. The test was administered at baseline and at the end of the intervention period (ie, at 6 mo).

The antigen score was calculated with the use of a well-established image analysis apparatus at Unilever Health Institute (Vlaardingen, Netherlands). This instrument measures the area of each individual induration; an induration of ≥ 2 mm² was considered positive. If a positive reaction to the glycerine control was observed, the area of this induration was subtracted from that of each of the other positive reactions. Results were calculated as the total number of positive reactions and the cumulative score (calculated as the total area of induration of all positive reactions).

Sample size, data presentation, and statistical analysis

Sample size (ie, number of subjects per treatment group) was calculated on the basis of the previously reported effect of dietary fish oil on the proliferation of lymphocytes in PBMC cultures stimulated with Con A (25). It was calculated that a sample size of 30 would have a power of 80% to detect a difference in lymphocyte proliferation of 30% at $P < 0.05$. In addition, it was anticipated that the amount of EPA provided in the high-EPA+DHA treatment would increase the EPA content of PBMC phospholipids by $\geq 100\%$ (25). According to data from other investigators (9), this indicated that the sample size of 30 would be sufficient to detect a difference of 30% in the production of at least some cytokines being studied (eg, TNF-α and IL-1β). To allow for an anticipated dropout rate of 25%, we recruited 40 subjects into each intervention arm.

Fatty acid intake, PBMC fatty acid composition, PBMC subsets, lymphocyte proliferation, and cytokine production were determined for all subjects who completed the study ($n = 29$ – 31 /intervention arm). For logistical reasons, phagocytosis and oxidative burst were determined only for cohorts 2 and 3 ($n = 20$ /intervention arm). For logistical reasons, the DTH response was determined only for cohorts 2 and 3; a small number of subjects declined to undergo this test, and these data are for $n = 16$ – 19 /intervention arm. Data for each intervention arm at each time point and data for the change from baseline (ie, 6 mo – 0 mo) in each intervention arm were tested for

TABLE 3

Fatty acid composition of peripheral blood mononuclear cell (PBMC) phospholipids at baseline (0 mo) and after 3 and 6 mo of dietary intervention¹

