Role of reactive oxygen species in rheumatoid arthritis synovial T lymphocytes

Remans, Philip Herman Jozef

CHAPTER 5

Nutrient supplementation with poly-unsaturated fatty acids and micronutrients in rheumatoid arthritis: clinical and biochemical effects.

P.H.J. Remans[1], J.K. Sont[2], L.W. Wagenaar[3], W. Wouters-Wesseling[3], W.M. Zuijderduin[1], A. Jongma[1], F.C. Breedveld[1], J.M. van Laar[1].

Departments of [1]Rheumatology, [2]Medical Decision Making, Leiden University Medical Center, Leiden, The Netherlands, and [3]Numico Research, Wageningen, The Netherlands.

Eur J Clin Nutr. 2004; 58: 839-45

Abstract

Objective: To investigate in a double blind placebo-controlled, parallel group study the effects of a nutrient supplement, containing, among other ingredients, the omega-3 fatty acids eicosapentaenoic acid (1.4 g EPA), docosahexaenoic acid (0.211 g DHA), omega-6 fatty acid gamma-linolenic acid (0.5 g GLA) and micronutrients in patients with active RA.
Design, subjects and intervention: RA patients were randomized to receive either daily liquid nutrient supplementation or placebo for 4 months. The primary endpoint was the change in tender joint count at 2 and 4 months. Other clinical variables included swollen joint count, visual analogue scales for pain and disease activity, grip strength, functionality score, and morning stiffness. Biochemical parameters included plasma concentrations of PUFA and vitamins C and E.
Setting: Outpatient university clinic.
Results: *S*ixty-six patients enrolled, 55 completed the study. No significant change from baseline in tender joint count or any of the other clinical parameters was detected in either group. Patients receiving nutrient supplementation but not those receiving placebo had significant increases in plasma concentrations of vitamin E (p=0.015), and EPA, DHA and docosapentaenoic acid (DPA) concomitant with decreases of arachidonic acid (AA)(p = 0.01). Intergroup differences for PUFA and vitamin E were significantly different (p=0.01 and 0.03 respectively).
Conclusions: This double-blind, placebo-controlled study in RA patients did not show superior clinical benefit of daily nutrient supplementation with EPA, GLA and micronutrients at the doses tested as compared to placebo. The study adds information regarding doses of omega-3 fatty acids below which anti-inflammatory effects in RA are not seen.

Introduction

Nutrient supplementation as add-on therapy in rheumatoid arthritis (RA) has witnessed a resurgence of scientific interest due to preclinical and clinical studies on supplementation with polyunsaturated fatty acids (PUFA) or micronutrients.
Initial studies, demonstrating beneficial effects of dietary fatty acids, made use of fish oils, that contains the omega-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). When supplemented these lipids compete with arachidonic acid (AA) for incorporation in cellular membranes, resulting in decreased synthesis of specific leukotrienes and prostaglandins (1). Furthermore, downmodulatory effects on pro-inflammatory cytokines have been described. Similar clinical results have been published with the omega-6 fatty acid gamma-linolenic acid (GLA), isolated from certain plant and seed oils (2-4). Administration of GLA leads to an increased incorporation of its metabolite dihomogammalinolenic acid (DGLA) in cell membranes, thus blocking the metabolism of AA and changing the balance of lipid mediators towards the production of less potent eicosanoids. Several clinical studies in RA patients have demonstrated modest but reproducable beneficial effects on joint tenderness of supplements with EPA + DHA at doses 2.3 – 7.1 g per day (5). Perhaps due to the high number of capsules needed to achieve clinical effects combined with their fishy aftertaste, however, nutritional supplements with PUFA have not received the same status as conventional antiphlogistic and analgesic drugs in the management of RA (6).
Micronutrient supplementation too has been propagated for its potential benefit in RA, based on the low antioxidant status in RA patients as compared to healthy controls, the role of chronic oxidative stress in functional hyporesponsiveness of synovial T lymphocytes, and the effects of reactive oxygen species (ROS) in cartilage degradation, inflammation and cell signaling (7-10). Additionally, dietary intake of several micronutrients has been reported to be inadequate in RA patients (11). In animal models, high doses of vitamin C, vitamin E and N-acetyl-cysteine had

