Fish oil and antioxidants alter the composition and function of circulating mononuclear cells in Crohn disease[1–3]

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ABSTRACT

Background: Crohn disease (CD) is associated with osteoporosis and other extraintestinal manifestations that might be mediated by cytokines from circulating (peripheral blood) mononuclear cells (PBMCs). Fish oil rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) reduces disease activity in patients with CD with raised laboratory markers of inflammation and in healthy subjects alters PBMC function.

Objective: We investigated the effect of fish oil plus antioxidants on cytokine production by PBMCs from patients with CD with raised C-reactive protein concentrations (≥6.9 mg/L) or erythrocyte sedimentation rates (≥18 mm/h).

Design: A randomized placebo-controlled trial of fish oil (2.7 g EPA and DHA/d; $n = 31$) or placebo (olive oil; $n = 31$) for 24 wk was conducted in patients with CD. The fish-oil group additionally received an antioxidant preparation (vitamins A, C, and E and selenium). Exclusion criteria included corticosteroid use. Fatty acid composition was measured by gas chromatography. Production of tumor necrosis factor α, interferon γ (IFN-γ), and prostaglandin E_2 (PGE_2) was measured by enzyme-linked immunosorbent assays after stimulation with mitogen and endotoxin (lipopolysaccharide).

Results: Fish-oil plus antioxidant dietary supplementation was associated with higher EPA and DHA incorporation into PBMCs ($P < 0.001$) and lower arachidonic acid ($P = 0.006$) and lower production of IFN-γ by mitogen-stimulated PBMCs ($P = 0.012$) and of PGE_2 by lipopolysaccharide-stimulated PBMCs ($P = 0.047$).

Conclusion: Dietary supplementation with fish oil plus antioxidants is associated with modified PBMC composition and lower production of PGE_2 and IFN-γ by circulating monocytes or macrophages. The response of extraintestinal manifestations of CD should be investigated in a randomized controlled trial. *Am J Clin Nutr* 2004;80:1137–44.

KEY WORDS Mononuclear cell, cytokines, polyunsaturated fatty acids, Crohn disease

INTRODUCTION

Crohn disease (CD) is an inflammatory condition of the gastrointestinal tract associated with extraintestinal manifestations (1) that include altered bone turnover (2, 3) and poor nutritional status (4). The mechanism through which localized inflammation in the CD-affected gastrointestinal tract is associated with systemic manifestations remains unconfirmed but can involve activated mononuclear cells that migrate by way of the peripheral blood (PBMCs) to bone, muscle, liver, and other tissues. PBMCs release inflammatory mediators (5–7), including tumor necrosis factor α (TNF-α), prostaglandin E_2 (PGE_2), and interferon γ (IFN-γ), with independent effects on bone turnover (8, 9) or nutritional status (10). Altered production of cytokines by PBMCs is noted in patients with CD when compared with healthy subjects (11, 12).

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are n−3 polyunsaturated fatty acids (PUFAs), whereas arachidonic acid (AA) is an n−6 PUFA. These PUFAs are acquired directly from the diet or by metabolism of dietary essential fatty acids (**Figure 1**) and are substrates for the production of the eicosanoid family of inflammatory mediators (prostaglandins and leukotrienes) by mononuclear cells. Fish oil is a rich source of EPA and DHA and, in healthy subjects, alters the n−3 PUFA composition of PBMCs (13) and their production of inflammatory mediators, including TNF-α, IFN-γ, and PGE_2 (14). The proportions of EPA and DHA in plasma phospholipid and PBMCs are altered in patients with CD compared with healthy subjects but can also be influenced by CD activity (15–19). Evidence exists that fish oil inhibits clinical disease relapse in patients with CD with raised laboratory markers of systemic inflammation [ie, erythrocyte sedimentation rate (ESR)] (20). This response is accompanied by a significant reduction in inflammatory markers, consistent with a systemic anti-inflammatory effect.

We propose that, in CD, dietary supplementation with fish oil plus antioxidants will result in systemic anti-inflammatory effects associated with modulated cytokine production by PBMCs. The demonstration of such an effect would support the need for a clinical study of fish oil plus antioxidants on bone turnover, nutritional status, and other extraintestinal manifestations in CD.

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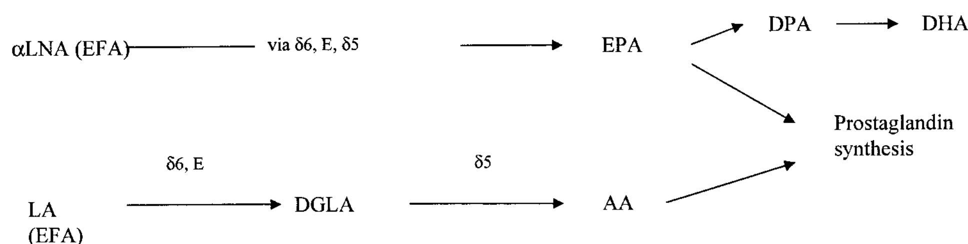

FIGURE 1. Schematic representation of synthetic pathways of n−3 and n−6 long-chain polyunsaturated fatty acids (PUFAs) from dietary essential fatty acids (EFAs). n−3 PUFAs: αLNA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. n−6 PUFAs: LA, linoleic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid. δ5, δ5-desaturase; δ6, δ6-desaturase; E, elongase.

