DIETARY FISH OIL AND OLIVE OIL SUPPLEMENTATION IN PATIENTS WITH RHEUMATOID ARTHRITIS

Clinical and Immunologic Effects

JOEL M. KREMER, DAVID A. LAWRENCE, WILLIAM JUBIZ, RALPH DiGIACOMO, RICHARD RYNES, LEE E. BARTHOLOMEW, and MALCOLM SHERMAN

Forty-nine patients with active rheumatoid arthritis completed a 24-week, prospective, double-blind, randomized study of dietary supplementation with 2 different dosages of fish oil and 1 dosage of olive oil. Clinical evaluations were performed at baseline and every 6 weeks thereafter, and immunologic variables were measured at baseline and after 24 weeks of study. The 3 groups of patients were matched for age, sex, disease severity, and use of disease-modifying antirheumatic drugs (DMARDs). Subjects continued receiving DMARDs and other background medications without change during the study. Twenty patients consumed daily dietary supplements of n3 fatty acids containing 27 mg/kg eicosapentaenoic acid (EPA) and 18 mg/kg docosahexaenoic acid (DHA) (low dose), 17 patients ingested 54 mg/kg EPA and 36 mg/kg DHA (high dose), and 12 patients ingested olive oil capsules containing 6.8 gm of oleic acid. Significant improvements from baseline in the number of tender joints were noted in the low-dose group at week 24 ($P = 0.05$) and in the high-dose group at weeks 18 ($P = 0.04$) and 24 ($P = 0.02$). Significant decreases from baseline in the number of swollen joints were noted in the low-dose group at weeks 12 ($P = 0.003$), 18 ($P = 0.002$), and 24 ($P = 0.001$) and in the high-dose group at weeks 12 ($P = 0.0001$), 18 ($P = 0.008$), and 24 ($P = 0.02$). A total of 5 of 45 clinical measures were significantly changed from baseline in the olive oil group, 8 of 45 in the low-dose fish oil group, and 21 of 45 in the high-dose fish oil group during the study ($P = 0.0002$). Neutrophil leukotriene B_4 production decreased by 19% from baseline in the low-dose fish oil group ($P = 0.0003$) and 20% in the high-dose group ($P = 0.03$), while macrophage interleukin-1 production decreased by 38.5% in the olive oil group (P not significant), 40.6% in the low-dose group ($P = 0.06$), and 54.7% in the high-dose group ($P = 0.0005$). Tritiated thymidine incorporation in peripheral blood mononuclear cells after stimulation with concanavalin A increased significantly in all 3 groups after 24 weeks, compared with baseline values. We conclude that the clinical benefits of dietary supplementation with omega-3 fatty acids are more commonly observed in patients consuming higher dosages of fish oil for time intervals that are longer than those previously studied. Dietary supplementation with olive oil is also associated with certain changes in immune function, which require further investigation.

Fish oil contains the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (1). EPA (20:5 n3) competitively inhibits the utilization of arachidonic acid (20:4 n6) and becomes a substrate for the production of alternative biologically active products through the cyclooxygenase and 5-lipoxygenase cellular metabolic pathways (2,3). Omega-3 fatty acid ingestion results in the production of thromboxane A_3 and prostacyclin I_3 (4) and a net physiologic shift that favors decreased platelet aggregation (since prostacyclin I_3 retains its vasodilatory

From the Division of Rheumatology, Department of Medicine, and the Department of Microbiology and Immunology, Albany Medical College; Medical Service, Albany Veterans Administration Medical Center; and the Department of Statistics, State University of New York, Albany.

Joel M. Kremer, MD; David A. Lawrence, PhD; William Jubiz, MD (current address: Veterans Administration Central Office, Washington, DC); Ralph DiGiacomo, MD; Richard Rynes, MD; Lee E. Bartholomew, MD; Malcolm Sherman, PhD.

Address reprint requests to Joel M. Kremer, MD, Division of Rheumatology, Albany Medical Center, Albany, NY 12208.

Submitted for publication May 4, 1989; accepted in revised form January 11, 1990.

and antiaggregatory properties, whereas thromboxane A_3 does not retain its proaggregatory activity). Fish oil ingestion results in the decreased production of leukotriene B_4 (LTB_4), derived from arachidonate through the 5-lipoxygenase pathway, with the new production of leukotriene B_5 from EPA (5). Since LTB_4 is a potent inflammatory and chemotactic compound, a decrease in its production could favorably affect the clinical manifestations of an inflammatory disease like rheumatoid arthritis (RA). We have reported that certain clinical manifestations of RA improved in patients receiving fish oil, and that this improvement was significantly correlated with decreased production of neutrophil LTB_4 (6).

Leukotrienes are also potent modulators of immune reactivity (7–10), which can significantly affect T cell and B cell activity through modulation of the production of certain cytokines, including interleukin-1 (IL-1) (11). IL-1 could potentially influence the course of an autoimmune inflammatory disease such as RA through several different mechanisms, including its effects on synovial tissue and cartilage metabolism (12) as well as immunomodulation (13).

Previous studies of fish oil fatty acid ingestion in patients with RA have used fixed daily doses of dietary supplements without regard to the patient's individual body weight (6,14,15). The possibility that there could be dose-dependent clinical and laboratory effects of fish oil supplementation in these subjects was not examined. It was not certain whether the observed benefits would be sustained, since ω3 fatty acid dietary supplements were not administered for periods longer than 14 weeks. Salutary effects have also been ascribed to oleic acid, a monounsaturated fatty acid found in olive oil, in an epidemiologic study of death rates from coronary heart disease (16). Immune effects of oleic acid ingestion have also been demonstrated (17).

We report here that different doses of ω3 fatty acid dietary supplements (fish oil) ingested over a period of 24 weeks results in some dose-related clinical benefits, which are maximal after 18 and 24 weeks of daily ingestion. These changes are accompanied by significant decreases in the production of both neutrophil LTB_4 and macrophage IL-1, with associated statistically significant ex vivo alterations in T and B cell reactivity.

PATIENTS AND METHODS

Study design. This was a prospective, randomized, double-blind, parallel study. Three groups were studied: 2 groups received different doses of fish oil dietary supplements, and 1 group received olive oil supplements. We chose to compare fish oil with olive oil because of the previously reported salutary effects of the oleic acid component of olive oil. Patients were randomized for age, sex, treatment with slow-acting antirheumatic drugs (SAARDs), and disease severity. For the purposes of randomization, the following system of rating disease activity was used: category 1 = total joint count 6–10 (total of the number of tender and swollen joints), category 2 = total joint count 11–20, and category 3 = total joint count ≥21.

