Prostaglandin E_2 production and T cell function after fish-oil supplementation: response to antioxidant cosupplementation[1–3]

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ABSTRACT

Background: Prostaglandin E_2 (PGE_2) inhibits lymphocyte proliferation and the production of interferon-γ (IFN-γ) by peripheral blood mononuclear cells, but the effect of PGE_2 on interleukin 4 (IL-4) production is unclear. Fish oil, which contains eicosapentaenoic and docosahexaenoic acids, inhibits production of PGE_2. The effects of fish oil on lymphocyte proliferation and production of IFN-γ and IL-4 are unclear and may be influenced by the availability of antioxidants.

Objective: We investigated the effect of dietary fish oil with and without antioxidant cosupplementation on lymphocyte proliferation and the production of PGE_2, IFN-γ, and IL-4 by peripheral blood mononuclear cells.

Design: Sixteen healthy men received dietary fish-oil supplements providing 0.3, 1, and 2 g eicosapentaenoic acid plus docosahexaenoic acid/d for 4 consecutive weeks each (total of 12 wk). All subjects were randomly assigned to daily cosupplementation with either antioxidants (200 μg Se, 3 mg Mn, 30 mg *RRR*-α-tocopheryl succinate, 90 mg ascorbic acid, 450 μg vitamin A) or placebo.

Results: Fish-oil supplementation decreased PGE_2 production and increased IFN-γ production and lymphocyte proliferation from baseline values. Cosupplementation with antioxidants did not affect cytokine production or lymphocyte proliferation.

Conclusion: Dietary fish oil modulates production of IFN-γ and lymphocyte proliferation in a manner consistent with decreased production of PGE_2, but this effect is not modified by antioxidant cosupplementation. *Am J Clin Nutr* 2003;78:376–82.

KEY WORDS Fish oil, lymphocyte proliferation, prostaglandin E_2, interferon-γ, interleukin 4

INTRODUCTION

Prostaglandin E_2 (PGE_2) is synthesized by monocytes from arachidonic acid (AA), an n−6 polyunsaturated fatty acid (PUFA) present in cell membranes and plasma phospholipids (1). In vitro studies showed that PGE_2 inhibits lymphocyte proliferation (2) and the production of cytokines [interferon-γ (IFN-γ) and interleukin 2 (IL-2)] by T helper 1 (Th1)–type cells (3–5), with an indirect stimulatory effect on cytokine production (IL-4, IL-5, and IL-10) by T helper 2 (Th2)–type cells (3, 5).

Fish oil is rich in the n−3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In healthy subjects, dietary supplementation with fish oil increases EPA and DHA concentrations and decreases AA concentrations within plasma and cell-membrane phospholipids (6–12). Fish oil also decreases production of PGE_2 by peripheral blood mononuclear cells (PBMCs) (6, 13). Therefore, fish oil would be expected to result in increased lymphocyte proliferation and increased production of Th1-type cytokines such as IL-2 and IFN-γ. However, studies indicate that supplementation of the diet of healthy humans with fish oil decreases lymphocyte proliferation (8, 12–14) and decreases production of IL-2 (8, 13, 15) and IFN-γ (15). Thus, there is a discrepancy between the proposed biological relation between PGE_2 production and T lymphocyte function and the responses of those functions observed after dietary fish-oil supplementation. One explanation for this discrepancy is that an increased intake of EPA and DHA leads to a reduction in the availability of antioxidants and an increased generation of lipid peroxides (16, 17) that may independently suppress lymphocyte proliferation and Th1-type cytokine production. The effect of dietary fish oil, with or without antioxidants, on cytokine production by Th2-type cells is unknown.

The purpose of the present study was to investigate the effect of dietary fish oil, with and without antioxidant cosupplementation, on lymphocyte production and the production of PGE_2, IFN-γ, and IL-4 by PBMCs in healthy volunteers. In conducting this investigation, we hoped to determine the influence of increased antioxidant availability on PGE_2 production and T lymphocyte (Th1- and Th2-type cells) function during dietary fish-oil provision.