anti-arthritic effects related to their anti-oxidative potential (12-14). Other candidate antioxidants have been identified with potential benefit including selenium, zinc, manganese, niacinamide, bioflavonoids and ß carotene. Nevertheless, clinical trials with anti-oxidants in RA patients have been disappointing. A randomized double-blind comparison of high-dose vitamin E (1200 mg daily), the most powerful naturally occurring lipid soluble antioxidant, did not show any anti-inflammatory effect as compared to placebo (15). Two placebo-controlled trials with selenium-enriched yeast (200µg/d and 156µg/d) did not result in detectable clinical benefit (16, 17).
Given the lack of evidence to date demonstrating anti-arthritic effects of supplementation with a single anti-oxidant, it has been suggested that combination of several such nutrients might be efficacious (18, 19). We therefore developed a liquid nutritional supplement as a vehicle to deliver supplementary concentrations of EPA, GLA and selected micronutrients to investigate its clinical and biochemical effects in a prospective, double-blind, placebo controlled, parallel group trial in patients with active rheumatoid arthritis.

Subjects and methods

Patients

Sixty-six patients with an established diagnosis of RA enrolled in a single center study (outpatient clinic LUMC) between August 2000 and September 2001. The study was approved by the Medical Ethical Committee and informed consent was obtained from all patients. All had active disease defined as 6 or more tender or swollen joints, morning stiffness > 1 hour, or erythrocyte sedimentation rate (ESR) > 25 mm/h. Concurrent treatment for RA with corticosteroids and/or disease modifying antirheumatic drugs was required to be stable for at least 2 months. Exclusion criteria were serious gastro-intestinal disease (inflammatory bowel disease, atrophic gastritis, stoma), malignancy, previously established intolerance of fish oil, or regular use of dietary supplements containing fish oil and/or antioxidants.

Study design, randomization, and supplementation

This was a placebo-controlled, randomized, double-blind, parallel group intervention trial. Patients were randomly allocated to receive either a liquid nutritional supplement containing polyunsaturated fatty acids and micronutrients or the placebo, one serving daily for the duration of 4 months. The product was specially produced for this study. The composition of the supplement is shown in **Table 1**. Placebo-drinks with same taste, odor and color were produced with water, sweetener (sodiumsaccharine/acesulpham-k), cloudifier, flavor and colorant. Both drink-feeds were provided in 200-ml tetrapacks (white) in two fruit flavors (forest and citrus fruit). The formulae were randomized and the tetrapacks were labeled with the appropriate number before delivery, and the patients were allocated a number on enrolment of the study. Patients and physicians were kept blinded to treatment assignment during the entire study. Patients were instructed to continue their dietary habits. Clinical parameters were evaluated at baseline and after 2 and 4 months by the same observer.

Sample size

The primary outcome parameter was the change of tender joint count. With alpha 0.05 (two-sided), beta 0.8 and allowing for a 20% drop-out rate a sample size of 32 patients per group was needed to detect a difference in tender joint count of 4, given a baseline tender joint count of 13 and standard deviation of 5.1.

Table 1 Contents of nutrient supplement per 200 ml

Energy (kcal)	150
Protein (whey) (kcal)	7.5 (20% of En)
Cysteine (mg)	170
Glutamin/mate (μg)	412
Carbohydrates (g)	19.4 (52% of En)
Fat (g)	4.7 (28% of En)
n-3 PUFA	
Eicosapentaenoic acid (mg)	1400
Docosahexaenoic acid (mg)	211
Docosapentaenoic acid (mg)	40
Alpha-linolenic acid (mg)	16
n-6 PUFA	
Gamma-linolenic acid (mg)	500
Linoleic acid (mg)	440
Dietary fibre (g)	3
Minerals	
Calcium (mg)	235
Phosphorus (mg)	235
Magnesium (mg)	37.5
Trace elements	
Iron (mg)	9.0
Zinc (mg)	9.0
Copper (mg)	0.75
Manganese (μg)	0.75
Selenium (μg)	150.0
Molybdenum (μg)	16.6
Chromium (μg)	11.0
Iodide (μg)	34.0
Fluoride (mg)	0.3
Vitamins	
Vit A (μg)	200
Vit D (μg)	3.75
Vit E (mg)	37.5
Vit K (μg)	12
Vit C (mg)	150
Thiamin (mg)	0.3
Riboflavin (mg)	0.75
Niacin (mg)	6.0
Pyridoxin (B_6) (mg)	1.3
Vit B_{12} (μg)	1.6
Folic acid (μg)	200
Panthothenic acid (mg)	1.2
Biotin (μg)	11.3
Others	
Choline (mg)	60
Coenzyme Q10 (mg)	2
Flavonoids (mg)	10
Carotenoids (mg)	1.5

