Dietary Supplementation with γ-Linolenic Acid or Fish Oil Decreases T Lymphocyte Proliferation in Healthy Older Humans[1]

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ABSTRACT Animal and human studies have shown that greatly increasing the amounts of flaxseed oil [rich in the (n-3) polyunsaturated fatty acid (PUFA) α-linolenic acid (ALNA)] or fish oil [FO; rich in the long chain (n-3) PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] in the diet can decrease mitogen-stimulated lymphocyte proliferation. The objective of this study was to determine the effect of dietary supplementation with moderate levels of ALNA, γ-linolenic acid (GLA), arachidonic acid (ARA), DHA or FO on the proliferation of mitogen-stimulated human peripheral blood mononuclear cells (PBMC) and on the production of cytokines by those cells. The study was randomized, placebo-controlled, double-blinded and parallel. Healthy subjects ages 55–75 y consumed nine 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 with oils rich in ALNA, GLA, ARA or DHA or FO. Subjects in these groups consumed 2 g of ALNA or 770 mg of GLA or 680 mg of ARA or 720 mg of DHA or 1 g of EPA plus DHA (720 mg of EPA + 280 mg of DHA) daily from the capsules. Total fat intake from the capsules was 4 g/d. The fatty acid composition of PBMC phospholipids was significantly changed in the GLA, ARA, DHA and FO groups. Lymphocyte proliferation was not significantly affected by the placebo, ALNA, ARA or DHA treatments. GLA and FO caused a significant decrease (up to 65%) in lymphocyte proliferation. This decrease was partly reversed by 4 wk after stopping the supplementation. None of the treatments affected the production of interleukin-2 or interferon-γ by PBMC and none of the treatments affected the number or proportion of T or B lymphocytes, helper or cytotoxic T lymphocytes or memory helper T lymphocytes in the circulation. We conclude that a moderate level GLA or EPA but not of other (n-6) or (n-3) PUFA can decrease lymphocyte proliferation but not production of interleukin-2 or interferon-γ. J. Nutr. 131: 1918–1927, 2001.

KEY WORDS: • *fish oil* • *immunity* • *lymphocyte* • *cytokine* • *polyunsaturated fatty acids* • *humans*

Lymphocytes can be classified as either T or B lymphocytes by the presence of CD3 or CD19, respectively, on their surface. When T lymphocytes are presented with antigen, they become activated, secrete cytokines and ultimately enter the cell cycle and divide (1). This so-called proliferation of lymphocytes leads to an increase in the number of antigen-specific lymphocytes and, as such, is a key component of the regulation, amplification and memory of the cell-mediated immune response (1). In cell culture, the stimulation and subsequent proliferation of T lymphocytes can be achieved by mitogens, such as concanavalin A (Con A)[4], which bind to the T cell receptor-CD3 complex (2). T lymphocytes are classified into helper T (Th) cells, distinguished by the presence of the molecule CD4 on their surface, and cytotoxic T (Tc) cells, distinguished by the presence of CD8 on their surface. Th lymphocytes can be further subdivided according to the expression of CD45 isoforms on their cell surface. It is believed that naive Th cells (i.e., those that have not yet encountered an activating stimulus) express CD45RA, whereas memory Th cells (i.e., those that have been produced as a consequence of exposure to an activating stimulus) express CD45RO (1). Both Th and Tc lymphocytes can also be subdivided functionally according to the pattern of cytokines that they produce. Type-1 helper or cytotoxic T lymphocytes produce interleukin (IL)-2 and interferon-γ (IFN-γ), whereas type-2

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[4] Abbreviations used: ALNA, α-linolenic acid; ARA, arachidonic acid; Con A, concanavalin A; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FACS, fluorescence-activated cell sorter; FAME, fatty acid methyl esters; FITC, fluorescein isothiocyanate; FO, fish oil; GLA, γ-linolenic acid; IFN-γ, interferon-γ; IL, interleukin; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; RPMI, Roswell Park Memorial Institute; Tc, cytotoxic T; Th, helper T.

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helper or cytotoxic T lymphocytes produce IL-4, IL-5 and IL-10 (1), although T cells are not the only sources of these cytokines.

Addition of PUFA to animal or human lymphocytes in culture leads to decreased ability of the cells to proliferate in response to Con A (3–5) and to produce IL-2 (6–8). The long chain (n-3) PUFA, eiocosapentaenoic acid [EPA; 20:5 (n-3)] and docosahexaenoic acid [DHA; 22:6 (n-3)] seem to be particularly potent inhibitors of these processes, but several other fatty acids, including α-linolenic acid [ALNA; 18:3 (n-3)], γ-linolenic acid [GLA; 18:3 (n-6)] and arachidonic acid [ARA; 20:4 (n-6)] are also active in cell culture (3–7).

Animal feeding studies show that the type of fatty acid within the diet affects lymphocyte proliferation; in accordance with their effects in vitro, (n-3) PUFA seem to have particularly potent effects. Increasing the amount of ALNA in the rodent diet decreases spleen lymphocyte proliferation compared with linoleic acid-rich diets (9–11), whereas feeding laboratory animals diets containing large amounts of fish oil (FO), which is rich in EPA and DHA, results in suppressed lymphocyte proliferation compared with feeding low fat diets or high fat diets rich in saturated fat or linoleic acid (12–18). Feeding rats diets rich in GLA also decreases spleen lymphocyte proliferation compared with feeding some other diets (19). There have been relatively few studies of dietary fatty acids and cytokine production by animal lymphocytes, although long chain (n-3) PUFA decreased IL-2 production by lymphocytes from pigs (20) and mice (16,21) and decreased IFN-γ production by lymphocytes from mice (21).

One human study has reported the effect of including an increased amount of ALNA in the human diet on lymphocyte proliferation: 18 g of ALNA/d for 8 wk resulted in a significant decrease in Con A-stimulated lymphocyte proliferation (22). Supplementation of the diet of healthy humans with FO providing 1.2 to 5.2 g of EPA plus DHA/d has been reported to decrease lymphocyte proliferation (23–26) and the production of IL-2 (23,24,27) and IFN-γ (27). The habitual intake of ALNA among adults in the United Kingdom is 1–2 g/d, while that of the long chain (n-3) PUFA is <150 mg/d (28,29). Thus, the amounts of these fatty acids provided in the supplementation studies performed to date are greatly in excess of habitual intakes and greatly in excess of intakes that are recommended or that could be achieved in most individuals through dietary change. The influence that lower levels of these fatty acids have on human immune function is not clear. Therefore, in the current study the effect of moderate supplementation of the diet of healthy, free-living subjects ages 55–75 y with encapsulated oil blends rich in ALNA, GLA, ARA, DHA or EPA upon lymphocyte proliferation and the production of IL-2 and IFN-γ was investigated.