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TABLE 1
Intakes of individual polyunsaturated fatty acids (PUFAs) and of total n−3 and n−6 PUFAs from fish-oil capsules[1]

		Fatty acid[2]							
Week	Fish-oil capsules	LA	LNA	AA	EPA	DPA	DHA	Total n−3 PUFAs	Total n−6 PUFAs
	no./d				*mg/d*				
0–4	1	12	9	11	208	24	101	342	26
4–8	3	36	26	33	624	72	303	1025	78
8–12	6	72	52	66	1248	144	606	2050	156

[1] LA, linoleic acid; LNA, α-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
[2] $\bar{x}$.

SUBJECTS AND METHODS

Subjects

Healthy white men (n = 16) were recruited from the staff of Southampton General Hospital and of the University of Southampton. The subjects completed a brief questionnaire regarding their lifestyle and medical history and gave written informed consent. Subjects were excluded if they took any regularly prescribed medication; had a history of hyperlipidemia, hypertension, or diabetes mellitus; or had a history of taking n−3 PUFA supplements or antioxidant vitamin supplements. A food-frequency questionnaire (18) was completed to give an estimate of habitual nutrient intake. The study was approved by the Southampton and South West Hants Joint Research Ethics Committee.

Study design

The subjects received a 12-wk course of fish oil, which contained EPA and DHA in the form of triacylglycerol (ratio of EPA to DHA = 2:1) (RP Scherer, Swindon, United Kingdom) and was administered in 1-g capsules. The subjects received the same dose of fish oil whether or not they also received antioxidant cosupplementation. The subjects received 1 g fish oil/d for the first 4 wk, 3 g fish oil/d for the second 4 wk, and 6 g fish oil/d for the third 4 wk, which were equivalent to total EPA plus DHA intakes of 0.3, 1, and 2 g/d, respectively (**Table 1**). All the subjects were randomly assigned in a double-blind manner to receive daily either an antioxidant preparation, which contained 200 μg Se, 3 mg Mn, 30 mg vitamin E as *RRR*-α-tocopheryl succinate, 450 μg vitamin A (300 μg retinol equivalents as retinol and 150 μg retinol equivalents as β-carotene), and 90 mg vitamin C as ascorbic acid (Wassen International, Leatherhead, United Kingdom), or a placebo (maltose and lactose) that was identical in appearance. Each fish-oil capsule contained 1 mg vitamin E to stabilize the PUFAs. The subjects were asked to maintain their habitual diet throughout the intervention period and to take the supplements every day before breakfast. After the subjects had fasted overnight, peripheral venous blood samples (30 mL) were taken from the antecubital fossa into heparin-containing tubes at baseline (week 0) and after each 4-wk period (weeks 4, 8, and 12). The subjects were reviewed every 4 wk for side effects and were issued fresh supplements at those times. Assessment of compliance was by self-reporting.

Cell and plasma preparation

Blood was layered over 20 mL Histopaque (density of 1.077 g/mL; Sigma Chemical Co, Poole, United Kingdom) and centrifuged for 15 min at 720 × g and 20 °C. The plasma layer was removed and stored at −70 °C. The PBMC layer was collected from the interface, washed, and resuspended in medium (RPMI containing 1.875 mmol glutamine/L and antibiotics). A second cycle was performed to reduce erythrocyte contamination. Cells were resuspended in 1 mL medium, counted, and removed for culture. For preparation of erythrocytes, 10 mL blood was centrifuged for 15 min at 720 × g and 4 °C. The plasma layer was removed, and the cells were washed with 9 g NaCl/L.

Fatty acid composition analysis

Internal standards (dipentadecanoyl phosphatidylcholine and diheptadecanoyl phosphatidylethanolamine) were added to all samples before analysis. Total plasma (1 mL) and erythrocyte (2 mL) lipids were isolated by extraction with chloroform-methanol (2:1, vol:vol) (19) containing 50 mg butylated hydroxytoluene/L, and phosphatidylcholine and phosphatidylethanolamine were purified by solid-phase extraction on aminopropylsilica cartridges (Varian, Surrey, United Kingdom) (20). Phosphatidylcholine and phosphatidylethanolamine fatty acids were converted to methyl esters by incubation with methanol containing 2% (vol:vol) sulfuric acid at 50 °C for 18 h (20). Fatty acid methyl esters were separated, redissolved in hexane, and analyzed by using a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with an HP7686 GC autosampler (Hewlett-Packard), a BPX-70 fused silica capillary column (50 m × 0.25 mm × 0.32 μm; SGE, Milton Keynes, United Kingdom), and a flame ionization detector. Peaks were identified by comparing their retention times to those of peaks in standards. Fatty acids are reported as proportionate values (g/100 g total fatty acids). The CV for determination of phospholipid fatty acid composition was < 5%.