The 3 study groups consisted of 1 group of RA patients who ingested 9 olive oil capsules/day containing a total of 6.84 gm of oleic acid (18:1 n9), 0.93 gm of palmitic acid (16:0), 0.53 gm of linoleic acid (18:2 n6), and 0.13 gm of stearic acid (18:0); 1 group of RA patients who took a "low dose" of fish oil, which consisted of 27 mg/kg/day EPA and 18 mg/kg/day DHA; and 1 group of RA patients who took a "high dose" of fish oil, 54 mg/kg/day EPA and 36 mg/kg/day DHA. All patients on the fish oil regimen ingested capsules containing 330 mg EPA and 240 mg DHA per capsule, in an ethyl ester form, provided by Pharmacaps, Inc. (Elizabeth, NJ). Each fish oil capsule also contained 40 mg of docosapentaenoic acid (22:5 n3), 110 mg of oleic acid, 100 mg of palmitoleic acid, 30 mg of palmitic acid, 30 mg of linoleic acid, and 20 mg or less of stearic acid, linolenic acid, eicosenic acid, tetracosaenoic acid, and arachidonic acid. Patients' weights often required that they receive a fraction of a fish oil capsule (i.e., 7¼, 8¼, etc.). When this occurred, the number of capsules dispensed was rounded to the next whole number if the fraction was ≥0.50 (calculated on the basis of body weight) and to the previous whole number if the fraction was ≤0.49.

The total daily caloric supplement provided by the capsules was 81 kcal (9 capsules) in all patients in the olive oil group. The mean ± SD daily caloric supplement was 52 ± 9.7 kcal (range 36–72) in the low-dose fish oil group and 103 ± 20.5 kcal (range 81–153) in the high-dose fish oil group.

Patient population. Patients were recruited from the outpatient clinic population of the Division of Rheumatology at Albany Medical College. Sixty-four patients with definite or classic RA, according to the American Rheumatism Association criteria (18), were enrolled in the study. All patients had active disease, based upon the presence of 3 of the following 4 criteria: 1) ≥6 tender joints on palpation, 2) ≥6 swollen joints, 3) morning stiffness ≥30 minutes, and 4) Westergren erythrocyte sedimentation rate (ESR) ≥28 mm/hour.

Patients had been receiving a stable dosage of a SAARD for at least 6 months prior to study entry, as well as a stable dosage of corticosteroids and/or nonsteroidal anti-inflammatory drugs (NSAIDs) for at least 1 month prior to study entry. Any change in medications for RA, including NSAIDs, SAARDs, oral corticosteroids, or intraarticular steroid injections, during the entire duration of the study was considered a reason to withdraw a patient from the study and data analysis. The study was approved by the Institutional Review Board of the Albany Medical College.

Clinical assessment. Clinical evaluations were performed by the same rheumatologist at baseline and every 6 weeks thereafter, through 30 weeks (6 visits). Patients re-

ceiving fish oil ingested these supplements for 24 weeks. All 3 groups ingested olive oil supplements during weeks 24–30. Patients were not aware of the change in dietary supplement during this period. Physicians were aware of the change, but remained blinded to the original dietary supplement group assignments through the 30 weeks of study. Patients received no dietary instructions. A 3-day, detailed, dietary history was obtained from all patients at baseline and at the 30-week visit, and the information was analyzed for consistency of nutrient intake with an IBM AT computer using a Short Report software package (Health Development, Columbus, OH).

The clinical evaluations consisted of the following: blood pressure, weight, duration of morning stiffness (in minutes), time interval to the onset of fatigue (in minutes; the interval from first awakening to the first experience of noticeable fatigue), grip strength (using a sphygmomanometer cuff rolled in a standard sleeve and inflated to 20 mm Hg; average of 3 determinations for each hand), patient and physician evaluation of both pain and global arthritis activity (5-point scale, where 0 = absent, 1 = mild, 2 = moderate, 3 = severe, and 4 = very severe), and the number of tender and swollen joints (among 66 diarthrodial joints on physical examination).

Compliance. Compliance was monitored by pill counts and gas chromatographic analysis of plasma fatty acids at each visit. Any patient in either fish oil group who failed to demonstrate an increase in plasma EPA levels was not included in the data analysis.

Laboratory and immunologic evaluations. Laboratory determinations included a complete blood cell count, platelet count, and Westergren ESR at each visit. In addition, the following laboratory determinations were made at baseline and at 24 weeks (maximum duration of fish oil ingestion): ionophore-stimulated neutrophil production of LTB_4 and LTB_5, IL-1 and IL-2 production, T cell proliferation after stimulation with concanavalin A (ConA) and phytohemagglutinin (PHA), T cell–dependent B cell proliferation after stimulation with pokeweed mitogen (PWM), B cell proliferation after stimulation with formalin-fixed *Staphylococcus aureus*, immunoglobulin production (IgG, IgM, and IgA) after PWM stimulation, and rheumatoid factor titer (latex fixation test).

Measurement of leukotrienes. Leukotrienes B_4 and B_5 were measured in stimulated neutrophils from peripheral blood at baseline and at 24 weeks. Neutrophils were isolated from 30 ml of peripheral blood by dextran sedimentation, hypotonic hemolysis of erythrocytes, and Ficoll-Hypaque centrifugation (Winthrop Laboratories, New York, NY) as previously described (19). After preincubation of the neutrophils (10^7 cells in 1 ml of Hanks' balanced salt solution containing 0.6% HEPES) at 37°C for 5 minutes, the cells were stimulated with 5 μM ionophore A23187 (Calbiochem-Behring, San Diego, CA) and incubated for an additional 5 minutes. The reaction was stopped by the addition of 0.5 ml of methanol.

Cells were centrifuged at 600*g* for 2 minutes, and the supernatant containing the leukotrienes was treated with 5 μl of glacial acetic acid and injected into a reverse-phase, high performance liquid chromatograph (Waters, Milford, MA). The instrument was equipped with a Z-module and a 5-μm radial-PAK C_{18} cartridge (10-cm long and 0.8-cm wide). The Z-module and the radial-PAK are used to expedite the processing of the samples and to improve separation of the leukotrienes. Leukotrienes were eluted with methanol, water, and acetic acid (75:25:0.01 volume/volume) at a pump rate of 2 ml/minute, and they were detected by ultraviolet spectroscopy at 270 nm. The leukotriene concentrations were calculated by comparing the area under the peak of a known amount of LTB_4 or LTB_5 standard with that of the patient's sample. Values are expressed as nanograms of leukotriene B_4 or B_5 produced by 10^6 neutrophils in 5 minutes.