METHODS

Materials. PBS tablets were obtained from Unipath, Basingstoke, United Kingdom. Histopaque, bovine serum albumin (fatty acid-free), HEPES-buffered Roswell Park Memorial Institute (RPMI) medium, glutamine, antibiotics (penicillin and streptomycin), Con A, boron trifluoride, propidium iodide, phycoerythrin-cyanin 5.1-labeled mouse anti-human CD19 (clone SJ25-C1) and phycoerythrin-cyanin 5.1-labeled mouse anti-human CD4 (clone Q4120) were purchased from Sigma Chemical Ltd. (Poole, UK). Fluorescein isothiocyanate (FITC)-labeled mouse anti-human CD8 (clone DK25) was purchased from DAKO (Ely, UK). FITC-labeled mouse anti-human CD45 (clone Immu19.2) in combination with phycoerythrin-labeled mouse anti-human CD14 (clone RMO52), FITC-labeled mouse anti-human CD3 (clone UCHT1) and phycoerythrin-labeled mouse anti-human CD (16 + 56; clones 3G8 + NKH1) were purchased from Coulter Corp. (Hialeah, FL). Fluorescence-activated cell sorter (FACS)-lysing solution was purchased from Becton Dickinson (Mountain View, CA). [^{3}H] Thymidine was purchased from Amersham International (Amersham, UK) and solvents were purchased from Fisher Scientific (Loughborough, UK). Cytokine EASIA enzyme-linked immunosorbent assay kits were obtained from BioSource (Fleurus, Belgium).

Subjects and study design. Ethical permission for all procedures involving humans was obtained from the Central Oxford Research Ethics Committee (No. 96.182). All volunteers completed a health and lifestyle questionnaire before entering the study and doctor's consent for inclusion into the study was obtained. Subjects were excluded if they were taking any prescribed medication; had diagnosed hypercholesterolemia, hypertriglyceridemia, coronary heart disease, diabetes or a chronic inflammatory disease; took aspirin regularly; were vegetarian; consumed FO, evening primrose oil or vitamin capsules; were blood donors; had undergone recent weight loss or smoked > 10 cigarettes/d. The characteristics of the 46 subjects who completed in the study are given in **Table 1**; mean age and body mass index did not differ among the treatment groups. All subjects were white and free-living; all lived in their own homes and none were disabled or immobile in any way. Twenty-seven subjects were in full-time employment and 19 were retired. All female subjects were postmenopausal and none were taking hormone-replacement therapy. None of the subjects participated in intense or vigorous exercise. Subjects height was measured to the nearest millimeter and weight to the nearest kilogram.

Forty-eight subjects were randomly allocated to receive one of the six types of encapsulated oil blends in a double-blind manner ($n = 8$ per treatment group); these blends are referred to as placebo, ALNA, GLA, ARA, DHA and FO. The placebo was an 80:20 mixture of palm and sunflower seed oils (Loders Croklaan, Wormeveer, The Netherlands). The ALNA blend was a 50:13:37 mixture of palm, sunflower seed and super refined flaxseed oils (flaxseed oil supplied by Loders Croklaan). The GLA blend was a 21:5:74 mixture of palm oil, sunflower seed oil and a GLA-rich triacylglycerol (Scotia Pharmaceuticals, Stirling, UK). The ARA and DHA blends were a 43:11:46 mixture of palm oil, sunflower seed oil and ARASCO or DHASCO, respectively (ARASCO and DHASCO; Martek, Columbia, MD).

TABLE 1

Characteristics of the treatment groups

Treatment	Subjects, *n*	Males:females	Smokers, *n*	Age,[1] *y*	Body mass index,[1] *kg/m²*
Placebo	8	5:3	1	62 (56–69)	25.1 (21.3–29.7)
ALNA	8	4:4	0	66 (60–74)	25.5 (22.2–28.0)
GLA[2]	7	3:4	0	64 (55–71)	23.3 (18.6–27.1)
ARA	8	4:4	0	61 (56–70)	24.1 (21.3–27.3)
DHA	8	5:3	0	65 (58–71)	23.5 (19.6–28.4)
FO[2]	7	3:4	0	62 (60–68)	26.7 (22.8–31.1)

[1] Values are mean values with ranges shown in parentheses.
[2] Excludes values for one female subject who dropped out during the study.

TABLE 2

Fatty acid composition of oil blends used in the study

Treatment	Fatty acid										
	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	GLA	ALNA	ARA	EPA	DHA
	g/100 g total fatty acids										
Placebo	0	37.4	0	4.0	36.0	22.7	0	0.2	0	0	0
ALNA	0	9.9	0	2.8	18.0	15.8	0	53.5	0	0	0
GLA	0	9.6	0	1.1	10.4	58.6	20.3	0.3	0	0	0
ARA	0	25.1	0	4.9	34.2	16.3	1.5	0.2	17.9	0	0
DHA	8.0	24.5	1.1	2.4	29.8	13.4	0	0.3	0	0	19.1
FO	10.7	19.1	9.8	3.2	11.9	1.2	0.5	0.9	1.4	18.8	7.4

The FO used was a Chilean FO (Loders Croklaan) containing 26 g of EPA plus DHA/100 g of total fatty acids. The fatty acid composition of the oil blends (determined by gas chromatography on total lipid extracts; see below) is shown in **Table 2**. The capsules were gelatin-coated, nontransparent and green-colored. Each capsule contained 445 mg of the oil blend. Subjects consumed 9 capsules/d (i.e., 4 g of encapsulated oil/d) for 12 wk. Therefore, subjects in the ALNA group consumed an extra 2.0 g of ALNA/d, subjects in the GLA, ARA and DHA groups consumed an extra ~700 mg of GLA, ARA or DHA/d, respectively, and subjects in the FO group consumed an extra 1 g of EPA plus DHA/d (720 mg of EPA + 280 mg of DHA). All capsules contained 300 μg of α-tocopherol-equivalents plus 180 μg of ascorbyl-palmitate/g of oil. Thus, all subjects consumed an extra 1.2 mg of α-tocopherol/d.

Two female subjects dropped out of the study once it was underway, one from the GLA group, due to illness, and one from the FO group, due to inability to comply because of stomach upsets.

Blood was sampled immediately before beginning supplementation, every 4 wk during supplementation and after a 4-wk washout period. Throughout this manuscript, wk 0 represents the baseline measurements, wk 4, 8 and 12 represent the supplementation period and wk 16 represents the end of the washout period. All treatment groups completed the study in parallel. The study was conducted from June 1997 (early summer) to December 1997 (early winter).

The capsules were provided to the subjects in blister packs (nine capsules/pack) with seven blister packs/box along with clear instructions of how they should be administered (three capsules three 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 a self-reporting questionnaire and, biochemically, by determining the plasma phospholipid fatty acid composition.