Treatment and time	LA	ALA	AA	EPA	DPA	DHA
	% by wt of total fatty acids					
Placebo						
0 mo	8.6 ± 0.3	0.30 ± 0.05	17.5 ± 0.6	0.57 ± 0.04	3.3 ± 0.4	2.8 ± 0.2
3 mo	9.0 ± 0.2	0.25 ± 0.04	15.6 ± 0.8	0.61 ± 0.07	3.2 ± 0.3	2.4 ± 0.2
6 mo	8.3 ± 0.3	0.24 ± 0.05	15.7 ± 0.6	0.42 ± 0.07	2.5 ± 0.2	2.3 ± 0.3
6 mo - 0 mo	-0.3 ± 0.3	-0.09 ± 0.06	-2.0 ± 0.1	-0.25 ± 0.15	-1.2 ± 0.5	-0.7 ± 0.2
Low-ALA						
0 mo	9.2 ± 0.3	0.28 ± 0.05	18.5 ± 0.8	0.51 ± 0.05	3.5 ± 0.4	3.2 ± 0.2
3 mo	9.2 ± 0.3	0.24 ± 0.04	15.9 ± 0.8	0.55 ± 0.06	2.8 ± 0.2	2.4 ± 0.2 ²
6 mo	8.5 ± 0.3	0.24 ± 0.03	16.2 ± 0.5	0.49 ± 0.05	3.7 ± 0.2	2.3 ± 0.1 ²
6 mo - 0 mo	-0.4 ± 0.3	0.08 ± 0.05	-2.6 ± 0.7	0.02 ± 0.08	-0.7 ± 0.4	-0.7 ± 0.3
High-ALA						
0 mo	9.3 ± 0.4	0.25 ± 0.05	17.7 ± 0.7	0.62 ± 0.06	3.2 ± 0.4	2.5 ± 0.2
3 mo	8.7 ± 0.3	0.31 ± 0.04	15.6 ± 0.6	0.81 ± 0.07 ²	3.3 ± 0.3	2.1 ± 0.2
6 mo	7.8 ± 0.4	0.24 ± 0.04	14.9 ± 0.5	0.84 ± 0.08 ²⁻⁴	3.2 ± 0.3	2.0 ± 0.1 ^{2,5}
6 mo - 0 mo	-1.1 ± 0.7	0.03 ± 0.06	-2.9 ± 0.7	0.21 ± 0.11	-0.3 ± 0.4	-0.6 ± 0.2
Low-EPA+DHA						
0 mo	8.7 ± 0.3	0.27 ± 0.03	19.1 ± 0.7	0.51 ± 0.05	2.8 ± 0.4	2.9 ± 0.2
3 mo	9.3 ± 0.2	0.24 ± 0.04	16.3 ± 0.7	0.50 ± 0.06	2.8 ± 0.2	2.8 ± 0.2
6 mo	8.5 ± 0.3	0.34 ± 0.06	15.9 ± 0.6	0.56 ± 0.09	2.3 ± 0.3	2.7 ± 0.2
6 mo - 0 mo	-0.1 ± 0.4	0.11 ± 0.07	-3.9 ± 0.8	0.15 ± 0.10	-0.6 ± 0.5	0.2 ± 0.3
High-EPA+DHA						
0 mo	9.2 ± 0.3	0.22 ± 0.05	19.0 ± 0.9	0.57 ± 0.05	2.9 ± 0.4	2.6 ± 0.3
3 mo	9.3 ± 0.3	0.26 ± 0.04	14.5 ± 0.7	0.97 ± 0.11 ²⁻⁵	3.1 ± 0.3	3.0 ± 0.2 ⁶
6 mo	9.3 ± 0.3	0.22 ± 0.03	15.5 ± 0.4	1.08 ± 0.06 ²⁻⁵	2.7 ± 0.3	3.3 ± 0.2 ³⁻⁵
6 mo - 0 mo	0.3 ± 0.6	-0.01 ± 0.04	-4.0 ± 0.9	0.40 ± 0.13 ^{7,8}	-0.5 ± 0.6	0.6 ± 0.3 ⁷⁻⁹

¹ $\bar{x} \pm \text{SEM}$; $n = 29-31$ per treatment group. LA, linoleic acid; ALA, α -linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. Two-factor ANOVA: significant effects of time for AA, EPA, and DHA ($P < 0.001$, $P = 0.004$, and $P = 0.013$, respectively), of treatment for EPA and DHA ($P < 0.001$ in both cases), and treatment \times time interactions for EPA and DHA ($P < 0.001$ and $P = 0.007$, respectively).

²Significantly different from baseline, $P < 0.04$ (one-factor ANOVA with post hoc Tukey test).

³Significantly different from placebo, $P < 0.03$ (one-factor ANOVA with post hoc Tukey test).

⁴Significantly different from low-ALA, $P < 0.015$ (one-factor ANOVA with post hoc Tukey test).

⁵Significantly different from low-EPA+DHA, $P < 0.03$ (one-factor ANOVA with post hoc Tukey test).

⁶Significantly different from high-ALA, $P < 0.009$ (one-factor ANOVA with post hoc Tukey test).

⁷Significantly different from placebo, $P \leq 0.015$ (one-factor ANCOVA with post hoc Tukey test).

⁸Significantly different from low-ALA, $P \leq 0.035$ (one-factor ANCOVA with post hoc Tukey test).