Lymphoproliferation assay. Peripheral blood mononuclear cells (PBMC) were isolated from EDTA-treated blood by Ficoll-Hypaque fractionation (20). The PBMC were washed twice with Tris-saline (pH 7.4) and resuspended in RPMI 1640 containing 10% fetal bovine serum. The mitogens included Con A (5 μg/ml; Sigma, St. Louis, MO), PHA (1:200; Burroughs Wellcome, Research Triangle Park, NC), PWM (1:200; Gibco, Grand Island, NY), and formalin-fixed *S aureus* (1:10,000; Bethesda Research, Gaithersburg, MD). Cells (1×10^5/100 μl) were cultured in 96-well plates at 37°C in an atmosphere of 5% CO_2 for 3 days (T cell mitogens) or for 5 days (B cell mitogens), and then assessed for DNA synthesis by addition of 0.5 μCi ^{3}H-thymidine (6.7 Ci/mmole) for a 6-hour pulse. Cells were harvested and analyzed for ^{3}H-thymidine incorporation as previously described (21).

IL-1 production. PBMC (1×10^6/ml) were cultured for 24 hours in the presence or absence of 1 μg/ml of lipopolysaccharide ([LPS] *Escherichia coli* 055:B5; Calbiochem, La Jolla, CA). The levels of IL-1 were below the limits of quantitation in the absence of LPS. By 24 hours, the monocytes have the characteristics of macrophages. Cell-free supernatants were stored at −70°C prior to quantitation of IL-1 in the C3H/HeJ thymocyte costimulation assay (22).

IL-2 production. PBMC (1×10^6/ml) were cultured for 24 hours in the presence or absence of PHA (1:200). Cell-free supernatants were stored at −70°C prior to quantitation of IL-2 in the HT-2 bioassay (23).

Ig production. PBMC were cultured for 7 days in the presence or absence of PWM (1:200). Cell-free supernatants were stored at −70°C prior to quantitation of IgG, IgA, or IgM in an enzyme-linked immunosorbent assay (24).

Statistical analysis. Clinical data were analyzed by multivariate (repeated measures) analysis of variance. The SAS general linear models procedure was used to test for the multivariate hypothesis of no treatment effects, and tests were also run for time–treatment interactions within subjects. Univariate analyses of variance were also performed to test all clinical variables for treatment effects at each visit. In addition, we performed univariate analyses of the changes from baseline values for each of visits 2–6 (weeks 6–30) and for visit 6 less visit 5 (week 30 less week 24) values. Laboratory variables were measured at baseline and at 24 weeks, and both the actual values and the changes from baseline were analyzed by treatment group, using univariate error of the mean tests. All data are presented as mean values, with 95% confidence intervals (CI) and *P* values. Significance values were calculated using Student's *t*-test and Wilcoxon signed rank test where appropriate.

RESULTS

Six patients withdrew from the study prior to its completion. Reasons for withdrawing included inconvenience of making study appointments (3 patients in the olive oil group) and gastrointestinal side effects (3 patients, 1 in each of the 3 study groups). Nine other patients were administratively withdrawn, leaving 49 patients who successfully completed the study with documented compliance and without medication change. The reasons for the 9 administrative withdrawals included a new diagnosis of lung cancer (1 in the olive oil group), change of SAARD because of increased clinical manifestations of disease (3 in the olive oil group), increase in oral corticosteroid dose (2 in the olive oil group), intraarticular steroid injection (1 in the olive oil group and 1 in the low-dose fish oil group), and lack of evidence of compliance based on gas chromatographic analysis of plasma (1 in the high-dose fish oil group). All other patients were compliant, based upon the results of pill counts and gas chromatographic analysis of plasma fatty acids.

The mean percentage of EPA in plasma over the entire study was 0.78% in the olive oil group, 4.48% in the low-dose fish oil group, and 6.29% in the high-dose fish oil group. The mean compliance demonstrated by pill counts for all patients successfully completing the study was 89.6%. A summary of patients withdrawn from the study is shown in Table 1. Demographic characteristics of patients successfully completing the study are shown in Table 2. Twenty-one patients (43%) were receiving prednisone at a mean ± SD dosage of 4.4 ± 3.1 mg/day, which was maintained throughout the study.

Table 1. Reasons for patient withdrawal from the study

	Group		
	Olive oil (n = 11)	Low-dose fish oil (n = 2)	High-dose fish oil (n = 2)
Gastrointestinal intolerance	1	1	1
Changes in medication	6*	1	0
Intraarticular steroid injection	1	1	0
Prednisone	2	0	0
Slow-acting antirheumatic drug	3	0	0
Inconvenience	3	0	0
Poor compliance	0	0	1
Other	1	0	0

* $P = 0.008$ versus fish oil groups.

Table 2. Demographic characteristics of the 49 rheumatoid arthritis patients who successfully completed the study

	Group		
	Olive oil (n = 12)	Low-dose fish oil (n = 20)	High-dose fish oil (n = 17)
Age, mean (range)	58 (22–81)	59 (32–81)	58 (30–80)
Disease duration, mean (range)	13.5 (2–28)	12.8 (1–30)	15 (3–36)
Females/males	8/4	11/9	14/3
Medications*			
NSAID or aspirin	11 (91)	19 (95)	17 (100)
Hydroxychloroquine	6 (50)	8 (40)	4 (23)
Sodium aurothioglucose	2 (17)	1 (5)	2 (12)
Auranofin	2 (17)	6 (30)	1 (6)
D-penicillamine	3 (25)	4 (20)	1 (6)
Methotrexate	2 (17)	2 (10)	6 (35)
Sulfasalazine	0 (0)	0 (0)	1 (6)
Prednisone†	4 (33)	9 (45)	8 (47)

* Values are the number (%) of patients. NSAID = nonsteroidal antiinflammatory drug.
† The mean ± SD prednisone dosages were 3.25 ± 3.09 mg/day for the olive oil group, 4.12 ± 1.31 mg/day for the low-dose fish oil group, and 4.64 ± 2.93 mg/day for the high-dose fish oil group.

Changes in clinical parameters from baseline. Changes in clinical parameters with time are shown in Table 3. Significant decreases from baseline values in the mean number of tender joints in the group ingesting low-dose fish oil were observed at the 24-week visit, when the number of tender joints was found to have decreased by 1.9 ($P = 0.05$), and in the high-dose fish oil group at the 18-week visit, when tender joint counts decreased by 2.6 ($P = 0.04$) and at the 24-week visit, when the tender joint count had decreased by 1.7 ($P = 0.02$). A trend toward significant improvement was observed in the high-dose fish oil group after 12 weeks, when tender joint counts had decreased by 2.4 ($P = 0.06$).

As seen in Table 3, significant decreases from baseline in the mean number of swollen joints were observed in the group ingesting low-dose fish oil at 12 weeks, when joint counts had decreased by 2.7 ($P = 0.003$), at 18 weeks ($P = 0.002$), and at 24 weeks ($P = 0.001$). The significant decrease in swollen joints was also noted at the time of the week-30 "washout" visit, when joint counts had decreased by 3.6 ($P = 0.007$) in this group of patients.

