Changes in plasma phospholipid fatty acids and their relationship to disease activity in rheumatoid arthritis patients treated with a vegetarian diet

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In a controlled clinical trial we have recently shown that patients with rheumatoid arthritis (RA) improved after fasting for 7–10 d and that the improvement could be sustained through 3·5 months with a vegan diet and 9 months with a lactovegetarian diet. Other studies have indicated that the inflammatory process in RA can be reduced through manipulation of dietary fatty acids. A switch to a vegetarian diet significantly alters the intake of fatty acids. Therefore, we have analysed the changes in fatty acid profiles of the plasma phospholipid fraction and related these changes to disease activity. The concentrations of the fatty acids 20:3*n-6* and 20:4*n-6* were significantly reduced after 3·5 months with a vegan diet ($P < 0·0001$ and $P < 0·01$ respectively), but the concentration increased to baseline values with a lactovegetarian diet. The concentration of 20:5*n-3* was significantly reduced after the vegan diet ($P < 0·0001$) and the lactovegetarian diet periods ($P < 0·01$). There was no significant difference in fatty acid concentrations between diet responders and diet non-responders after the vegan or lactovegetarian diet periods. Our results indicate that the changes in the fatty acid profiles cannot explain the clinical improvement.

Fatty acid profile: Vegetarian diet: Rheumatoid arthritis

The concentrations of fatty acids in serum phospholipids and phosphatidylcholine have been found to be altered in patients with rheumatoid arthritis (RA; Bruderlein *et al.* 1981; Jacobsson *et al.* 1990) and in patients with juvenile chronic arthritis (JCA; Johansson *et al.* 1986) compared with healthy controls. In these studies the proportion of the saturated fatty acids was found to be significantly increased, and the proportion of linoleic acid (18:2*n*-6) significantly reduced. This altered fatty acid profile is most likely to be a result of the inflammatory process, since no difference in dietary intake has been found between groups of patients with RA or JCA and healthy controls or patients with osteoarthritis (Kowsari *et al.* 1983; Johansson *et al.* 1986; Haugen *et al.* 1992). This notion is also supported by the negative correlation between the proportion of 18:2*n*-6 and acute-phase reactants (C-reactive protein and orosomucoid; Jacobsson *et al.* 1990).

It has been shown that the fatty acid composition of plasma and tissue phospholipids with change to reflect dietary intake. This has stimulated rheumatologists to undertake supplementation studies with γ-linolenic acid (18:3*n*-6) and polyunsaturated long-chain fatty acids of the *n*-3 series in attempts to modulate the immune system through a reduction of arachidonic acid (20:4*n*-6; Ziff, 1983). Arachidonic acid is the precursor of the

proinflammatory eicosanoids, whereas dihomo-γ-linolenic acid (20:3*n*-6) and eicosapentaenoic acid (20:5*n*-3) are the precursors of less inflammatory eicosanoids. Thus, during an inflammatory stimulus the macrophages will produce less of the prostaglandins and leukotrienes with high inflammatory potential (Endres *et al.* 1989; Santoli *et al.* 1990). Dietary supplementation with 20:3*n*-6 (Belch *et al.* 1988; Pullman-Mooar *et al.* 1990) and with 20:5*n*-3 together with docosahexaenoic acid (22:6*n*-3; Kremer *et al.* 1987; Kjeldsen-Kragh *et al.* 1992) has been shown to reduce clinical symptoms in patients with RA.

Attention has also been paid to oleic acid (18:1*n*-9), which constitutes 72% of the fatty acids in olive oil. Reductions of pain and articular index have been found in a study in which olive oil was used as placebo (Brzeski *et al.* 1991). Furthermore, a reduced incidence of RA has been reported to be associated with an increased intake of olive oil (Linos *et al.* 1991). The mechanism by which oleic acid exerts its effects in RA patients has yet to be elucidated but, since oleic acid can substitute for the unsaturated fatty acids in the phospholipid fractions, it is possible that oleic acid exerts its effect through reduced availability of the substrates for eicosanoid production (Vossen *et al.* 1993).