SUBJECTS AND METHODS

Subjects

The study was approved by the Southampton and South West Hampshire Joint Research Ethics Committee. Patients were identified from a database of outpatients with CD under active and recent follow-up at a large university hospital. All patients gave informed consent. The diagnosis of CD was based on endoscopic, histologic, or radiologic findings, and patients with a disease distribution, including small or large bowel or perianal disease, were recruited. Inclusion criteria included laboratory markers of inflammation above the locally determined normal range for serum C-reactive protein (CRP) concentration (≥6.9 mg/L) or ESR (≥18 mm/h) or both within the previous 4 wk. Exclusion criteria included oral or intravenous corticosteroid medication within the previous 4 wk; introduction of other immunosuppressant medication within the previous 8 wk; awaiting or immediately after surgery; use of nutritional support in any form; considerable small bowel intestinal resection, total or subtotal colectomy, or features of short bowel syndrome; inflammatory disease unrelated to CD, hyperlipidemia or diabetes mellitus, liver or renal impairment, cancer, or any other serious acute medical condition; pregnancy or lactation; recent consumption of n−3 PUFA dietary supplements; age <18 y.

At baseline all patients were assessed for CD activity with the use of a clinical disease score [Crohn disease activity index (CDAI) (21)] and completed a questionnaire-based assessment of previous gastroenterologic and medical history. Medical notes were reviewed for further information. Nutritional assessment included body height with the use of a free-standing CMS Stadiometer (Chasmore Ltd, London), body weight with the use of electronic scales (Seca, Hamburg, Germany), body mass index [body weight (in kg)/height^2 (in m)], and a validated semiquantitative self-administered food-frequency questionnaire (22) to estimate habitual dietary nutrient intake.

Study design

This was a randomized, double-blind, placebo-controlled trial of the effect of fish oil plus antioxidants compared with placebo on the composition and function of PBMCs. Randomization was in permutated blocks of 6, with patients stratified for immunosuppressant use, sex, and, in women, menopausal status. Randomization codes were held by a designated pharmacist with no other direct involvement in the study. Patients consumed 9 capsules of fish oil/d (MaxEPA; Seven Seas Ltd, Hull, England) or placebo oil in addition to their habitual diet. Placebo was olive oil containing the monounsaturated fatty acid (MUFA), oleic acid (OA). The fish-oil intervention was equivalent to an additional intake of 2.7 g EPA and DHA/d (**Table 1**). Patients in the fish-oil group additionally received a compound antioxidant dietary supplement containing 200 μg selenium, 3 mg manganese, 30 mg vitamin E as d-α-tocopheryl succinate, 450 μg vitamin A (300 μg RE as retinol and 150 μg RE as β-carotene), and 90 mg vitamin C as ascorbic acid. The placebo group received an identical capsule containing maltose and lactose (both antioxidant and placebo supplied by Wassen International, Leatherhead, United Kingdom). Patients were assessed at baseline and at 8, 16, and 24 wk for treatment compliance and side effects, laboratory markers of disease activity, plasma and mononuclear cell fatty acid composition, and PBMC function and for issuing of fresh capsules. Compliance to the study was assessed by direct questioning and confirmed by analysis of plasma phospholipid composition.

Cell and plasma preparation

Peripheral venous blood samples (30 mL) were taken into heparinized bottles from the antecubital fossa after an overnight fast. Blood was layered over 20 mL Histopaque (density, 1.077 g/mL; Sigma Chemical Co, Poole, United Kingdom) and centrifuged (720 × *g*) for 15 min at 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/L glutamine and antibiotics). A second cycle was performed to reduce erythrocyte contamination. Cells were resuspended in 1 mL medium, counted, and used either for cell culture or for fatty acid composition analysis.

Fatty acid composition analysis

Internal standards (phosphatidylcholine 15:0/15:0 and phosphatidylethanolamine 17:0/17:0) were added to all samples before analysis. Total plasma (1 mL) and PBMC lipids were isolated by extraction with chloroform:methanol (2:1 by vol) (23)

TABLE 1
Total supplementary intake of fatty acids in the fish oil plus antioxidants and placebo groups[1]

	MA	PA	PoA	SA	OA	LA	EPA	DHA
				g/d				
Fish oil group	0.63	1.44	0.9	0.41	1.28	0.41	1.62	1.08
Placebo group	0	1.02	0.09	0.24	6.62	0.85	0	0

[1] Saturated fatty acids: MA, myristic acid; PA, palmitic acid; SA, stearic acid. Monounsaturated fatty acids: PoA, palmitoleic acid; OA, oleic acid. n−6 polyunsaturated fatty acid: LA, linoleic acid. n−3 polyunsaturated fatty acids: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