We also observed significant decreases from baseline in the mean number of swollen joints in the high-dose fish oil group at 12 weeks ($P = 0.0001$), 18 weeks ($P = 0.008$), and 24 weeks ($P = 0.02$). No significant decreases from baseline in the number of

Table 3. Mean intragroup change from baseline in the clinical parameters assessed*

		Mean change (95% confidence interval)				
	Baseline	6 weeks	12 weeks	18 weeks	24 weeks	36 weeks
Tender joint count						
Olive oil	5.8 (2.0, 9.0)	−0.7 (−2.6, 1.3)	0.4 (−1.9, 2.8)	−0.8 (−2.4, 0.8)	0.4 (−2.3, 3.2)	−0.3 (−2.8, 2.2)
Low-dose fish oil	6.0 (3.3, 8.7)	0.1 (−2.1, 2.2)	−1.7 (−3.9, 0.5)	−1.1 (−2.8, 0.7)	−1.9† (−3.7, 0.0)	−1.0 (−3.2, 1.2)
High-dose fish oil	5.4 (1.8, 8.9)	−1.1 (−3.3, 1.2)	−2.4 (−4.8, 0.1)	−2.6† (−5.1, 0.0)	−1.7† (−3.1, −0.2)	−1.8 (−4.0, 0.3)
Joint swelling count						
Olive oil	16.3 (10.6, 22.2)	−0.8 (−3.5, 1.9)	−2.8 (−6.3, 0.6)	−2.6 (−6.5, 1.3)	−2.4 (−5.8, 0.9)	−2.4 (−6.3, 1.6)
Low-dose fish oil	14.4 (11.3, 17.5)	−0.8 (−2.5, 0.8)	−2.7† (−4.4, −1.0)	−3.6† (−5.6, 1.5)	−4.1† (−6.9, −1.8)	−3.6† (−6.1, −1.1)
High-dose fish oil	13.0 (10.7, 15.3)	−0.4 (−3.4, 1.7)	−2.9† (−4.0, −1.8)	−2.3† (−3.9, −0.7)	−2.8† (−5.0, −0.7)	−1.4 (−3.5, 0.8)
Morning stiffness (minutes)						
Olive oil	41.3 (11.6, 70.8)	−7.9 (−25.5, 9.7)	−15 (−33.5, 3.5)	−13.3 (−34.7, 8.1)	−1.2 (−43.0, 40.5)	−17.9 (−44.2, 8.4)
Low-dose fish oil	44.0 (17.1, 70.9)	−15.3 (−43.3, 12.8)	−8.5 (−36.8, 19.8)	−17.8 (−46.4, 10.7)	−4.8 (−39.6, 30.1)	−1.3 (−45.1, 92.6)
High-dose fish oil	69.4 (34.6, 104.2)	−14.4 (−35.4, 6.6)	−30.3 (−66.1, 5.5)	−41.7† (−73.9, −9.6)	−46.5† (−76.3, −16.6)	−30.0† (−63.7, 3.7)
Interval to onset of fatigue (hours)						
Olive oil	8.4 (6.1, 10.6)	0.8 (−0.7, 2.4)	−0.4 (−1.5, 0.6)	−0.9 (−3.8, 1.0)	−0.5 (−2.5, 1.6)	−0.3 (−2.5, 1.9)
Low-dose fish oil	8.4 (7.0, 9.8)	−0.1 (−0.9, 1.1)	−1.0 (−2.5, 0.5)	−1.8 (−1.9, 1.6)	0.4 (−0.8, 1.5)	−0.2 (−1.7, 1.3)
High-dose fish oil	8.4 (6.7, 10.0)	0.4 (−0.8, 1.7)	0.2 (−1.7, 2.2)	1.1 (−0.3, 2.6)	0.6 (−0.6, 1.8)	1.0 (−0.1, 2.1)
Grip strength (mm Hg)						
Olive oil	126.1 (92.0, 160.3)	5.9 (−2.7, 14.6)	11.8 (1.5, 22.2)	6.9 (−2.4, 16.4)	2.9 (−8.6, 14.4)	2.8 (−15.1, 9.5)
Low-dose fish oil	122.8 (95.5, 150.1)	0.2 (−8.0, 8.4)	8.0 (−3.6, 19.7)	12.2† (2.7, 21.8)	12.0† (0.9, 23.2)	8.5 (−1.1, 18.1)
High-dose fish oil	92.5 (75.0, 110.0)	11.7 (−5.8, 29.3)	12.2† (0.4, 24.1)	17.8† (2.0, 33.6)	21.1† (6.2, 35.9)	19.6† (2.5, 36.7)
Patient evaluation of pain						
Olive oil	1.6 (1.2, 2.0)	−0.1 (−0.5, 0.3)	0.0 (−0.4, 0.4)	0.0 (−0.3, 0.3)	−0.3 (−0.7, 0.2)	−0.2 (−0.5, 0.2)
Low-dose fish oil	1.8 (1.4, 2.1)	−0.1 (−0.5, 0.3)	−0.2 (−0.4, 0.1)	0.0 (−0.4, 0.4)	−0.2 (−0.6, 0.3)	0.0 (−0.5, 0.5)
High-dose fish oil	1.6 (1.3, 1.9)	−0.2 (−0.5, 0.2)	−0.2 (−0.6, 0.2)	−0.3† (−0.5, 0.1)	−0.2 (−0.6, 0.2)	−0.1 (−0.4, 0.2)
Patient evaluation of global disease						
Olive oil	1.9 (1.5, 2.4)	−0.4† (−0.7, −0.1)	−0.1 (−0.4, 0.2)	−0.4† (−0.8, 0.0)	−0.4† (−0.7, −0.1)	−0.4† (−0.7, −0.1)
Low-dose fish oil	1.7 (1.4, 2.0)	−0.1 (−0.4, 0.3)	−0.1 (−0.5, 0.3)	−0.1 (−0.4, 0.2)	−0.4 (−0.7, 0.0)	−0.2 (−0.6, 0.3)
High-dose fish oil	1.7 (1.4, 2.0)	−0.2 (−0.4, 0.1)	0.1 (−0.4, 0.3)	−0.1 (−0.4, 0.2)	−0.1 (−0.5, 0.3)	0.0 (−0.3, 0.3)
Physician evaluation of pain						
Olive oil	1.4 (1.1, 1.7)	0.1 (−0.2, 0.4)	0.1 (−0.3, 0.5)	0.0 (−0.3, 0.3)	0.1 (−0.3, 0.5)	0.0 (−0.3, 0.3)
Low-dose fish oil	1.5 (1.1, 1.8)	0.0 (−0.3, 0.3)	−0.2 (−0.5, 0.2)	−0.1 (−0.3, 0.4)	−0.3 (−0.6, 0.1)	−0.1 (−0.3, 0.2)
High-dose fish oil	1.5 (1.1, 1.9)	−0.3 (−0.6, 0.1)	−0.4† (−0.7, 0.1)	−0.4† (−0.7, 0.2)	−0.4† (−0.7, 0.2)	−0.1 (−0.4, 0.3)
Physician evaluation of global disease						
Olive oil	2.0 (1.5, 2.5)	−0.2 (−0.5, 0.0)	−0.2 (−0.6, 0.2)	−0.2 (−0.5, 0.2)	−0.4† (−0.7, −0.1)	−0.2 (−0.5, 0.1)
Low-dose fish oil	1.9 (1.5, 2.2)	−0.1 (−0.4, 0.2)	−0.3 (−0.6, 0.1)	−0.3 (−0.6, 0.1)	−0.4† (−0.8, −0.1)	−0.3 (−0.6, 0.0)
High-dose fish oil	1.9 (1.5, 2.2)	−0.4† (−0.7, −0.1)	−0.4† (−0.7, 0.0)	−0.4† (−0.7, 0.0)	−0.5† (−0.9, 0.2)	−0.3† (−0.6, −0.1)

