

Biochemical effects of a diet containing foods enriched with n-3 fatty acids¹⁻³

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ABSTRACT

Background: Results of many studies indicate that consumption of n-3 fatty acids can benefit persons with cardiovascular disease and rheumatoid arthritis. However, encapsulated fish oil is unlikely to be suited to lifetime daily use and recommendations to increase fish intake have not been effective.

Objective: The objective was to examine the effectiveness of a diet that incorporates foods rich in n-3 fatty acids in elevating tissue concentrations of eicosapentaenoic acid and in suppressing the production of inflammatory mediators.

Design: Healthy male volunteers were provided with foods that were enriched in α -linolenic acid (cooking oil, margarine, salad dressing, and mayonnaise) and eicosapentaenoic and docosahexaenoic acids (sausages and savory dip) and with foods naturally rich in n-3 fatty acids, such as flaxseed meal and fish. Subjects incorporated these products into their food at home for 4 wk. Fatty acid intakes, cellular and plasma fatty acid concentrations, and monocyte-derived eicosanoid and cytokine production were measured.

Results: Analyses of dietary records indicated that intake of eicosapentaenoic acid plus docosahexaenoic acid averaged 1.8 g/d and intake of α -linolenic acid averaged 9.0 g/d. These intakes led to an average 3-fold increase in eicosapentaenoic acid in plasma, platelet, and mononuclear cell phospholipids. Thromboxane B₂, prostaglandin E₂, and interleukin 1 β synthesis decreased by 36%, 26%, and 20% ($P < 0.05$), respectively.

Conclusions: Foods that are strategically or naturally enriched in n-3 fatty acids can be used to achieve desired biochemical effects without the ingestion of supplements or a change in dietary habits. A wide range of n-3-enriched foods could be developed to support large-scale programs on the basis of the therapeutic and disease-preventive effects of n-3 fatty acids. *Am J Clin Nutr* 2000;72:42-8.

KEY WORDS α -Linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, n-3 fatty acids, n-3 fatty acid-rich foods, cytokines, eicosanoids, men

INTRODUCTION

The health benefits of the dietary consumption of foods rich in n-3 fatty acids in persons with cardiovascular disease and rheumatoid arthritis have been well documented (1, 2). Accordingly, health authorities in Canada (3), the United Kingdom (4),

and Australia (5) have recommended increases in the consumption of n-3 fatty acids, namely, α -linolenic acid (ALA; 18:3n-3) and its long-chain metabolites eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). ALA is present in certain vegetable oils (flaxseed and canola), whereas EPA and DHA are present in fish and in more concentrated amounts in fish oil. Consumption of ALA leads to significant increases in tissue EPA but not in DHA (6, 7). However, the consumption of fish or fish oil (which contain both EPA and DHA) yields higher concentrations of both EPA and DHA (8, 9).

Although health authorities may recommend increased intakes of n-3 fatty acids, the studies on which the recommendations are based do not necessarily provide guidelines for achieving this goal. For example, in controlled clinical studies of rheumatoid arthritis, the sole strategy for increasing cellular EPA and DHA concentrations has been dietary supplementation with fish-oil capsules (1). Twelve double-blind, placebo-controlled studies provided evidence that n-3 fatty acid supplementation of the diet has a beneficial effect in patients with rheumatoid arthritis (1). However, encapsulated fish oil is not suitable for daily use; thus, the results of rheumatoid arthritis studies generally have not led to changes in clinical practice that incorporate advice to increase intakes of n-3 fatty acids.

Evidence that dietary n-3 fatty acids can prevent cardiovascular disease has led to recommendations to increase fish consumption (2). However, modern Western societies consume little fish and an increase in fish consumption would involve major dietary changes. Therefore, this dietary approach is not an effective way to achieve an increase in the consumption of n-3 fatty acids at a community or clinical level.

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TABLE 1
n-3 and n-6 Fatty acid content of the food items provided to the subjects during the study¹

Food	LA	ALA	EPA	DHA
<i>% of total fatty acids</i>				
Oil ²	9.2	16.0	0	0
Margarine ²	10.5	22.1	0	0
Salad dressing ²	3.0	9.7	0	0
Mayonnaise				
Herb ²	2.7	5.2	0	0
Plain ²	4.7	15.3	0	0
Flaxseed-meal mixture	5.4	16.7	0	0
Sardines	0.14	0.05	0.50	1.68
Pink salmon	0.20	0.15	0.89	1.66
Deep-sea bream	0	0	0.30	0.40
Flathead	0	0	0.05	0.29
Mullet	0	0	0.73	0.52
Sausages ³	0.70	0.17	0.52	0.31
French onion dip ³	0	0.28	0.65	0.74

¹LA, linoleic acid; ALA, α -linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

²Supplemented with flaxseed oil.

³Supplemented with fish oil.

