

Reduction of Cardiovascular Risk Factors with Longterm Fish Oil Treatment in Early Rheumatoid Arthritis

LESLIE G. CLELAND, GILLIAN E. CAUGHEY, MICHAEL J. JAMES, and SUSANNA M. PROUDMAN

ABSTRACT. *Objective.* Rheumatoid arthritis (RA) is associated with increased risk for cardiovascular (CV) events through multiple factors. Fish oil has been shown to reduce symptoms in RA and to reduce CV risk. We assessed the effect of an antiinflammatory dose of fish oil on CV risk factors within a program of combination chemotherapy for patients with early RA.

Methods. Patients who chose not to take fish oil ($n = 13$) were compared with patients who achieved a sustained elevation of eicosapentaenoic acid (EPA) in plasma phospholipid fatty acids ($> 5\%$ total fatty acids) while taking fish oil over a 3-year period ($n = 18$). We examined cellular content of arachidonic acid (AA), synthesis of thromboxane A_2 and prostaglandin E_2 , use of nonsteroidal antiinflammatory drugs (NSAID), traditional CV lipid risk factors, and disease activity at 3 years.

Results. At 3 years, AA (as a proportion of AA plus long-chain n-3 fatty acids that can compete with AA for cyclooxygenase metabolism) was 30% lower in platelets and 40% lower in peripheral blood mononuclear cells in subjects taking fish oil. Serum thromboxane B_2 was 35% lower and lipopolysaccharide-stimulated whole-blood prostaglandin E_2 was 41% lower with fish oil ingestion compared to no fish oil. NSAID use was reduced by 75% from baseline with fish oil ($p < 0.05$) and by 37% without fish oil (NS). Favorable changes in fasting blood lipids were seen with, but not without fish oil. Remission at 3 years was more frequent with fish oil use (72%) compared to no fish oil (31%).

Conclusion. Fish oil reduces cardiovascular risk in patients with RA through multiple mechanisms. (First Release Aug 1 2006; J Rheumatol 2006;33:1973-9)

Key Indexing Terms:

FISH OIL EARLY RHEUMATOID ARTHRITIS COMBINATION THERAPY
CARDIOVASCULAR RISK REMISSION CYCLOOXYGENASE INHIBITION

The symptomatic benefit of fish oil in rheumatoid arthritis (RA) is well established¹⁻⁴. This symptomatic response can be explained by the inhibitory effect of fish oil upon synthesis by the cyclooxygenase (COX) pathway of nociceptive eicosanoid prostaglandin E_2 (PGE₂)^{5,6}, which mediates pain experienced when inflamed joints are subjected to stretch and pressure during everyday activities. A similar biochemical and symptomatic effect is achieved with nonsteroidal antiinflammatory drugs (NSAID), which, like the long-chain n-3 fatty acids (also known as omega-3 fats) eicosapentaenoic acid (EPA; C20:5n-3), docosapentaenoic acid (DPA; C22:5n-3), and docosahexaenoic acid (DHA; C22:6n-6) found in fish oil, inhibit metabolism of arachidonic acid (AA; 20:4n-6) by

COX^{7,8}. NSAID have a clear advantage in short-term efficacy compared to fish oil, with which symptomatic benefit can be delayed up to 3 months. However, this delay is acceptable in longterm therapy, where fish oil may allow NSAID to be withdrawn or reduced^{2,9-11}.

Unlike NSAID, fish oil has not been associated with serious upper gastrointestinal complications and has been shown to reduce cardiovascular (CV) risk through multiple mechanisms¹². The CV benefits of fish oil have achieved greater import following the observations of increased risk for serious CV events, first seen with COX-1-sparing NSAID but now considered to be, to some degree, an unwanted effect of NSAID generally^{13,14}. This propensity to CV risk with NSAID is especially problematic in patients with RA, where disease activity appears to increase CV risk^{15,16}. However, published studies of fish oil in RA have been relatively short-term, most studies being 3 to 6 months in duration and only 2 studies extending to 12 months¹⁷. We examined biochemical markers relevant to inflammatory symptoms and CV risk in patients with RA following advice to take an antiinflammatory dose of fish oil over a 3-year observation period. The investigation took place within the context of a contemporary program of early introduction of combination chemotherapy for recent onset RA.

From the Rheumatology Unit, Royal Adelaide Hospital, Adelaide, Australia.

Supported by the National Health and Medical Research Council of Australia. Berg LipidTech, Aalesund, Norway, provided a proportion of the fish oil used in the study.

L.G. Cleland, MD, FRACP, Director of Rheumatology; G.E. Caughey, PhD, Research Officer; M.J. James, PhD, Chief Medical Scientist; S.M. Proudman, MB BS, FRACP, Senior Visiting Rheumatologist.

Address reprint requests to Prof. L.G. Cleland, Rheumatology Unit, Royal Adelaide Hospital, North Terrace, Adelaide, Australia 5000. E-mail: lcleland@mail.rah.sa.gov.au

Accepted for publication April 11, 2006.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2006. All rights reserved.