Analysis of cytokine and eicosanoid production

Purified PBMCs at a concentration of 1 × 10^6 cells/mL were incubated for 24 h in 2 mL RPMI medium containing 5% autologous plasma, either with or without the monocyte- and macrophage-stimulant lipopolysaccharide at a concentration of 15 mg/L (for PGE_2) or the T cell stimulant concanavalin A at a concentration of 25 mg/L. After this, culture plates were centrifuged for 10 min at 180 × g and 20 °C, and the supernatant fluid was removed and frozen at −30 °C. IL-4 and IFN-γ concentrations were measured by using EASIA enzyme-linked immunosorbent assay kits (Biosource Europe SA, Nivelles, Belgium). PGE_2 concentrations were measured by using NEOGEN enzyme-linked immunosorbent assay kits (Neogen Corporation, Lexington, KY). The kits were used according to the manufacturers' instructions. The CVs for both the cytokine and prostaglandin

TABLE 2
Baseline characteristics and habitual dietary intakes estimated from a food-frequency questionnaire for subjects who received fish oil plus antioxidants or fish oil only[1]

	Fish oil only (n = 8)	Fish oil plus antioxidants (n = 8)
Age (y)	30.6 ± 4.5[2]	30.3 ± 6.1
Cigarette smokers (n)	1	1
Dietary intake		
Total n−6 PUFAs (g/d)	13.5 ± 4.1	13.4 ± 7.0
Total n−3 PUFAs (g/d)	1.9 ± 0.2	1.8 ± 0.7
Carotene (μg/d)	3542 ± 1174	2819 ± 1385
Retinol (μg/d)	435 ± 282	450 ± 250
Vitamin C (mg/d)	181 ± 90.0	167 ± 95.8
Vitamin E (mg/d)	11.0 ± 3.8	11.1 ± 4.8
Selenium (μg/d)	77.6 ± 33.0	66.3 ± 19.2
Manganese (mg/d)	4.8 ± 2.04	4.4 ± 1.49

[1] PUFAs, polyunsaturated fatty acids. There were no significant differences between the 2 groups.
[2] $\bar{x}$ ± SD.

assays were < 10%, and the limits of detection were 30 IU/L for IFN-γ and 2 ng/L for IL-4. Production of IL-4 by unstimulated PBMCs was below the level of detection and was therefore excluded from analysis.

Analysis of lymphocyte proliferation

Purified PBMCs at a concentration of 1×10^6 cells/mL were incubated in RPMI medium supplemented with 2 mmol glutamine/L and 5% autologous serum with and without the T cell stimulant concanavalin A at a concentration of 25 mg/L. The total volume of 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. Triplicate values were averaged and expressed as incorporation into unstimulated and concanavalin A–stimulated PBMCs. The CV was < 10%.

Statistical analysis

Sample size was calculated on the basis of existing data from the literature indicating significant effects of fish-oil supplementation on cytokine production by PBMCs (6, 8, 12–14). We determined that a sample size of 8 would detect a difference in lymphocyte proliferation of > 25% with a two-sided significance level of 5% and 80% power.

EXCEL version XP (Microsoft Corp, Redmond, WA) was used for data handling, and SPSS version 11 (SPSS Inc, Chicago) was used for statistical analysis and graph generation. Data are summarized for each arm containing 8 subjects and for the pooled group of 16 subjects where appropriate. The effects of supplementary n−3 PUFA intake and antioxidant cosupplementation and of their interaction were determined by repeated-measures analysis of variance (n−3 PUFA intakes equivalent to 0.3, 1.0, and 2.0 g/d compared with baseline intake, ie, 0 g/d). When the effect of antioxidant cosupplementation was not significant, the 2 groups were pooled. The assumption of sphericity was tested by using Mauchly's test. The Greenhouse-Geisser correction factor was applied when the sphericity assumption was not met. Post hoc pairwise comparisons (based on the SEs of the differences in individual paired comparisons) were Bonferroni corrected.