* Positive values for interval to onset of fatigue indicate improvement over baseline. Patient and physician evaluation of pain and global disease were graded on a scale of 0–4 (see Patients and Methods).
† $P \leq 0.05$ versus baseline.

tender or swollen joints were noted at any time in the patients ingesting the olive oil supplement.

Significant decreases in the mean rating of morning stiffness from baseline were noted only in the high-dose fish oil group. They occurred at 18 weeks ($P = 0.01$) and 24 weeks ($P = 0.004$), with a tendency toward a carryover benefit noted at the 30-week visit ($P = 0.07$). Significant improvements in morning stiffness did not occur in the group ingesting low-dose fish oil or olive oil.

Mean grip strength increased in the low-dose fish oil group at 18 weeks ($P = 0.01$) and 24 weeks ($P = 0.01$). In the high-dose fish oil group, mean grip strength improved at 12 weeks ($P = 0.04$), 18 weeks ($P = 0.02$), and 24 weeks ($P = 0.008$), and a carryover benefit was seen at the 30-week visit ($P = 0.02$). Significant improvements in grip strength were not observed in the group ingesting olive oil. Grip strength was the only clinical parameter that showed significant improvement in all patients ingesting fish oil, compared with those ingesting olive oil ($P = 0.04$).

Improvement in patient evaluation of pain was seen only at week 18 in the high-dose fish oil group ($P = 0.02$). Patient evaluation of global arthritis activity significantly improved only in the olive oil–supplemented group at week 6 ($P = 0.02$), week 18 ($P = 0.05$), week 24 ($P = 0.02$), and week 30 ($P = 0.02$). The physician evaluation of pain experienced by patients in the high-dose fish oil group improved after 12 weeks ($P = 0.01$), 18 weeks ($P = 0.004$), and 24 weeks ($P = 0.004$). No significant improvements in the interval to the onset of fatigue were noted at any time in any group. We also observed decreases from baseline in the physician evaluation of global arthritis activity at the time of the 24-week visit in the group ingesting olive oil ($P = 0.02$) and in the low-dose fish oil group ($P = 0.03$). Significant improvements in physician evaluation of global arthritis activity were seen in the high-dose fish oil group after week 6 ($P = 0.03$), week 12 ($P = 0.05$), week 18 ($P = 0.05$), and week 24 ($P = 0.008$), with a carryover improvement noted at 30 weeks ($P = 0.02$).

A reduction in mean systolic blood pressure was noted in the low-dose fish oil group after 12 weeks (−8.1 mm Hg; 95% CI −16.4, 0.3, $P = 0.05$), 24 weeks (−10.5 mm Hg; 95% CI −20.3, −0.6, $P = 0.03$), and 30 weeks (−9.9 mm Hg; 95% CI −18.7, −1.0, $P = 0.03$). No other significant changes in systolic or diastolic blood pressure were observed.

Laboratory evaluations. No significant changes in hemoglobin levels, Westergren ESR, or rheumatoid factor titer were observed in any group. Ionophore-stimulated neutrophil LTB_4 levels decreased by 19% in the low-dose fish oil group ($P = 0.0003$) and 20% in the high-dose fish oil group ($P = 0.03$) (Table 4).

Table 4. Ionophore-stimulated neutrophil leukotriene B_4 production by peripheral blood mononuclear cells from the 3 study groups*

Group	n	Change from baseline at week 24, ng/10^6 cells/5 minutes
Olive oil	12	−1.81 (−4.43, −0.81)
Low-dose fish oil	20	−3.88 (−5.73, −2.03)†
High-dose fish oil	17	−4.13 (−7.73, −0.53)‡

* Values are the mean (95% confidence interval).
† $P = 0.0003$ versus baseline.
‡ $P = 0.03$ versus baseline.

Changes in IL-1 and IL-2 levels from baseline measurements. IL-1 production and release decreased by 40.6% from baseline measurements ($P = 0.059$) in the low-dose fish oil group after 24 weeks and decreased by 54.7% from baseline ($P = 0.0005$) in the high-dose fish oil group (Table 5). Although IL-1 decreased by 38.5% in the olive oil group, the change was not statistically significant. IL-2 increased by 32.8% from baseline after 24 weeks of fish oil consumption in the low-dose group, and increased by 16% from baseline in the high-dose group (Table 6). Neither of these changes from baseline measurements were statistically significant.

^{3}H-thymidine incorporation in mitogen-stimulated PBMC. Tritiated thymidine incorporation in PBMC after stimulation with Con A increased in all 3 groups after 24 weeks compared with baseline measurements (Table 7). Incorporation in the olive oil group increased by 70.9% ($P = 0.004$) compared with an increase of 82% ($P = 0.001$) in the low-dose fish oil group and an increase of 51.6% ($P = 0.002$) in the high-dose fish oil group. Increased thymidine incorpo-

Table 5. Lipopolysaccharide-induced interleukin-1 production by peripheral blood mononuclear cells from the 3 study groups*

Group	n	Change from baseline at week 24, units/ml
Olive oil	11	−243.1 (−540.4, 54.2)
Low-dose fish oil	18	−239.9 (−490.4, 10.6)†
High-dose fish oil	17	−416.2 (−623.0, 209.4)‡

* Values are the mean (95% confidence interval). One unit represents 50% maximal proliferation in the C3H/HeJ costimulation assay (see Patients and Methods).
† $P = 0.059$ versus baseline.
‡ $P = 0.0005$ versus baseline.