Assessment of habitual nutrient intakes. Subjects completed a 7-d food diary (during July and August 1997, midsummer). None of the subjects ate outside of their home during the week in which the food diaries were completed. Habitual nutrient intakes were determined using FOODBASE, Version 1.3 (Institute of Brain Chemistry, London, UK).

Preparation of peripheral blood mononuclear cell (PBMC). Blood samples were collected into heparinized vacutainer tubes between 0800 and 1000 h after a fast of at least 12 h 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 $\times$ *g* at 20°C. The cells (PBMC) were collected from the interphase, washed once with PBS, resuspended in 2.5 mL of PBS and layered onto 5 mL of Histopaque. They were centrifuged once more to achieve a lower degree of erythrocyte contamination, washed with PBS and finally resuspended.

Analysis of leukocyte numbers. Lymphocyte numbers and subsets were analyzed only in the blood samples collected at wk 0 (baseline), 12 (end of supplementation) and 16 (washout).

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

For the determination of lymphocyte subsets, whole blood (100 μL) was incubated with various combinations of fluorescently labeled monoclonal antibodies (20 μL) for 40 min at 12°C. Monoclonal antibody combinations used were anti-CD45/anti-CD14 (to distinguish lymphocytes as $CD45^+CD14^-$), anti-CD3/anti-CD16/anti-CD56 (to distinguish T lymphocytes as $CD3^+CD16^-$), anti-CD19/anti-CD16/anti-CD56 (to distinguish B lymphocytes as $CD19^+CD16^-CD56^-$), anti-CD4/anti-CD8/anti-CD45RO (to distinguish Th cells as $CD4^+CD8^-$, Tc cells as $CD4^-CD8^+$ and memory Th cells as $CD4^+CD8^-CD45RO^+$). Erythrocytes were then lysed and leukocytes fixed with 3 mL of Becton Dickinson FACS-lysing solution, 10 min. Leukocytes were collected by centrifugation (250 $\times$ *g* for 5 min), resuspended in 3 mL of PBS and then centrifuged again. Finally, they were resuspended in 1 mL of PBS and analyzed in a Coulter XL/MCL flow cytometer (Coulter Corp.). Fluorescence data were collected on 10^4 cells and were analyzed using System II software.

Analysis of PBMC phospholipid fatty acid composition. Lipid was extracted from PBMC with chloroform/methanol (2:1 v/v) and phospholipids isolated by thin layer chromatography using a mixture of hexane:diethyl ether:acetic acid (90:30:1 v/v/v) as the elution phase. Fatty acid methyl esters (FAME) were prepared by incubation with 140 g/L of boron trifluoride at 80°C for 60 min. FAME were isolated by solvent extraction, dried and separated by gas chromatography in a Hewlett-Packard 6890 gas chromatograph (Hewlett Packard, Avondale, PA) fitted with a 30-m $\times$ 0.32-mm BPX70 capillary column (film thickness: 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 was maintained at 170°C for 12 min after sample injection and was programmed to then increase from 170 to 210°C at 5°C/min before being maintained at 210°C for 15 min. The separation was recorded with Hewlett Packard gas chromatography Chem Station software (Hewlett Packard). FAME were identified by comparison with standards assayed previously.

Measurement of lymphocyte proliferation in PBMC cultures. PBMC (2×10^5) were cultured in HEPES-buffered RPMI medium, supplemented with 2 mmol/L of glutamine, 25 mL/L of autologous plasma, antibiotics and Con A at final concentrations of 5, 15, 25 and 50 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 [^{3}H]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 stimulation index where:

Stimulation index

$$= \frac{[^3H]\text{thymidine incorporation in the presence of Con A}}{[^3H]\text{thymidine incorporation in the absence of Con A}}.$$

Measurement of the production of cytokines by PBMC cultures. PBMC (2×10^6) were cultured for 24 h in HEPES-buffered RPMI medium, supplemented with 2 mmol/L of glutamine, 25 mL/L of autologous plasma, antibiotics and 15 mg/L of Con A; the final culture volume was 2 mL. At the end of the incubation, the plates were centrifuged and the culture medium was collected and frozen in aliquots. The concentrations of IL-2 and IFN-γ were measured by specific EASIA enzyme-linked immunosorbent assays. Limits of detection for these assays were 100 U/L (IL-2) and 30 U/L (IFN-γ; data supplied by the manufacturer of the kits). The CV was <10% for both assays. One unit of IL-2 is equivalent to 1.1 IU as defined by the National Institutes of Health standard NIBSC 86/504. One unit of IFN-γ is equivalent to 1 IU as defined by the National Institutes of Health standard Gg 23-901-530.

Statistical analysis. Sample size (i.e., number of subjects per treatment group) was calculated on the basis of measurements made previously in our laboratory using the same methods as those used in this study and of existing data from the literature (23–25). It was determined that a sample size of eight would detect a difference in lymphocyte proliferation and cytokine production of ≥25% at $P \leq 0.05$ with 80% power.

Unless otherwise indicated, results are expressed as mean ± SEM for 7 or 8 subjects per treatment group. One-factor ANOVA was used to determine differences among treatment groups at baseline (wk 0) and to determine differences in absolute change over the treatment period (i.e., wk 12 − wk 0). Statistical significances of treatment, of time and of their interaction were determined using two-factor repeated-measures ANOVA. If the interaction between treatment and time was significant, the effects of treatment and of time were further analyzed by one-factor ANOVA. Bonferroni's correction for multiple comparisons was used in all cases. All statistical tests were performed using SPSS, Version 6.0 (SPSS, Chicago, IL), and a value of $P \leq 0.05$ was taken to indicate statistical significance.

RESULTS

Fatty acid intakes of the subjects. Habitual intakes of individual fatty acids did not differ among the treatment groups. Habitual intakes of fatty acids among all subjects (g/d) were: myristic acid (14:0) 3.5 ± 0.2, palmitic acid (16:0) 14.9 ± 0.5, palmitoleic acid [16:1 (n-7)] 1.27 ± 0.05, stearic acid (18:0) 7.2 ± 0.3, oleic acid [18:1 (n-9)] 19.8 ± 0.7, linoleic acid [18:2 (n-6)] 9.8 ± 0.6, GLA 0.005 ± 0.001, ALNA 0.89 ± 0.05, ARA 0.15 ± 0.01, EPA 0.09 ± 0.01 and DHA 0.15 ± 0.01. Habitual intakes of total saturated fatty acids, total monounsaturated fatty acids, total PUFA, total (n-6) PUFA and total (n-3) PUFA did not differ among treatment groups (data not shown); the (n-6):(n-3) PUFA ratio of the habitual diet also did not differ among treatment groups (9.0 ± 0.7).