The proportion of 20:4*n*-6 in different phospholipid fractions has been found to be either changed or unaltered as a result of vegan and lactovegetarian diets (Kirkeby & Bjerkedal, 1968; Sanders *et al.* 1978; Phinney *et al.* 1990; Sanders & Roshanai, 1992). These diets contain more 18:2*n*-6 than omnivorous diets, but they are devoid of 20:4*n*-6, 20:5*n*-3, 20:6*n*-3 and 22:6*n*-3 (Roshanai & Sanders, 1984). This implies that the eicosanoid precursors must be produced endogenously from 18:2*n*-6 and 18:3*n*-3 respectively. If the endogenous production of 20:4*n*-6 from 18:2*n*-6 cannot compensate for the absence of 20:4*n*-6 in the diet, the precursor of the proinflammatory eicosanoids would be reduced. It is possible that this could explain the beneficial effect of vegetarian diets in patients with RA.

Lipid peroxides are believed to be generated from unsaturated fatty acids by O radicals during the inflammatory process (Rowley *et al.* 1984). The O radicals are thought to play a part in the disease process by mediating oxidative damage (Lunec *et al.* 1981). It has been reported that fasting and consumption of a vegetarian diet for 3 weeks reduced the concentration of lipid peroxidation products measured as thiobarbituric acid-reacting substances (TBARS) in patients with fibromyalgia (Høstmark *et al.* 1993). Antioxidants, which are abundantly present in vegetarian diets, may explain the reduced concentration of the TBARS (Abdulla *et al.* 1981). This could perhaps also contribute to the alleviation of the disease symptoms in patients with RA during treatment with a vegetarian diet.

In a controlled single-blind clinical trial we found that the disease activity decreased in patients with RA who ate a vegetarian diet (Kjeldsen-Kragh *et al.* 1991). This study has been extended by determining the changes in the fatty acid profile of the plasma phospholipids during the vegetarian diets and by examining whether the changes of the fatty acid concentrations were associated with concomitant changes in subjective and/or objective variables of disease activity. For the same purpose we also measured the concentrations of the serum lipid peroxidation products.

SUBJECTS AND METHODS

Study design

The study was a prospective, single-blind, controlled clinical trial designed to investigate possible impact on disease activity of fasting and a 1-year vegetarian diet in patients with RA (Kjeldsen-Kragh *et al.* 1991). The patients were randomized to either a diet group or a control group. The study was approved by the regional scientific ethical committee.

Subjects

Fifty-three omnivorous RA patients with active disease were enrolled in the study (forty-five female and eight male; Kjeldsen-Kragh *et al.* 1991). All patients satisfied the criteria of the American Rheumatism Association for classical or definite RA (Ropes *et al.* 1958). Twenty-seven patients were allocated to the diet group (mean age 51 years) and twenty-six patients to the control group (mean age 55 years). In the diet group, one patient terminated the study after 1 month and a further three patients after 4 months. Altogether twenty-three patients continued on the lactovegetarian diet for more than 3 months. In the control group, one patient terminated the study after 1 month and a further four patients after 4 months in the study. Blood was drawn and dietary and clinical assessments were carried out at inclusion, after 1 month, after 4 months, and at the time point at which the patients left the study.

Treatment

The patients in the diet group began the study with a 4-week stay at a health farm where they fasted for 7–10 days. The fast consisted of herb teas, garlic, vegetable broths, decoction of potatoes and parsley and vegetable juices of carrots, beets and celery. Thereafter, the patients reintroduced a 'new' food item every 2nd day, but kept a strict gluten-free vegan diet during the following 3·5 months. After 3·5 months they switched to a lactovegetarian diet. The patients in the control group began the study with a 4-week stay at a convalescent home. They were told to continue their normal diet and were not given any dietary instructions, except for two patients who were given low-fat diet guidelines because of elevated serum cholesterol values (over 8 mmol/l).

Since it is known that a vegan diet leads to a low intake of vitamin D, the patients were told to take vitamin-D supplementation (10 μg cholecalciferol/d). The patients who took cod-liver oil supplementation on a regular basis before entering the study were told to continue with the cod-liver oil supplementation throughout the study (5 ml cod-liver oil/d = 10 μg cholecalciferol/d). Altogether five patients in the diet group and five patients in the control group used cod-liver oil supplementation on a daily basis during the whole study period.