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containing 50 mg/L butylated hydroxytoluene. Plasma phosphatidylcholine was purified by solid-phase extraction on aminopropylsilica cartridges (Varian, Surrey, United Kingdom). Plasma phosphatidylcholine and total PBMC fatty acids were converted to methyl esters by incubation with methanol containing 2% (by vol) sulfuric acid at 50 °C for 18 h. Fatty acid methyl esters were isolated, redissolved in hexane, and analyzed by capillary gas chromatography with the use of a Hewlett-Packard 5890 GC (Hewlett-Packard, Stockport, United Kingdom) equipped with an HP7686 GC autosampler with the use of a BPX-70 fused silica capillary column (50 m × 0.25 mm × 0.32 μm) with flame ionization detection. Peaks were identified by retention times relative to standards. Fatty acids are reported as proportionate values (g/100g total fatty acids). CV was <5% for determination of fatty acid composition.

Analysis of cytokine and eicosanoid production by peripheral blood mononuclear cells

Purified PBMCs at a concentration of 1×10^6 cells/mL were incubated in 2 mL medium containing 5% autologous plasma, with and without the monocyte or macrophage stimulant lipopolysaccharide (LPS; also known as endotoxin) at a concentration of 15 mg/L (for TNF-α and PGE_2) or the T cell mitogenic stimulant concanavalin A (ConA) at a concentration of 25 mg/L (IFN-γ), both for 24 h. After this, culture plates were centrifuged (180*g*) for 10 min at 20 °C, and supernatant was removed and frozen at −30 °C. TNF-α and IFN-γ concentrations were determined with the use of EASIA enzyme-linked immunosorbent assay kits (Biosource Europe SA, Nivelles, Belgium). PGE_2 concentrations were determined with the use of NEOGEN enzyme-linked immunosorbent assay kits (Neogen Corporation, Lexington, KY). Kits were used according to the manufacturer's instructions. CV was <10% for both cytokine and prostaglandin assays, and the limits of detection were to 3 ng/L for TNF-α and 0.03 kIU/L for IFN-γ.

Statistical analysis

The primary outcome variables, compared between fish-oil plus antioxidant and placebo groups, included change from baseline to 24 wk in concentrations of n−3 and n−6 PUFAs in PBMCs and in plasma phosphatidylcholine and TNF-α, IFN-γ, and PGE_2 production by PBMCs. Analysis of covariance was used for statistical comparisons between the groups for outcomes at 24 wk adjusted for both baseline values and use of corticosteroids by patients during the study. Baseline characteristics and values for dietary intakes, laboratory investigations, and clinical disease scores were compared between the groups with the use of the 2-sample *t* tests for independent variables for continuous (nonparametric variables were log-transformed before analysis) and the chi-square test for categorical variables. Analyses were preformed with the use of SPSS for Windows, version 11 (SPSS Inc, Chicago).

RESULTS

Subjects

A total of 77 patients were recruited and randomly assigned to fish oil plus antioxidants or placebo. Fifteen patients were excluded from the outcome analysis. Six patients withdrew from the study (2 in the fish-oil plus antioxidant group and 4 in the placebo group). Nine patients were retrospectively identified with laboratory markers of inflammation (ESR or CRP) that were within the locally accepted normal ranges for the healthy population and, therefore, below the threshold for recruitment. There were no reported serious side effects of treatment with either fish oil plus antioxidants or placebo.

TABLE 2
Baseline characteristics of patients with Crohn disease in the fish oil plus antioxidants and placebo groups[1]

	Fish oil group (n = 31)	Placebo group (n = 31)
Sex (n)		
Male	11	9
Female		
Premenopausal	13	14
Postmenopausal	7	8
Age (y)	45.4 ± 13.1[2]	40.5 ± 13.8
Disease duration (y)	10 (5, 19)[3]	7 (2, 17)
Disease site (n)		
Small bowel	8	6
Large bowel	8	13
Small and large bowels	14	12
Perianal only	1	0
Previous intestinal resection (n)	10	6
Current drug history (n)		
Azathioprine-methotrexate	10	7
5-Aminosalicylic acid	4	4
Corticosteroids	0	0
Markers of disease activity		
Crohn disease activity index	158 ± 89	175 ± 82
C-reactive protein (mg/L)	6.6 (3.7, 14.1)	10.2 (3.6, 16.9)
Erythrocyte sedimentation rate (mm/h)	14 ± 2	17 ± 2
Nutritional status		
Weight (kg)	68.8 ± 10.7	73.6 ± 14.1
BMI (kg/m^2)	27.8 ± 6.7[4]	25.0 ± 2.9
Current smoking (n)	10	12

[1] There were no significant differences between the fish oil and placebo groups except where indicated.
[2] $\bar{x}$ ± SD (all such values).
[3] Median; interquartile range in parentheses (all such values).
[4] Significantly different from placebo group, P = 0.048 (two-sample *t* test).