⁹Significantly different from high-ALA, $P < 0.03$ (one-factor ANCOVA with post hoc Tukey test).

normality with the Kolmogorov-Smirnov test. When data were not normally distributed, they were log transformed. Data that were normally distributed either before or after log transformation are presented as means \pm SEMs. The statistical significance of treatment and time and of the treatment \times time interaction was determined with the use of two-factor analysis of variance (ANOVA). When the treatment \times time interaction was significant, the effects of treatment and of time were further analyzed by one-factor ANOVA; post hoc analysis was done with Tukey's test. Data for change from baseline (ie, 0 mo) were analyzed by analysis of covariance with the use of age, sex, and baseline value as covariates; post hoc analysis was done with Tukey's test. Some MFI data were not normally distributed even after log transformation. Data for the number of positive DTH responses were not normally distributed and are expressed as medians. For data that were not normally distributed, two-factor ANOVA was performed on the ranked data to determine the statistical significance of treatment and time and of the treatment \times time interaction. All statistical tests were performed with SPSS software, version 10.0 (SPSS Inc, Chicago), and a value of $P < 0.05$ was considered to indicate significance.

RESULTS

Fatty acid composition of PBMC phospholipids

The fatty acid composition of PBMC phospholipids was not affected by the placebo treatment (Table 3). There were significant effects of time on the proportions of arachidonic acid (AA; 20:4n-6), EPA, and DHA in PBMC phospholipids ($P < 0.001$, $P = 0.004$, and $P = 0.013$, respectively; two-factor ANOVA). Significant effects of treatment and significant treatment \times time interactions were observed only for EPA and DHA (two-factor effect of treatment, $P < 0.001$ in both cases; treatment \times time interaction, $P < 0.001$ and $P = 0.007$, respectively). These effects were investigated further by one-factor ANOVA. The proportion of ALA in PBMC phospholipids did not change even in those subjects consuming ALA. However, consumption of ALA at the higher dose significantly increased the proportion of EPA in PBMC phospholipids ($P = 0.036$ and $P = 0.02$ at 3 and 6 mo compared with baseline; one-factor ANOVA), with the increase (<35%) not being significantly different between 3 and 6 mo. The proportion of EPA in PBMC phospholipids in the high-ALA group at 6 mo was significantly higher than that in the placebo

TABLE 4Cytokine production by peripheral blood mononuclear cells (PBMCs) in response to lipopolysaccharide in the treatment groups¹

Treatment and time	TNF- α	IL-6	IL-1 β	IL-10
	$\mu\text{g/L}$			
Placebo				
0 mo	11.1 \pm 1.3	37.2 \pm 4.5	5.6 \pm 0.6	577 \pm 118
6 mo	12.4 \pm 1.6	28.7 \pm 4.4	6.9 \pm 0.7	785 \pm 124
6 mo – 0 mo	1.1 \pm 1.9	-7.0 \pm 5.0	1.3 \pm 0.8	241 \pm 120
Low-ALA				
0 mo	12.3 \pm 1.4	33.4 \pm 3.7	5.7 \pm 0.6	469 \pm 69
6 mo	9.6 \pm 1.2	36.7 \pm 4.8	6.9 \pm 0.8	687 \pm 115
6 mo – 0 mo	-2.7 \pm 1.6	2.0 \pm 6.5	0.9 \pm 0.8	269 \pm 123
High-ALA				
0 mo	11.5 \pm 1.3	39.4 \pm 4.4	5.3 \pm 0.7	361 \pm 62
6 mo	10.7 \pm 1.5	37.1 \pm 3.4	5.6 \pm 0.7	573 \pm 107
6 mo – 0 mo	-0.7 \pm 1.5	-2.5 \pm 7.1	0.2 \pm 0.8	273 \pm 114
Low-EPA+DHA				
0 mo	8.4 \pm 1.2	35.5 \pm 4.8	4.7 \pm 0.8	490 \pm 96
6 mo	9.5 \pm 1.4	40.2 \pm 4.2	7.3 \pm 0.9	622 \pm 84
6 mo – 0 mo	1.2 \pm 1.8	2.9 \pm 6.0	2.6 \pm 1.0	152 \pm 127
High-EPA+DHA				
0 mo	11.0 \pm 1.8	31.2 \pm 4.1	4.9 \pm 0.7	663 \pm 169
6 mo	12.2 \pm 1.4	42.6 \pm 4.1	6.7 \pm 0.7	800 \pm 107
6 mo – 0 mo	1.3 \pm 1.4	11.1 \pm 6.4	1.8 \pm 0.9	152 \pm 115