Clinical evaluation
Patients participating in the study were evaluated at the start of the trial, and after 2 and 4 months. The clinical evaluation encompassed a 28-tender joint count and a swollen joint count (using a dichotomous scale: 0 = absent, 1= present for both counts), patient's visual analogue scale (VAS) for pain, disease activity, general health and physician's VAS for disease activity. Based on these data the 20% response rate as defined by the American College of Rheumatology (ACR20-response) criteria (20) and Disease Activity Score (DAS28, 28-joint count)(21) were calculated. Gripstrength was measured using a manometer (kPa). Duration of morning stiffness (in minutes), anthropometric (weight and height) data and concomitant drugs were recorded. Additionally, patients were asked to document their NSAID intake during the first week after initiation and the week prior to termination in a diary, and to complete a Health Assessment Questionnaire (HAQ) and Arthritis Impact Measurement Scale (AIMS).

Laboratory evaluation
All mentioned variables were performed on fasting blood samples at baseline and after 4 months. Plasma concentrations of fatty acids and vitamin C and E were measured in a subset of both groups (11 supplement + 12 placebo for fatty acids, and 20 supplement + 19 placebo for vitamins C and E). The fatty acid composition of the phospholipid fraction was determined in plasma as follows: after total lipid extraction by the method of Bligh and Dyer (22), phospholipids were separated from the total lipid fraction by column chromatography using bonded phase columns (23). Fatty acids were then methylated with boron trifluoride in methanol according to the method of Morrison and Smith (24). The methyl esters (FAME) were separated and quantified by capillary gas chromatography (Shimadzu GC-17A) with a CPSIL88 column (Chrompack; Middelburg, Netherlands).
Vitamin C and E were analyzed by HPLC as previously described (25, 26).

Data analysis
All statistical analyses were done with the statistical software package STATA 6.0 (StataCorp, College Station, TX, USA). For both treatment groups, Student's paired t-test was applied to assess whether the outcome at 2 and 4 months differed from the baseline value. Unpaired t-tests were used to test whether baseline values and/or the
changes from baseline were different between the groups. Statistical significance was accepted at a probability level of 0.05. Correlation analysis was performed in order to assess whether changes after 4 months of treatment were consistent among biochemical parameters.

Results

Sixty-six patients enrolled in the trial: 33 in the placebo group and 33 receiving the nutrient supplement. The two groups did not differ with respect to clinical and demographic variables at baseline, except for a higher age in the experimental group. Eleven subjects (17%) dropped out during the 4 months treatment period: 3 (5%) due to gastro-intestinal intolerance (2 in the experimental group, 1 in the control group), 8 (12%) due to lack of efficacy (5 in the experimental group and 3 in the control group). Baseline characteristics of completers in both groups are shown in **Table 2**.
Fifty-five patients completed the 4 months of treatment: 29 receiving placebo and 26 the supplement. Changes in clinical parameters are listed in **Table 3**. Mean tender joint count, the primary study parameter, did not change significantly between the groups nor within each group. Disease activity as measured with DAS28 deteriorated in both groups after 4 months, but this attained statistical significance only in the control group, but not in the experimental group or between the groups. Two patients receiving the supplement and 2 receiving placebo achieved an

Table 2 Baseline characteristics of patients who completed the study (data expressed as mean ± s.d.)