Baseline characteristics of the remaining patients with CD in fish-oil plus antioxidant (n = 31) and placebo (n = 31) groups are shown (**Table 2**). Values for CDAI, CRP, and ESR were comparable between the 2 groups and consistent with mild-to-moderate disease activity, but there was evidence of a higher body mass index in the fish-oil plus antioxidants group. No significant differences were observed between fish-oil plus antioxidants and placebo groups in dietary fat or antioxidant intake, quantitatively or qualitatively (**Table 3**), or baseline plasma vitamin E ($\bar{x}$± SD fish-oil compared with placebo group, respectively; 29.4 ± 7.9 compared with 28.8 ± 9.3, P = 0.802), vitamin A (1.8 ± 0.6 compared with 2.1 ± 0.8, P = 0.174), selenium (0.9 ± 0.2 compared with 0.9 ± 0.2, P = 0.994), zinc (13.7 ± 2.7 compared with 14.3 ± 2.4, P = 0.378), or copper (20.5 ± 7.2 compared with 22.0 ± 8.8, P = 0.924).

No significant differences were observed in the response to fish oil plus antioxidants compared with placebo of CDAI (P = 0.388), CRP (P = 0.684), or ESR (P = 0.594). Six patients in the

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TABLE 3
Habitual dietary intake of fat and antioxidants in the fish oil plus antioxidants and placebo groups[1]

	Fish oil group (n = 31)	Placebo group (n = 31)	Mean difference (95% CI)
Energy (kcal/d)	2608 ± 987[2]	2304 ± 572	303 (−107, 713)
Saturated fat (g/d)	29.1 ± 14.5	26.7 ± 11.3	2.4 (−4.17, 9.04)
Monounsaturated fat (g/d)	26.3 ± 10.6	22.3 ± 7.1	4.0 (−0.63, 8.55)
n−6 Polyunsaturated fat (g/d)	11.5 ± 6.4	9.4 ± 4.2	2.2 (−0.59, 4.90)
n−3 Polyunsaturated fat (g/d)	1.75 ± 0.76	1.47 ± 0.75	0.28 (−0.10, 0.66)
Carotene (μg/d)	3948 ± 6405	2125 ± 1073	1823 (−510, 4156)
Retinol (μg/d)	628 ± 547	502 ± 403	126 (−118, 370)
Selenium (μg/d)	68 ± 32	65 ± 31	2.9 (−13.0, 18.9)
Vitamin C (mg/d)	140 ± 95	114 ± 64	26 (−14.8, 67.8)
Vitamin E (mg/d)	10.0 ± 7.8	7.4 ± 3.8	2.5 (−0.63, 5.69)

[1] Determined by food-frequency questionnaire. None of the differences were significant by two-sample t test.
[2] $\bar{x}$ ± SD (all such values).

fish-oil plus antioxidants group (19.4%) and 7 patients in the placebo group (22.6%) required corticosteroid medication during the intervention period. No significant differences were observed in the response to fish oil plus antioxidants compared with placebo of plasma vitamin A (P = 0.244), vitamin E (P = 0.244), or copper (P = 0.209) at 24 wk. However, plasma selenium was higher in the fish-oil plus antioxidants group (1.26 ± 0.33 μmol/L) than in the placebo group (0.86 ± 0.2 μmol/L; $P < 0.001$).

Fatty acid composition in peripheral blood mononuclear cells and plasma phosphatidylcholine

Fish oil plus antioxidants was associated with significantly greater incorporation of EPA ($P < 0.001$), docosapentaenoic acid (DPA; P = 0.001), and DHA ($P < 0.001$) within PBMCs than with placebo and a significantly lower incorporation of AA (P = 0.006) (**Table 4**). No significant differences were observed for other fatty acids, including OA (Table 4).

TABLE 4
Fatty acid composition of total phospholipids from peripheral blood mononuclear cells from patients with Crohn disease at baseline and after dietary supplementation with fish oil plus antioxidants or placebo at 8, 16, and 24 wk[1]