MATERIALS AND METHODS

Patients attending the Royal Adelaide Hospital Early Arthritis Clinic were recruited to the study with their written informed consent. All subjects were over 18 years old, fulfilled the American College of Rheumatology (ACR) criteria for RA¹⁸, and had symptoms for less than 12 months at baseline. The treatment was open-label and undertaken according to predefined rules that specified the agents to be used, their order of introduction, and dosage adjustment, withdrawal and substitution according to measures of disease activity and unwanted effects. Details of this program will be published separately (Proudman SM, *et al* unpublished observations). Briefly, patients were treated at the outset with the combination of methotrexate (MTX), hydroxychloroquine, and sulfasalazine (unless sulfonamide-intolerant) and given advice to take bottled fish oil daily on juice in order to provide 4 to 4.5 g EPA plus DHA daily. The fish oil was from Berg LipidTech (Aalesund, Norway) and was obtained either directly or through Melrose Laboratories (Mitcham, Victoria, Australia). Patients unwilling or unable to take bottled fish oil were advised to take the equivalent dose of fish oil as capsules (7 × 1 g capsules twice daily). When disease suppression criteria were not fulfilled, doses of sulfasalazine then MTX were increased, followed by sequential addition of leflunomide and gold sodium thiomalate. Oral glucocorticoids were not used, but intraarticular or intramuscular steroid injection was allowed if required for prompt control of symptoms. Paracetamol was recommended for first-line analgesia. An NSAID was allowed as second-line analgesia for symptomatic relief if required. All medications used were recorded in questionnaires and case files. Clinical review was 3 to 6-weekly initially and in the event of a disease flare. Patients with well controlled disease were assessed 3-monthly. Analyses are confined to patients who had completed at least 3 years of treatment.

The study was approved by the Royal Adelaide Hospital Research Ethics Committee.

Plasma and cell separation. NSAID were stopped at least 10 plasma half-lives before the whole-blood assay for eicosanoid synthesis. Paracetamol was not taken from the day prior to the assay. Peripheral venous blood samples (up to 40 ml total) were collected; 9 ml was added to Vacuette tubes (Greiner Bio-One, Canberra, Australia) containing EDTA and centrifuged at 320 g to obtain the plasma. The erythrocyte pellet was washed in normal saline. Another 20 ml of blood was added to tubes containing saline 4 ml with EDTA 4.5% (wt by vol) and 4 ml dextran (6% wt by vol) in saline. Erythrocytes were allowed to settle at 37°C for 30 min. The red cell-depleted blood was layered onto Lymphoprep (Nycomed Pharma, Oslo, Norway) and centrifuged at 110 g for 10 min to separate leukocytes from platelets. The platelet-rich supernatant was removed and treated with hypotonic saline to lyse contaminating erythrocytes. Platelets were then washed in normal saline. After further centrifugation at 200 g for 20 min, the peripheral blood mononuclear cell (PBMC) fraction was removed from the interface of the Lymphoprep gradient and washed in normal saline. Plasma and washed cell fractions were stored at -80°C until processing for fatty acid analysis.

Fatty acid analysis. Erythrocyte, platelet, and PBMC fractions were processed for fatty acid analysis as described⁶. Briefly, total lipids were extracted in chloroform-methanol. Phospholipids were isolated by thin-layer chromatography and transesterified by methanolysis. The fatty acid methyl esters were separated by gas liquid chromatography (Hewlett-Packard 5890, Hewlett-Packard, Palo Alto, CA, USA) and quantified using a flame ionization detector, with identification based on retention times of authentic standards. The erythrocyte omega-3 index of CV risk was computed as described¹⁹.

Whole-blood assay for eicosanoid synthesis. A whole-blood assay was used to measure synthesis of COX-1-derived thromboxane A₂ (TXA₂) and COX-2-derived prostaglandin E₂ (PGE₂) essentially as described⁶, with minor modifications. Briefly, 5 ml blood was collected into a plain tube and allowed to clot at 37°C for 60 min. Serum was separated by centrifugation and stored at -80°C until assay for TXB₂, the stable metabolite of TXA₂. Another 5 ml blood was collected into a Vacuette tube containing EDTA and incubated at 37°C with lipopolysaccharide (LPS) at a final concentration of 200 ng/ml for

24 h. Plasma was then taken and stored at -80°C until assay for PGE₂. Radioimmunoassay was used for TXB₂ and PGE₂ analyses⁶.

Clinical assessment. For each visit, patients were required to complete a disease activity questionnaire developed by Pincus, *et al*²⁰. Minor modifications were made to capture additional details of interest, including fish oil intake. Fields within the form include a modified Health Assessment Questionnaire (mHAQ), visual analog scores for pain, disease activity and fatigue, and details of medications and unwanted events. Joints were examined for tenderness and swelling using a proforma examination sheet. Choice of 28 joints for scoring tenderness and swelling and the formula for computing Disease Activity Score (DAS) were according to European League Against Rheumatism (EULAR) criteria. Plasma triglycerides and cholesterol fractions were determined on fasting samples using routine assay procedures at the Clinical Chemistry Laboratory, Institute of Medical and Veterinary Sciences, Adelaide.