Blood analyses

Venous peripheral blood samples were collected before the morning meal, between 08.00 and 10.00 hours. Serum samples were frozen (−20°) for subsequent analysis of serum TBARS and plasma samples were frozen (−70°) for subsequent analysis of the fatty acids in the plasma phospholipids. The TBARS were measured as malondialdehyde-reactive products (Kosugi *et al.* 1989). The concentration of plasma phospholipid acids was measured according to the method described by Bønaa *et al.* (1990). These results are presented as mmol/l and as a percentage of the total phospholipid fatty acids. During the lactovegetarian diet period the data were collected from five patients after 3 months, one patient after 6 months and seventeen patients after 9 months on a lactovegetarian diet. All these data were treated as if they had been collected at the same time point. The justification for this is that fatty acid concentrations in plasma phospholipids respond rapidly to changes in the dietary intake (Grønn *et al.* 1991).

Dietary assessments

Food intake was assessed by 24 h recalls at the same time as the blood samples were drawn. The amount consumed was given in household measurements. Energy, total fat and fatty acid intake were calculated using the software package 'FIBER' based on the Norwegian Food Composition Tables (Statens Ernæringsråd, 1988).

Characterization of responders/non-responders

The following variables were used to distinguish between diet responders and diet non-responders: number of swollen joints, Stanford Health Assessment Questionnaire Index (HAQ; Fries *et al.* 1980), pain score on a visual analogue scale, number of tender joints, patients global assessment and erythrocyte sedimentation rate (ESR). These variables have been assigned as core measures for clinical trials in RA (Tugwell *et al.* 1993). A two-grade improvement on the scale for patient's global assessment was defined as a substantial improvement and for the other five variables $\geqslant 20\%$ improvement compared with baseline values was required (Paulus *et al.* 1990). To be characterized as diet responders the patients had to have improved substantially in at least three of these core variables at each of the last three clinical examinations.

Statistical analysis

Data are expressed as means and standard deviations. For the fatty acids 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3 only the data from the patients not using cod-liver oil is included, since the concentrations of these fatty acids were significantly higher in the group using cod-liver oil. To test within-group differences of plasma phospholipid fatty acids and serum lipid TBARS a paired *t* test was used. The distributions of plasma phospholipid 18:2*n*-6, 18:3*n*-3, 20:5*n*-3 and 22:6*n*-3 were skewed, and log transformed values were used in these analyses. To test between-group differences an unpaired *t* test was used. Since the dietary intakes of most of the fatty acids during the treatment period were non-normal and since no suitable transformation could be found, the Wilcoxon rank-sum test was used to test for possible differences in fatty acid intake. The Mann–Whitney test was used to analyse the possible differences between the patients who used or did not use cod-liver oil supplementation at the time of inclusion. This test was also used to compare the concentrations of fatty acids between diet responders and diet non-responders after the vegetarian diet periods.

To test the overall group differences for TBARS an analysis of covariance (ANACOVA) was used with the baseline values as covariate. This was done because the serum concentrations of the lipid peroxidation products in the diet and control groups were significantly different at the time of inclusion (Montgomery, 1984). To study a possible association between the different fatty acids and disease activity, ANACOVA was performed. In these models the number of swollen joints, the number of tender joints and ESR were run as dependent variables respectively. Either the concentration or the proportion of the fatty acids were run as covariates (Kleinbaum *et al.* 1988). *P* values below 0·05 were considered to be statistically significant. The NCSS software package was used (Hintze, J. L., Kaysville, Utah, USA).

RESULTS

Compliance with the diet could not be evaluated by any objective means. However, during the health farm stay the patients had scarce opportunities to deviate from the diet, living in a close community with other patients and the health farm personnel. During the strict gluten-free vegan diet, four patients confessed intake of dairy products once or twice. The infringement consisted of sour cream as dressing and one patient had one glass of skimmed milk once. One male patient who found the vegan diet very hard to follow ate elk meat four times. During the modified lactovegetarian diet period, five patients admitted to having eaten meat on two occasions, but the diet was mostly broken by intake of fish at special occasions such as weddings and anniversaries.

Table 1. *Intakes of energy (MJ/d), protein and fat (% energy) by rheumatoid arthritis patients consuming a vegetarian diet, compared with controls*†

(Mean values and standard deviations)

	At inclusion		After 1 months		After 4 months		After 7–13 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Diet group‡								
n	27		27		26		23	
Energy intake (MJ/d)	7·1	1·5	5·0***	2·1	6·8	3·0	6·1	1·6
Protein (% energy)	14·5	2·4	10·2***	2·8	10·7***	2·4	13·5	3·9
Fat (% energy)	35·8	7·4	27·2**	13·4	29·3**	11·0	31·2	8·5
Control group								
n	26		26		25		21	
Energy intake (MJ/d)	6·2	2·4	6·8	2·8	6·2	2·6	6·6	1·7
Protein (% energy)	16·8	5·0	16·9	3·6	16·9	4·8	16·0	2·9
Fat (% energy)	36·6	7·2	34·8	7·2	36·5	9·6	33·7	7·5

Mean values were significantly different from those at inclusion into the study: ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

† For details of subjects and procedures, see pp. 556–557.