Intakes of individual fatty acids during the period of treatment with the supplements were calculated by adding habitual intakes to intakes due to the supplements. Intakes of myristic, palmitic, palmitoleic, stearic, oleic and linoleic acids during supplementation were not significantly different from habitual intakes, and during supplementation, intakes of these fatty acids were not different among the treatment groups (data not shown). In contrast, supplementation affected the intakes of ALNA, GLA, ARA, EPA and DHA, such that there were significant differences in the intakes of these fatty acids among the different treatment groups ($P < 0.0001$ for ALNA, GLA, ARA and EPA; $P = 0.0003$ for DHA). The intake of ALNA in the ALNA group was 2.94 ± 0.17 g/d; the intake of GLA in the GLA group was 775 ± 1 mg/d; the intake of ARA in the ARA group was 820 ± 20 mg/d; the intake of DHA in the DHA group was 850 ± 20 mg/d; and the intakes of EPA and DHA in the FO group were 800 ± 20 and 400 ± 30 mg/d, respectively.

Fatty acid composition of PBMC phospholipids. The fatty acid composition of PBMC phospholipids was not affected by the placebo or ALNA treatments. ALNA and GLA did not appear in PBMC phospholipids even in those subjects supplementing their diets with the ALNA or GLA capsules. Two-factor ANOVA did not detect any significant effects of time or treatment on the proportions of palmitic acid (~21 g/100 g of total fatty acids), stearic acid (~22 g/100 g of total fatty acids) oleic acid (~14 g/100 g of total fatty acids) or linoleic acid (see **Table 3**) in PBMC phospholipids. In contrast, there were significant time-dependent effects of treatment upon the proportions of dihomo-γ-linolenic acid [DGLA; 20:3 (n-6)], ARA, EPA and DHA in PBMC phospholipids (two-factor ANOVA effects of both time and treatment $P < 0.001$ in all cases and time × treatment interaction $P = 0.05$ for DGLA and $P < 0.001$ for ARA, EPA and DHA). These effects were investigated further by one-factor ANOVA.

Effects of GLA supplementation. The proportion of DGLA in PBMC phospholipids was significantly higher after 8 and 12 wk of GLA supplementation than at baseline and after washout (Table 3). After 4, 8 and 12 wk of GLA supplementation, the proportion of DGLA in PBMC phospholipids was significantly higher in the GLA group than in the each of the other groups, including the placebo group (Table 3).

Effects of ARA supplementation. The proportion of ARA in PBMC phospholipids was significantly higher after 8 and 12 wk of ARA supplementation than at baseline and after washout (Table 3). After 4 wk of washout, the proportion of ARA in PBMC phospholipids was not different from that observed at baseline (Table 3). The proportion of ARA in PBMC phospholipids was significantly higher at wk 8 and 12 in the ARA group than in the DHA and FO groups (Table 3). ARA supplementation resulted in a significant decrease in the proportion of DGLA in PBMC phospholipids (Table 3).

Effects of DHA supplementation. The proportion of DHA in PBMC phospholipids was higher after 4, 8 and 12 wk of DHA supplementation than at baseline and after washout (Table 3). After 4 wk of washout, the proportion of DHA in PBMC phospholipids was not different from that observed at baseline (Table 3). The proportion of DHA was significantly higher at wk 4, 8 and 12 in the DHA group than in each of the other groups, including the placebo group (Table 3).

Effects of FO supplementation. There was a significant increase in the proportion of EPA in the PBMC 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 than that in each of other groups, including the placebo, after 4, 8 or 12 wk of supplementation (Table 3). This increase in the proportion of EPA in PBMC phospholipids was maximal after 4 wk of FO supplementation (Table 3). The maximal increase in the proportion of EPA after FO supplementation was approximately twofold above baseline (Table 3). The proportion of ARA was significantly lower after 4 or 12 wk of FO supplementation than after washout, although it was not different from the proportion observed at baseline (Table 3).

Effect of treatments on lymphocyte numbers and subsets. The total number of lymphocytes and the proportions of T lymphocytes, B lymphocytes, Th lymphocytes, Tc lymphocytes and memory Th cells (defined by CD45RO expression) in the circulation did not differ among the treatment groups at baseline or at the end of supplementation (**Table 4**). There was no effect of any of the treatments on the total number of

lymphocytes or on the proportions of T lymphocytes, B lymphocytes, Th lymphocytes, Tc lymphocytes or memory Th cells in the circulation (Table 4). The changes in total lymphocyte numbers and in the proportions of the lymphocyte subsets over the course of the treatment period did not differ among the groups (Table 4).

Effect of treatments on lymphocyte proliferation. There was larger than expected variation among individuals in the proliferative response of lymphocytes to Con A. Among all subjects the stimulation index (mean ± SD) was 181.5 ± 14.4, 234.4 ± 15.7, 161.0 ± 12.5 and 51.3 ± 4.6 at Con A concentrations of 5, 15, 25 and 50 mg/L, respectively. Despite this variation, lymphocyte proliferation determined as either thymidine incorporation (data not shown) or as stimulation index measured at each concentration of Con A did not differ among the treatment groups at baseline (**Table 5**).

There were significant effects of time and treatment and significant time × treatment interactions for stimulation index in response to Con A concentrations of 5, 15 and 25 mg/L but not at 50 mg/L (P for time × treatment interaction = 0.023, 0.043 and 0.025 at Con A concentrations of 5, 15 and 25 mg/L, respectively). These effects were investigated further by one-factor ANOVA. There were no significant effects of placebo, ALNA, ARA or DHA treatment on stimulation index, although the stimulation index did decline in each of these treatment groups (Table 5). The average decreases after 12 wk of treatment and across the four concentrations of Con A used were 11% (placebo group), 32% (ALNA group), 35% (ARA group) and 29% (DHA group). Treatment with GLA or FO significantly affected lymphocyte proliferation (Table 5). FO resulted in a significant time-dependent decline in lymphocyte proliferation at all Con A concentrations, such that after 12 wk of treatment, stimulation index was significantly lower than at wk 0 (baseline) and wk 4 (**Fig. 1**). The change in stimulation index after 12 wk of FO treatment was significantly different from the change in the placebo group (Table 5). The mean decline in stimulation index after 12 wk of FO treatment was 55%, 65%, 59% and 60% at Con A concentrations of 5, 15, 25 and 50 mg/L, respectively (Table 5). After 4 wk of washout (i.e., wk 16), stimulation index increased but did not return to baseline values (Fig. 1). In the GLA group stimulation index in response to Con A concentrations of 5, 15 and 25 mg/L was significantly lower at wk 12 than at wk 0 and 4 (Table 5; **Fig. 2**). The change in stimulation index after 12 wk of GLA treatment was significantly different from the change in the placebo group (Table 5). The mean decline in

TABLE 3

Effect of supplementation of the diet of healthy older humans with different PUFA on the fatty acid composition of PBMC phospholipids[1]