¹ $\bar{x} \pm \text{SEM}$; $n = 29\text{--}31$ per treatment group. TNF- α , tumor necrosis factor α ; IL, interleukin; ALA, α -linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Two-factor ANOVA: no significant effects of time or treatment on production of TNF- α or IL-6; significant effects of time, but not of treatment, on the production of IL-1 β and IL-10 ($P = 0.002$ and $P = 0.009$, respectively); no significant treatment \times time interactions for the production of any of these cytokines ($P > 0.05$).

and low-ALA groups ($P = 0.004$ and $P = 0.014$, respectively; one-factor ANOVA). The proportion of DHA also decreased when ALA was consumed ($P = 0.008$ and $P = 0.002$ at 3 and 6 mo compared with baseline for the low-ALA diet and $P = 0.025$ at 6 mo compared with baseline for the high-ALA diet; one-factor ANOVA). The higher dose of EPA+DHA significantly increased the proportion of EPA in PBMC phospholipids by about 100% ($P = 0.006$ and $P < 0.001$ for 3 and 6 mo compared with baseline; one-factor ANOVA). The increase observed at 3 mo was not significantly different from that observed at 6 mo, and, at both of these time points, the proportion of EPA was significantly greater than that in the placebo, low-ALA, and low-EPA+DHA intervention groups ($P = 0.027$, $P = 0.003$, and $P < 0.001$, respectively, at 3 mo; $P < 0.001$ in all cases at 6 mo; one-factor ANOVA). The change in the proportion of EPA in the high-EPA+DHA intervention group was significantly different from that in the placebo and low-ALA intervention groups ($P = 0.015$ and $P = 0.035$, respectively; one-factor analysis of covariance). There was a trend for the proportion of DHA to increase in the high-EPA+DHA intervention group (20%), but the increased proportion did not differ significantly from baseline. However, the proportion of DHA at 6 mo was significantly higher in this group than in the placebo, low-ALA, and low-EPA+DHA intervention groups ($P = 0.002$, $P < 0.001$, and $P < 0.001$, respectively; one-factor ANOVA). The change in the proportion of DHA in the high-EPA+DHA intervention group differed

significantly from that in the placebo, low-ALA, and high-ALA intervention groups ($P = 0.006$, $P = 0.015$, and $P = 0.027$, respectively; one-factor analysis of covariance).

PBMC subsets

There were no effects of time or treatment on the proportion of PBMCs as T lymphocytes, helper T cells, cytotoxic T cells, B lymphocytes, or monocytes (data not shown). However, there was a significant effect of time ($P < 0.002$; two-factor ANOVA) but not of treatment and no significant treatment \times time interaction on the proportion of PBMCs as natural killer cells. The proportion of B lymphocytes or monocytes expressing intercellular adhesion molecule-1 and the level of that expression did not differ significantly between the groups and were not affected by the interventions (data not shown).

Phagocytosis by neutrophils and monocytes

The percentage of neutrophils and monocytes carrying out phagocytosis of *E. coli* ($\approx 75\%$ of neutrophils and $\approx 25\%$ of monocytes) and the activity of those cells (MFI) did not differ among the groups at baseline or at the end of intervention (data not shown). There was, however, a significant effect of time ($P < 0.001$ in both cases; two-factor ANOVA) but not of treatment and no significant treatment \times time interaction on MFI for both neutrophils and monocytes.