‡ The diet group followed a 'fasting' regimen for 7–10 d, a vegan diet for the next 3·5 months, and a lactovegetarian diet for the remainder of the study.

Energy and fatty acid intake

Total energy and fat intakes were reduced during the vegan and lactovegetarian diet periods (Table 1). The dietary intake of the saturated and monoenic fatty acids, except for 18:1, was significantly reduced during the vegan and the lactovegetarian diet periods. The intake of 18:2*n*-6 was significantly increased during the vegan diet period and it constituted approximately 50% of the fatty acid intake during this period. In the control group the intake of 18:2*n*-6 was increased during the convalescent home stay but during the remaining study period the fatty acid intake was unaltered (Table 2).

At the time of recruitment the intake of 20:4*n*-6 was calculated to be 0·14 (range 0·0–0·41) g/d in the diet group, whereas during the vegan and lactovegetarian diet periods the calculated intake of this fatty acid was zero. The Norwegian Food Composition Tables do not give any values for 20:4*n*-6 in milk and dairy products, but the Norwegian Dairy Company (Norwegian Dairy Company, personal communication) estimates 20:4*n*-6 to constitute 0·003% of the fat content in milk. However, these values would be too minute to be registered in the database (i.e. 10 mg 20:4*n*-6/l full-fat milk). In the control group the mean daily intake of 20:4*n*-6 was 0·12 (range 0·0–0·35) g/d.

At recruitment the ten patients who used cod-liver oil supplementation had a mean daily calculated intake of 20:5*n*-3 of 0·44 (range 0·21–0·89) g/d, whereas in the group not taking cod-liver oil supplementation the intake of 20:5*n*-3 was 0·15 (range 0·0–1·81) g/d. During the dietary treatment the five patients in the diet group taking cod-liver oil had an intake of 20:5*n*-3 of 0·22 g/d, whereas the patients not taking cod-liver oil supplementation had an intake of 0·0 g/d.

The fatty acid profile in plasma phospholipids

The concentrations of most of the fatty acids were significantly reduced in plasma phospholipids after the vegan diet period (Table 3). After the lactovegetarian diet the concentrations of most of the fatty acids had returned to baseline values. The concentration of 18:2*n*-6, however, was stable during the whole study period, and accordingly the

Table 2. *Intake of fatty acids (g/d) by rheumatoid arthritis patients following a vegetarian diet, compared with controls*‡

(Mean values and standard deviations)

	At inclusion		After 1 month		After 4 months		After 7–13 months	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Diet group§								
n	27		27		26		23	
14:0	3·7	2·1	0·1†	0·1	0·6†	1·6	2·3**	1·7
16:0	13·0	4·7	3·1†	2·3	5·9***	4·5	8·2**	4·3
16:1	1·9	0·7	0·1†	0·2	0·3†	0·4	0·9***	0·6
18:0	6·1	2·3	1·6†	1·3	3·0***	2·3	3·9***	2·1
18:1	19·1	6·6	10·6**	8·1	16·4	11·9	14·5**	6·3
18:2*n*-6	7·0	3·0	18·0***	17·9	22·8†	13·6	13·2	7·5
18:3*n*-3	1·0	0·6	1·2	1·4	1·4	1·4	1·0	1·2
Total SFA	27·9	10·7	5·0†	3·7	10·4***	9·9	17·4***	9·9
Total MUFA	24·2	7·4	11·0†	8·4	17·0**	12·3	16·7**	7·2
Total PUFA	9·6	3·8	19·6**	18·5	24·4***	14·4	14·9	8·0
Control group								
n	26		26		25		21	
14:0	3·4	1·6	3·6	1·9	3·4	1·8	3·3	1·4
16:0	11·0	3·2	12·3	6·6	11·5	6·2	11·1	4·5
16:1	1·9	0·7	1·9	1·2	1·7	0·9	1·9	0·8
18:0	4·9	1·7	5·7	3·2	5·3	3·3	4·9	2·1
18:1	15·6	5·3	17·8	11·9	16·4	10·1	14·9	6·7
18:2*n*-6	6·0	4·7	9·9**	11·9	6·2	5·8	6·1	3·3
18:3*n*-3	0·8	0·6	1·0	0·8	0·9	0·8	0·9	0·5
Total SFA	23·9	8·3	26·3	13·7	24·6	13·1	23·0	8·9
Total MUFA	20·9	7·5	22·8	14·5	21·1	11·8	21·0	9·6
Total PUFA	8·8	6·0	9·7	7·9	9·0	7·1	9·9	5·6

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Mean values were significantly different from those at inclusion into the study. ** $P < 0·01$; *** $P < 0·001$; † $P < 0·0001$.