Treatment	Wk	Fatty acid				
		Linoleic	DGLA	ARA	EPA	DHA
		g/100 g total fatty acids				
Placebo	0 (baseline)	8.1 ± 0.5	2.4 ± 0.1	20.4 ± 0.6	0.46 ± 0.09	3.2 ± 0.2
	4	7.8 ± 0.4	2.1 ± 0.2	18.8 ± 0.8	0.56 ± 0.11	3.0 ± 0.2
	8	7.9 ± 0.4	2.3 ± 0.1	21.1 ± 0.3	0.73 ± 0.12	2.9 ± 0.3
	12	7.9 ± 0.5	2.0 ± 0.2	20.8 ± 0.5	0.53 ± 0.09	2.9 ± 0.2
	16 (washout)	7.7 ± 0.6	2.3 ± 0.2	21.4 ± 0.8	0.50 ± 0.07	2.9 ± 0.2
ALNA	0 (baseline)	8.4 ± 0.6	2.3 ± 0.1	20.7 ± 0.9	0.65 ± 0.15	3.2 ± 0.2
	4	7.9 ± 0.8	1.9 ± 0.2	20.7 ± 1.2	0.49 ± 0.06	2.8 ± 0.2
	8	9.0 ± 1.0	1.9 + 0.2	22.3 ± 1.0	0.53 ± 0.03	2.5 ± 0.1
	12	7.8 ± 0.4	2.0 ± 0.2	20.4 ± 0.7	0.63 ± 0.09	2.4 ± 0.1
	16 (washout)	8.3 ± 0.9	2.0 ± 0.2	22.0 ± 1.3	0.48 ± 0.08	2.9 ± 0.2
GLA	0 (baseline)	8.7 ± 0.8	2.2 ± 0.3	19.8 ± 0.8	0.83 ± 0.34	2.9 ± 0.5
	4	7.6 ± 0.5	2.8 ± 0.2[4]	18.9 ± 1.1	0.55 ± 0.12	3.1 ± 0.5
	8	7.9 ± 0.3	3.2 ± 0.4[2,3,4]	21.3 ± 0.9	0.78 ± 0.15	2.5 ± 0.3
	12	8.3 ± 0.9	3.1 ± 0.2[2,3,4]	20.7 ± 0.8	0.57 ± 0.07	2.3 ± 0.4
	16 (washout)	8.0 ± 0.7	2.4 ± 0.2	21.4 ± 1.0	0.58 ± 0.14	2.3 ± 0.2
ARA	0 (baseline)	9.3 ± 0.5	2.1 ± 0.2	19.9 ± 0.5	0.55 ± 0.15	3.2 ± 0.3
	4	7.0 ± 0.5	1.1 ± 0.1[2,4]	20.2 ± 0.8	0.60 ± 0.15	2.5 ± 0.2
	8	6.9 ± 0.5	1.4 ± 0.2[4]	23.4 ± 1.0[2,3,5]	0.40 ± 0.09	2.4 ± 0.2
	12	6.6 ± 0.5	1.5 ± 0.2	22.8 ± 0.9[2,3,5]	0.60 ± 0.10	2.2 ± 0.2
	16 (washout)	8.2 ± 0.2	1.5 ± 0.2	21.4 ± 0.9	0.30 ± 0.09	2.3 ± 0.1
DHA	0 (baseline)	8.4 ± 0.6	2.3 ± 0.2	20.2 ± 0.3	1.00 ± 0.10	2.9 ± 0.3
	4	8.1 ± 0.6	1.8 ± 0.1	18.6 ± 0.3	0.66 ± 0.15	5.0 ± 0.3[2,3,4]
	8	8.6 ± 0.8	2.2 ± 0.2	19.6 ± 0.7	0.65 ± 0.09	4.5 ± 0.4[2,3,4]
	12	8.2 ± 0.8	2.0 ± 0.2	19.3 ± 0.6	0.66 ± 0.06	5.2 ± 0.3[2,3,4]
	16 (washout)	8.4 ± 0.6	2.3 ± 0.2	20.7 ± 0.7	0.48 ± 0.13	3.0 ± 0.1
FO	0 (baseline)	7.6 ± 0.4	2.0 ± 0.1	19.6 ± 1.0	0.48 ± 0.09	2.5 ± 0.4
	4	7.6 ± 0.3	1.8 ± 0.1	18.8 ± 0.8[3]	1.54 ± 0.18[2,4]	3.1 ± 0.3
	8	8.2 ± 0.4	2.0 ± 0.1	19.4 ± 0.5	1.37 ± 0.17[2,4]	2.9 ± 0.3
	12	8.4 ± 0.3	2.0 ± 0.1	18.8 ± 0.2[3]	1.54 ± 0.19[2,4]	2.9 ± 0.2
	16 (washout)	7.5 ± 0.4	2.0 ± 0.1	21.5 ± 0.5	0.84 ± 0.12	2.7 ± 0.2

[1] Values shown are mean ± SEM, n = 7 or 8.
[2] Significantly different from baseline (wk 0).
[3] Significantly different from washout (wk 16).
[4] Significantly different from all other groups, including placebo, at the same time point.
[5] Significantly different from DHA and FO groups at the same time point.

TABLE 4

Circulating total lymphocyte number and lymphocyte subpopulations in older humans undergoing dietary supplementation with different PUFA[1]

