

Influence of diet with different lipid composition on neutrophil chemiluminescence and disease activity in patients with rheumatoid arthritis

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SUMMARY Neutrophil chemiluminescence was determined in patients with active rheumatoid arthritis. Twelve patients were randomly assigned either to a diet high in polyunsaturated fatty acids supplemented with eicosapentaenoic and docosahexaenoic acids or to a diet high in saturated fatty acids. A correlation with clinical and laboratory parameters is also reported. No statistical difference was observed in neutrophil chemiluminescence and in clinical parameters in the group of patients treated with a diet high in saturated fatty acids. Fish oil ingestion resulted in subjective alleviation of active rheumatoid arthritis and reduction of neutrophil chemiluminescence. This study corroborates the hypothesis of an anti-inflammatory role for polyunsaturated fatty acids in patients with chronic inflammatory diseases.

Dietary composition has been shown to influence the immune response both in animals and in humans.¹ Therefore dietary manipulations may form the basis for a nutritional approach to treating autoimmune disorders.

The regulatory effect of dietary lipid composition on many biological processes is well known.²⁻⁴ The ratio polyunsaturated:saturated fatty acids (P:S ratio) and the n-3 fatty acid amount are the important factors. Whereas the P:S ratio regulates the concentration of plasma cholesterol,⁵ the n-3 fatty acids (eicosapentaenoate 20:5, docosahexaenoate 22:6) are mainly involved in the regulation of arachidonate metabolism.⁶

The main source of eicosapentaenoic acid and docosahexaenoic acid is fish derived oil and an adequate dietary content seems to be 2-5 g/day.

The relation between dietary factors and rheumatoid arthritis is becoming more evident.^{7,8} Furthermore, patients with rheumatoid arthritis (RA) show a notable increase of arachidonate metabolism via lipoxygenase, leading to leucotrienes B4 or D4.⁹

Recently, Lee *et al* presented evidence for an inhibitory effect on polymorphonuclear activity of a diet rich in n-3 fatty acids.¹⁰

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Neutrophils stimulated by particulate or soluble stimuli generate a photon emission process (chemiluminescence) that is associated with NADPH-oxidase activation. Chemiluminescence can be easily measured either with purified cells or with whole blood.^{11,12}

The aim of this study was to test the effect of a diet rich in eicosapentaenoic and docosahexaenoic acids on neutrophil chemiluminescence in a group of patients with active rheumatoid arthritis. In addition, a correlation between clinical and laboratory parameters is reported.

Patients and methods

Twelve female patients with definite or classical rheumatoid arthritis as defined by the American Rheumatism Association (ARA) diagnostic criteria¹³ were studied. All patients had active disease as defined by the following criteria: morning stiffness of at least 30 minutes' duration, six or more tender joints, three or more swollen joints, and an erythrocyte sedimentation rate of at least 30 mm/h.

No patient had received systemic steroids or immunosuppressive or disease modifying drugs in the three months before enrolment.

The patients were randomly divided into two groups and assigned to a one month isoenergetic regimen of either a diet high in saturated fatty acids

(P:S ratio 1.33) (group A) or to a diet high in polyunsaturated fatty acids (P:S ratio 5.0) with a daily consumption of nine capsules of Max Epa (group B) (Table 1).

Max Epa consists mainly of triglycerides in which 34% of the total fatty acids and 86% of the polyunsaturated fatty acids are eicosapentaenoic acid and docosahexaenoic acid; the arachidonic acid precursor, linoleic acid, constitutes less than 2% of the fatty acids and arachidonic acid is not detectable. The amount of Max Epa orally administered was equivalent to 1.6 g of eicosapentaenoic acid, 1.1 g of docosahexaenoic acid, and to 339 kJ daily.

All the patients were maintained on a stable dosage of non-steroidal anti-inflammatory drug. Each patient was assessed before enrolling in the study and after 30 days.

Clinical evaluation included duration of morning stiffness (in minutes), grip strength, and Ritchie's index.

At each assessment fasting blood samples were collected from the antecubital veins of patients for measurement of neutrophil chemiluminescence and for routine laboratory assessment.

Chemiluminescence was measured in whole blood as reported by De Sole *et al*¹² with some modifications, using a commercial kit (Fagolux; Bouty, Milan, Italy). Briefly, 50 μ l of 1:200 diluted blood was added to 1.0 ml final volume of the luminescent medium (Krebs-Ringer phosphate medium pH 7.4 with 0.3 mM Ca^{++} , 5.5 mM glucose, and 20 μ M luminol). Chemiluminescence was monitored at seven-minute intervals after addition of opsonised zymosan in a Packard Picolite luminometer, model 6500. Specific activity (counts per minute/polymorphonuclear cells (cpm/PMN)) was obtained by dividing the maximum chemiluminescence activity (cpm) by the number of neutrophils (PMN) present in the test vial.