‡ For details of subjects and procedures, see pp. 556–557.

§ The diet group followed a 'fasting' regime for 7–10 d, a vegan diet for the next 3·5 months, and a lactovegetarian diet for the remainder of the study.

proportion of this fatty acid increased significantly (Table 4). The concentrations as well as the proportions of 20:5*n*-3 and 22:6*n*-3 were reduced during the study period. The concentration of 20:4*n*-6 was significantly reduced after the vegan diet periods, but was increased again after the lactovegetarian diet. However, the proportion of 20:4*n*-6 was not changed during the study period. In the control group the total amount of fatty acids in the plasma phospholipid fraction was stable during the whole study period (data not shown).

The patients who used cod-liver oil supplementation had significantly higher concentrations of 20:5*n*-3 and 22:6*n*-3 in the phospholipid fraction compared with the patients who did not supplement their diet with cod-liver oil ($P = 0·02$ for both fatty acids). However, there was no difference in the disease activity variables between the group who took cod-liver oil supplementation and those who did not, either at the time of inclusion or at any of the subsequent clinical evaluations (data not shown).

Ten patients in the diet group used linseed oil during the vegan diet period. These patients had a higher concentration of 18:3*n*-3 after the health farm stay ($P < 0·001$), but this difference was not detectable after 3 months on a vegan diet.

Table 3. *Fatty acid composition of plasma phospholipids (mmol/l) in rheumatoid arthritis patients consuming vegan and lactovegetarian diets*§‖

(Mean values and standard deviations)

	At inclusion (*n* 27)		After the health farm stay (*n* 27)		After the vegan diet (*n* 26)		After the lactovegetarian diet (*n* 23)	
Fatty acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	4·5	1·6	2·5†	0·8	3·1***	1·1	4·5	1·6
16:0	308·3	53·4	279·2***	49·4	254·1†	44·6	288·2	57·6
16:1	5·5	2·3	4·9***	1·6	4·0†	1·4	5·2	2·4
18:0	162·3	26·2	137·2†	25·5	134·3†	16·9	150·1	30·5
18:1	114·0	25·9	93·4***	18·7	84·6†	16·6	95·7**	23·4
18:2*n*-6	247·9	53·5	248·9	43·9	253·9	48·9	259·7	59·8
18:3*n*-3	2·5	0·7	1·9	2·1	2·0**	1·2	2·5	1·0
20:0	8·3	1·5	8·3	2·1	7·7	1·6	8·1	1·6
20:1	3·4	1·6	2·4***	1·2	2·3***	0·7	2·6**	1·4
20:3*n*-9	2·3	1·1	0·6†	0·7	0·6†	0·6	1·6**	1·1
20:2*n*-6	6·0	1·4	4·7***	1·1	5·5	1·6	6·2	1·8
20:3*n*-6	36·5	10·0	19·5†	5·8	25·5†	6·8	36·1	10·6
20:4*n*-6	102·5	23·3	93·9**	29·2	87·2**	24·4	95·9	23·9
20:5*n*-3¶	22·7	11·2	8·2†	5·8	8·4†	5·2	17·4**	13·4
22:0	29·5	6·2	21·7†	4·8	24·3**	4·8	27·1	5·8
22:1*n*-11	9·7	4·2	4·8†	2·9	3·9†	3·5	6·2**	3·5
22:1*n*-9	3·1	1·4	1·8***	1·5	1·9†	1·2	2·3**	1·2
22:4*n*-6	3·3	2·4	1·6***	1·2	1·1***	1·1	2·3	2·0
22:5*n*-6	1·8	0·7	1·5**	0·8	1·3**	0·8	1·9	1·2
22:5*n*-3¶	15·7	3·4	14·3**	3·6	14·2**	3·0	16·6	4·7
22:6*n*-3¶	78·2	19·7	66·7**	20·1	54·1†	16·0	66·1**	22·6
24:0	13·8	3·0	11·7***	2·8	14·6	3·0	14·5	4·1
24:1	30·2	4·8	35·0**	9·3	31·0	8·6	28·5	6·8
Total (*n*-6)	408·0	75·3	369·4**	56·3	374·3‡	60·5	402·3	81·7
Total (*n*-3)	133·1	42·5	109·3†	45·6	92·4†	37·4	92·5†	30·6
Total saturated	526·7	84·9	460·7†	76·1	438·2†	57·3	492·7	91·9