Statistical analysis. Results are presented as mean ± SD, unless otherwise stated. Statistical analyses were performed using GraphPad InStat[®]. Fisher's exact test was used to analyze nonparametric data.

RESULTS

Defining groups for non-use and regular use of fish oil. Questionnaire and plasma fatty acid data were informative with regard to fish oil use. Of 73 patients fulfilling entry and duration of followup criteria, 13 patients either declined fish oil entirely or took a single dose and chose not to continue. Their plasma phospholipid fatty acids, which were assayed 3-monthly for 12 months then 6-monthly, did not change from baseline during the 3-year observation period (EPA as percentage total fatty acids: baseline 1.0 ± 0.4; 3-yr 0.9 ± 0.3; Table 1). Of the remainder, 18 patients were found to have plasma EPA > 5% of total plasma phospholipid fatty acids throughout the study period; they had recorded regular fish oil intake at the recommended dose. The mean EPA in this group increased from 1.2 ± 0.5% of phospholipid fatty acids at baseline to 8.2 ± 1.7% at 3 years (Table 1). For the purpose of the study, the 2 groups were regarded as being nonusers (No fish oil group) or fully compliant users (Fish oil group), respectively. Although the remaining 42 patients stated they took fish oil, in some this was intermittent and some chose to take fish oil capsules, generally at doses less than equivalent to the recommended dose of bottled fish oil. These patients with intermediate fish oil ingestion were not included in this investigation of vascular risk factor. The salient clinical characteristics, including disease activity and NSAID use, of the No fish oil group and the Fish oil group were similar at baseline (Table 2).

Cell membrane AA, competitor n-3 fatty acids, and eicosanoid synthesis. After 3 years of regular fish oil use, the n-6 fatty acid, AA, decreased whereas the n-3 fatty acids EPA, DPA, and DHA increased in platelet and PBMC phospholipids (data not shown). The three n-3 fatty acids compete with AA for metabolism to eicosanoids by COX. Thus, the relative availability of AA substrate for COX can be expressed as a percentage of the sum proportions of AA+EPA+DPA+DHA in the cellular phospholipids. In between-group comparisons at 3 years, this index of AA availability for eicosanoid synthesis

Table 1. Plasma phospholipid fatty acids in the No fish oil and the Fish oil groups. Data represent mean \pm SD.

	No Fish Oil		Fish Oil	
	Baseline, n = 13	3 Years, n = 13	Baseline, n = 15	3 Years, n = 18
Total saturates	43.7 \pm 1.0	43.4 \pm 0.7	43.4 \pm 1.4	44.2 \pm 0.9
Oleic acid, 18:1 n-9	9.9 \pm 1.4	10.5 \pm 1.9	10.4 \pm 1.6	8.2 \pm 0.61*#
Total monounsaturates	14.0 \pm 1.8	14.1 \pm 1.9	14.5 \pm 2.0	12.2 \pm 0.9*#
Linoleic acid, 18:2 n-6	18.8 \pm 2.0	19.1 \pm 2.8	18.8 \pm 2.8	13.6 \pm 2.8*#
Dihomo- γ -linolenic acid, 20:3 n-6	4.0 \pm 0.5	4.3 \pm 0.7	3.6 \pm 0.5	1.5 \pm 0.5*#
AA, 20:4 n-6	11.0 \pm 1.9	10.6 \pm 1.3	10.9 \pm 2.2	7.1 \pm 1.0*#
Total n-6	35.3 \pm 2.1	35.2 \pm 2.7	34.2 \pm 3.4	22.9 \pm 3.38*#
α -Linolenic acid, 18:3 n-3	0.16 \pm 0.1	0.19 \pm 0.12	0.2 \pm 0.09	0.18 \pm 0.1
EPA, 20:5 n-3	1.0 \pm 0.4	0.9 \pm 0.3	1.2 \pm 0.5	8.2 \pm 1.7*#
DPA, 22:5 n-3	1.1 \pm 0.3	1.14 \pm 0.2	1.1 \pm 0.3	1.8 \pm 0.41*#
DHA, 22:6 n-3	3.5 \pm 1.0	3.7 \pm 0.7	4.3 \pm 1.2	8.6 \pm 1.4*#
Total n-3	5.8 \pm 1.3	6.2 \pm 0.9	6.8 \pm 1.9	19.0 \pm 2.9*#

* Significantly different from baseline ($p < 0.001$, t test); # significantly different at 3 years by comparison with the No fish oil group ($p < 0.001$, t test).

Table 2. Baseline characteristics of patients in the No fish oil and the Fish oil groups.