RESULTS

Subjects

All the subjects completed the trial and reported that they had complied with the intervention regimen. There were no major side effects of treatment; 3 subjects (2 in the group who received fish oil plus antioxidants and 1 in the group who received fish oil only) reported mild allergic rhinitis (1 subject as a first presentation). The habitual intakes of n−3 and n−6 PUFAs and of antioxidants were not significantly different between the subjects who received fish oil only and those who received fish oil plus antioxidants (**Table 2**). At baseline, n−6 and n−3 PUFA concentrations in plasma and erythrocyte phospholipids; production of PGE_2, IL-4, and IFN-γ by PBMCs; and lymphocyte proliferation were not significantly different between the 2 groups.

Fatty acid composition of plasma and erythrocyte phospholipids

Antioxidant cosupplementation had no significant effect on the proportions of linoleic acid (LA), AA, EPA, or DHA in plasma or erythrocyte phospholipids after dietary fish-oil supplementation, and there was no significant interaction between antioxidant cosupplementation and dietary fish oil. Therefore, the 2 groups (ie, fish oil plus antioxidants and fish oil only) were pooled (n = 16) for analysis of the response of phospholipid composition to increased dietary intakes of EPA and DHA. Dietary supplementation with EPA and DHA, as fish oil, was associated with dose-responsive increases and decreases in the proportionate concentrations of EPA and AA, respectively, in plasma and erythrocyte phospholipids (**Table 3**). Proportionate concentrations of LA decreased in response to dietary fish oil only in erythrocyte phosphatidylethanolamine, and DHA increased only in plasma phosphatidylcholine and erythrocyte phosphatidylethanolamine (Table 3). The increasing proportionate concentrations of EPA and DHA in phospholipid, as well as the decreasing concentrations of LA and AA, tended toward a plateau response when plotted against supplementary EPA and DHA intake.

Antioxidant cosupplementation and PBMC function and proliferation

Antioxidant cosupplementation had no significant effect on the production of PGE_2, IFN-γ, or IL-4 by unstimulated or stimulated PBMCs or on lymphocyte proliferation, and there was no significant interaction between antioxidant cosupplementation and dietary fish oil. Therefore, the subjects who received fish oil plus antioxidants were pooled with those who received fish oil only (n = 16) for analysis of the response of phospholipid composition to increased dietary intakes of EPA and DHA.

Prostaglandin E_2 production by PBMCs

Production of PGE_2 by unstimulated and lipopolysaccharide-stimulated PBMCs decreased significantly from baseline values for all doses of n−3 PUFAs, with a trend toward a dose-response relation to supplementary EPA and DHA intake (**Table 4**). PGE_2 production by PBMCs was negatively correlated with the proportion of EPA in plasma phosphatidylcholine (r = 0.6, P = 0.001 for unstimulated cells; r = 0.46, P = 0.001 for lipopolysaccharide-stimulated cells). Furthermore, PGE_2

TABLE 3
Proportionate concentrations of linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in plasma phosphatidylcholine (PC) and erythrocyte phosphatidylethanolamine (PE) and PC in healthy subjects after dietary supplementation with fish oil[1]