Variable	*Placebo (n=29)*	*Nutrient supplement (n=26)*
Sex (m:f)	7:22	3:23
Age (y)*	52.9 ± 11.2	59.5 ± 11.0
Disease duration (y)	11.7 ± 11.1	13.6 ± 11.9
DMARD users[a]	28/29	22/26
DAS 28[b]	5.14 ± 1.05	5.43 ± 0.94

*Significantly higher in supplement group ($P<0.05$).
[a]DMARD=disease-modifying antirheumatic drug. Placebo group: methotrexate 16, methotrexate + sulfasalazine + hydroxychloroquine 1, sulfasalazine 9, gold 1, d-penicillamine 1, prednisone 3; supplement group: methotrexate 16, methotrexate + sulfasalazine + hydroxychloroquine 1, sulfasalazine 4, gold 1, prednisone 1.
[b]DAS28=disease activity score, 28-joint count.

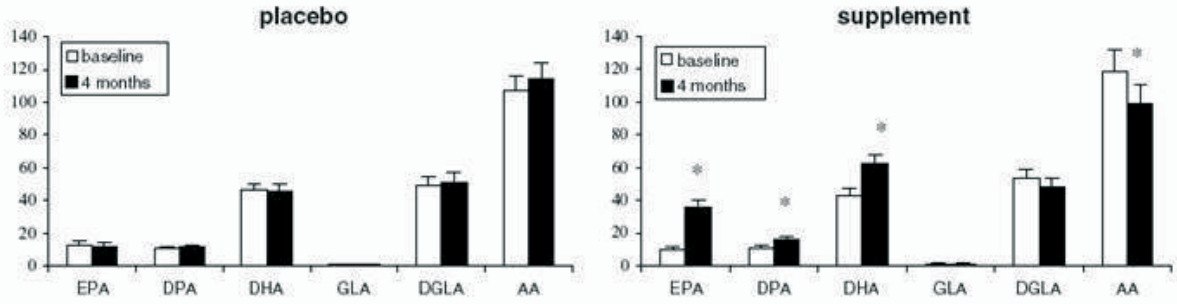
Table 3 Changes in clinical parameters (mean ± s.d.) in patients who completed the study

	Placebo (n=29)	*Supplement (n=26)*		*Placebo*	*Supplement*
Parameter			*Parameter*		
Tender joint count			Morning stiffness (min)		
Baseline ± s.d.	8.7 ± 5.3	10.7 ± 4.9	Baseline ± s.d.	83 ± 100	63 ± 68
Δ Mean 2 months ± s.d.	1.0 ± 3.0	−0.8 ± 4.4	Δ Mean 2 months ± s.d.	−9 ± 67	2 ± 69
Δ Mean 4 months ± s.d.	1.0 ± 5.1	0.0 ± 4.1	Δ Mean 4 months ± s.d.	−12 ± 40	13 ± 70
Swollen joint count			Grip str. R. (kPa)		
Baseline ± s.d.	8.8 ± 3.5	9.4 ± 3.4	Baseline ± s.d.	193 ± 79	183 ± 72
Δ Mean 2 months ± s.d.	−0.3 ± 3.8	−0.6 ± 4.9	Δ Mean 2 months ± s.d.	7 ± 56	1 ± 29
Δ Mean 4 months ± s.d.	−0.7 ± 4.14	0.5 ± 4.6	Δ Mean 4 months ± s.d.	6 ± 61	−7 ± 27
DAS28[a]			Grip str. L. (kPa)		
Baseline ± s.d.	5.14 ± 1.05	5.36 ± 0.92	Baseline ± s.d.	192 ± 80	169 ± 69
Δ Mean 2 months ± s.d.	0.22 ± 0.74	−0.01 ± 0.82	Δ Mean 2 months ± s.d.	−4 ± 55	−5 ± 27
Δ Mean 4 months ± s.d.	0.21 ± 0.93	0.22 ± 0.77	Δ Mean 4 months ± s.d.	−2 ± 45	−2 ± 32
VAS[a] health (mm)			HAQ[a]		
Baseline ± s.d.	48 ± 18	47 ± 18	baseline ± s.d.	1.16 ± 0.64	1.23 ± 0.62
Δ Mean 2 months ± s.d.	−1 ± 21	4 ± 25	Δ Mean 2 months ± s.d.	ND	ND
Δ Mean 4 months ± s.d.	3 ± 9	7 ± 23	Δ Mean 4 months ± s.d.	0.06 ± 0.23	−0.06 ± 0.33
VAS[a] patient (mm)			AIMS[a]		
Baseline ± s.d.	55 ± 18	50 ± 18	Baseline ± s.d.	5.12 ± 1.40	4.83 ± 1.47
Δ Mean 2 months ± s.d.	−3 ± 21	2 ± 21	Δ Mean 2 months ± s.d.	ND	ND
Δ Mean 4 months ± s.d.	−4 ± 17	5 ± 18	Δ Mean 4 months ± s.d.	0.01 ± 1.37	0.46 ± 1.48
VAS[a] physician (mm)			Weight (kg)		
Baseline ± s.d.	35 ± 17	39 ± 18	Baseline ± s.d.	77.9 ± 15.6	71.6 ± 11.0
Δ Mean 2 months ± s.d.	2 ± 23	−2 ± 17	Δ Mean 2 months ± s.d.	0.3 ± 1.3	0.9 ± 1.3*
Δ Mean 4 months ± s.d.	3 ± 18	−1 ± 7	Δ Mean 4 months ± s.d.	0.0 ± 2.0	0.8 ± 1.5
C-reactive protein (mg/l)			BMI (kg/m^2)		
Baseline ± s.d.	18.6 ± 19.8	14.8 ± 12.4	Baseline ± s.d.	26.4 ± 4.9	25.6 ± 3.3
Δ Mean 2 months ± s.d.	0.0 ± 10.5	1.0 ± 10.6	Δ Mean 2 months ± s.d.	0.1 ± 0.4	0.31 ± 0.46
Δ Mean 4 months ± s.d.	−0.4 ± 11.2	2.6 ± 10.0	Δ Mean 4 months ± s.d.	0.0 ± 0.7	0.31 ± 0.59
ESR (mm/first hour)			NSAID (tablets/week)		
Baseline ± s.d.	29 ± 23	30 ± 21	Baseline ± s.d.	12.5 ± 11.5	12.0 ± 3.9
Δ Mean 2 months ± s.d.	4 ± 12	1 ± 10	Δ Mean 2 months ± s.d.	ND	ND
Δ Mean 4 months ± s.d.	2 ± 9	4 ± 10	Δ Mean 4 months ± s.d.	0.8 ± 3.6	0.6 ± 2.9