Table 6. Concanavalin A–induced interleukin-2 production by peripheral blood mononuclear cells from the 3 study groups*

Group	n	Change from baseline at week 24, units/ml
Olive oil	9	34 (−7.6, 92.9)
Low-dose fish oil	15	25 (−18.7, 68.7)
High-dose fish oil	11	1.5 (−21.3, 24.2)

* Values are the mean (95% confidence interval). One unit represents 50% maximal proliferation in the HT-2 cell bioassay.

ration was also noted after PHA stimulation, but was significant only in the high-dose fish oil group, which showed an increase of 9.8% from baseline ($P = 0.04$). An increase from baseline levels of thymidine incorporation after PWM stimulation was observed in all groups: 33.2% ($P = 0.0007$) in the olive oil group, 38.9% ($P = 0.02$) in the low-dose fish oil group, and 19.9% ($P = 0.06$) in the high-dose fish oil group.

Ig production. Production of IgG in vitro in the absence of mitogen stimulation decreased by 28.1% ($P = 0.02$) in the low-dose group and by 17.6% ($P = 0.05$) in the high-dose group at 24 weeks (Table 8). IgG production after mitogen stimulation was not statistically significantly changed. Production of IgA in non–mitogen-stimulated cultures decreased by 36.1% ($P = 0.02$) (results not shown). No other significant changes in IgA, IgG, or IgM production were observed.

DISCUSSION

We have previously demonstrated significant improvements in selected clinical variables in RA patients who ingested omega-3 fatty acids for 12–14 weeks (6,14). We report here that the beneficial effects of omega-3 fatty acids on the clinical manifestations of RA, including fewer tender and swollen joints, decreased duration of morning stiffness, improvements in grip strength, and physician assessments of pain and disease activity, are sustained and more commonly observed after 18–24 weeks of treatment.

The improvement from baseline in the patients ingesting fish oil were usually not statistically significant compared with the patients taking olive oil supplements. It should be noted, however, that the clinical outcomes in the olive oil group may have been biased toward more favorable results because of the withdrawal of 11 of the original 23 patients in this study group. Six of these 11 patients were withdrawn for reasons relating to increased disease activity and the concomitant need for an increased oral steroid dose, change in DMARD, or intraarticular steroid injection. The distribution of withdrawals because of increased disease activity in the olive oil group compared with both fish oil groups was significant ($P = 0.008$) (Table 1).

Conversely, olive oil itself could have beneficial effects in RA patients due to cell membrane (lipid) changes in lymphocytes resulting in altered immune

Table 7. Mitogen-induced proliferation of lymphocytes from the 3 study groups, as determined by ^{3}H-thymidine incorporation*

Culture condition, duration	^{3}H-thymidine incorporation, $\times 10^3$ cpm/culture					
	Olive oil (n = 12)		Low-dose fish oil (n = 20)		High-dose fish oil (n = 17)	
	Baseline	24 weeks	Baseline	24 weeks	Baseline	24 weeks
Medium, 5 days	1.9 (0.3, 3.5)	2.2 (0.3, 4.0)	2.0 (0.9, 3.0)	1.6 (0.7, 2.4)	1.1 (0.7, 1.5)	1.8 (0.3, 3.2)
Mitogen						
Con A, 3 days	24.8 (19.2, 30.5)	42.4† (22.4, 63.7)	25.1 (20.0, 30.1)	45.7† (37.9, 53.4)	28.4 (22.1, 34.7)	43.1† (36.2, 50.0)
PHA, 3 days	71.5 (58.2, 84.9)	83.6 (63.3, 10.4)	66.8 (55.4, 78.3)	74.3 (62.4, 86.2)	69.7 (56.6, 82.7)	76.5† (64.2, 88.8)
PWM, 5 days	32.3 (25.0, 39.6)	43.1† (34.7, 50.2)	35.2 (28.6, 41.8)	48.9† (36.3, 61.4)	38.3 (30.4, 46.2)	45.9 (35.9, 55.9)
SA, 5 days	6.6 (5.5, 7.8)	6.9 (2.3, 10.8)	7.8 (6.1, 9.3)	9.8 (6.9, 12.5)	8.5 (5.9, 10.9)	9.1 (6.9, 11.3)

* Values are the mean (95% confidence interval) of triplicate cultures (<15% variance) for each patient. Peripheral blood mononuclear cells (1×10^5) were cultured for 3 or 5 days with or without mitogen and pulsed with ^{3}H-thymidine. Con A = concanavalin A; PHA = phytohemagglutinin; PWM = pokeweed mitogen; SA = *Staphylococcus aureus* (formalin-fixed).
† $P \leq 0.04$ versus baseline.

Table 8. Spontaneous and pokeweed mitogen–induced in vitro IgG production by peripheral blood mononuclear cells from the 3 study groups*

		IgG production, ng/ml			
		Baseline		24 weeks	
Group	n	Spontaneous	PWM-induced	Spontaneous	PWM-induced
Olive oil	12	185 (129, 290)	744 (234, 1,252)	168 (115, 222)	653 (189, 1,117)
Low-dose fish oil	19	191 (147, 234)	764 (426, 1,103)	136† (118, 156)	849 (520, 1,179)
High-dose fish oil	17	185 (139, 232)	903 (461, 1,344)	152† (119, 186)	813 (338, 1,288)

* Values are the mean (95% confidence interval). Cells were cultured for 7 days in the presence and absence of pokeweed mitogen (PWM; 1:200).
† $P < 0.05$ versus baseline.

function through a variety of mechanisms (25–28). Dietary lipid modulation of immune responses has been recently reviewed (28). Olive oil has been demonstrated to induce immune changes in the MLR/*lpr* mouse model of autoimmune disease (17) and to result in significant clinical improvements when ingested by RA patients over a period of several weeks (29). In the present investigation, significantly increased reactivity was observed in lymphocytes from patients ingesting olive oil after Con A stimulation and after PWM stimulation. IL-1 also decreased by 38% from baseline in these patients, but did not achieve statistical significance. Whether these effects have clinical significance is unclear. Further studies of the effects of monounsaturated fatty acids on clinical and immune variables in patients with RA are warranted.

We have previously noted a correlation between the magnitude of the decrease in LTB_4 and the reduction in the number of tender joints on physical examination in patients ingesting fish oil (6), and it would be tempting to link the decrease in this potent inflammatory substance to clinical improvements. In the current investigation, there was a highly significant decrease in the production of IL-1 after fish oil ingestion. LTB_4 augments IL-1 production by human monocytes (30). Thus, diminished production of IL-1 might occur in association with an omega-3 fatty acid–induced decrease in LTB_4. In vitro studies have shown that lipoxygenase inhibitors also decrease the production of IL-1 (30), suggesting that modulation of LTB_4 influences IL-1 production.

Decreased IL-1 production has recently been observed in normal subjects ingesting dietary supplements of omega-3 fatty acids (31). Interestingly, those authors reported that the decrease in IL-1 was greatest 10 weeks after discontinuing the supplements. We have noted sustained clinical benefits after discontinuing fish oil supplements in a previous crossover investigation (6). In the current study, we observed that significant benefits continued in several clinical parameters 6 weeks after discontinuing fish oil supplements, and it is possible that the previously reported prolonged decrease in IL-1 contributes to these effects after discontinuing the supplements.