	No Fish Oil, n = 13	Fish Oil, n = 18
Female, %	76	67
Age, yrs, mean \pm SD	51.1 \pm 15.9	61.8 \pm 9.9
Weeks of polyarthritis, mean (range)	17.2 (4–34)	12.9 (2–40)
Rheumatoid factor-positive, %	62	50
Anti-CCP antibody-positive, %	39	44
Shared epitope-positive, %	69	61
DAS28, mean \pm SD	5.7 \pm 0.9	5.0 \pm 1.5
Periarticular erosions, %	38.5	38.9
NSAID use, %	86	89

Anti-CCP: anti-cyclic citrullinated peptide; DAS: Disease Activity Score. No statistically significant differences were found between measures in either group.

was 30% lower in platelets and 40% lower in PBMC of the Fish oil group compared to the No fish oil group (Figure 1). There was a corresponding difference in serum TXB₂, which is produced through platelet COX-1, with the mean 35% lower in the Fish oil group ($p < 0.01$) (Figure 1). Similarly, PGE₂ produced via PBMC COX-2 in LPS-stimulated whole blood was 41% lower in the Fish oil group ($p < 0.01$; Figure 1).

NSAID use. NSAID use was determined from the questionnaires and patients were classified as NSAID users if they recorded taking an NSAID at any time during the third year of the study. NSAID use was lower at 3 years in the Fish oil group than in the No fish oil group (22% vs 54%; $p < 0.05$, between-group comparison). Discontinuation of NSAID in 75% of those taking NSAID at baseline in the Fish oil group was significant ($p < 0.01$, within-group comparison), whereas the discontinuation by 37% of NSAID users in the No fish oil group was not significant. A significant difference in use of other agents was not seen.

Lipid CV risk factors. Favorable differences in plasma triglycerides, HDL cholesterol, and total cholesterol/HDL ratio were seen in the Fish oil group at 3 years compared to baseline and compared to the No fish oil group (Table 3). LDL cholesterol did not change. All patients in the Fish oil group had an erythrocyte omega-3 index above the threshold for low risk¹⁹, whereas none achieved this level in the No fish oil group (Table 3).

Effect of fish oil on disease control. Because persisting disease activity is also a risk factor for CV disease, comparisons were made between the 2 groups. As expected with a cohort of patients receiving intensive combination therapy for early RA, significant improvements were seen in both individual and composite measures of disease activity in both groups (Table 4). Comparisons between groups at 3 years favored the fish oil-compliant group for mHAQ score, tender joint count, erythrocyte sedimentation rate, and DAS28 ($p < 0.05$), with the exceptions being swollen joint score and C-reactive protein, where substantial reductions from baseline were seen in both groups ($p < 0.01$). The proportion in remission at 3 years was greater in the fish oil-compliant group (72% vs 31%; $p < 0.05$).

DISCUSSION

Defining groups with regard to nonuse and regular use of antiinflammatory doses of fish oil. Previous randomized controlled trials established the symptomatic benefit of fish oil in RA¹⁷. Increases in n-3 fatty acids in plasma lipids have been examined in some studies^{21,22}. Cellular studies have shown reduced synthesis of leukotriene B₄ by neutrophils from patients randomized to take fish oil^{1,21,23}. Our study differs in design from the previous in being a cohort study, with groups defined by compliance with the dietary supplementation advice. Thus, it has similarities with a per-protocol analysis. The groups so defined by both questionnaire responses and