Oxidative burst by neutrophils and monocytes

The percentages of neutrophils and monocytes engaging in oxidative burst in response to *E. coli* ($\approx 90\%$ of neutrophils and $\approx 50\%$ of monocytes) or to phorbol ester ($\approx 90\%$ of neutrophils and $\approx 60\%$ of monocytes) and the activity of those cells (MFI) did not differ among the intervention arms at baseline or at the end of intervention and were not affected by the treatments (data not shown). There were no differences among the intervention arms with respect to the absolute changes in oxidative burst by monocytes or neutrophils (data not shown).

Cytokine production by PBMCs

Production of TNF- α , IL-1 β , IL-6, and IL-10 by PBMCs stimulated with 15 mg LPS/L did not differ among the groups at baseline or at the end of the intervention (Table 4). There were no significant effects of treatment on the production of these cytokines (two-factor ANOVA), but there was a significant effect of time on the production of IL-1 β and IL-10 ($P = 0.002$ and $P = 0.009$, respectively; two-factor ANOVA). In contrast, there was no significant treatment \times time interaction on the production of any of these cytokines. Production of IL-2, IL-4, and IFN- γ by PBMCs stimulated with 25 mg ConA/L did not differ among the intervention arms at baseline or at the end of intervention (Table 5). There were no significant effects of time or treatment on the production of these cytokines.

Lymphocyte proliferation

For all subjects, peak incorporation of thymidine occurred at a Con A concentration of 25 mg/L. There was no significant effect of time or treatment or a significant treatment \times time interaction on the incorporation of thymidine in response to any concentration of Con A used (data for 25 mg/L are shown in Table 5).

Delayed-type hypersensitivity response

Most subjects responded positively to one or two antigens, most frequently tetanus and tuberculin, with a mean cumulative

TABLE 5
Proliferation and cytokine production by peripheral blood mononuclear cells (PBMCs) in response to concanavalin A in the treatment groups¹

Treatment and time	Thymidine incorporation	IL-2	Interferon γ	IL-4
	<i>cpm/well</i>	<i>kU/L</i>	<i>kU/L</i>	<i>ng/L</i>
Placebo				
0 mo	29 345 \pm 2880	8.9 \pm 1.0	138 \pm 27	56 \pm 11
6 mo	31 288 \pm 1952	9.4 \pm 1.5	167 \pm 33	45 \pm 11
6 mo - 0 mo	1898 \pm 3776	0.5 \pm 1.0	18 \pm 29	-10 \pm 6
Low-ALA				
0 mo	33 086 \pm 2669	9.0 \pm 1.2	148 \pm 28	58 \pm 12
6 mo	34 637 \pm 1980	8.5 \pm 1.2	162 \pm 37	63 \pm 15
6 mo - 0 mo	1143 \pm 3546	-0.5 \pm 1.3	5 \pm 34	5 \pm 8
High-ALA				
0 mo	31 503 \pm 3402	8.3 \pm 1.7	98 \pm 18	46 \pm 9
6 mo	31 659 \pm 2347	7.2 \pm 1.1	126 \pm 24	46 \pm 11
6 mo - 0 mo	606 \pm 3863	-1.1 \pm 1.7	30 \pm 19	-6 \pm 12
Low-EPA+DHA				
0 mo	35 768 \pm 2880	7.8 \pm 1.0	117 \pm 25	77 \pm 22
6 mo	34 013 \pm 1797	10.5 \pm 2.0	133 \pm 19	50 \pm 10
6 mo - 0 mo	-1678 \pm 3493	2.0 \pm 1.5	21 \pm 21	-22 \pm 13
High-EPA+DHA				
0 mo	31 230 \pm 2911	8.0 \pm 1.0	113 \pm 24	67 \pm 13
6 mo	29 637 \pm 1542	8.5 \pm 1.2	155 \pm 40	57 \pm 11
6 mo - 0 mo	-2404 \pm 3053	0.6 \pm 1.0	46 \pm 39	-11 \pm 7