An effective strategy for implementing a long-term increase in n-3 fatty acid consumption has yet to be developed. A suitable approach will be one that offers a selection of foods and ingredients that can be substituted in the diet for common everyday food items. In this study we developed a range of foods and ingredients enriched with ALA, EPA, and DHA and examined the effect of these foods and ingredients on n-3 fatty acid tissue concentrations and on the synthesis of eicosanoids and cytokines in healthy volunteers.

SUBJECTS AND METHODS

Subjects

Fifteen healthy male subjects were recruited from volunteers who responded to an advertisement about dietary intervention studies. The specifics of the study were explained to the subjects before they were enrolled to ensure that the subjects' schedules were compatible with the requirements of the study. The mean (\pm SD) age of the subjects was 37.7 ± 6.5 y and their mean body mass index (BMI; in kg/m^2) was 25.6 ± 2.9 . None of the subjects had a history of hyperlipidemia or inflammatory disease and all were apparently healthy, as indicated by a medical questionnaire completed at enrollment. The study was approved by the Royal Adelaide Hospital Research Ethics Committee and written, informed consent was obtained from all participants.

Diets and experimental design

The foods and ingredients selected for the diet were designed to increase the dietary content of n-3 fatty acids and to decrease the content of n-6 fatty acids and were supplied to the subjects in quantities sufficient for their entire families. Subjects maintained the n-3-enriched, n-6-poor diet for 4 wk. Foods were either enriched with ALA (from flaxseed oil) or with EPA and DHA. Food items that were enriched with ALA included cooking oil, margarine, salad dressing, and mayonnaise (Meadow Lea

Foods Pty Ltd, Mascot, Australia) (Table 1). Foods enriched with EPA and DHA via fish oil (ROPUFA-30; Roche Vitamins Pty Ltd, Sydney, Australia) included sausages (4% by wt) and French onion dip (5% by wt). Subjects were also supplied with muffin mix (Meadow Lea Foods Pty Ltd), from which the shortening had been omitted so that the ALA-enriched oil could be added before the muffins were baked. Subjects were supplied with canned fish (pink salmon and sardines; SAFCOL, Adelaide, Australia) and fresh fish (mullet, flathead, and deep-sea bream). A flaxseed-meal mixture naturally rich in ALA (Alena Energy Drink; ENRECO, Manitowoc, WI) was also provided and subjects were instructed to use it in orange juice, in food preparation (eg, cakes, muffins, casseroles, Mornay sauce, and soups), and with their breakfast cereal. Advice was provided on avoidance, when practical, of foods containing high amounts of n-6 polyunsaturated fatty acids (PUFAs), eg, certain nuts, potato chips, and certain cooking oils and their derivative products. Although subjects were discouraged from dining away from home, they were educated about suitable choices that they could make (including suitable fast-food take-out choices) when they did dine away from home.

Subjects were not asked to eat any set quantity of any food, but rather to use the provided foods and ingredients in desired quantities during food preparation. A target value for EPA of 1.5% of total fatty acids in the mononuclear cells was set. Mononuclear cell phospholipid fatty acids were analyzed 2 wk into the study and, if the percentage of EPA was below the target value, the subjects were contacted and encouraged to increase their use of the provided n-3-enriched foods in place of other foods if this was still practical.

All subjects were asked to record their dietary intakes on 2 weekdays and 1 weekend day of every week during the trial. Subjects were instructed to maintain a diet diary by using weighed measurements and standard household measures at the commencement of the trial. All subjects were supplied with digital electronic scales. Diet diaries were analyzed for total energy intakes; the percentages of energy derived from protein, carbohydrate, fat, and alcohol; and intakes of oleic acid (OA; 18:1n-9), linoleic acid (LA; 18:2n-6), ALA, EPA, and DHA with DIET-1 (Xyris Software Pty Ltd, Brisbane, Australia), which is based on the Australian Nutrient Database NUTTAB 91-92 (Department of Community Services and Health). We modified this database to include the fatty acid content of Australian foods and ingredients as well as of the provided foods.

Fasting venous blood samples were taken at each of the visits (0, 2, and 4 wk). Subjects were instructed to fast for 12 h and to avoid abnormally high intakes of alcohol 24 h before the clinic visits. Nonsteroidal antiinflammatory medications that inhibit the prostaglandin-endoperoxide synthase (cyclooxygenase; COX) pathway were proscribed (10). Paracetamol (acetaminophen) was allowed for pain relief if required.