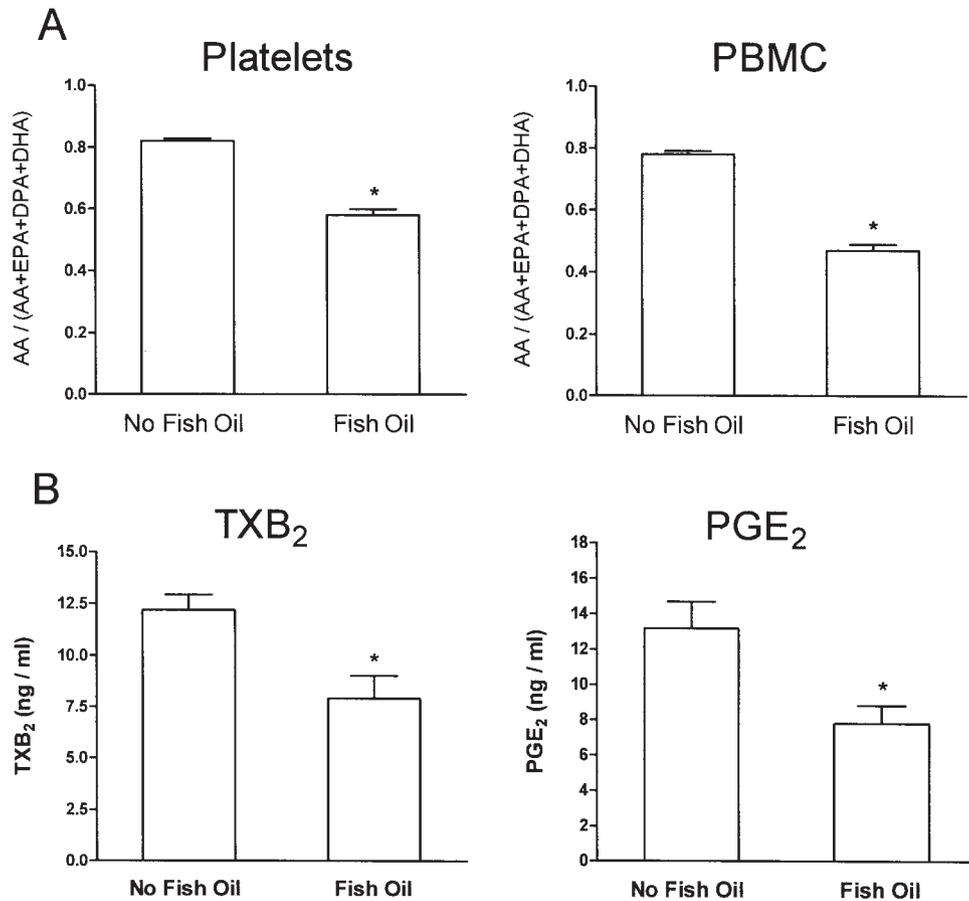


Figure 1. A. Arachidonic acid (AA) as a proportion of AA plus competitor n-3 fatty acids (AA+EPA+DPA+DHA) (mean \pm SEM) at 3 years in platelets and peripheral blood mononuclear cells (PBMC). B. Eicosanoid formation in whole-blood assay at 3 years (mean \pm SEM). TXB₂ measured in serum (formed through platelet COX-1). PGE₂ measured in supernatants after 24 h incubation of anticoagulated blood with LPS (formed through COX-2). *p < 0.01, unpaired t test.

Table 3. Lipid cardiovascular risk factors in plasma and erythrocytes from fasting blood samples. RBC Omega-3 Index (EPA+DHA) is an independent index that correlates inversely with CV risk¹⁹. Data represent mean \pm SD.

	No Fish Oil		Fish Oil	
	Baseline, n = 11	3 Years, n = 13	Baseline, n = 10	3 Years, n = 18
Total triglycerides	1.4 \pm 0.7	1.4 \pm 0.5	1.6 \pm 0.5	0.6 \pm 0.2*#
Total cholesterol	5.4 \pm 0.6	5.3 \pm 0.9	5.6 \pm 1.5	5.3 \pm 0.8
HDL	1.2 \pm 0.5	1.4 \pm 0.4	1.6 \pm 0.5	1.9 \pm 0.4*#
LDL	3.6 \pm 0.7	3.3 \pm 1.0	3.4 \pm 1.2	3.1 \pm 0.9
Total cholesterol/HDL	4.5 \pm 2.4	3.5 \pm 1.5	3.6 \pm 0.8	3.0 \pm 0.8*#
RBC omega-3 index (EPA+DHA)	—	5.06 \pm 0.8	—	13.8 \pm 1.8#

* Significantly different from baseline (p < 0.01, paired t test); # significantly different at 3 years compared with the No fish oil group (p < 0.05, t test).

blood fatty acid analyses provide a sound basis for examining the biochemical effects of antiinflammatory doses of fish oil taken within the context of structured chemotherapy. The group of compliant users constituted the upper quartile for

change in EPA in plasma phospholipids. It is acknowledged that this measure may be influenced by individual differences in efficiency of incorporation of ingested n-3 fatty acids into phospholipids. Notwithstanding, the defining value of EPA >

Table 4. Clinical indices of disease activity at baseline and 3 years. Data are mean \pm SD unless stated otherwise.

	No Fish Oil		Fish Oil	
	Baseline, n = 13	3 Years, n = 13	Baseline, n = 18	3 Years, n = 18
mHAQ	7.1 \pm 4.2	3.3 \pm 3.2*	6.6 \pm 3.2	1.2 \pm 1.7*#
Tender joint count 28	8.8 \pm 3.6	3.5 \pm 3.9*	6.4 \pm 6.2	0.7 \pm 1.1*#
Swollen joint count 28	6.9 \pm 4.7	0.3 \pm 0.6*	5.4 \pm 5.5	0.9 \pm 1.8*
ESR, mm/h, mean (range)	36.5 (4–80)	21.5 (8–46)*	43.1 (1–91)	8.5 (2–34)*#
CRP, mg/l, mean (range)	17.2 (4–34)	6.6 (3–15)*	30.8 (1–140)	4.0 (0.3–19)*
DAS28	5.7 \pm 0.9	3.3 \pm 1.0*	5.0 \pm 1.5	2.1 \pm 0.9*#
NSAID Use, % [†]	86	54	89	22
Remission Rates at 3 years, % ^{††}	—	31	—	72

* Significantly different from baseline ($p < 0.01$, except for ESR in No fish oil group, where $p < 0.05$).