Results are expressed as mean (SE). Student's *t* test was used for statistical evaluation.

Table 1 Baseline characteristics of patients in groups A and B

	Group A	Group B
Mean age in years (range)	37 (20-55)	36 (20-50)
Mean disease duration in years (range)	2.6 (1-5)	2.1 (1-5)
ARA class (%)		
I	50	16
II	16	50
III	34	34
Radiological stage (%)		
II	66	66
III	34	34

Results

No statistical differences in clinical variables were observed between the two groups of patients at the beginning of the study.

No significant differences in grip strength, Ritchie's index, or morning stiffness were observed in group A patients at the end of the study as compared with the basal values (Fig. 1).

In group B patients a tendency towards benefit was observed for each clinical variable measured. A significant decrease as compared with the basal value was observed in Ritchie's index after 30 days (10.6 (3.48) ν 17.2 (3.38); $p < 0.01$) and in morning stiffness (22 (8.45) ν 33 (7.34) min; $p < 0.01$) while a significant increase was recorded in grip strength (136 (12.88) ν 116 (13.26) mmHg; $p < 0.01$) after 30 days (Fig. 2). Furthermore, a significant difference between the two groups of patients at the end of the study was observed in Ritchie's index (10.6 (3.48) ν 21.4 (3.2); $p < 0.005$), in morning stiffness (22 (8.45)

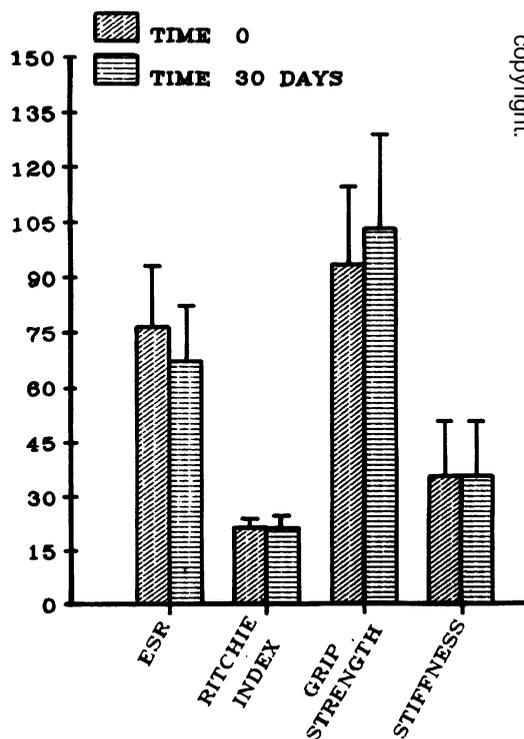


Fig. 1 Erythrocyte sedimentation rate (ESR) (mm/1st h), Ritchie's index, grip strength (mmHg), and morning stiffness (minutes) at the start and after 30 minutes in group A patients.

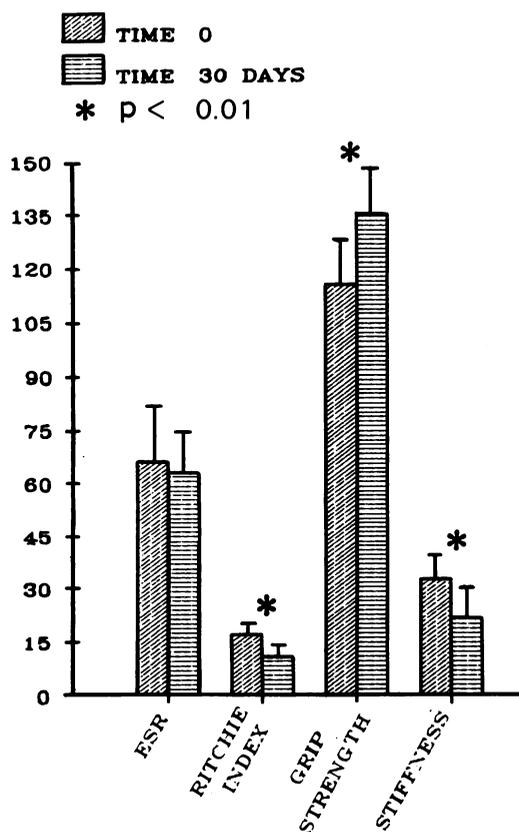


Fig. 2 ESR (mm/1st h), Ritchie's index, grip strength (mmHg), and morning stiffness (minutes) at the start and after 30 minutes in group B patients.

v 36 (10.17) min; $p < 0.01$), and in grip strength (136 (12.88) v 104 (21.58) mmHg; $p < 0.01$).