[a]DAS28 denotes disease activity score, VAS visual analogue scale, HAQ health-assessment questionnaire, AIMS arthritis impact measurement scale.For both treatment groups, Student's paired *t*-test was applied to assess whether the outcome at 2 and 4 months differed from the baseline value. Unpaired *t*-tests were used to test whether baseline values and/or the changes from baseline were different between the groups. With statistical significance accepted at a probability level of 0.05, none of the changes reached significance except for the change in body weight in the supplement group at 2 months ($P=0.002$).

ACR20 response at 4 months. When the individual components of the ACR20 response criteria and DAS28 outcome criteria were analyzed, no significant changes within the groups nor differences in changes between the groups were detected. Functional assessment by means of the AIMS and HAQ questionnaire also showed no changes. No differences in the taking of NSAIDs were found. A statistically significant increase in body weight was detected in the experimental group at 2 months, but the changes in weight and BMI were not significant between the groups. **Figure 1** shows the changes in plasma concentrations of the key PUFA. All patients receiving nutrient supplementation had significant increases in the omega-3 PUFA (EPA, DPA, DHA) when compared to baseline. These changes were also significantly higher when compared to changes in the placebo group. The mean concentration of omega-6 PUFA (GLA, DGLA) did not change in either group. Plasma arachidonic acid (AA) concentrations significantly decreased in the patients taking the nutrient supplement, but not in the placebo group. Changes in plasma concentrations of EPA correlated inversely with AA (r –0.66, p=0.001) and positively with DPA and DHA (r 0.83 for both, p<0.0001), while those of DPA with DHA and GLA with DGLA also significantly correlated (r 0.75, p<0.0001, r 0.59, p=0.003 respectively). Increases in serum concentrations of vitamin C (mean±SD at baseline 51.7±25.1, delta mean 6.9±28.3; p 0.74) and vitamin E (mean±SD at baseline 33.4±7.5 mM, delta mean 4.3±6.5; p=0.015) were observed in the experimental arm, and decreases in the placebo-group of vitamin C (mean±SD at baseline 42.7±21.6 mM, delta mean –3.8±15.1; p=0.98) and vitamin E (mean±SD at baseline 34.6±8.6, delta mean –0.4±6.3; p=0.76). The intergroup difference in vitamin E was significant (p = 0.03).