Analytic methods

Cell separation and fatty acids

Peripheral blood samples (20 mL) were added to tubes containing 4 mL 4.5% EDTA in water and 4 mL 6.0% dextran (Pharmacia LKB, Uppsala, Sweden) in normal saline, pH 7.0. Erythrocytes were allowed to sediment under gravity at 37°C for 30 min. The resulting leukocyte-enriched plasma was loaded onto a Ficoll-Paque gradient with a density of 1.077 kg/L

(Lymphoprep; Nycomed Pharma, Oslo) and centrifuged at $110 \times g$ for 10 min at 25°C to separate the plasma and platelets. The gradient was further centrifuged at $200 \times g$ for 20 min to separate the mononuclear cells. The plasma and cellular fractions were extracted in chloroform:methanol (11) and stored at -70°C before fatty acid analysis. The total lipid extract from the plasma and cellular fractions was fractionated by thin-layer chromatography and the phospholipid fractions retained. The cellular and plasma fractions were then transesterified by methanolysis (1% H_2SO_4 in methanol at 70°C for 3 h). Fatty acid methyl esters were separated and quantified with a Hewlett-Packard 6890 gas chromatograph equipped with a 50-m capillary column (0.33 mm internal diameter) coated with BPX-70 (0.25- μm film thickness; SGE Pty Ltd, Victoria, Australia). The injector temperature was set at 250°C and the detector (flame ionization) temperature was set at 300°C . The initial oven temperature was 140°C and was programmed to rise to 220°C at $5^\circ\text{C}/\text{min}$. Helium was used as the carrier gas at a velocity of 35 cm/s. Fatty acid methyl esters were identified based on the retention time to authentic lipid standards obtained from NuChek Prep Inc (Elysian, MN) (12). All organic solvents contained butylated hydroxy anisole (0.005%) as an antioxidant.

Cyclooxygenase activity

Blood was processed to distinguish eicosanoids produced by COX-1 and COX-2 according to the method of Patrignani et al (13) as follows.

COX-1 activity. After subjects fasted overnight, a 10-mL venous blood sample was obtained, of which 5 mL was allowed to clot at 37°C for 60 min. Cell-free serum was separated by centrifugation and stored at 0°C until analyzed for thromboxane B_2 (TXB_2), a measure of constitutive COX-1 activity in human whole blood (13).

COX-2 activity. The remainder of the blood sample was added to a lithium heparin-containing tube to which freshly prepared aspirin (AsproClear, Roche, Australia) (10 mg/L) was added immediately (at time 0) to inhibit constitutive COX-1 activity. Lipopolysaccharide (serotype 0111:B4; Sigma Chemical Co, St Louis) was then added (final concentration: 0.2 mg/L) followed by incubation for 24 h at 37°C . Cell-free plasma was then separated by centrifugation (10 min at $800 \times g$ at 4°C) and stored at

0°C until analyzed for interleukin 1β (IL- 1β), tumor necrosis factor α (TNF- α), prostaglandin E_2 (PGE_2), and TXB_2 . Because aspirin is rapidly hydrolyzed in whole blood ($t_{1/2} = 39$ min), the activity of COX-2, which is induced by lipopolysaccharide stimulation after 3–4 h, is not affected (13).

Immunoassays

TNF- α and IL- 1β were measured by enzyme-linked immunosorbent assay (ELISA) by using commercially developed matched-pair antibodies and recombinant TNF- α and IL- 1β standards (Endogen, Woburn, MA). The range of detection was 0.07–5 $\mu\text{g}/\text{L}$ for both TNF- α and IL- 1β . PGE_2 and TXB_2 were measured in triplicate by radioimmunoassay with use of [^3H]PGE₂ and [^3H]TXB₂ (Amersham Australia Pty Ltd, Sydney, Australia), PGE₂ and TXB₂ (Cayman Chemical Co, Ann Arbor, MI), and rabbit anti-PGE₂ (Sigma Chemical Co); rabbit anti-TXB₂ was prepared by inoculating rabbits with TXB₂-thyroglobulin conjugates (14). Cross-reactivities for the PGE₂ anti-serum were <0.001% for TXB₂, 4.6% for 6-keto PGF_{1 α} , and 3.8% for PGF_{2 α} . Cross-reactivities for the TXB₂ anti-serum were 0.06% for PGE₂, 0.05% for 6-keto PGF_{1 α} , and <0.05% for PGF_{2 α} .

Statistics

The data were analyzed with repeated-measures analysis of variance to determine any differences between visits. Where there was a significant difference ($P < 0.05$), the analysis of variance was followed by Tukey's honestly significantly different multiple comparisons analysis to identify differences. WINKS 4.5 (1997; TexaSoft, CedarHill, TX) was used for the analyses. Data are given as means \pm SDs.

RESULTS

Subjects and compliance

Subjects' weights did not change significantly during the period of the trial and there were no withdrawals from the study. Behavioral responses to the dietary advice were monitored from the dietary records, which were maintained during the 4-wk period and at the dietetic interviews at 0, 2, and 4 wk. All subjects appeared to make the appropriate substitutions in their foods in line with the dietary advice.