¹ $\bar{x} \pm$ SEM; $n = 29-31$ per treatment group. IL, interleukin; ALA, α -linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. There were no significant effects of time or treatment or significant treatment \times time interactions on lymphocyte proliferation or on production of IL-2, interferon γ , or IL-4, $P > 0.05$ (two-factor ANOVA).

area of response of ≈ 20 mm². There were no significant differences among the groups at baseline or at the end of intervention in terms of the number of positive indurations or the cumulative area of induration (Table 6). There was no significant effect of treatment or time or a significant treatment \times time interaction on the number of positive indurations or the cumulative area of induration.

DISCUSSION

This study investigated the effect of increased intakes of n-3 PUFAs of plant (ALA) or marine (EPA+DHA) origin on a range of immune measures representative of key functional activities of neutrophils, monocytes, and lymphocytes. The effects of n-3 PUFAs on many of these variables were studied previously (see Introduction for references), but the effects on the production of IL-4 and IL-10 by human cells were not investigated. Many of the previous studies involved the provision of large doses of the fatty acids in capsules rather than that of more modest doses through foods, did not investigate more than a single dose of the fatty acid under study, were of relatively short duration, studied a small number of subjects, and did not attempt to normalize the amount of vitamin E provided relative to fatty acid double bonds (4). The current study was placebo controlled, involved a larger number of subjects and was of longer duration than most previous studies of this type, provided the n-3 PUFAs at 2 concentrations and largely through food, and normalized the amount of vitamin E provided relative to the number of fatty acid double bonds.

The amounts of ALA provided through the interventions in the current study (4.5 or 9.5 g/d when combined with intake from the background diet) increased total ALA intake 3- to 6-fold (27). The proportion of ALA in PBMC phospholipids did increase in the

subjects who consumed additional ALA. The product of ALA elongation and desaturation (EPA) was significantly elevated in PBMC phospholipids in the high-ALA group, whereas there was a trend toward a decrease in DHA (also a product of ALA elongation and desaturation) over the 6-mo intervention period. Such changes in the proportions of EPA and DHA after greatly increased ALA consumption were shown previously (9, 28). Thus, it appears that, when ALA is included in the diet, it is not incorporated into PBMC phospholipids in significant amounts. Furthermore, if it is elongated and desaturated, the products of those processes are differentially incorporated into PBMC phospholipids.

The amount of EPA+DHA provided in the EPA+DHA interventions in the current study (0.77 or 1.7 g/d when combined with intake from the background diet) increased total EPA+DHA intake 3- and 6-fold, respectively (27). EPA intake in these 2 groups was < 0.3 and 0.7 g/d, respectively. There was a 2-fold increase in the proportion of EPA and a 25% increase in the proportion of DHA in PBMC phospholipids in the high-EPA+DHA group. These changes are consistent with those reported previously for PBMCs in subjects given EPA+DHA (25, 29, 30). Increased intake of EPA+DHA resulted in a decrease ($< 20\%$) in AA over the 6-mo intervention in both EPA+DHA treatment groups. Thus, this study shows that n-3 PUFAs of plant and marine origin can modulate the content of AA, EPA, and DHA in PBMC phospholipids. Furthermore, it shows that 9.5 g ALA/d induces a greater increase in PBMC phospholipid EPA content than does 0.3 g EPA/d, but a lesser increase than does 0.7 g EPA/d.