Figure 1



- p<0.01 compared to baseline and compared to placebo;
- EPA denotes eicosapentaenoic acid, DPA docosapentaenoic acid, DHA docosahexaenoic acid, GLA gamma-linolenic acid, DGLA dihomogammalinolenic acid, AA arachidonic acid

Discussion

This is the first double-blind, placebo-controlled study to investigate the clinical efficacy of a daily nutrient supplementation containing polyunsaturated fatty acids (PUFA) and micronutrients in RA patients. Based on preclinical data it was postulated that the combination of these nutrients would produce synergistic or additive effects without compromising safety (27, 28). An odorless liquid nutritional supplement was developed as a vehicle to enhance compliance. Compared to placebo, supplementation resulted in significant increases in serum concentrations EPA, DPA, DHA, and vitamin E and a modest increase in body weight indicating that compliance was indeed achieved. This was further corroborated by correlations between individual PUFA. However, no statistically significant difference was found in the primary outcome parameter, the change in tender joint counts (TJC), nor in the other clinical parameters measured.

Several factors may have influenced the outcome of our study. First, our trial was designed as a pragmatic trial to evaluate the add-on therapeutic effect of nutrient supplementation in RA patients with persistent disease activity despite antiphlogistic and antirheumatic medication. This may have introduced bias by selection of patients in whom marked improvement of (symptoms of) disease activity is difficult to achieve. The probability to detect significant changes in TJC (but not the other clinical parameters) may have also been compromised because baseline values were lower than anticipated. Second, the metabolism and effects of PUFA and micronutrients may have been affected by continued use of NSAIDs and dietary factors. Recent studies reported that the beneficial effects of EPA are reduced when the diet is high in essential omega-6 PUFA by the intake of margarine and polyunsaturated oil (29, 30). With an average intake of omega n-6 PUFA in a Dutch diet of 12-13 g/day (31) (approximately 10x the amount in the supplement) such interaction cannot be ruled out. On the other hand, in most published studies patient populations did not differ from ours with respect to background medication and diet. Third, the concentrations of EPA, GLA and micronutrients may have been too low, even though marked and significant effects on plasma concentrations of EPA and AA were detected in the present study. Although we did not measure membrane fatty acid levels, changes in plasma fatty acids have been extensively used as surrogate markers. Previous reports demonstrated efficacy of slightly higher doses of omega-3 PUFA, but not of lower doses than used in our study. One study comparing low and high dose omega-3 PUFA (27 mg/kg EPA + 18 mg/kg DHA versus 54 mg/kg EPA + 36 mg/kg DHA respectively) showed equal effectiveness on tender and swollen joint counts, but only effects on other variables in the high dose group (32). Another study comparing daily supplements with either 2.6 gm of omega-3 PUFA, 1.3 gm omega-3 PUFA plus 3 gm olive oil, or 6 gm olive oil in patients with active RA only found significant clinical benefit in the first group (33). GLA has been shown to be clinically effective in RA at doses higher than 0.45 g/day, but the possibility of a dose dependence of these effects could explain the lack of efficacy in our trial. Food technological constraints precluded the use of higher concentrations of PUFA in our supplement. With respect to micronutrients little is known about the doses needed to induce immunomodulation (34). Studies with single antioxidants failed to demonstrate a significant clinical effect of high doses selenium, or vitamin E in RA patients (15-17).