Dietary intakes

Dietary intakes were estimated by analyzing the diet diaries (Tables 2 and 3). The mean energy intake throughout the study was 10.3 ± 1.9 MJ/d. The mean percentage of energy derived from protein was $18.4 \pm 2.6\%$, that from fat was $35.3 \pm 4.6\%$, that from carbohydrate was $42.5 \pm 6.7\%$, and that from alcohol was $4.2 \pm 6.2\%$ (Table 2). The mean intake of LA was 9.2 ± 2.5 g/d and the mean intake of ALA was 9.2 ± 4.2 g/d (Table 3). The mean intake of EPA was 0.8 ± 0.4 g/d and the mean intake of DHA was 1.03 ± 0.6 g/d. The combined mean total intake of EPA and DHA from all dietary sources was 1.83 g/d.

Fatty acids

Fatty acids in the plasma phospholipid fraction, mononuclear cell phospholipids and platelet phospholipids (Tables 4–6, respectively) of the samples obtained at the onset of the trial (0 wk) and 2 and 4 wk after consumption of the n-3 enriched foodstuffs were analyzed.

TABLE 2

Estimates of the subjects' daily intakes of energy, protein, fat, carbohydrate, and alcohol¹

	Value
Energy (MJ/d)	10.3 ± 1.9 (7.6–15.0)
Protein	
(g/d)	109.7 ± 15.1 (85.6–156.4)
(% of energy)	18.4 ± 2.6 (15.0–24.3)
Fat	
(g/d)	98.4 ± 23.9 (62.9–149.4)
(% of energy)	35.3 ± 4.6 (26.1–46.6)
Carbohydrate	
(g/d)	275 ± 76.5 (172.0–426.0)
(% of energy)	42.5 ± 6.7 (26.4–53.8)
Alcohol	
(g/d)	14.7 ± 21.8 (0.0–92.1)
(% of energy)	4.2 ± 6.19 (0–24.8)

¹ $\bar{x} \pm \text{SD}$; range in parentheses.

TABLE 3Estimates of the subjects' daily intakes of selected n-9, n-6, and n-3 fatty acids¹

	Value
18:1n-9	
(g/d)	31.7 ± 8.5 (15.9–51.8)
(% of energy)	11.4 ± 2.1 (7.2–17.3)
18:2n-6	
(g/d)	9.2 ± 2.5 (6.0–15.5)
(% of energy)	3.3 ± 0.6 (2.3–5.2)
18:3n-3	
(g/d)	9.2 ± 4.2 (2.9–18.4)
(% of energy)	3.3 ± 1.3 (1.3–5.5)
20:5n-3	
(g/d)	0.8 ± 0.4 (0.16–1.82)
(% of energy)	0.29 ± 0.13 (0.07–0.65)
22:6n-3	
(g/d)	1.03 ± 0.61 (0.15–2.95)
(% of energy)	0.37 ± 0.20 (0.07–0.96)

¹ $\bar{x} \pm SD$; range in parentheses. OA, oleic acid; LA, linoleic acid; ALA, α -linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Cellular and plasma n-3 fatty acids

α -Linolenic acid. After consumption of the n-3-enriched diet for 2 wk, there was a significant increase (≈ 3 –4-fold) in ALA concentrations in all of the fractions analyzed (except for the mononuclear cells, in which the increase was ≈ 5 -fold; NS). After another 2 wk of the diet, there was little further change in ALA concentrations.

Eicosapentaenoic acid. After consumption of the n-3-enriched diet for 2 wk, there was a significant increase (≈ 2.5 -fold) in EPA concentrations in all of the fractions analyzed. After another 2 wk of the diet, there were further increases in EPA (≈ 3 -fold increase from baseline), but the difference from values at 2 wk was not significant.

Docosapentaenoic acid. There was a significant increase (30%) in docosapentaenoic acid (DPA; 22:n-3) concentrations in all of the fractions analyzed after 2 wk of consumption of the n-3-enriched diet, with no further increase observed after 4 wk.

Docosahexaenoic acid. After consumption of the n-3-enriched diet for 2 wk, there was a significant increase (≈ 1.5 -fold) in DHA concentrations in all of the fractions analyzed. DHA concentrations did not increase further in the following 2 wk.

Cellular and plasma n-6 fatty acids

Linolenic acid. After 2 wk of the prescribed dietary regimen, there were significant decreases in LA concentrations in the plasma phospholipid fraction, which were sustained at 4 wk. LA concentrations decreased in the mononuclear cell fraction only at the end of the 4-wk period. A similar proportionate decrease ($\approx 8\%$) in platelet LA concentrations was seen at 2 and 4 wk, but it was not significant.

Arachidonic acid. Significant decreases ($\approx 7\%$) in arachidonic acid (AA; 20:4n-6) concentrations were observed in the plasma phospholipid and mononuclear cell phospholipid fractions. There were no significant changes in AA concentrations in the platelet phospholipid fraction.

Cellular and plasma monounsaturated fatty acids

Oleic acid. There was a small but significant increase in OA concentrations and in the total amount of monounsaturated fatty

acids at the 2-wk time point in the mononuclear cell phospholipid and platelet phospholipid fractions. Concentrations of OA and total monounsaturated fatty acids did not change significantly between 2 and 4 wk.