	Supplementary n−3 PUFA intake (g/d)				
	0	0.3	1.0	2.0	P^2
	g/100 g total fatty acids				
Plasma PC					
LA	24.7 ± 0.5	24.0 ± 0.8	23.5 ± 0.6	22.8 ± 0.6	0.083
AA	9.8 ± 0.4	9.1 ± 0.3	8.4 ± 0.3[3]	8.2 ± 0.3[3,4]	<0.001
EPA	1.1 ± 0.1	1.7 ± 0.2[3]	2.9 ± 0.4[3]	3.7 ± 0.3[3,4]	<0.001
DHA	3.0 ± 0.2	3.5 ± 0.2[3]	4.4 ± 0.3[3,4]	4.8 ± 0.3[3,4]	<0.001
Erythrocyte PE					
LA	8.2 ± 0.3	7.9 ± 0.3	7.5 ± 0.3[3,4]	7.3 ± 0.3[3,4]	<0.001
AA	24.5 ± 0.5	24.1 ± 0.6	23.1 ± 0.7[4]	21.8 ± 0.4[3,4]	<0.001
EPA	1.8 ± 0.2	2.1 ± 0.2[3]	2.8 ± 0.2[3,4]	3.9 ± 0.3[3,4,5]	<0.001
DHA	7.0 ± 0.5	7.2 ± 0.4	7.8 ± 0.4[4]	8.5 ± 0.4[3,4,5]	<0.001
Erythrocyte PC					
LA	16.3 ± 0.5	16.8 ± 0.3	16.1 ± 0.5	16.0 ± 0.6	0.275
AA	9.4 ± 0.4	7.1 ± 0.2[3]	6.5 ± 0.2[3,4]	5.7 ± 0.4[3,4]	<0.001
EPA	0.8 ± 0.1	1.0 ± 0.1	1.4 ± 0.1[3,4]	1.9 ± 0.1[3,4,5]	<0.001
DHA	2.4 ± 0.2	2.2 ± 0.3	2.3 ± 0.2	2.7 ± 0.1	0.458

[1] $\bar{x}$ ± SEM; n = 15. PUFA, polyunsaturated fatty acid.
[2] Repeated-measures ANOVA. Greenhouse-Geisser correction factor was applied when the sphericity assumption was not met.
[3] Significantly different from baseline (0 g/d), $P < 0.05$ (Bonferroni correction).
[4] Significantly different from 0.3 g/d, $P < 0.05$ (Bonferroni correction).
[5] Significantly different from 1.0 g/d, $P < 0.05$ (Bonferroni correction).

production by PBMCs was positively correlated with the proportion of AA in plasma phosphatidylcholine ($r = 0.24$, $P = 0.04$ for unstimulated cells; $r = 0.35$, $P = 0.04$ for lipopolysaccharide-stimulated cells). Production of PGE_2 by unstimulated PBMCs was not significantly correlated with production of IFN-γ or IL-4.

PBMC proliferation

Proliferation of unstimulated PBMCs and concanavalin A–stimulated T lymphocytes increased significantly from baseline in response to increased dietary intakes of EPA and DHA as fish oil (Table 4), with a trend toward a dose-response relation.

Effect of fish oil on IFN-γ and IL-4 production by PBMCs

IFN-γ production by concanavalin A–stimulated PBMCs increased from baseline in a dose-responsive manner after dietary fish-oil supplementation (Table 4). Production of IL-4 by concanavalin A–stimulated PBMCs did not change significantly from baseline after dietary fish-oil supplementation, but a trend toward an increase was noted (Table 4). EPA concentrations in

TABLE 4
Ex vivo synthesis of prostaglandin E_2 (PGE_2), interferon-γ (IFN-γ), and interleukin-4 (IL-4) and lymphocyte proliferation by unstimulated and stimulated peripheral blood mononuclear cells from healthy subjects after dietary supplementation with fish oil[1]

	Supplementary n−3 PUFA intake (g/d)				
	0	0.3	1.0	2.0	P^2
PGE_2 (ng/L)					
Unstimulated	33.9 ± 7.2	6.7 ± 2.6[3]	1.4 ± 0.2[3]	1.7 ± 0.4[3]	<0.001
Stimulated	99.3 ± 20.1	23.9 ± 11.5[3]	4.0 ± 0.7[3]	2.0 ± 0.7[3]	<0.001
IFN-γ (kIU/L)					
Unstimulated	1.10 ± 0.3	0.74 ± 0.2	0.68 ± 0.2	1.34 ± 0.3	0.067
Stimulated	49.3 ± 10.4	29.5 ± 11.4	87.4 ± 20.4	157.4 ± 35.2[3,4]	0.005
IL-4 (ng/L)					
Stimulated	18.1 ± 3.9	23.9 ± 9.1	42.1 ± 8.6	42.0 ± 11.8	0.158
Cell proliferation (cpm/well)					
Unstimulated	392 ± 93	380 ± 66	510 ± 99	800 ± 157	0.035
Stimulated	22 809 ± 3737	30 366 ± 6433	36 686 ± 4053	43 698 ± 2184[3]	0.028