Significantly different at 3 years by comparison with the No fish oil group ($p < 0.05$). [†] NSAID use at 3 years defined as any use of NSAID for rescue analgesia within 3rd year of the study. ^{††} Remission rate at 3 years is based on DAS28 < 2.6 according to EULAR criteria. DAS: Disease Activity Score; mHAQ: modified Health Assessment Questionnaire.

5% of total fatty acids in plasma phospholipids, which was more than 4-fold greater than mean levels seen in fish oil nonusers (including the compliant group at baseline), could only be achieved and sustained with continuous ingestion of an antiinflammatory dose of fish oil or, within the Western setting, a remarkably high daily fish intake on the order of at least 2 large fish meals per day for 3 years. This group of longterm compliant fish oil takers is thus an appropriate group in which to assess the effects of prolonged fish oil ingestion on the laboratory measures of interest. Such a compliant group also provides the sample for a sound measure of the efficacy of optimal fish oil treatment. It is recognized that an intention-to-treat analysis of a randomized intervention group that is heterogeneous with respect to compliance can provide an important measure of optimum effectiveness of treatments for which efficacy is established, as is the case with fish oil in RA^{1-3,17}.

Cell membrane AA, competitor n-3 fatty acids, and eicosanoid synthesis. The biochemical changes found in this study are of particular interest as the duration of fish oil ingestion substantially exceeds that of previous studies in RA, or indeed that of most therapeutic studies of antiinflammatory doses of fish oil in any setting. The observed change in AA in relation to potential competitor n-3 substrates for COX metabolism, as inferred from the ratio of AA relative to AA plus EPA, DPA, and DHA, can be taken as a guide to what is realistically achievable with longterm fish oil treatment. The results of the whole-blood assay for eicosanoid synthesis are interesting from several perspectives. This relatively recently developed assay has not been reported previously in studies of longterm antiinflammatory treatment with fish oil. As well, it provides evidence for inhibition of COX-1 (serum TXA₂) and COX-2 (PGE₂ in LPS-stimulated blood) activity. The observed reduction in prothrombotic TXA₂ is important in providing a further mechanism through which fish oil in antiinflammatory doses may reduce CV risk. The reduced PGE₂ provides a mechanism for the symptomatic benefit and NSAID-sparing effect of longterm fish oil treatment, confirmed in this study.

Advantages compared to NSAID. That fish oil achieved only ~30% reduction in eicosanoid synthesis may seem modest compared to the more complete inhibition seen with NSAID. However, the extreme stimulus provocation used with *in vitro* assays may override the effects of inhibitory agents and may not reflect the situation *in vivo*. Also, the effect of regular fish oil treatment has the advantage of continuous inhibition, in contrast to the fluctuating levels of inhibition expected with short-acting COX inhibitors such as ibuprofen and diclofenac. Further, while fish oil inhibits COX-1 and COX-2, unlike NSAID, it does not inhibit synthesis of the vascular patency factor, prostacyclin (PGI₂), by endothelial cells. Indeed, antiinflammatory doses of fish oil have been associated with increased PGI₂ synthesis by arterial and venous vascular explants *in vitro*²⁴. PGI₂ and its stable analogs are gastroprotective^{25,26}, and increased PGI₂ synthesis within the gastric vascular endothelium could be a desirable effect of fish oil treatment. Such an effect could explain the lack of serious upper gastrointestinal events associated with fish oil in spite of reduced synthesis of putatively gastroprotective PGE₂. The continuous desirable changes in patterns of eicosanoid synthesis seen with fish oil contrast with those of NSAID, among which agents with half-lives sufficient to maintain continuous COX inhibition have particularly high toxicity. Examples include rofecoxib (long half-life, COX-1-sparing, high CV risk) and piroxicam (long half-life, relatively nonselective, high gastrototoxicity)²⁷. Another unwanted effect of at least some NSAID is increased synthesis of tumor necrosis factor- α (TNF- α) and interleukin 1 β (IL-1 β) synthesis²⁸, probably mediated by inhibition of synthesis of PGE₂, which is an inhibitor of inflammatory cytokine synthesis^{29,30}. By contrast, fish oil inhibits synthesis of the inflammatory cytokines TNF- α and IL-1 β , through mechanisms that appear to include inhibition of translocation of nuclear factor- κ B to the nucleus³¹. TNF- α and IL-1 β are present in both atheroma and RA synovium, and their expression thus has relevance to outcomes of both conditions. The favorable effect of fish oil on synthesis

of these cytokines may be partly through direct action and partly through reduction in NSAID use.

Cardiovascular risk. RA is associated with a 2-fold increase in CV mortality, including a 2-fold increase in sudden death. Some of the risk is accounted for by traditional CV risk factors^{16,32}. The favorable effect of fish oil on plasma triglycerides, HDL cholesterol, and the total cholesterol:HDL cholesterol ratio is therefore helpful. Fish oil has been shown to have multiple other beneficial CV effects. These include a reduction in blood pressure of a similar degree but opposite in direction to the rise in blood pressure associated with NSAID³³. While the basis for these effects on blood pressure control is not well established, the respective effects of these treatments on vascular PGI₂ synthesis (increased by fish oil, inhibited by NSAID) may contribute. Reduced arterial stiffness and increased heart rate variability are other benefits of antiinflammatory doses of fish oil³⁴. Fish oil has an antiarrhythmic effect and has been shown to reduce sudden cardiac death in secondary prevention studies following myocardial infarction³⁵.