Cellular and plasma saturated fatty acids

Total saturated fatty acid concentrations changed significantly after the test diet only in plasma and platelet phospholipids, in which concentrations decreased at the 2-wk time point. The decrease was maintained at 4 wk in the platelet but not in the plasma phospholipid fraction.

Target values

The notional target value that was set for EPA in the mononuclear cells was 1.5% of total fatty acids. At the 2-wk analysis, 5 of the 15 subjects had attained this level in the mononuclear cell phospholipids. The remaining 10 subjects were contacted by telephone within 2 d of this assessment and counseled on ways to further increase their intake of n-3 fatty acids from the foods that were provided for the remaining 2-wk period. At 4 wk, only 1 of these 10 subjects had achieved the target value. One of the 5 subjects who achieved the target value at 2 wk failed to do so at the 4-wk assessment.

Eicosanoid and cytokine synthesis

As described above, serum TXB₂ concentrations reflect COX-1 activity, whereas eicosanoids in lipopolysaccharide-stimulated, aspirin-pretreated whole blood reflect COX-2 activity (13). There was no significant change in serum TXB₂ synthesis during the study (0 wk: 362 ± 208 nmol/L; 2 wk: 300 ± 178 nmol/L; and 4 wk: 297 ± 126 nmol/L). In lipopolysaccharide-stimulated, aspirin-pretreated whole blood, the synthesis of TXB₂ and PGE₂ decreased significantly from baseline by 36% and 26%, respectively, after consumption of the diet for 2 wk (**Table 7**). There was no further change in eicosanoid concentrations between 2 and 4 wk.

The synthesis of IL-1 β decreased significantly by 20% after the n-3-enriched diet was consumed for 2 wk and decreased further by 4 wk, but not significantly so (**Table 7**). The synthesis

TABLE 4Fatty acids in plasma phospholipids at 0 (baseline), 2, and 4 wk of the diet¹

Fatty acid	Duration of diet		
	0 wk	2 wk	4 wk
	% of total fatty acids		
Total saturated	44.51 ± 0.89 ^a	43.59 ± 1.10 ^b	44.60 ± 0.90 ^a
18:1n-9	10.04 ± 1.44 ^a	9.62 ± 0.98 ^a	9.70 ± 1.16 ^a
Total monounsaturated	13.72 ± 1.66 ^a	13.83 ± 1.13 ^a	13.52 ± 1.16 ^a
n-6			
18:2	21.00 ± 4.12 ^a	18.00 ± 3.02 ^b	18.03 ± 2.36 ^b
20:4	9.54 ± 2.05 ^a	9.37 ± 1.67 ^{a,b}	8.88 ± 1.76 ^b
Total	35.30 ± 1.93 ^a	31.29 ± 1.63 ^b	30.54 ± 1.50 ^b
n-3			
18:3	0.19 ± 0.07 ^a	0.68 ± 0.35 ^b	0.55 ± 0.23 ^b
20:5	0.98 ± 0.39 ^a	2.55 ± 1.08 ^b	2.98 ± 1.08 ^b
22:5	1.14 ± 0.29 ^a	1.47 ± 0.29 ^b	1.37 ± 0.24 ^b
22:6	3.16 ± 0.90 ^a	5.54 ± 1.13 ^b	5.44 ± 1.14 ^b
Total	5.55 ± 1.14 ^a	10.24 ± 2.19 ^b	10.37 ± 2.01 ^b

¹ $\bar{x} \pm SD$. Values with different superscript letters are significantly different, $P < 0.05$.

TABLE 5Fatty acids in mononuclear cell phospholipids at 0 (baseline), 2, and 4 wk of the diet¹

Fatty acid	Duration of diet		
	0 wk	2 wk	4 wk
	% of total fatty acids		
Total saturated	43.46 ± 0.74 ^a	43.72 ± 0.85 ^a	43.49 ± 0.64 ^a
18:1n-9	11.76 ± 0.95 ^a	12.43 ± 0.74 ^b	12.98 ± 0.82 ^c
Total monounsaturated	15.53 ± 1.21 ^a	16.41 ± 0.78 ^b	16.36 ± 1.22 ^b
n-6			
18:2	6.40 ± 1.66 ^a	6.24 ± 0.80 ^{ab}	5.93 ± 0.94 ^b
20:4	22.04 ± 1.09 ^a	20.50 ± 1.24 ^b	20.79 ± 1.40 ^b
Total	34.23 ± 0.87 ^a	31.31 ± 1.08 ^b	31.01 ± 1.29 ^b
n-3			
18:3	0.02 ± 0.08 ^a	0.10 ± 0.11 ^a	0.10 ± 0.11 ^a
20:5	0.40 ± 0.10 ^a	0.93 ± 0.32 ^b	1.11 ± 0.44 ^b
22:5	2.79 ± 0.28 ^a	3.02 ± 0.37 ^b	3.06 ± 0.43 ^b
22:6	2.36 ± 0.48 ^a	3.21 ± 0.52 ^b	3.33 ± 0.62 ^b
Total	5.59 ± 0.59 ^a	7.26 ± 0.79 ^b	7.67 ± 1.05 ^b

¹ $\bar{x} \pm SD$. Values with different superscript letters are significantly different, $P < 0.05$.

of TNF- α decreased significantly by 40% after 2 wk of the diet. However, after 4 wk of the diet, TNF- α synthesis was not significantly different from that at 2 wk or at baseline.