The observed lack of effect of the treatments on circulating inflammatory or immune cell proportions suggests that modest increases in the consumption of n-3 PUFAs will not alter the numbers and types of inflammatory and immune cells in the circulation. This finding agrees with a previous study providing 1 g EPA+DHA/d, 0.7 g DHA/d, or 2 g ALA/d, in which no changes in

TABLE 6
Delayed-type hypersensitivity response in subjects in the treatment groups¹

Treatment and time	Indurations ²	Cumulative area ³ <i>mm</i> ²
Placebo		
0 mo	2.0 (1.5, 3.0)	26.8 ± 6.7
6 mo	1.0 (1.0, 3.0)	21.8 ± 5.8
6 mo - 0 mo	-1.0 (-1.0, 0.0)	-4.9 ± 6.2
Low-ALA		
0 mo	1.0 (0.0, 2.0)	19.2 ± 4.5
6 mo	1.0 (1.0, 2.0)	14.5 ± 4.9
6 mo - 0 mo	0.0 (0.0, 0.0)	-4.7 ± 4.9
High-ALA		
0 mo	1.0 (1.0, 2.0)	20.9 ± 7.5
6 mo	1.0 (1.0, 2.0)	19.4 ± 6.7
6 mo - 0 mo	0.0 (0.0, 0.0)	-1.5 ± 9.9
Low-EPA+DHA		
0 mo	2.0 (1.0, 2.5)	22.6 ± 8.8
6 mo	1.0 (1.0, 2.0)	18.6 ± 5.2
6 mo - 0 mo	0.0 (-1.0, 1.0)	-4.0 ± 7.2
High-EPA+DHA		
0 mo	2.0 (1.0, 3.0)	21.1 ± 4.5
6 mo	1.0 (1.0, 1.0)	16.1 ± 3.3
6 mo - 0 mo	0.0 (-0.5, 0.0)	-5.0 ± 3.0

¹ALA, α -linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. There were no significant effects of time or treatment or a significant treatment \times time interaction on the number of indurations or the cumulative area of the delayed-type hypersensitivity response, $P > 0.05$ (two-factor ANOVA).

²Median; 25th and 75th percentile in parentheses. $n = 16$ – 19 per treatment group.

³ $\bar{x} \pm$ SEM; $n = 16$ – 19 per treatment group.

the numbers of leukocytes or the proportions of different leukocytes were observed (31). However, Kelley et al (17) reported that 6 g DHA/d induced a decrease in circulating total leukocyte numbers, which was largely due to a decrease in granulocyte numbers; neither the total numbers of monocytes and lymphocytes nor the proportion of leukocytes as monocytes changed, but the proportion of lymphocytes increased. Combined, these observations suggest that high intakes of either DHA or long-chain $n-3$ PUFAs in general but not of ALA [because 4.5, 9.5, or 18 g ALA/d did not alter the numbers of granulocytes, lymphocytes, or monocytes in the circulation (present study and 19)] might affect the numbers of circulating leukocytes, especially granulocytes. Most of the studies using fish oil have not reported leukocyte numbers, and so it is not clear whether this finding is an effect of both EPA and DHA or of DHA alone.

The present study did not identify major effects of ALA on any of the immune functions assessed. Previous studies using high doses of ALA (14 or 18 g/d) observed decreased TNF- α and IL-1 β production (9) and decreased lymphocyte proliferation, IL-2 production, and DTH response (19). In contrast, a study using 2 g ALA/d found no effect on the phagocytic activity or oxidative burst of monocytes or neutrophils (31); the production of TNF- α , IL-1 β , or IL-6 by monocytes (31); the production of IL-2 or IFN- γ by lymphocytes (25); or lymphocyte proliferation (25). Furthermore, 4 g ALA/d did not affect neutrophil oxidative burst (32). The present study extends these earlier observations by showing that an increase in ALA intake to 9.5 g/d does not induce alterations in human immune cell function. Because the present

study involved a controlled dietary intervention, the increased intake of ALA was largely at the expense of linoleic acid, and thus the ratio of $n-6$ to $n-3$ PUFAs in the diet differed markedly between subjects receiving the placebo intervention and those receiving the ALA interventions (a 3- or 8-fold difference). Because increased ALA intake did not affect the immune functions measured, it can be concluded that these functions are not influenced by significant alterations in the ratio of linoleic acid to ALA in the diet.