Although our study was primarily designed to investigate potential add-on effects of PUFA and micronutrient supplementation on symptoms and disease activity, other beneficial clinical effects cannot be excluded, cardioprotective in particular. RA patients suffer from excessive cardiovascular morbidity and mortality when compared to age- and sex-matched individuals (35). Epidemiological and interventional studies have shown protective effects of fish oil and PUFA on cardiovascular events in various populations, in part through modulation of serum lipids (36). Interestingly, a recent study showed significant effects of supplementation with fish oil-derived PUFA and GLA on circulating plasma lipids and fatty acid profiles in healthy women, estimated to result in a 43% reduction in the risk of myocardial infarction (37). Whether

these results apply to patients with a chronic inflammatory disorders remains to be proven. Extrapolating results from these studies, large-scale clinical trials would be needed to demonstrate cardioprotective effects in RA.
We conclude that nutritional supplementation with micronutrients and PUFA at the doses tested does not ameliorate signs and symptoms in RA patients. More needs to be learned on the role of anti-oxidants in chronic inflammatory conditions such as RA, possible interactions (synergistic or antagonistic) between various anti-oxidants and PUFA, and dose-response relationships.

ACKNOWLEDGEMENTS
The authors thank Dr. M. Froelich (LUMC) and Dr. J. van den Berg (Numico Research) for laboratory analyses.

Reference list

1. Kremer JM. n-3 fatty acid supplements in rheumatoid arthritis. Am. J. Clin. Nutr. 2000; 71:349S-51S.
2. Zurier RB, Rossetti RG, Jacobson EW, DeMarco DM, Liu NY, Temming JE, White BM, Laposata M. Gamma-linolenic acid treatment of rheumatoid arthritis. A randomized, placebo-controlled trial. Arthritis Rheum. 1996; 39:1808-17.
3. Johnson MM, Swan DD, Surette ME, Stegner J, Chilton T, Fonteh AN, Chilton FH. Dietary supplementation with gamma-linolenic acid alters fatty acid content and eicosanoid production in healthy humans. J. Nutr. 1997; 127:1435-44.
4. Belch JJ, Hill A. Evening primrose oil and borage oil in rheumatologic conditions. Am. J. Clin. Nutr. 2000; 71:352S-56S.
5. Cleland LG, James MJ, Proudman SM . The role of fish oils in the treatment of rheumatoid arthritis. Drugs 2003; 63:845-53.
6. Sköldstam L, Börjesson O, Kjällman A, Seiving B, Åkesson 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.
7. Lunec J, Halloran SP, White AG, Dormandy TL. Free radical oxidation (peroxidation) products in serum and synovial fluid in rheumatoid arthritis. J. Rheumatol. 1981; 8:233-44.
8. Burkhardt H, Schwingel M, Menninger H, Macartney HW, Tschesche H . Oxygen radicals as effectors of cartilage destruction. Arthritis Rheum 1986; 29:379-87.
9. Taysi S, Polat F, Gul M, Sari RA, Bakan E. Lipid peroxidation, some extracellular antioxidants, and antioxidant enzymes in serum of patients with rheumatoid arthritis. Rheumatol. Int. 2002; 21:200-4.
10. Jikimoto T, Nishikubo Y, Koshiba M, Kanagawa S, Morinobu S, Morinobu A, Saura R, Mizuno K, Kondo S, Toyokuni S, Nakamura H, Yodoi J, Kumagai S. Thioredoxin as a biomarker for oxidative stress in patients with rheumatoid arthritis. Mol. Immunol. 2002; 38:765-72.
11. Stone SJ, Doube A, Dudson D, Wallace J. Inadequate calcium, folic acid, vitamin E, zinc, and selenium intake in rheumatoid arthritis patients: results of a dietary survey. Semin. Arthritis Rheum. 1997; 27:180-5.
12. Kheir-Eldin AA, Hamdy MA, Motawi TK, Shaheen AA, Abd el Gawad HM. Biochemical changes in arthritic rats under the influence of vitamin E. Agents Actions 1992; 36:300-5.