Mononuclear cell fatty acid, eicosanoid, and cytokine concentrations

Mononuclear cell EPA concentrations were inversely associated with the lipopolysaccharide-stimulated, aspirin-pretreated whole-blood synthesis of TXB₂, PGE₂, and IL-1 β . The correlation coefficients (r) were -0.48 ($P < 0.05$), -0.33 ($P < 0.05$), and -0.48 ($P < 0.05$), respectively. No relation was observed between TNF- α synthesis and mononuclear cell EPA concentrations.

DISCUSSION

The major aim of this dietary study was to determine the feasibility of using novel foods enriched in n-3 fatty acids (ALA, EPA, and DHA) to increase tissue n-3 fatty acids to concentrations known to be associated with a low production of proinflammatory cytokines and eicosanoids. Generally, fish oil and, to a lesser extent, fish were used as the source of n-3 fatty acids in clinical and biochemical studies in which reduced eicosanoid and cytokine production were observed (1, 15-17). To explore strategies designed to increase n-3 fatty acid intakes that would be suited more realistically to long-term daily use throughout a community, healthy volunteers were provided with a variety of foods naturally rich in or enriched in ALA, EPA, and DHA.

To be practical for long-term daily use, it was essential that the selected foods be core items in the typical Australian diet, ie, foods that are consumed regularly and not intermittently. Most importantly, the food items had to withstand enrichment with n-3 fatty acids (either flaxseed oil or fish oil) without effects on palatability or the extent of their use in food preparation. In addition, the foods needed to have broad appeal across age groups so that they would be consumed by everyone within a household; overall use would have been less than optimal if replacement foods needed to be prepared for other family members. With these factors in mind, savory foods were chosen because they

were more likely to form the core items of the diet and were more likely to accommodate the addition of the n-3-enriched oils without taste being compromised.

Lovegrove et al (18) used a similar dietary approach to increase dietary EPA and DHA, but they chose mainly sweet foods to supplement with fish oil, including bread, biscuits, cake, ice cream, orange drink, milkshakes, low-fat spreads, pasta, mayonnaise, vinaigrette, and milk shake powder. Although the subjects in that study consumed an average of 1.4 g EPA and DHA daily, there was a mean increase in body weight of 1.2 kg over the 3-wk study period. By contrast, in our dietary study there was no significant change in weight over the 4-wk study period. In the study by Lovegrove et al (18), many of the supplemental foods used were not core food items. In the present study, the supplemental foods used were savory and were used by subjects in place of their usual foods. This is an important consideration over the long term because weight gain would be an undesirable and unnecessary side effect of consuming n-3-enriched foods.

This 4-wk study showed a 3-fold increase in EPA concentrations and 1.5-2.0-fold increases in DHA concentrations in the tissue phospholipids that were examined. ALA, the precursor to EPA, rose by 3-4-fold during this period. These tissue n-3 PUFA concentrations were attained by the 2-wk midpoint analysis, thereby indicating the relatively short time required for n-3 PUFA concentrations to rise. The increases were achieved with an average ALA intake of 9.0 g/d, an average EPA intake of 0.80 g/d, and an average DHA intake of 1.03 g/d over the 4-wk period. The ALA intake achieved was 9-fold higher than the usual intake in the community (6). The LA intake averaged ≈ 9.0 g/d over the 4-wk period, which is $\approx 50\%$ lower than the intakes of the general community (6). A reduction in the intake of dietary LA is important because high LA intakes have been shown to reduce tissue concentrations of EPA and DHA resulting from dietary sources (19).

The total average intake of EPA and DHA was 1.83 g/d, which is equivalent to the amount provided by taking 6 menhaden fish-oil capsules/d. The equivalent number of fish meals needed to achieve these intakes is more difficult to compute because of the different content of long-chain n-3 fatty acids in different fish species. In comparison, the average EPA and DHA intakes in the present study equated to amounts that could be achieved from

TABLE 6Fatty acids in platelet phospholipids at 0 (baseline), 2, and 4 wk of the diet¹