Erythrocyte fatty acid index. Case-control studies have shown that, compared with the lowest quartiles, erythrocyte or whole-blood n-3 fatty acids in the highest quartile were associated with a 90% reduction in risk of primary cardiac arrest³⁶ or sudden cardiac death³⁷. These observations have been extended with correlations between risk in cohort and intervention studies and estimates of erythrocyte EPA+DHA calculated from intake values¹⁹. The authors concluded that erythrocyte EPA+DHA > 8% correlates with low CV risk. In our study, longterm compliance with fish oil treatment was associated with erythrocyte n-3 index values well into the low-risk range, in contrast to levels seen in patients not taking fish oil.

Effect of fish oil on disease suppression. The effectiveness of fish oil in reducing symptoms in late RA is well established. Our study extends observations with fish oil to early RA, but was not designed primarily to assess efficacy. It was a pilot study to assess the feasibility of using high-dose fish oil in early RA as a component of longterm combination therapy, and is a precursor to a randomized trial to assess the contribution of fish oil to longterm outcomes with combination therapy in early RA. The primary objectives of this analysis were to examine changes that relate to CV risk. In this context it is important to consider the issue of disease control because disease activity correlates with CV risk¹⁶. Suppression of disease activity was more pronounced in the fish oil-compliant group. On the basis of available evidence, this apparently favorable effect cannot necessarily be ascribed to the fish oil treatment, since compliance with fish oil may be a sign of a more general propensity to comply with the various components of the combination therapy. However, the findings can be interpreted as showing the potential of optimal compliance with multifaceted low-cost chemotherapy in early RA. For perspective, the remission rate of 72% observed in the fish oil-compliant group is numerically greater than the rates for ACR 20%

improvements seen in early RA with both MTX and etanercept as monotherapy in the ERA study³⁸. As the contribution of fish oil to the observed outcomes has not been defined, one cannot assume equivalent success without including fish oil.

In summary, use of fish oil in longterm combination treatment for early RA was associated with reduced synthesis of platelet TXA₂ and reduced synthesis of PGE₂ by LPS-stimulated PBMC in whole blood. Favorable changes were seen in multiple lipid risk factors for CV disease, and recourse to symptomatic use of NSAID was reduced. Measures of disease activity were generally lower in the compliant fish oil users. Antiinflammatory doses of fish oil should be considered in patients with RA as a means of reducing CV risk.

ACKNOWLEDGMENT

We thank Leah McWilliams, our Early Arthritis Clinic trial coordinator, and Cindy Hall for database management. We thank Dr. Robert Gibson (Infant Nutrition Research Unit, Child Health Research Institute, Women's and Children's Hospital) and Kate Boyd (Department of Paediatrics and Child Health, Flinders Medical Centre) for fatty acid analyses. We thank Dr. Anita Lee and physician trainees involved in the Early Arthritis Clinic.

REFERENCES

1. 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.
2. Kremer JM, Lawrence DA, Pettilo GF, et al. Effects of high-dose fish oil on rheumatoid arthritis after stopping nonsteroidal antiinflammatory drugs. *Arthritis Rheum* 1995;38:1107-14.
3. Fortin PR, Lew RA, Liang MH, et al. Validation of a meta-analysis: the effects of fish oil in rheumatoid arthritis. *J Clin Epidemiol* 1995;48:1379-90.
4. Cleland LG, James MJ, Proudman SM. Fish oil: what the prescriber needs to know. *Arthritis Res Ther* 2005;8:202 [Epub ahead of print].
5. 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.
6. Mantzioris E, Cleland LG, Gibson RA, et al. Biochemical effects of a diet containing foods enriched with n-3 fatty acids. *Am J Clin Nutr* 2000;72:42-8.
7. Lands WEM. Biosynthesis of prostaglandins. *Annu Rev Nutr* 1991;11:41-60.
8. Akiba S, Murata T, Kitatani K, Sato T. Involvement of lipoxygenase pathway in docosapentaenoic acid-induced inhibition of platelet aggregation. *Biol Pharm Bull* 2000;23:1293-7.
9. Skoldstam L, Borjesson O, Kjallman A, Seiving B, Akesson B. Effect of six months of fish oil supplementation in stable rheumatoid arthritis. A double blind, controlled study. *Scand J Rheumatol* 1992;21:178-85.
10. Lau CS, Morley KD, Belch JFF. Effects of fish oil supplementation on non-steroidal anti-inflammatory drug requirement in patients with mild rheumatoid arthritis — a double blind placebo controlled study. *Br J Rheumatol* 1993;32:982-9.
11. Geusens P, Wouters C, Nijs J, Jiang Y, Dequeker J. Long-term effect of omega-3 fatty acid supplementation in active rheumatoid arthritis. *Arthritis Rheum* 1994;37:824-9.
12. O'Keefe JH Jr, Harris WS. From Inuit to implementation: omega-3 fatty acids come of age. *Mayo Clin Proc* 2000;75:607-14.
13. Solomon DH. Selective cyclooxygenase 2 inhibitors and cardiovascular events. *Arthritis Rheum* 2005;52:1968-78.
14. Hippisley-Cox J, Coupland C. Risk of myocardial infarction in