Mean values were significantly different from those at inclusion into the study: ** $P < 0·01$; *** $P < 0·001$; † $P < 0·0001$; ‡ $P = 0·02$.

§ For details of subjects and procedures, see pp. 556–557.

‖ The patients followed a 'fasting' regimen for 7–10 d, a vegan diet for the next 3·5 months and a lactovegetarian diet for the remainder of the study.

¶ Only the patients who did not take cod-liver oil supplements.

TBARS

Because the concentration of TBARS was significantly different between the patient groups at baseline ANACOVA test was used with the baseline values as covariate. ANACOVA revealed that there was an overall significant reduction in the concentration of TBARS in the diet group compared with the control group during the study period ($P = 0·03$; Table 5). The TBARS were not significantly different in the diet responder group compared with the diet non-responder group.

Association between fatty acid concentration and disease activity

No significant differences were found in fatty acid concentrations between diet responders and diet non-responders. ANACOVA was used to investigate if the concentration of the fatty acids in plasma phospholipids varied in accordance with disease activity variables

Table 4. *Percentage distribution of fatty acids in plasma phospholipids in rheumatoid arthritis patients consuming vegan and lactovegetarian diets*‡§

(Mean values and standard deviations)

	At inclusion (*n* 27)		After the health farm stay (*n* 27)		After the vegan diet (*n* 26)		After the lactovegetarian diet (*n* 23)	
Fatty acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	0·36	0·36	0·23†	0·06	0·30**	0·12	0·38	0·11
16:0	24·85	0·98	25·81**	1·24	24·51	1·68	25·03	1·11
16:1	0·45	0·14	0·45	0·12	0·39**	0·11	0·44	0·16
18:0	13·19	0·69	12·71	1·18	13·01	1·16	13·06	0·79
18:1	9·18	1·23	8·64	1·10	8·18	1·14	8·41	1·30
18:2*n*-6	20·97	3·23	23·31**	4·47	24·71†	4·26	22·72**	3·43
18:3*n*-3	0·20	0·05	0·18	0·22	0·19	0·11	0·21	0·08
20:0	0·68	0·10	0·76**	0·13	0·75**	0·13	0·73	0·11
20:1	0·27	0·10	0·21**	0·08	0·22**	0·11	0·23	0·11
20:3*n*-9	0·18	0·08	0·05†	0·06	0·05†	0·05	0·12**	0·08
20:2*n*-6	0·48	0·07	0·44**	0·09	0·54	0·10	0·55**	0·11
20:3*n*-6	2·94	0·61	1·80†	0·41	2·48**	0·64	3·13	0·64
20:4*n*-6	8·32	1·19	8·56	1·76	8·40	1·77	8·36	1·38
20:5*n*-3‖	1·86	1·00	0·76†	0·44	0·81***	0·45	1·48**	0·98
22:0	2·41	0·39	2·00†	0·32	2·38	0·49	2·43	0·50
22:1*n*-11	0·79	0·31	0·43†	0·24	0·38†	0·32	0·53**	0·34
22:1*n*-9	0·25	0·11	0·16**	0·12	0·11†	0·11	0·19	0·11
22:4*n*-6	0·26	0·17	0·14***	0·11	0·09***	0·09	0·18	0·13
22:5*n*-6	0·14	0·05	0·13	0·06	0·12	0·07	0·16	0·09
22:5*n*-3‖	1·35	0·21	1·40	0·19	1·44	0·26	1·38	0·21
22:6*n*-3‖	6·58	1·60	6·33	1·27	5·80**	1·27	5·88**	1·71
24:0	1·13	0·25	1·09	0·21	1·45†	0·35	1·31**	0·38
24:1	2·47	0·42	3·21†	0·52	3·00**	0·72	2·54	0·62

Mean values were significantly different from those at inclusion into the study: ** $P < 0·01$; *** $P < 0·001$; † $P < 0·0001$.