Fig. 3 shows the chemiluminescence specific activities both for the patients receiving Max Epa and those on a diet high in saturated fatty acids. Patients treated with the diet high in saturated fatty acids showed a non-significant alteration in neutrophil chemiluminescence after 30 days (2.66 (0.38) v 3.25 (0.58) cpm/PMN; $p = NS$). In contrast, the Max Epa diet produced a notable reduction of neutrophil chemiluminescence after 30 days (2.04 (0.3) v 3.67 (0.87) cpm/PMN; $p < 0.05$).

No statistically significant changes in sedimentation rate, haemoglobin concentration, triglyceride, or cholesterol dietary levels were observed between the two dietary regimens.

Discussion

Alterations in essential fatty acids have been shown

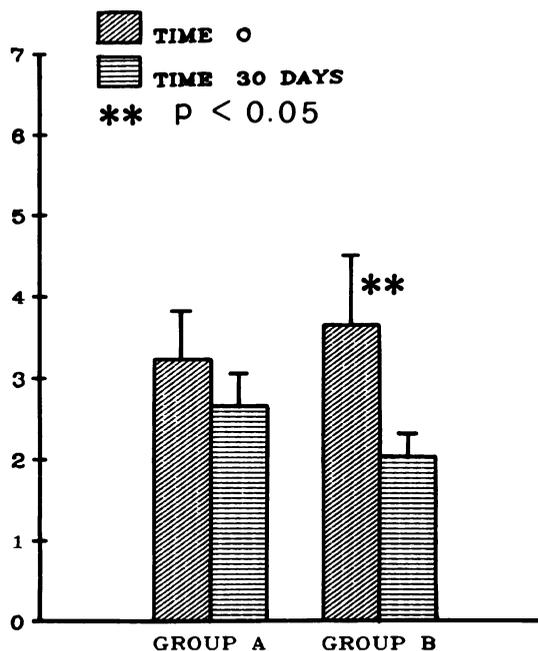


Fig. 3 Neutrophil chemiluminescence (obtained by dividing the maximum chemiluminescence activity by the number of neutrophils) at the start and after 30 days in group A and group B patients.

to affect immune function and the inflammatory response in animal models by various mechanisms.^{14, 15} The cyclo-oxygenase and lipoxygenase products of n-3 fatty acids are less proinflammatory than those derived from arachidonic and other n-6 fatty acids,^{10, 16} suggesting a possible anti-inflammatory role for fish oil. Eicosapentaenoic acid is a precursor for the 5 series of leucotrienes (LTB), and when the diet of rats is supplemented with this fatty acid there is a significant increase in the production of LTB₅, which is 30 times less potent than LTB₄ in causing neutrophil activation.¹⁶

The effect of a 30 day Max Epa diet on neutrophil chemiluminescence (Fig. 3) is clearly evident. Because the chemiluminescence in our system is dependent on a hydrophobic chemilumigenic probe (luminol), the decrease after a Max Epa diet is probably due to a change in neutrophil membrane lipid composition.

These results are in agreement with those of Lee *et al.*¹⁰ who found a reduction of LTB₄ and of chemotactic activity with an increase of membrane polarisation value in leucocytes of normal subjects receiving Max Epa.

The favourable effects observed with the administration to patients with RA of a diet high in

polyunsaturated fatty acids derived from fish oil are in agreement with the results of Kremer *et al*,^{17 18} who demonstrated a subjective alleviation of active RA and a reduction in neutrophil LTB₄ production in these patients.

It is noteworthy that patients treated with a Max Epa diet were in a more severe class of RA (fewer in ARA class I—Table 1). This could mean that the improvement in this group of patients was even more significant.

Diets high in polyunsaturated fatty acids containing n-3 acids are generally well tolerated and produce a statistically significant reduction of disease activity.^{16 17}

The improvement noted in each clinical variable studied and in neutrophil chemiluminescence may be attributed to the same factors: cyclo-oxygenase and lipoxygenase products of n-3 fatty acids, which are less proinflammatory than those derived from arachidonic and other n-6 acids.

Further studies will, however, be necessary for a better understanding of the precise mechanism of action of fish oil supplementation in patients with RA.

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