- patients taking cyclo-oxygenase-2 inhibitors or conventional non-steroidal anti-inflammatory drugs: population based nested case-control analysis. *BMJ* 2005;330:1366.
15. Krause D, Schleusser B, Herborn G, Rau R. Response to methotrexate treatment is associated with reduced mortality in patients with severe rheumatoid arthritis. *Arthritis Rheum* 2000;43:14-21.
 16. Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE. Cardiovascular death in rheumatoid arthritis: a population-based study. *Arthritis Rheum* 2005;52:722-32.
 17. Cleland LG, James MJ, Proudman SM. The role of fish oils in the treatment of rheumatoid arthritis. *Drugs* 2003;63:845-53.
 18. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
 19. Harris WS, Von Schacky C. The omega-3 index: a new risk factor for death from coronary heart disease? *Prev Med* 2004;39:212-20.
 20. Pincus T, Brooks RH, Callahan LF. A proposed 30-45 minute 4 page standard protocol to evaluate rheumatoid arthritis (SPERA) that includes measures of inflammatory activity, joint damage, and longterm outcomes. *J Rheumatol* 1999;26:473-80.
 21. Cleland LG, French JK, Betts WH, Murphy GA, Elliott M. Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. *J Rheumatol* 1988;15:1471-5.
 22. Volker D, Fitzgerald P, Major G, Garg M. Efficacy of fish oil concentrate in the treatment of rheumatoid arthritis. *J Rheumatol* 2000;27:2343-6.
 23. van der Tempel H, Tulleken JE, Limburg PC, Muskiet FAJ, van Rijswijk MH. Effects of fish oil supplementation in rheumatoid arthritis. *Ann Rheum Dis* 1990;49:76-80.
 24. De Caterina R, Giannesi D, Mazzone A, et al. Vascular prostacyclin is increased in patients ingesting omega-3 polyunsaturated fatty acids before coronary artery bypass graft surgery. *Circulation* 1990;82:428-38.
 25. Boku K, Ohno T, Saeki T, et al. Adaptive cytoprotection mediated by prostaglandin I(2) is attributable to sensitization of CRGP-containing sensory nerves. *Gastroenterology* 2001;120:134-43.
 26. Takeuchi K, Kato S, Takeeda M, et al. Facilitation by endogenous prostaglandins of capsaicin-induced gastric protection in rodents through EP2 and IP receptors. *J Pharmacol Exp Ther* 2003;304:1055-62.
 27. Henry D, Lim LL, Garcia Rodriguez LA, et al. Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: results of a collaborative meta-analysis. *BMJ* 1996;312:1563-6.
 28. Endres S, Whitaker RE, Ghorbani R, Meydani SN, Dinarello CA. Oral aspirin and ibuprofen increase cytokine-induced synthesis of IL-1 beta and of tumour necrosis factor-alpha ex vivo. *Immunology* 1996;87:264-70.
 29. Demasi M, Cleland LG, Cook-Johnson RJ, Caughey GE, James MJ. Effects of hypoxia on monocyte inflammatory mediator production: dissociation between changes in cyclooxygenase-2 expression and eicosanoid synthesis. *J Biol Chem* 2003;278:38607-16.
 30. Caughey GE, Pouliot M, Cleland LG, James MJ. Regulation of tumor necrosis factor alpha and interleukin-1 beta synthesis by thromboxane A₂ in non-adherent human monocytes. *J Immunol* 1997;158:351-8.
 31. Zhao Y, Joshi-Barve S, Barve S, Chen LH. Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappa B activation. *J Am Coll Nutr* 2004;23:71-8.
 32. Maradit-Kremers H, Crowson CS, Nicola PJ, et al. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. *Arthritis Rheum* 2005;52:402-11.
 33. Geleijnse JM, Giltay EJ, Grobbee DE, Donders AR, Kok FJ. Blood pressure response to fish oil supplementation: meta-regression analysis of randomized trials. *J Hypertens* 2002;20:1493-9.
 34. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 2002;106:2747-57.
 35. GISSI Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet* 1999;354:447-55.
 36. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of cardiac arrest. *JAMA* 1995;274:1363-7.
 37. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. *N Engl J Med* 2002;346:1113-8.
 38. Bathon JM, Genovese MC. The Early Rheumatoid Arthritis (ERA) trial comparing the efficacy and safety of etanercept and methotrexate. *Clin Exp Rheumatol* 2003;21:S195-7.