13. Sakai A, Hirano T, Okazaki R, Okimoto N, Tanaka K, Nakamura T. Large-dose ascorbic acid administration suppresses the development of arthritis in adjuvant-infected rats. Arch. Orthop. Trauma Surg. 1999; 119:121-6.
14. Tsuji F, Miyake Y, Aono H, Kawashima Y, Mita S. Effects of bucillamine and N-acetyl-L-cysteine on cytokine production and collagen-induced arthritis (CIA). Clin. Exp. Immunol. 1999; 115:26-31.
15. Edmonds SE, Winyard PG, Guo R, Kidd B, Merry P, Langrish-Smith A, Hansen C, Ramm S, Blake DR. Putative analgesic activity of repeated oral doses of vitamin E in the treatment of rheumatoid arthritis. Results of a prospective placebo controlled double blind trial. Ann Rheum Dis. 1997; 56:649-50.
16. Tarp U, Overvad K, Thorling EB, Graudal H, Hansen JC. Selenium treatment in rheumatoid arthritis. Scand. J. Rheumatol. 1985; 14:364-8.
17. Peretz A, Siderova V, Neve J. Selenium supplementation in rheumatoid arthritis investigated in a double blind, placebo-controlled trial. Scand. J. Rheumatol. 2001; 30:208-12.
18. Darlington LG, Stone TW. Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. Br. J. Nutr. 2001; 85:251-69.
19. Calder PC, Kew S. The immune system: a target for functional foods? Br. J. Nutr. 2002; 88:5165-77.
20. Felson DT, Anderson JJ, Boers M, Bombardier C, Chernoff M, Fried B, Furst D, Goldsmith C, Kieszak S, Lightfoot R. The American College of Rheumatology preliminary core set of disease activity measures for rheumatoid arthritis clinical trials. The Committee on Outcome Measures in Rheumatoid Arthritis Clinical Trials. Arthritis Rheum1993; 36:729-40.
21. Prevoo ML, van ‘t Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum. 1995; 41:44-8.
22. Bligh EG, Dyer WT. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 1959; 37:911-20.
23. Kaluzny MA, Duncan LA, Merritt MV, Epps DE. Rapid separation of lipid classes in high yield and purity using bonded phase columns. J. Lipid Res. 2000; 26:135-40.
24. Morrison WR, Smith LM. Preparation of fatty acids methyl esters and dimethylacetals from lipids with boron fluoride methanol. J. Lipid Res. 1964; 5:600-7.
25. Stump DD, Roth EF Jr, Gilbert HS. Simultaneous determination by high-performance liquid chromatography of tocopherol isomers, alpha-tocopherol quinone, and cholesterol in red blood cells and plasma. J. Chromatogr. 1984; 306:377-82.
26. Liau LS, Lee BL, New AL, Ong CN. Determination of plasma ascorbic acid by high-performance liquid chromatography with ultraviolet and electrochemical detection. J. Chromatogr. 1993; 612:63-70.
27. Chilton FH, Patel M, Fonteh AN, Hubbard WC, Triggiani M. Dietary n-3 fatty acid effects on neutrophil lipid composition and mediator production. Influence of duration and dosage. J. Clin. Invest. 1993; 91:115-22.
28. Venkatraman JT, Chu WC. Effects of dietary omega-3 and omega-6 lipids and vitamin E on serum cytokines, lipid mediators and anti-DNA antibodies in a mouse model for rheumatoid arthritis. J. Am. Coll. Nutr. 1999; 18:602-13.
29. 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.

30. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmacother. 2002; 56:365-79.
31. de Vries JHM, Jansen A, Kromhout D, van de Bovenkamp P, van Staveren WA, Mensink RP, Katan MB. The fatty acid and sterol content of food composites of middle-aged men in seven countries. J. Food Compos. Anal. 1997; 10:115-41.
32. Kremer JM, Lawrence DA, Jubiz W, DiGiacomo R, Rynes R, Bartholomew LE, Sherman M. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. Clinical and immunologic effects. Arthritis Rheum 1990; 33:810-20.
33. Geusens P, Wouters C, Nijs J, Jiang Y, Dequeker J. Long-term effect of omega-3 fatty acid supplementation in active rheumatoid arthritis. A 12-month, double-blind, controlled study. Arthritis Rheum 1994; 37:824-9.
34. Rosenstein ED, Caldwell JR. Trace elements in the treatment of rheumatoid arthritis. Rheum. Dis. Clin. North Am. 1999; 25:929-35.
35. del Rincon ID, Williams K, Stern MP, Freeman GL, Escalante A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. Arthritis Rheum 2001; 44:2737-45.
36. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002; 106:2747-57.
37. Laidlaw M, Holub BJ. Effects of supplementation with fish oil-derived n-3 fatty acids and γ-linolenic acid on circulating plasma lipids and fatty acid profiles in women. Am. J. Clin. Nutr. 2003; 77:37-42.