Fatty acid and intervention group	Fatty acid composition				Mean difference (95% CI)	P[2]
	Baseline	8 wk	16 wk	24 wk		
	g/100 g total fatty acids					
OA					−0.51 (−2.37, 1.36)	0.589
Fish oil	17.9 ± 2.8[3]	19.6 ± 3.0	18.2 ± 2.5	17.0 ± 3.8		
Placebo	18.4 ± 2.4	19.3 ± 2.9	19.9 ± 1.98	17.7 ± 4.0		
LA					−0.25 (−1.75, 1.24)	0.735
Fish oil	9.4 ± 3.8	9.1 ± 3.1	8.9 ± 2.9	8.9 ± 2.5		
Placebo	8.9 ± 1.5	9.2 ± 2.7	11.2 ± 6.1	9.1 ± 3.1		
αLNA					−0.05 (−0.10, 0.01)	0.100
Fish oil	0.56 ± 0.48	0.49 ± 0.58	0.45 ± 0.62	0.10 ± 0.09		
Placebo	0.38 ± 0.32	0.43 ± 0.68	0.20 ± 0.17	0.15 ± 0.11		
DGLA					−0.15 (−0.48, 0.19)	0.381
Fish oil	2.4 ± 0.8	1.9 ± 0.5	2.1 ± 0.5	2.2 ± 0.6		
Placebo	2.5 ± 0.66	2.4 ± 0.6	2.4 ± 0.5	2.4 ± 0.8		
AA					−2.90 (−4.92, −0.88)	0.006
Fish oil	18.8 ± 3.4	14.9 ± 4.0	16.4 ± 3.3	17.8 ± 3.7		
Placebo	20.1 ± 2.4	18.8 ± 3.8	17.8 ± 4.0	20.7 ± 3.7		
EPA					2.07 (1.50, 2.64)	<0.001
Fish oil	1.1 ± 0.6	1.9 ± 1.1	2.3 ± 1.3	2.7 ± 1.5		
Placebo	1.0 ± 0.6	0.5 ± 0.3	0.8 ± 0.8	0.7 ± 0.4		
DPA					0.93 (0.39, 1.46)	0.001
Fish oil	3.2 ± 1.5	3.8 ± 1.0	3.1 ± 0.8	4.0 ± 1.1		
Placebo	2.6 ± 0.6	1.9 ± 0.7	2.1 ± 0.7	2.9 ± 0.9		
DHA					1.31 (0.86, 1.77)	<0.001
Fish oil	2.6 ± 0.9	5.3 ± 3.0	4.6 ± 1.4	3.5 ± 1.1		
Placebo	2.8 ± 1.0	2.9 ± 0.9	2.8 ± 0.9	2.3 ± 0.7		

[1] OA, oleic acid. n−6 polyunsaturated fatty acids: LA, linoleic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid. n−3 polyunsaturated fatty acids: LNA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA; docosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Analysis of covariance for differences between the fish oil and placebo groups in change from baseline to 24 wk, adjusted for corticosteroid use.
[3] $\bar{x}$ ± SD (all such values).

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TABLE 5
Fatty acid composition of plasma phosphatidylcholine from patients with Crohn disease at baseline and after dietary supplementation with fish oil plus antioxidants or placebo at 8, 16, and 24 wk[1]

	Fatty acid composition					
Fatty acid and intervention group	Baseline	8 wk	16 wk	24 wk	Mean difference (95% CI)	P^2
	g/100 g total fatty acids					
OA					−1.75 (−2.44, −1.05)	<0.001
Fish oil	11.3 ± 1.1[3]	10.6 ± 1.4	10.5 ± 1.2	10.2 ± 1.5		
Placebo	11.3 ± 2.0	12.6 ± 2.3	12.2 ± 1.8	11.9 ± 1.8		
LA					−2.77 (−4.26, −1.29)	<0.001
Fish oil	20.9 ± 2.4	17.8 ± 3.6	18.4 ± 10.0	18.7 ± 3.7		
Placebo	22.0 ± 2.9	21.3 ± 3.7	22.2 ± 3.7	22.2 ± 3.0		
αLNA					0.01 (−0.03, 0.05)	0.617
Fish oil	0.27 ± 0.13	0.25 ± 0.15	0.23 ± 0.12	0.25 ± 0.09		
Placebo	0.28 ± 0.11	0.24 ± 0.12	0.26 ± 0.15	0.25 ± 0.09		
DGLA					−1.26 (−1.70, −0.82)	<0.001
Fish oil	3.9 ± 1.0	2.6 ± 0.9	2.8 ± 1.0	2.8 ± 1.3		
Placebo	3.7 ± 0.9	3.8 ± 1.0	3.7 ± 0.9	3.9 ± 1.0		
AA					−1.36 (−1.92, −0.79)	<0.001
Fish oil	9.3 ± 1.5	7.6 ± 1.3	7.5 ± 1.5	7.7 ± 1.1		
Placebo	8.7 ± 1.7	8.7 ± 1.7	8.8 ± 1.8	8.6 ± 1.9		
EPA					3.55 (2.61, 4.49)	<0.001
Fish oil	1.2 ± 0.7	4.8 ± 2.1	4.8 ± 2.2	4.7 ± 2.5		
Placebo	1.2 ± 0.7	1.7 ± 2.1	1.3 ± 0.8	1.2 ± 0.9		
DPA					0.51 (0.34, 0.69)	<0.001
Fish oil	1.1 ± 0.3	1.6 ± 0.5	1.6 ± 0.4	1.6 ± 0.4		
Placebo	0.97 ± 0.37	1.04 ± 0.38	0.95 ± 0.29	1.01 ± 0.33		
DHA					2.95 (2.11, 3.79)	<0.001
Fish oil	3.5 ± 1.3	6.8 ± 1.8	6.5 ± 1.6	6.5 ± 2.0		
Placebo	3.7 ± 1.3	3.8 ± 1.8	3.5 ± 1.0	3.6 ± 1.3		

[1] OA, oleic acid. n−6 polyunsaturated fatty acids: LA, linoleic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid. n−3 polyunsaturated fatty acids: LNA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA; docosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Analysis of covariance for differences between the fish oil and placebo groups in change from baseline to 24 wk, adjusted for corticosteroid use.
[3] $\bar{x}$ ± SD (all such values).