[1] $\bar{x}$ ± SEM; n = 15. PUFA, polyunsaturated fatty acid; cpm, counts per minute.
[2] Repeated-measures ANOVA. Greenhouse-Geisser correction factor was applied when the sphericity assumption was not met.
[3] Significantly different from baseline (0 g/d), $P < 0.05$ (Bonferroni correction).
[4] Significantly different from 0.3 g/d, $P < 0.05$ (Bonferroni correction).

plasma phosphatidylcholine were not significantly correlated with production of IL-4 or IFN-γ by unstimulated PBMCs. EPA concentrations in plasma phosphatidylcholine were positively correlated with production of IL-4 ($r = 0.29$, $P = 0.016$) and IFN-γ ($r = 0.48$, $P < 0.001$) by concanavalin A–stimulated PBMCs. Production of IL-4 was positively correlated with production of IFN-γ ($r = 0.31$, $P = 0.008$).

DISCUSSION

The present study found dose-responsive increases in the proportions of EPA and DHA in plasma and erythrocyte phospholipids after dietary supplementation with EPA and DHA as fish oil. In addition, there were simultaneous dose-responsive decreases in the proportion of AA in plasma and erythrocyte phospholipids and in the proportion of LA in erythrocyte phospholipids, and these decreases were not influenced by coadministration of antioxidants.

The mean habitual dietary intakes of n−3 and n−6 PUFAs, selenium, manganese, and vitamin E in the study population were comparable with values for the adult population in the United Kingdom (21). Dietary fish-oil supplementation increased n−3 PUFA intake between 15% (lowest dose) and 100% (highest dose) from that of the subjects' habitual diet. However, habitual dietary n−3 PUFA intake in the United Kingdom comprises mainly α-linolenic acid, with relatively small amounts of EPA and DHA (21), and therefore the fish-oil dietary supplements represented considerable increases in the intake of these longer-chain PUFAs (Table 1). Dietary antioxidant supplementation increased vitamin E and selenium intakes 3–4-fold and vitamin A and C intakes 1–2-fold.

Cosupplementation of antioxidants with fish oil did not have an effect on lymphocyte proliferation. Therefore, these findings do not support the hypothesis that a failure to show increased lymphocyte proliferation after dietary fish-oil supplementation (8, 12–14) may reflect insufficient availability of antioxidants (ie, vitamin E) (13, 22). Furthermore, antioxidant cosupplementation did not modify the effect of fish oil on the production of PGE_2, IFN-γ, or IL-4. The results of the present study, therefore, do not support the hypothesis that fish oil inhibits the production of Th1-type cytokines because of an increased production of lipid peroxides and an associated reduction in the availability of antioxidants (13).

The production of PGE_2 in PBMC cultures is largely due to monocytes, because lymphocytes have very low cyclooxygenase activity (23). Lipopolysaccharide selectively stimulates monocyte function because these cells express CD14, the lipopolysaccharide receptor. In the present study, dietary fish oil decreased the production of PGE_2 by monocytes, a finding that is consistent with dietary supplementation studies in humans (6, 13) and nonhuman primates (24). However, the present study also showed, for the first time, that dietary fish oil simultaneously increased lymphocyte proliferation and the production of IFN-γ, a cytokine produced by Th1-type cells (25). These findings conflict with the results of dietary fish-oil supplementation studies in humans, in which a decrease in PGE_2 production is associated with decreases in lymphocyte proliferation and production of IL-2 (also produced by Th1-type cells) (8, 13, 26), but are consistent with the effects of dietary supplementation in nonhuman primates (24). A possible explanation for the contrast in results between the present study and other reported studies of dietary fish-oil supplementation may relate to the intakes of EPA and DHA involved. The present study used lower doses of EPA and DHA than were used in those studies that showed inhibitory effects of fish oil on cytokine production by Th1-type cells (8, 13–15). We propose, therefore, that supplementary EPA and DHA intakes of ≤2 g/d for ≤4 wk may result in increased cytokine production by Th1-type cells. There may be an inhibitory effect of EPA and DHA at higher intakes or after longer periods of such intakes. The dose-response relation between EPA and DHA intake and cytokine production may therefore follow a "bell-shaped" curve.