The present study did not identify major effects of EPA+DHA on any of the immune cell functions assessed. The observed lack of effect on leukocyte phagocytosis is consistent with a study in which neither EPA nor DHA at 3.8 g/d affected the percentage of monocytes engaged in phagocytosis (33). In previous studies reporting that EPA+DHA decreased oxidative burst by neutrophils (5–7) or monocytes (8); decreased TNF- α , IL-1 β , and IL-6 production by monocytes (9–12); decreased IL-2 and IFN- γ production by lymphocytes (11, 12, 14); and decreased lymphocyte proliferation (11–14), supplementary EPA+DHA doses of >2.4 g/d and ≤ 9.6 g/d have generally been used. The present study identifies an intake of EPA+DHA (1.7 g/d) that is substantially above the habitual intake but that does not have detrimental immunologic effects in healthy subjects. The lack of a significant effect of EPA+DHA on the DTH response accords with the lack of effect on lymphocyte proliferation and cytokine production. The increased intake of EPA+DHA in the low- and high-EPA+DHA groups was achieved at the same time that the linoleic acid and ALA intakes were kept constant. Thus, the EPA+DHA interventions resulted in an alteration in the ratio of $n-6$ to $n-3$ PUFAs in the diet but against a background of a constant ratio of linoleic acid to ALA. The findings of the present study suggest that even a marked change in the ratio of $n-6$ to $n-3$ PUFAs in the diet due to increased intake of long-chain $n-3$ PUFAs will not significantly alter immune cell function in healthy subjects.

Several important conclusions can be drawn from this study. First, increasing ALA intake to 9.5 g/d in subjects with a habitual intake of <1.5 g/d does not affect immune cell function. Second, an ALA intake of 9.5 g/d does not mimic previously reported effects of long-chain $n-3$ PUFAs that have been described as anti-inflammatory (eg, decreased superoxide and TNF- α production) and that may be desirable in certain settings (3, 4). Third, increasing EPA+DHA intake to 1.7 g/d in subjects with a habitual intake of <0.5 g/d does not affect immune cell function. Fourth, an EPA+DHA intake of 1.7 g/d is below the threshold required to exert anti-inflammatory effects in healthy subjects. Fifth, marked changes in the ratio of $n-6$ to $n-3$ PUFAs in the diet due to increased intake of either ALA or EPA+DHA at the expense of linoleic acid do not affect immune cell function among subjects with the characteristics of those studied here.

Increased consumption of $n-3$ PUFAs did not significantly alter immune cell functions examined under the conditions identified in preliminary experiments, such as stimulant concentration and incubation time. The present study did not investigate the effects of the $n-3$ PUFAs on immune cell responses to suboptimal concentrations of the various stimuli used (except for lymphocyte proliferation) or on the kinetics of those responses. Therefore, it is not clear from this study whether increased consumption of $n-3$ PUFAs might affect the time course of immune cell responses or the sensitivity of those responses (except for lymphocyte proliferation) to stimulants.

In summary, the present study shows that intakes of plant- or marine-derived $n-3$ PUFAs up to 9.5 or 1.7 g/d, respectively, do not influence circulating inflammatory and immune cell



numbers and do not alter the functional responses of inflammatory and immune cells (determined under the conditions used here) over a 6-mo period more than does the intake of an n-6 PUFA-rich control. Therefore, it appears that the consumption of n-3 PUFAs could be increased in line with recommendations without inducing adverse effects on the innate or acquired immune response and thus on the ability of persons to mount a successful immune response. 

AMM, RM, RA, CMW, and PCC were involved in the study design. AMM and YEF were involved in subject recruitment and carrying out the intervention under the supervision of CMW. SK, TB, AMM, and YEF were involved in data collection under the supervision of CMW and PCC. SK and PCC performed the data analysis. SK and PCC wrote the manuscript, with contributions from all other authors. RM is an employee of Roche Vitamins Ltd. RA is an employee of Unilever Health Institute Vlaardingen. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

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