IL-1 exerts many effects that could result in increased disease activity in patients with RA (32–41). Suppression of these varied biologic activities through decreased production of IL-1 after fish oil ingestion would be expected to result in improvement in the disease manifestations of RA. It should be emphasized, however, that although both neutrophil LTB_4 and macrophage IL-1 production significantly decreased after fish oil ingestion, specific correlations between these changes and improvement in individual clinical parameters were not observed.

The significant increases in T cell and B cell reactivity to mitogenic stimulation after fish oil ingestion, observed ex vivo, confirm previous observations in asthmatic patients ingesting 4.0 gm of EPA for 8 weeks (42). The investigators observed a substantial increase in PHA-induced T cell proliferation, with a concomitant decrease in the T helper:suppressor cell ratio. Although enhanced proliferation was observed after the olive oil as well as the fish oil supplements, the other immune-related assays were not significantly altered by olive oil ingestion. Since clinically significant improvements with olive oil supplements were less common than with fish oil, the lymphoproliferation assay does not appear to be a good correlate for efficacy determinations.

Lipids have been shown to have numerous immunomodulatory effects (43). It is possible that the monounsaturated fatty acid (oleic acid) found in olive oil may have an enhancing effect on lymphocyte proliferation through a variety of mechanisms, but may have less significant inhibitory effects on LTB_4, IL-1, or IgG production, which are more significantly inhibited by high-dose fish oil. Omega-3 fatty acids may preferentially reduce T helper cell function (41), or alternatively, several lines of in vitro evidence indicate that enhanced T suppressor cell activity could occur (43–45). Either of these effects would decrease activation both of macrophages, which are major producers of LTB_4 and IL-1, and of B cells, which produce autoantibodies in patients with RA. Down-regulation of LTB_4, IL-1, and spontaneous IgG production should result in significant beneficial effects in these patients. Since we observed that IL-2 production is not inhibited by fish oil ingestion, its major effect may be enhancement of T suppressor cell or T suppressor/inducer cell activity. Differential effects of fish oil on the functional reactivities of the T cell subsets will need to be assessed.

Statistically significant changes in clinical parameters were more commonly observed in the high-dose fish oil group (Table 3). It should also be noted that IL-1 levels decreased by 54.7% from baseline in the patients ingesting high-dose fish oil, compared with a 40.6% decrease in the low-dose group and a 38.5% decrease in the olive oil group. Of interest in this context is the recent observation that the antihypertensive effects of fish oil in patients with essential hypertension are dose-dependent (46).

With the demonstration of sustained clinical improvements and favorable alterations in neutrophil LTB_4 and macrophage IL-1 production, it is appropriate to examine the side effects of fish oil. The potential for toxicity should be judged in the context of the medications used for the treatment of RA. Minor gastrointestinal intolerance or eructation (6,14) is not clinically significant when measured against the potential significant toxicities of the other agents. We have previously observed that patients with RA who take aspirin or an NSAID and fish oil supplements have a statistically significant increase in bleeding time (Ivy method) (14). However, the bleeding time remained within the normal range and the increase was not considered to be clinically significant.

A reduction in the level of several mediators of inflammation (15) and an improvement in clinical disease activity after fish oil ingestion in patients with RA have been observed by other investigators (47). Significant decreases in IL-1 after fish oil consumption in normal volunteers have recently been reported (31). Based upon the frequency with which significant clinical improvements were noted in the high-dose fish oil group compared with patients in the low-dose group, it appears that an individual would be more likely to benefit from ingestion of higher doses of $\omega 3$ fatty acids for sustained periods. We therefore believe that further studies to elucidate the effects of dietary manipulations of $\omega 3$ and monounsaturated fatty acids over extended periods are both necessary and appropriate in patients with RA.

REFERENCES

1. Stansby ME: Nutritional properties of fish oils. World Rev Nutr Diet 11:46–105, 1969
2. Dyerberg J, Bang HO, Stoffersen G, Moncada S, Vane JR: Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. Lancet II:117–119, 1978
3. Lee TH, Mencia-Huerta J-M, Shih C, Corey EJ, Lewis RA, Austen KF: Effects of exogenous arachidonic, eicosapentaenoic, and docosahexaenoic acids on the generation of 5-lipoxygenase pathway products by ionophore-activated human neutrophils. J Clin Invest 74: 1922–1933, 1984
4. Fischer S, Weber PC: Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. Nature 307: 165–168, 1984
5. Lee TH, Hoover RL, Williams JD, Sperling RI, Ravalese JR III, Spur BW, Robinson DR, Corey EJ, Lewis RA, Austen KF: Effect of dietary enrichment with eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. N Engl J Med 312:1217–1224, 1985
6. Kremer JM, Jubiz W, Michalek A, Rynes RI, Bartholomew LE, Bigaouette J, Timchalk MA, Beeler D, Lininger L: Fish-oil fatty acid supplementation in active rheumatoid arthritis. Ann Intern Med 106:497–503, 1987
7. Payan DG, Goetzl EJ: Specific suppression of human T lymphocyte function by leukotriene B_4. J Immunol 131:551–553, 1983
8. Gualde N, Durgaprasadarao A, Goodwin JS: Effect of lipoxygenase metabolites of arachidonic acid on proliferation of human T cells and T cell subsets. J Immunol 134:1125–1129, 1985
9. Rola-Pleszczynski M, Bouvrette L, Gingras D, Girard M: Identification of interferon-γ as the lymphokine that mediates leukotriene B_4-induced immunoregulation. J Immunol 139:513–517, 1987
10. Blomgren H, Hammarstrom S, Wasserman J: Synergistic enhancement of mitogen responses of human lym-