Fish oil plus antioxidants was associated with significantly greater incorporation of EPA ($P < 0.001$), DPA ($P < 0.001$), and DHA ($P < 0.001$) in plasma phosphatidylcholine than with the placebo group, as well as significantly lower incorporation of the n−6 PUFA linoleic acid ($P < 0.001$), dihomo-γ-linolenic acid ($P < 0.001$), and AA ($P = 0.001$) and of the MUFA OA ($P = 0.001$) (**Table 5**).

Production of inflammatory mediators by peripheral blood mononuclear cells

Fish oil plus antioxidants was associated with significantly lower production of IFN-γ by ConA-stimulated PBMCs (**Figure 2**) and of PGE_2 by LPS-stimulated PBMCs (**Figure 3**) than with the placebo group. No significant difference was observed between groups in the response of TNF-α by LPS-stimulated PBMCs (**Figure 4**) or in the production of TNF-α ($P = 0.484$), IFN-γ ($P = 0.863$), or PGE_2 ($P = 0.480$) by unstimulated PBMCs (data not shown).

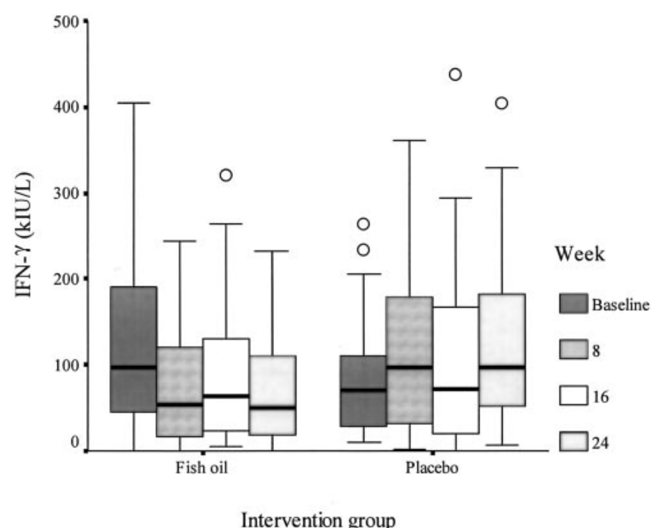

FIGURE 2. Box-and-whisker plots that show the median, spread, and interquartile ranges for interferon γ (IFN-γ) production by concanavalin A–stimulated peripheral blood mononuclear cells from patients with Crohn disease after fish oil plus antioxidants or placebo at baseline and at 8, 16, and 24 wk. Outliers (○) were included in the analysis. $P = 0.012$ for the difference between the fish oil and placebo groups in change from baseline to 24 wk, adjusted for corticosteroid use.

DISCUSSION

This was a randomized controlled study of fatty acid composition and function of PBMCs in patients with CD and their response to dietary supplementation with an EPA- and DHA-rich

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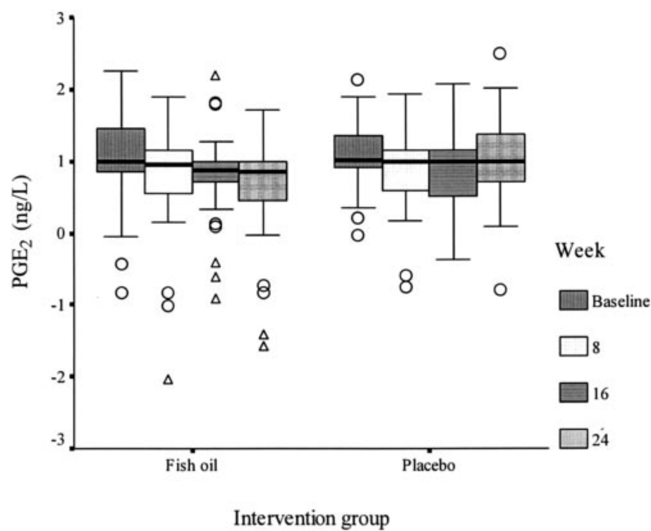

FIGURE 3. Box-and-whisker plots that show the median, spread, and interquartile ranges for prostaglandin E_2 (PGE_2) production (log transformed) by lipopolysaccharide-stimulated peripheral blood mononuclear cells from patients with Crohn disease after fish oil plus antioxidants or placebo at baseline and at 8, 16, and 24 wk. Outliers (○) were included in the analysis; △ indicates extreme value. $P = 0.047$ for the difference between the fish oil and placebo groups in change from baseline to 24 wk, adjusted for corticosteroid use.