Cell culture studies with human PBMCs showed inhibition of lymphocyte proliferation and Th1-type cell cytokine production after exposure to PGE_2 (2–5). The nature of this relation suggests a mechanism of action for dietary EPA and DHA that involves reduction in the production of PGE_2 by the eicosanoid synthetic pathway, and, as a consequence, reduction in the inhibition of lymphocyte proliferation and Th1-type cell cytokine production. However, in the present study, the production of PGE_2 was not correlated with the production of IFN-γ. Furthermore, moderate positive correlations were noted between the production of IFN-γ by concanavalin A–stimulated PBMCs and the proportionate concentrations of EPA within plasma phosphatidylcholine. This suggests that enhancement of cytokine production by Th1-type cells after increased dietary intakes of EPA and DHA represents a direct effect that is independent of altered PGE_2 production by monocytes. A direct effect of EPA on T cell function was previously shown in cell culture studies (27–32), and an effect of fish oil on T cell function that was independent of PGE_2 production was previously proposed in healthy subjects (13).

IL-4 is a cytokine produced by Th2-type cells (25). In the present study, dietary fish oil was not associated with significant changes from baseline in the production of IL-4 by PBMCs, although a trend toward a dose-responsive increase in production was noted. This contrasts with the results of cell culture studies in which increased cytokine production by Th2 cells occurs in the presence of increased PGE_2 release (5), rather than decreased PGE_2 as found in the present study. A possible explanation for these contrasting findings between cell culture and human intervention studies is that the response of Th2-type cells to dietary fish oil is independent of the effects of fish oil on PGE_2 production.

To investigate the effects of fish oil on cytokine production by PBMCs, the present study used an open trial design, as has been used previously (6, 8, 13, 15). This study design involved comparison of baseline values with values after each intervention period in each subject (ie, each subject acted as his own control) to decrease the confounding effects of wide between-individual variations in cytokine production (33, 34), which commonly complicate parallel studies. In comparison, variation in cytokine production within subjects over time is relatively small (33, 34). Washout periods between the intervention periods were omitted because the intake of EPA and DHA and their concentrations within plasma and erythrocyte phospholipids increased with each successive intervention period. Maximal changes in the fatty acid composition of plasma and PBMCs occur within 4 wk of increasing n−3 PUFA intake (11, 12, 35); therefore, 4 wk was chosen as the length of each intervention period for the 3 intakes of EPA and DHA used in the present study. The washout period required to return EPA concentrations to baseline values after each intervention may be as long as 20 wk (6), and such a washout period would have increased the time course of the study to ≈1 y and thereby

introduced additional variance because of the effects of seasonally-related variations in cytokine production (36, 37). Finally, baseline states represent habitual n−3 and n−6 PUFA intakes, not exclusion of PUFAs from the habitual diet, and in themselves do not represent true washouts.

In summary, increased dietary intakes of EPA and DHA (up to 2 g/d) were associated with decreases in PGE_2 production and simultaneous increases in lymphocyte proliferation and IFN-γ production and with a trend toward increased IL-4 production by PBMCs. Antioxidant cosupplementation did not modify the effects of fish oil on the production of PGE_2, IFN-γ, or IL-4 or on lymphocyte proliferation. In healthy persons, dietary supplementation with fish oil containing EPA plus DHA appears to be associated with the up-regulation of both Th1- and Th2-type cell function.

TT, SAW, NKA, MAS, and PCC were involved in the study design. TT was involved in subject recruitment and in carrying out the intervention under the supervision of MAS, SAW, and PCC. TT was involved in data collection under the supervision of SAW, MAS, EAM, GCB, and PCC. TT and NKA performed the data analysis. TT, MM, SAW, NKA, ABB, MAS, and PCC were involved in data interpretation. TT and PCC wrote the manuscript, with contributions from all other authors. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

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