Treatment	Wk	Lymphocytes[2]	T lymphocytes[3]	B lymphocytes[4]	Th cells[5]	Tc cells[6]	Memory Th cells[7]
		$10^{-6} \times$ n/L blood	% of total lymphocytes		% of T lymphocytes		
Placebo	0	1664 ± 235	71.1 ± 2.9	13.7 ± 1.5	68.1 ± 5.9	28.7 ± 4.7	42.9 ± 3.7
	12	1393 ± 195	74.7 ± 2.7	11.8 ± 0.9	67.5 ± 5.6	30.3 ± 4.8	45.1 ± 3.2
	12-0	−288 ± 82	3.6 ± 1.8	−1.8 ± 1.2	−0.6 ± 1.7	1.7 ± 1.6	2.1 ± 2.1
ALNA	0	1434 ± 133	71.9 ± 3.3	14.7 ± 2.4	67.1 ± 6.0	30.4 ± 5.9	47.2 ± 4.5
	12	1293 ± 231	72.7 ± 2.1	14.7 ± 2.4	67.5 ± 5.6	30.4 ± 5.4	49.3 ± 3.5
	12-0	−144 ± 210	0.7 ± 1.6	−0.1 ± 0.9	0.4 ± 0.7	−0.1 ± 0.9	2.1 ± 1.2
GLA	0	1372 ± 183	75.3 ± 4.3	9.7 ± 1.1	67.8 ± 4.3	30.5 ± 4.4	45.2 ± 4.0
	12	1181 ± 151	77.4 ± 2.8	8.3 ± 1.1	67.3 ± 3.7	30.9 ± 3.6	48.7 ± 4.4
	12-0	−192 ± 200	2.1 ± 1.7	−1.3 ± 0.6	−0.4 ± 1.1	0.5 ± 1.1	3.5 ± 1.0
ARA	0	1130 ± 158	75.5 ± 3.1	11.3 ± 1.8	59.7 ± 6.3	37.6 ± 5.3	38.8 ± 4.0
	12	1111 ± 128	76.2 ± 2.8	11.2 ± 2.0	59.3 ± 6.5	37.8 ± 5.4	40.4 ± 4.6
	12-0	−19 ± 69	0.7 ± 0.7	−0.1 ± 0.4	−0.4 ± 0.5	0.3 ± 0.4	1.6 ± 0.8
DHA	0	1375 ± 233	76.1 ± 3.7	9.1 ± 1.9	54.9 ± 4.6	40.6 ± 5.2	43.9 ± 4.1
	12	1350 ± 235	71.3 ± 4.4	9.0 ± 1.5	53.6 ± 3.1	42.1 ± 3.2	44.3 ± 2.8
	12-0	−36 ± 63	−4.8 ± 3.5	−0.1 ± 1.2	−1.4 ± 2.4	1.5 ± 2.4	0.3 ± 2.2
FO	0	1236 ± 90	71.1 ± 2.2	13.9 ± 2.2	59.1 ± 5.8	38.7 ± 5.7	43.5 ± 7.0
	12	1398 ± 101	70.4 ± 2.0	12.7 ± 2.4	57.7 ± 6.5	40.2 ± 6.6	42.9 ± 6.6
	12-0	162 ± 187	−0.7 ± 1.4	−1.3 ± 1.0	−1.4 ± 1.5	1.5 ± 1.8	−0.6 ± 1.6

[1] Values shown are mean ± SEM at wk 0 (baseline) and at wk 12 (end of supplementation) and mean ± SEM difference between wk 12 and 0, $n = 7$ or 8 per treatment group.
[2] Calculated from percentage of total leukocytes staining $CD45^+CD14^-$.
[3] Determined as percent of lymphocytes staining $CD3^+CD16^-$.
[4] Determined as the percent of lymphocytes staining $CD19^+CD16^-CD56^-$.
[5] Determined as percent of T lymphocytes staining $CD4^+CD8^-$.
[6] Determined as percent of T lymphocytes staining $CD4^-CD8^+$.
[7] Determined as percent of T lymphocytes staining $CD4^+CD8^-CD45RO^+$.

TABLE 5

Lymphocyte proliferation and cytokine production in older humans undergoing dietary supplementation with different PUFA[1]

Treatment	Wk	Con A (mg/L)	Lymphocyte proliferation				IL-2	IFN-γ
			5	15	25	50	15	15
			Stimulation index				kU/L	
Placebo	0		168.6 ± 24.0	232.4 ± 26.1	201.3 ± 39.6	73.4 ± 11.8	6.8 ± 1.8	6.4 ± 1.9
	12		148.5 ± 23.6	208.6 ± 25.3	179.6 ± 18.8	64.9 ± 8.2	7.3 ± 0.8	7.2 ± 1.1
	12-0		−21.3 ± 11.3	−24.5 ± 16.6	−22.1 ± 15.8	−8.1 ± 5.3	0.6 ± 0.9	0.9 ± 0.7
ALNA	0		184.2 ± 37.5	247.3 ± 52.5	177.1 ± 38.9	51.9 ± 16.3	8.4 ± 2.8	6.1 ± 1.9
	12		120.8 ± 35.6	167.8 ± 28.1	126.7 ± 36.4	34.5 ± 5.7	8.9 ± 2.4	5.8 ± 1.2
	12-0		−59.2 ± 23.5	−81.9 ± 24.3	−47.9 ± 19.6	−17.1 ± 8.3	0.4 ± 0.7	−0.3 ± 0.3
GLA	0		237.9 ± 34.9	288.8 ± 38.7	166.2 ± 31.7	56.4 ± 10.4	7.0 ± 1.1	6.8 ± 1.1
	12		89.4 ± 11.3†	110.9 ± 16.7†	73.4 ± 18.6†	24.2 ± 4.8	7.5 ± 1.4	7.2 ± 0.7
	12-0		−146.2 ± 31.6*	−178.3 ± 33.6*	−90.7 ± 20.4*	−30.7 ± 8.6	0.5 ± 0.8	0.4 ± 0.3
ARA	0		193.7 ± 46.9	220.1 ± 29.4	182.1 ± 27.7	56.8 ± 17.3	7.0 ± 2.1	6.5 ± 2.5
	12		119.7 ± 37.8	149.7 ± 18.9	118.3 ± 16.9	40.8 ± 7.2	7.3 ± 1.9	5.9 ± 1.3
	12-0		−66.6 ± 33.5	−70.3 ± 30.4	−67.6 ± 16.5	−16.1 ± 8.8	0.4 ± 0.7	−0.7 ± 0.7
DHA	0		179.6 ± 40.9	204.2 ± 40.1	99.5 ± 15.2	37.9 ± 7.2	6.5 ± 1.7	7.9 ± 1.5
	12		121.7 ± 26.7	136.2 ± 19.4	79.6 ± 11.1	24.7 ± 5.8	6.4 ± 0.7	7.2 ± 1.1
	12-0		−59.2 ± 23.3	−68.6 ± 29.1	−17.9 ± 7.1	−12.9 ± 5.5	−0.1 ± 0.6	−0.8 ± 0.5
FO	0		156.1 ± 28.1	216.1 ± 38.6	151.3 ± 26.1	43.7 ± 8.4	8.1 ± 3.3	6.6 ± 1.6
	12		70.3 ± 17.5†	77.2 ± 17.4†	62.8 ± 13.1†	17.3 ± 5.7	7.8 ± 2.9	6.1 ± 1.2
	12-0		−84.4 ± 24.5*	−139.7 ± 31.3*	−87.0 ± 14.8*	−25.3 ± 7.1	−0.4 ± 0.6	−0.5 ± 0.7

[1] Values shown are mean ± SEM at wk 0 (baseline) and at wk 12 (end of supplementation) and mean ± SEM difference between wk 12 and 0, $n = 7$ or 8 per treatment group.
* Significantly different from placebo treatment.
† Significantly different from wk 0.