Fatty acid	Duration of diet		
	0 wk	2 wk	4 wk
Total saturated	43.48 ± 0.54 ^a	42.60 ± 0.89 ^b	42.10 ± 1.01 ^b
18:1n-9	13.17 ± 0.57 ^a	14.00 ± 0.74 ^b	14.30 ± 0.78 ^b
Total monounsaturated	16.59 ± 0.85 ^a	17.50 ± 0.83 ^b	17.92 ± 1.10 ^b
n-6			
18:2	7.40 ± 1.76 ^a	6.71 ± 1.18 ^a	6.82 ± 1.45 ^a
20:4	22.17 ± 1.24 ^a	21.54 ± 1.37 ^a	21.27 ± 2.21 ^a
Total	34.53 ± 1.14 ^a	32.34 ± 1.00 ^b	31.95 ± 1.61 ^b
n-3			
18:3	0.08 ± 0.02 ^a	0.22 ± 0.09 ^b	0.20 ± 0.08 ^b
20:5	0.50 ± 0.12 ^a	1.19 ± 0.37 ^b	1.46 ± 0.46 ^b
22:5	1.88 ± 0.26 ^a	2.14 ± 0.33 ^b	2.27 ± 0.34 ^b
22:6	1.89 ± 0.40 ^a	2.86 ± 0.62 ^b	3.06 ± 0.61 ^b
Total	4.42 ± 0.47 ^a	6.47 ± 1.00 ^b	7.05 ± 1.06 ^b

¹ $\bar{x} \pm SD$. Values with different superscript letters are significantly different, $P < 0.05$.

TABLE 7

Eicosanoid [thromboxane B₂ (TXB₂) and prostaglandin E₂ (PGE₂)] and cytokine [interleukin 1 β (IL-1 β) and tumor necrosis factor α (TNF- α)] synthesis from bacterial lipopolysaccharide-treated, aspirin-pretreated whole blood at 0 (baseline), 2, and 4 wk of the diet¹

	Duration of diet		
	0 wk	2 wk	4 wk
TXB ₂ (nmol/L)	34.9 \pm 17.6 ^a	22.4 \pm 17.5 ^b	23.6 \pm 17.4 ^b
PGE ₂ (nmol/L)	15.3 \pm 11.6 ^a	11.3 \pm 11.1 ^{a,b}	10.4 \pm 8.6 ^b
IL-1 β (μ g/L)	40.93 \pm 12.17 ^a	32.97 \pm 9.18 ^b	32.31 \pm 7.63 ^b
TNF- α (pmol/L)	680 \pm 309 ^a	411 \pm 260 ^b	578 \pm 286 ^{a,b}

¹ $\bar{x} \pm$ SD. Values with different superscript letters are significantly different, $P < 0.05$.


the consumption of \approx 70 g canned pink salmon/d or 150 g fresh mullet/d. Daily fish intake at these levels is unlikely to be achievable in the long term because it would limit dietary variety. In Australia, the intake of fish did not increase in a recent 12-y period during which the health benefits of eating fish were promoted increasingly. The National Dietary Survey in 1983 reported average fish and seafood intakes of 21 g/d in South Australian males aged >19 y (20). The National Nutrition Survey, conducted in 1995, reported fish and seafood intakes of 23 g/d in the same reference group (21).

The increase in tissue phospholipid n-3 fatty acid concentrations was coincident with modest suppression of the cytokine IL-1 β and of the eicosanoids TXB₂ and PGE₂. Suppression of these inflammatory mediators was observed 2 wk after the n-3-enriched diet began and was maintained for the following 2 wk of the diet. Although TNF- α synthesis decreased significantly after 2 wk of the diet, values at 4 wk were not significantly different from those at baseline. The data indicate that TNF- α was not suppressed by the n-3-enriched diet as were the other inflammatory mediators. The mononuclear cell EPA concentrations correlated inversely with the synthesis of TXB₂, PGE₂, and IL-1 β , but not with the synthesis of TNF- α . These correlations were somewhat weaker than those observed in a previous study in which fish-oil capsules were given as a supplement (22).

The present findings suggest that there may be differences in the suppressive effects of n-3 fatty acids on COX-1- and COX-2-mediated eicosanoid synthesis. Although the COX-2-mediated synthesis of TXB₂ and PGE₂ was significantly reduced after consumption of the n-3-enriched diet, there was no significant reduction in COX-1-mediated TXB₂ synthesis. This may indicate that COX-2 activity is more sensitive to elevated n-3 concentrations than is COX-1 activity. However, because the comparison involved different cell types (platelets compared with monocytes) and different methods of stimulation (clotting compared with lipopolysaccharide stimulation), the differences observed may be unrelated to different sensitivities of the COX isotypes to n-3 fatty acids.

In the study by Lovegrove et al (18), target dietary intakes of n-3 fatty acids were used. In the present study, the concept of a target tissue concentration of EPA rather than a target dietary intake was introduced. We selected a provisional target value for EPA of 1.5% of total fatty acids in the mononuclear cells because this was the value at which we observed maximum suppression of cytokine synthesis in an earlier study (22). We acknowledge that there are insufficient published data to relate this target level to clinical benefits in rheumatoid arthritis. Nevertheless, the aim of

future studies should be to establish target values of tissue n-3 fatty acids that might be therapeutic for or considered preventive against cardiovascular disease and rheumatoid arthritis. This could be more appropriate than recommending a set dietary intake of n-3 PUFAs, which becomes problematic when n-3 PUFAs are delivered through many different foods. Additionally, target tissue EPA values could be met via the consumption of ALA as well as EPA. Thus, by setting a target EPA value, a wide variety of dietary strategies can be used to achieve a common objective.