phocytes by inhibitors of cyclo-oxygenase and 5,8,11-eicosatriynoic acid, an inhibitor of 12-lipoxygenase and leukotriene biosynthesis. Int Arch Allergy Appl Immunol 83:247–255, 1987
11. Rola-Pleszczynski M, Lemaire I: Leukotrienes augment interleukin-1 production by human monocytes. J Immunol 135:3958–3961, 1985
12. Dayer JM, de Rochemonteix B, Burrus B, Demczuk S, Dinarello CA: Human recombinant interleukin-1 stimulates collagenase and prostaglandin E_2 production by human synovial cells. J Clin Invest 77:645–648, 1986
13. Dinarello CA: An update on human interleukin-1: from molecular biology to clinical relevance. J Clin Immunol 5:287–297, 1985
14. Kremer JM, Michalek AV, Lininger L, Huyck C, Bigaouette J, Timchalk MA, Rynes RI, Zieminski J, Bartholomew ME: Effects of manipulation of dietary fatty acids on clinical manifestations of rheumatoid arthritis. Lancet I:184–187, 1985
15. Sperling RI, Weinblatt M, Robin J-L, Ravalese J III, Hoover RL, House F, Coblyn JS, Fraser PA, Spur BW, Robinson DR, Lewis RA, Austen KF: Effects of dietary supplementation with marine fish oil on leukocyte lipid mediator generation and function in rheumatoid arthritis. Arthritis Rheum 30:988–997, 1987
16. Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, Djordjevic BS, Dontas AS, Fidanza F, Keys MH, Kromhout D, Nedeljkovic S, Punsar S, Seccareccia F, Toshima H: The diet and 15-year death rate in the seven countries study. Am J Epidemiol 124:903–915, 1986
17. Godfrey DG, Stimson WH, Watson J, Belch JF, Sturrock RD: Effects of dietary supplementation on autoimmunity in the MRL/lpr mouse: a preliminary investigation. Ann Rheum Dis 45:1019–1024, 1986
18. Ropes MW, Bennett GA, Cobb S, Jacox R, Jessar RA: 1958 revision of diagnostic criteria for rheumatoid arthritis. Bull Rheum Dis 9:175–176, 1958
19. Jubiz W, Draper RE, Gale J, Nolan G: Decreased leukotriene B_4 synthesis by polymorphonuclear leukocytes from male patients with diabetes mellitus. Prostaglandins Leukotrienes Med 14:305–311, 1984
20. Mendelsohn J, Skinner A, Kornfeld S: The rapid induction by PHA of increased r-aminoisobutyric acid uptake by lymphocytes. J Clin Invest 50:818–826, 1971
21. Noelle RJ, Lawrence DA: Determination of glutathione in lymphocytes and possible association of redox state and proliferative capacity of lymphocytes. Biochem J 198:571–579, 1981
22. Rosenwasser LJ, Dinarello CA: Antibody of leukocyte pyrogen to enhance phytohemagglutinin induced murine thymocyte proliferation. Cell Immunol 63:134–142, 1981
23. Gillis S, Germ MM, Ou W, Smith KA: T-cell growth factor, parameters of production and a quantitative microassay for activity. J Immunol 120:2027–2032, 1978
24. Check IJ, Piper M: Quantitation of immunoglobulin, Manual of Clinical Laboratory Immunology. Edited by NR Rose, H Friedman, JL Fahey. Washington, DC, ASM Press, 1986
25. Traill KN, Wick G: Lipids and lymphocyte function. Immunol Today 5:70–75, 1984
26. Johnston PV: Dietary fat, eicosanoids, and immunity. Adv Lipid Res 21:103–141, 1985
27. Del Buono BJ, Williamson PL, Schlegel RA: Alterations in plasma membrane lipid organization during lymphocyte differentiation. J Cell Physiol 126:379–388, 1986
28. Erickson KL: Dietary fat modulation of immune response. Int J Immunopharmacol 8:529–543, 1986
29. Darlington LG, Ramsay NW: Olive oil for rheumatoid arthritis? (abstract). Br J Rheumatol 26 (suppl 2):215, 1987
30. Dinarello CA, Bishai I, Rosenwasser J, Coceani F: The influence of lipoxygenase inhibitors on the in vitro production of human leukocytic pyrogen and lymphocyte activity factor (IL-1). Int J Immunopharmacol 1:43–50, 1984
31. Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JMW, Cannon JG, Rogers TS, Klempner MS, Weber PC, Schaeffer EJ, Wolff SM, Dinarello CA: The effects 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 320:265–271, 1989
32. Dinarello CA: Interleukin-1. Rev Infect Dis 6:51–90, 1984
33. Dinarello CA: Interleukin-1 and the pathogenesis of the acute-phase response. N Engl J Med 311:1413–1418, 1984
34. Luger TA, Charon JA, Colot M, Micksche M, Oppenheim JJ: Chemotactic properties of partially purified human epidermal cell-derived thymocyte-activating factor for polymorphonuclear and mononuclear cells. J Immunol 131:816–820, 1983
35. Schmidt JA, Mizel SB, Cohen D, Green I: Interleukin-1: a potential regulator of fibroblast proliferation. J Immunol 128:2177–2182, 1982
36. Mizel SB, Dayer JM, Krane SM, Mergenhagen SE: Stimulation of rheumatoid synovial cell collagenase and prostaglandin production by partially purified lymphocyte-activating factor (interleukin-1). Proc Natl Acad Sci USA 78:2474–2477, 1981
37. Goto M, Sasano M, Yamanaka H, Miyasaka N, Kamatani N, Inoue K, Nishioka K, Miyamoto T: Spontaneous production of an interleukin 1–like factor by cloned rheumatoid synovial cells in long-term culture. J Clin Invest 80:786–796, 1987
38. Miyasaka N, Sato K, Goto M, Sasano M, Natsuyama M, Inoue K, Nishioka K: Augmented interleukin-1 production and HLA-DR expression in the synovium of

rheumatoid arthritis patients: possible involvement in joint destruction. Arthritis Rheum 31:480–486, 1988
39. Di Giovine FS, Malawista SE, Nuki G, Duff GW: Interleukin 1 (IL 1) as a mediator of crystal arthritis: stimulation of T cell and synovial fibroblast mitogenesis by urate crystal-induced IL 1. J Immunol 138:3213–3218, 1987
40. Gowen M, Wood DD, Ihrie EJ, McGuire MKB, Russell BGG: An IL-1 like factor stimulates bone resorption in vitro. Nature 306:378–380, 1983
41. Saklatvala J, Sarsfield SJ, Pilsworth LM: Characterization of proteins from human synovium and mononuclear leukocytes that induce resorption of cartilage proteoglycan in vitro. Biochem J 209:337–344, 1983
42. Goetzl EJ, Wong M, Payan DG, Chernof-Rogan T, Pickett WC, Blake VA: Effects of eicosapentaenoic acid on immune responses and inflammation in humans, Health Effects of Polyunsaturated Fatty Acids in Seafoods. Edited by FA Simopoulos, RR Kifer, RE Martin. New York, Academic Press, 1986
43. Hwang D: Essential fatty acids and immune response. FASEB J 3:2052–2061, 1989
44. Webb DR, Nowowiejski I: The role of prostaglandins in the control of the primary 19S immune response to SRBC. Cell Immunol 33:1–10, 1977
45. Payan DG, Goetzl EJ: Specific suppression of human T lymphocyte function by leukotriene B_4. J Immunol 131:551–553, 1985
46. Knapp HR, Fitzgerald GA: The antihypertensive effects of fish-oil: a controlled study of polyunsaturated fatty acid supplements in essential hypertension. N Engl J Med 320:1037–1043, 1989
47. Cleland LG, French JK, Betts WH, Murphy GA, Elliott MJ: Clinical and biochemical effects of dietary fish-oil supplements in rheumatoid arthritis. J Rheumatol 15: 1471–1475, 1988