fish oil, plus an antioxidant cosupplement. Fish-oil plus antioxidant dietary supplementation was associated with greater incorporation of the long chain n−3 PUFAs, EPA, DPA, and DHA, and lower incorporation in the n−6 PUFA, AA, in PBMCs (and plasma phospholipid). The habitual dietary intakes of n−3 and n−6 PUFAs among patients in fish-oil plus antioxidants and placebo groups were consistent with estimated mean values for the UK population of 1.8 and 10.2 g/d, respectively (24). However, patients randomly assigned to the fish-oil plus antioxidants group received a further 2.7 g EPA and DHA/d, which compares

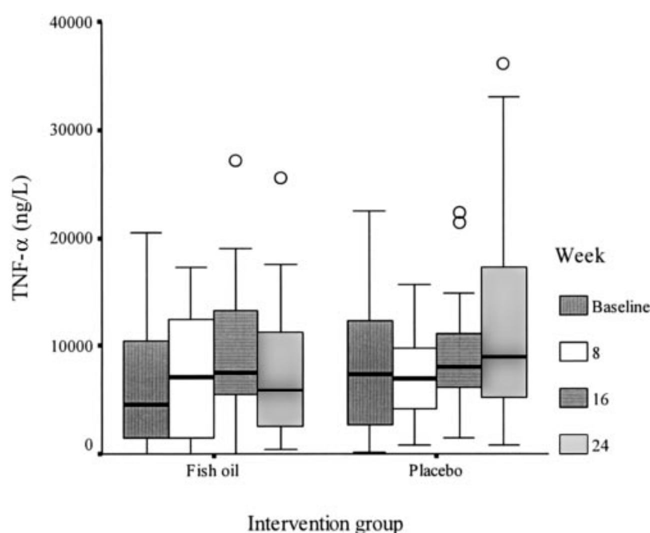

FIGURE 4. Box-and-whisker plots that show the median, spread, and interquartile ranges for tumor necrosis factor α (TNF-α) production by lipopolysaccharide-stimulated peripheral blood mononuclear cells from patients with Crohn disease after fish oil plus antioxidants or placebo at baseline and at 8, 16, and 24 wk. Outliers (○) were included in the analysis.

with an estimated mean intake in the UK population of <0.25 g/d (24). To our knowledge, the current study is the first to demonstrate altered PBMC fatty acid composition in response to dietary fish oil plus antioxidants in patients with CD. The effect of fish oil plus antioxidants on fatty acid composition of PBMCs and plasma in CD is consistent with that seen in studies in healthy humans receiving fish oil alone (13).

The alterations in PUFA incorporation into PBMCs and plasma phospholipid in the fish-oil plus antioxidants group, in the current study, were associated with lower production of IFN-γ by ConA-stimulated mononuclear cells. IFN-γ is produced by T-helper type 1 (Th1) cells (25), and ConA is a specific T cell stimulant. To our knowledge the current study is the first to demonstrate that fish oil, in this case combined with antioxidants, alters production of cytokines by PBMCs in CD and, more specifically, production of IFN-γ by activated T cells. The results of the current study contrast with those from a healthy human study demonstrating that fish oil (with or without antioxidant cosupplementation) is associated with greater production of IFN-γ by PBMCs (26). However, the results are consistent with other studies that demonstrate reductions in interleukin 2, a cytokine also produced by Th1 cells (27, 28). Activation of IFN-γ–producing Th1 cells in the lamina propria of CD-affected gut plays a pivotal role in the pathogenesis of gastrointestinal CD (29). The clinical (gastrointestinal) benefits of fish oil in CD demonstrated by Belluzzi et al (20) can be explained by inhibition of T cell function in the gut and are, therefore, consistent with the response of circulating T cells to fish oil in the current study. However, in the current study, the response of PBMC composition and function to fish oil plus antioxidants was not associated with a change in the CDAI. This observation suggests, therefore, that the effects on PBMCs cannot be simply explained as a result of resolving gastrointestinal CD but are more likely to reflect a direct effect of EPA and DHA.

In the current study, an antioxidant cosupplement was administered in the fish-oil arm to inhibit lipid peroxidation (30) and production of free radicals that can result from increased EPA and DHA in plasma (31) and cell membranes (32) after fish-oil consumption (33, 34). Evidence suggests that production of free radicals contributes toward the pathogenesis of inflammatory bowel disease and related cellular damage in the gut (35, 36). Dietary supplementation with antioxidants, including vitamin E, can reduce lipid peroxidation in CD (37), but the effect of coadministration with fish oil is uncertain in subjects with CD or in health subjects (31, 33). In the current study, dietary supplementation with the antioxidant preparation was associated with higher plasma selenium concentrations but not with changes in plasma concentrations of vitamin E or A. A possible criticism of the current study is that the antioxidant cosupplement, and in particular selenium, might account for the response noted in the intervention group. The effect of an identical antioxidant supplement on production of PGE_2 and IFN-γ by PBMCs in association with various intakes of fish oil is assessed in a randomized controlled trial, published elsewhere (26). That study of healthy volunteers suggested that there is not a significant independent effect of the antioxidant cosupplement on fatty acid composition of plasma or cell membrane or function of PBMCs at intakes of fish oil equivalent to 2 g EPA and DHA/d.