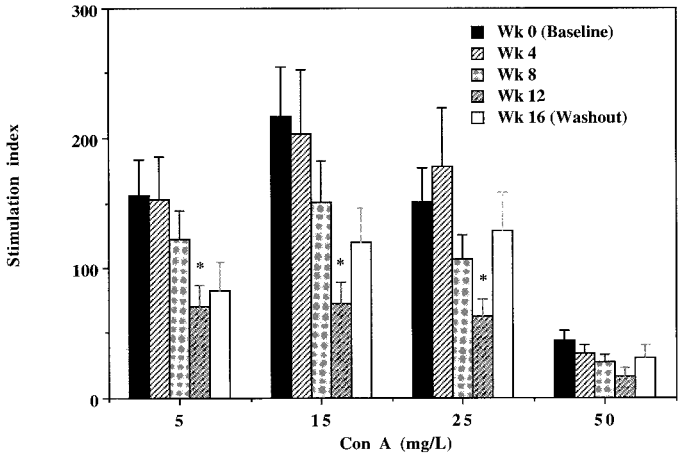

FIGURE 1 Proliferation of lymphocytes from older humans in the FO group in response to different concentrations of Con A. Data at each time point are mean ± SEM (n = 7). *Significantly different from wk 0 (baseline) and wk 4 ($P < 0.05$).

stimulation index after 12 wk of GLA treatment was 61%, 61%, 55% and 54% at Con A concentrations of 5, 15, 25 and 50 mg/L, respectively (Table 5). After 4 wk of washout (i.e., wk 16), stimulation index increased in the GLA group but had not returned to baseline values and remained significantly lower than at wk 4 (Fig. 2).

Effect of treatments on cytokine production. Production of IL-2 and IFN-γ by PBMC stimulated with 15 mg/L Con A did not differ among the treatment groups at baseline or at the end of supplementation (Table 5). There were no significant effects of treatment or of time or a time × treatment interaction upon production of these cytokines (Table 5). The changes in cytokine production over the treatment period did not differ among the treatment groups (Table 5).

DISCUSSION

Supplementation of the diet of healthy older subjects with ~700 mg of GLA, ARA or DHA or with 1 g of EPA plus DHA/d significantly altered PBMC phospholipid fatty acid composition, with changes being apparent after 4 wk of supplementation. Despite the marked changes in fatty acid composition caused by the ARA and DHA treatments, these did not significantly alter lymphocyte proliferation. In contrast, supplementation with GLA or FO for 12 wk significantly decreased lymphocyte proliferation.

The habitual intakes of ALNA, EPA and DHA among the subjects in the current study were in accordance with other reports in the adult population of the United Kingdom (28,29), while the habitual intake of ARA among these subjects was consistent with other reports in Western adults (30–32). The level of ALNA included in the supplement in the current study (2 g/d) increased total ALNA intake by twofold to threefold to 3 g/d. ALNA was absent from PBMC phospholipids in most subjects at baseline and was not increased in the subjects who supplemented their diet with ALNA. The products of ALNA elongation and desaturation (EPA and DHA) were not significantly elevated in PBMC phospholipids in the ALNA group. Thus, it seems that when ALNA is included in the diet at moderate levels, it is not incorporated into PBMC phospholipids in significant amounts. Furthermore, if it is elongated and desaturated, the products of this are not preferentially incorporated into PBMC phospholipids.

GLA did not appear in PBMC phospholipids. Levels of the elongation product of GLA, DGLA, were increased during GLA supplementation, however, suggesting that some of the GLA is elongated before incorporation into PBMC. These observations are consistent with those of Johnson et al. (33) who found that there was no appearance of GLA in neutrophil phospholipids after GLA consumption, but that the proportion of DGLA in neutrophil phospholipids increased according to the amount of GLA consumed by healthy subjects.

The level of ARA provided in the supplement in the current study (680 mg/d) increased ARA intake nearly fivefold. This resulted in significant enrichment of PBMC phospholipids with ARA, which was increased from ~20 to 23 g/100 g total fatty acids. An earlier study in which the immunological effects of providing healthy young men with 1.5 g of ARA/d for 7 wk was investigated did not report PBMC fatty acid composition (34,35). Thus, it seems that the current study is the first to report PBMC fatty acid composition after ARA supplementation of the human diet.

The level of EPA plus DHA provided in the FO supplement in the current study (1 g/d) increased total EPA plus DHA intake by fourfold. Intakes of both EPA and DHA were increased in the FO group (by 8- and 1.7-fold, respectively), and there was a twofold increase in the proportion of EPA in PBMC phospholipids and a nonsignificant trend for increased DHA in this group (P = 0.098). These changes are consistent with those reported previously for PBMC in subjects given FO (36,37). In the subjects supplemented with DHA, the intake of DHA increased ~5.5-fold, whereas that of EPA did not change. Supplementation with this level of DHA increased the proportion of DHA in PBMC phospholipids twofold without affecting that of EPA. This is in accordance with effect of a larger dose of DHA (6 g/d) given to healthy volunteers for 12 wk (38). Supplementation with FO, but not with DHA, decreased the proportion of ARA in PBMC phospholipids.

There was no significant effect of ALNA, ARA or DHA on lymphocyte proliferation (P = 0.064 to 0.191 depending upon the treatment group and the Con A concentration), although each of these treatments tended to decrease this response. There have been no studies reporting the effect of a moderate dose of ALNA, as used here, on human lymphocyte proliferation. Animals studies show that large amounts of ALNA in the diet suppress lymphocyte proliferation (9–11) and these are supported by a study in humans, which showed significant

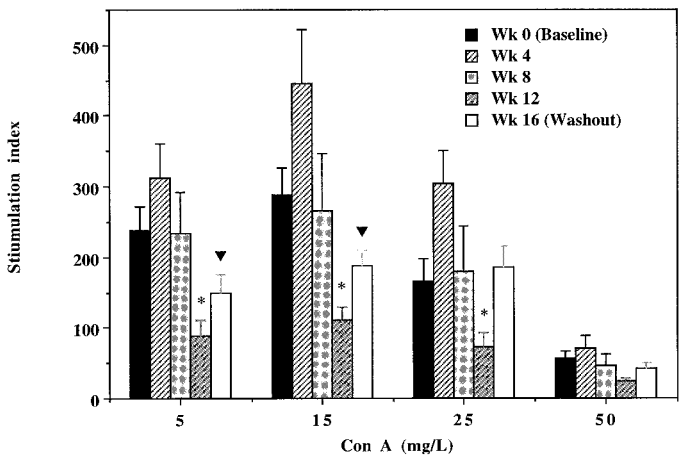

FIGURE 2 Proliferation of lymphocytes from older humans in the GLA group in response to different concentrations of Con A. Data at each time point are mean ± SEM (n = 7). *Significantly different from wk 0 (baseline) and wk 4; ▼significantly different from wk 4 ($P < 0.05$).

suppression of Con A-stimulated lymphocyte proliferation after providing human volunteers with 18 g ALNA/d (22). However, the current study indicates that increasing ALNA intake to 3 g/d will not significantly impair human lymphocyte proliferation. This is an important observation, because there are recommendations for humans to increase ALNA intake (29). The lack of a significant effect of ARA is consistent with the observations of no effect of 7-wk supplementation of the diet of young men with 1.5 g of ARA/d upon proliferation of lymphocytes stimulated by Con A, phytohemagglutinin (PHA) or pokeweed mitogen (34). Similarly, the lack of a significant effect of DHA is in accordance with the report that a larger dose of DHA than is used in the current study (6 g/d compared with 700 mg/d) as part of a low fat diet for 12 wk did not alter human lymphocyte proliferation in response to Con A or PHA (39).