Overall, there is substantial evidence of the health benefits of n-3 fatty acids (1, 2). However, practical clinical use of the evidence will require consumption of a variety of n-3 fatty acid-enriched foods that are easily incorporated into daily diets. Additionally, the development of target tissue n-3 fatty acid concentrations will provide a single outcome measure that accommodates a variety of dietary approaches to achieve such an outcome. 

REFERENCES

- James MJ, Cleland LG. Dietary n-3 fatty acids and therapy for rheumatoid arthritis. *Semin Arthritis Rheum* 1997;27:85-97
- Simopoulos AP. ω -3 fatty acids in the prevention-management of cardio-vascular disease. *Can J Physiol Pharmacol* 1997;75:234-9.
- Scientific Review Committee. Nutrition recommendations. Ottawa: Minister of National Health and Welfare, 1990. (H49-42/1990E.)
- The British Nutrition Foundation. Unsaturated fatty acids—nutritional and physiological significance: the report of the British Nutrition Foundation's Task Force. London: Chapman and Hall, 1992.
- National Health and Medical Research Council. Report of the NHMRC working party: the role of polyunsaturated fats in the Australian diet. Canberra, Australia: Australian Government Publishing Service, 1992.
- Mantzioris E, James MJ, Gibson RA, Cleland LG. Dietary substitution with an α -linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. *Am J Clin Nutr* 1994;59:1304-9.
- Sanders TAB, Younger KM. The effect of dietary supplements of ω 3 polyunsaturated fatty acids on the fatty acid composition of platelets and plasma choline phosphoglycerides. *Br J Nutr* 1981; 45:613-6.
- Brown AJ, Roberts DCK, Pritchard JE, Truswell AS. A mixed Australian fish diet and fish-oil supplementation: impact on the plasma lipid profile of healthy men. *Am J Clin Nutr* 1990;52:825-33.
- Cobiac L, Clifton PM, Abbey M, Belling GB, Nestel PJ. Lipid, lipoprotein, and hemostatic effects of fish vs fish-oil n-3 fatty acids in mildly hyperlipidemic males. *Am J Clin Nutr* 1991;53: 1210-6.
- Goodwin JS. Mechanisms of action of non-steroidal anti-inflammatory agents. *Am J Med* 1984;77:57-64.
- Folch J, Lees M, Sloane-Stanley JH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;226:497-509.
- Gibson RA, Neumann MA, James MJ, Hawkes JS, Hall C, Cleland LG. Effect of n-3 and n-6 dietary fats on the lipoxigenase products from stimulated rat neutrophils. *Prostaglandins Leukot Essent Fatty Acids* 1992;46:87-91.
- Patrignani P, Panara MR, Greco A, et al. Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. *J Pharmacol Exp Ther* 1994;271:1705-12.
- James MJ, Walsh JA. Inter-relationships between vascular thromboxane and prostacyclin synthesis. *Prostaglandins Leukot Essent Fatty Acids* 1988;31:91-6.

15. Kremer JM, Lawrence DA, Jubiz W, et al. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. *Arthritis Rheum* 1990;33:810–20.
16. Endres S, Ghorbani R, Kelley VE, et al. The effect of dietary supplementation with n–3 polyunsaturated fatty acids on the synthesis of interleukin 1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 1989;320:265–71.
17. Meydani SN, Lichtenstein AH, Cornwall S, et al. Immunologic effects of National Cholesterol Education Panel Step-2 diets with and without fish-derived n–3 fatty acid enrichment. *J Clin Invest* 1993;92:105–13.
18. Lovegrove JA, Brooks CN, Murphy MC, Gould BJ, Williams CM. Use of manufactured foods enriched with fish oils as a means of increasing long-chain n–3 polyunsaturated fatty acid intake. *Br J Nutr* 1997;78:223–36.
19. Cleland LG, James MJ, Neumann MA, D'Angelo M, Gibson RA. Linoleate inhibits EPA incorporation from dietary fish-oil supplements in human subjects. *Am J Clin Nutr* 1992;55:395–9.
20. Commonwealth Department of Community Services and Health. National dietary survey of adults: 1983 no.1 foods consumed. Canberra, Australia: Australian Government Publishing Service, 1983.
21. Australian Bureau of Statistics and Department of Health and Family Services. National Nutrition Survey, selected highlights. Canberra, Australia: Australian Government Publishing Service, 1997.
22. Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ. The effect on human tumor necrosis factor α and interleukin 1 β production of diets enriched in n–3 fatty acids from vegetable oil or fish oil. *Am J Clin Nutr* 1996;63:116–22.