The choice of placebo in the current study was olive oil, which contains MUFA in the form of OA. The immunologic properties of OA are investigated in several studies (38), but these studies

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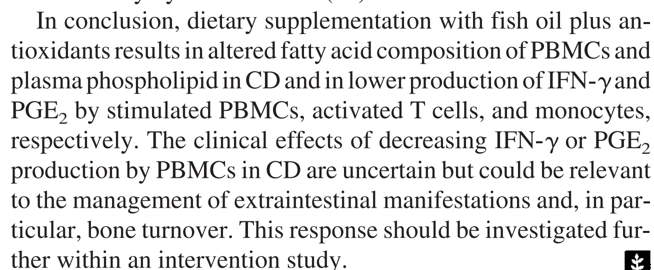
have not demonstrated a significant effect on the immune response in humans, including production of cytokines by Th1 cells (39). Yaqoob (38) explains the lack of immune effect of dietary supplementation with OA as a consequence of the high concentration of monounsaturated fat in the habitual Western diet. In the current study, the total intake of MUFA consumed by the patients in their habitual diet and from the fish-oil plus antioxidants and placebo capsules was 28.2 g/d in the intervention group and 28.4 g/d in the placebo group. The olive oil placebo was not associated with greater incorporation of OA within PBMCs (Table 4), which is consistent with the literature (13).

In the current study, fish oil plus antioxidants was also associated with a lower production of PGE_2 by LPS-stimulated PBMCs. PGE_2 is synthesized by monocytes (40), and LPS is a monocyte-specific stimulant. The response of PGE_2 production to fish oil plus antioxidants is consistent with, but less marked than, the response noted in some fish-oil dietary supplementation studies in healthy humans (26, 27, 41). In the current study, therefore, fish oil plus antioxidants was associated with lower production of both PGE_2 and IFN-γ. However, evidence suggests that release of PGE_2 by monocytes or macrophages inhibits production of IFN-γ by Th1 cells (5, 42, 43); therefore, the expected response to lower production of PGE_2 would be greater production of IFN-γ. The mechanism underlying the apparently paradoxical response of a simultaneous reduction in both IFN-γ and PGE_2 is uncertain but could be related to the relative rates of metabolism of EPA to PGE_3 and AA to PGE_2 or to other members of the eicosanoid family. For example, EPA and AA are competitive and mutually antagonistic substrates in the prostaglandin synthetic pathway (Figure 1), in which their rate of metabolism is related to their respective dietary intakes (44–46). PGE_2 and PGE_3 have similar effects on the mitogenic response and function of PBMCs (47), but the production of PGE_3 from EPA by prostaglandin synthase enzymes is inefficient (48). This finding suggests that total PGE production by PBMCs after fish oil plus antioxidants (ie, EPA and DHA) is altered in a quantitative and not qualitative manner. EPA can lower production of PGE_2, increase production of PGE_3, and, as a result of the sum of these effects, increase or decrease total production of PGE. The findings of the current study and conflicting results of studies in healthy humans [see (14)] might, therefore, be explained as a dose-response effect in which low supplementary intakes of EPA (eg, 1 g/d) decrease production of PGE_2 from AA but result in insufficient PGE_3 to compensate and, therefore, increase synthesis of IFN-γ [ie, as noted in healthy subject studies (26)]. By comparison, high intakes of EPA (eg, >2 g/d) that are used in the current study also decrease production of PGE_2 from AA but lead to sufficiently high production of PGE_3 to decrease production of IFN-γ. The dose of EPA and DHA administered in the current study was 2.7 g/d and similar to the supplementary intake in the clinical study by Belluzzi et al (20).

In conclusion, dietary supplementation with fish oil plus antioxidants results in altered fatty acid composition of PBMCs and plasma phospholipid in CD and in lower production of IFN-γ and PGE_2 by stimulated PBMCs, activated T cells, and monocytes, respectively. The clinical effects of decreasing IFN-γ or PGE_2 production by PBMCs in CD are uncertain but could be relevant to the management of extraintestinal manifestations and, in particular, bone turnover. This response should be investigated further within an intervention study.



The respective authors were responsible for the following contributions toward this study: TMT was responsible for trial design, patient recruitment and management, laboratory analysis, data analysis, and preparation of manuscript; NKA was involved in trial design; SAW was involved in trial design and nutritional assessment; PCC was involved in trial design, laboratory techniques, and preparation of manuscript; MAM was involved in statistical analysis; DRF and MAS were involved in trial design and patient recruitment; and MAS was additionally involved in preparation of the manuscript. None of the authors have a conflict of interest.

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