Several recent studies have studied the effect of increasing the amount of GLA in the human diet on lymphocyte proliferation (37,40,41). These studies present conflicting findings. Yaqoob et al. (37) found no significant effect of 1 g of GLA/d (provided as evening primrose oil) for up to 12 wk on lymphocyte proliferation in response to a range of Con A concentrations. Wu et al. (41) reported a small (20%), but significant, enhancing effect of 675 mg of GLA/d (provided as blackcurrant seed oil) for 8 wk on lymphocyte proliferation in response to some PHA concentrations, but not to others. Rossetti et al. (40) reported the effects of 2.4 g of GLA/d (provided as borage oil) given to two men. Data for one of these showed a time-dependent suppression of lymphocyte proliferation of 25%, 72% and 90% after 6, 12 and 24 wk, respectively. Data for the second subject showed a 90% decrease in proliferation after 6, 8 and 11 wk of supplementation and then a reversal of this effect 4 and 12 wk after stopping supplementation; proliferation remained suppressed by 75% after 4 wk of washout compared with baseline but had returned to the baseline value after 12 wk of washout (40). In the current study we found that 700 mg GLA/d (provided as a GLA-rich triacylglycerol) tended to enhance lymphocyte proliferation after 4 wk, but significantly suppressed it (by up to 60%) after 12 wk and that 4 wk of washout was insufficient to return the response fully to baseline. Thus, overall the data from this study support the data from the two men studied by Rossetti et al. (40). It is not clear why the results of this study and (40) are different from those of Wu et al. (41) and Yaqoob et al. (37) but this might relate to other characteristics of the subjects studied and/or to other components of the oils used.

The current study observed a significant time-dependent decline in lymphocyte proliferation in subjects given FO, which only partly returned to baseline values 4 wk after washout. Previous studies have reported that supplementation of the diet of healthy human volunteers with FO providing 1.2–3.4 g of EPA plus DHA/d results in decreased mitogen-stimulated lymphocyte proliferation (23–26). The current study provided 1 g of EPA plus DHA/d and this represents the lowest level of long chain (n-3) PUFA supplementation that has been demonstrated to decrease human lymphocyte proliferation. The observed significant reduction in lymphocyte proliferation after FO supplementation (providing 720 mg of EPA plus 280 mg of DHA/d) but not after supplementation with 720 mg DHA/d strongly suggests that EPA but not DHA is responsible for the effect of FO.

As indicated earlier, the ALNA, ARA and DHA treatments tended to decrease lymphocyte stimulation index by ~30%, although this effect did not reach statistical significance, whereas the GLA and FO treatments significantly decreased stimulation index by 55–65%. Before beginning the study, we estimated that eight subjects per treatment group would be sufficient to identify a 25% decrease in lymphocyte proliferation as significant. Seven of eight subjects who began on the GLA and FO treatments completed the study, and this was a sufficient number to identify the effects of these two treatments as statistically significant. In contrast, it is apparent that eight subjects was an insufficient number to identify a 30% decrease in stimulation index as statistically significant. However, the biological significance of a 30% decrease in stimulation index is unclear, because there was a very large variation in stimulation index among the subjects at entry to study and because the post-treatment (i.e., wk 12) responses of lymphocytes from individuals in the ALNA, ARA and DHA groups were all within the range of the responses seen among all individuals pretreatment.

Production of the cytokines IL-2 and IFN-γ was not affected by any of the treatments, including those that significantly decreased lymphocyte proliferation. Previous studies of ARA supplementation (1.5 g/d for 7 wk; 35) and GLA supplementation [675 mg/d for 8 wk (41) or 1 g/d for 12 wk (37)] reported no effect on IL-2 production, while Yaqoob et al. (37) also reported no effect of GLA on IFN-γ production. The findings of the lack of effect of ARA and GLA on IL-2 and IFN-γ production in the current study are in accordance with these previous observations. Studies that have reported that FO decreases IL-2 production have provided 2.4 (23,24) or 5.2 g (27) of EPA plus DHA/d. In contrast, some studies report no effects of FO, providing between 1.2 and 4.6 g of EPA plus DHA/d, on IL-2 production (25,37,42). Of the studies that have investigated the effect of dietary FO supplementation on IFN-γ production by human lymphocytes (27, 37 and the current study), only the study by Gallai et al. (27) found an effect (inhibition) and that study used a high dose of EPA plus DHA (5.2 g/d). The findings of the current study along with the existing literature suggest that increasing EPA plus DHA intake by up to 1.2 g/d will not alter IL-2 and IFN-γ production.

The main cytokine involved in promoting T lymphocyte proliferation is IL-2 (43) and so it might be expected that changes in proliferation might result from changes in IL-2 production. However, this is not always the case: both the current study and the study by Meydani et al. (25) reported a significant effect of FO on lymphocyte proliferation without a change in IL-2 production.

Lymphocyte proliferation and the production of IL-2 and IFN-γ are important in host defense against invading bacteria, viruses and fungi (1). Impairment in these activities might make the host more susceptible to infection. Animal studies that show that FO impairs lymphocyte proliferation and IL-2 and IFN-γ production (see Introduction for references) are paralleled by studies showing decreased resistance to pathogens (43–48), although some other animal studies report that FO does not alter (49), or even increases (50), resistance to some pathogens. The current study showing no effect on IL-2 and IFN-γ production after FO consumption by humans suggests that adverse immunological effects are unlikely at the level of EPA plus DHA provided. However, it will be important to conduct longer-term studies of the effect of long chain (n-3) PUFA on human immune function and on rates of infection in humans.

The mechanism by which GLA and EPA might decrease lymphocyte proliferation is unclear. In addition to not

affecting IL-2 production, these fatty acids did not affect the proportions of different lymphocyte subsets. Thus, the changed proliferative responses do not reflect a change in the numbers or relative proportions of the different classes of lymphocytes being cultured. The lack of effect of GLA and FO on lymphocyte subsets is in agreement with previous studies using long chain (n-3) PUFA (23,37,39) or oils rich in GLA (37,41). It is possible that these fatty acids might act to inhibit another component of the proliferative process. Jolly et al. (51) reported that dietary EPA (and DHA) decreased induction of messenger RNA for the IL-2 receptor α-subunit in Con A-stimulated murine splenocytes and inferred that this effect accounted, at least in part, for the inhibition of Con A-stimulated lymphocyte proliferation that they had reported previously (16). Additional studies are needed to identify the mechanism of action of selected dietary PUFA on functional responses of human lymphocytes.

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