‡ For details of subjects and procedures, see pp. 556–557.

§ The patients followed a 'fasting' regimen for 7–10 d, a vegan diet for the next 3·5 months and a lactovegetarian diet for the remainder of the study.

‖ Only the patients who did not take cod-liver oil supplements.

during the study. The concentrations of 20:4*n*-6 and 20:3*n*-6 were inversely associated with the number of swollen joints ($P = 0·05$ and $P = 0·01$ respectively).

DISCUSSION

We have conducted a clinical trial in which RA patients treated with a fast and a 1-year vegetarian diet improved significantly compared with RA patients using an omnivorous diet (Kjeldsen-Kragh *et al.* 1991). Since it has been shown that the inflammatory process can be modulated through fatty acid supplementation in patients with RA (Cleland *et al.* 1988; Baker *et al.* 1989; Tate *et al.* 1989), it is possible that the altered fatty acid profile following vegetarian diets (Phinney *et al.* 1990) can explain the measured improvement.

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the treatment of RA patients. As these drugs inhibit the production of prostaglandins it could be envisaged that treatment with such drugs may influence the concentration of fatty acids in the plasma phospholipids. Eleven patients did not use NSAIDs medication during the

Table 5. *Concentration of thiobarbituric acid-reacting substances (TBARS) in the plasma of patients with rheumatoid arthritis consuming a vegetarian diet, compared with controls**†*

(Mean values and standard deviations)

	TBARS (μmol/l)			
	Diet group‡		Control group	
	Mean	SD	Mean	SD
At inclusion	4·39	2·42	5·53	1·83
After 1 month	4·53	2·76	5·39	2·16
After 4 months	4·02	1·83	5·97	2·46
After 7–13 months	3·83	2·08	4·96	2·87

* For details of subjects and procedures, see pp. 556–557.

† Patients in the diet group followed a 'fasting' regimen for 7–10 d, a vegan diet for the next 3·5 months and a lactovegetarian diet for the remainder of the study.

‡ Overall difference between the diet group and the control group, $P = 0{\cdot}03$ (ANACOVA).

study period. Five patients reduced and one patient increased their intake of NSAID along the study course (Kjeldsen-Kragh *et al.* 1991). However, there was no significant difference with regard to fatty acid concentration or lipid peroxidation products between the patients who used NSAID and those who did not. The simultaneous use of corticosteroids (five patients), second-line drugs (four patients) and cyostatic drugs (two patients) might have obscured this result. As this was a longitudinal study and since the use of drugs was kept constant, except for the six patients, the measured within-group differences most likely express changes in the diet.

Increased intake of 18:2*n*-6 in the diet group did not result in an increased concentration of this fatty acid in the plasma phospholipids. The proportion of 18:2*n*-6, however, was significantly increased both after the vegan and the lactovegetarian diet periods (Table 4). Neither the concentration nor the proportion of 18:2*n*-6 was different in diet responders compared with diet non-responders and no association was found between this fatty acid and the disease activity variables. Therefore, an increased intake of 18:2*n*-6 does not seem to have any impact on disease activity in patients with RA. This result agrees with the conclusions of two other studies, where RA patents were treated with 18:2*n*-6 (Jäntti *et al.* 1985; Sköldstam *et al.* 1988).

Another way in which a vegetarian diet could have an impact on the inflammatory process in RA is through a reduced availability of 20:4*n*-6. The main source of this fatty acid in Western communities is the diet (Phinney *et al.* 1990; Adam, 1992). During a vegan diet period, which is devoid of 20:4*n*-6, and during a lactovegetarian diet period, which may only supply trace amounts of 20:4*n*-6, tissue 20:4*n*-6 must be supplied through endogenous production. This production of 20:4*n*-6 from 18:2*n*-6 is dependent on the activity of delta-6 and delta-5 desaturase enzymes. It has been shown that an intake of 20 g 18:2*n*-6/d inhibits the delta-6 desaturase activity (Adam, 1992). This would imply a reduced production of 20:4*n*-6 during the vegan diet period, since the mean dietary intake of 18:2*n*-6 exceeded this amount. The plasma concentration of phospholipid 20:4*n*-6 was significantly decreased during the vegan diet period but the proportion of 20:4*n*-6 in plasma phospholipids was unchanged. The observation that the concentration of 20:4*n*-6 was inversely associated with the number of swollen joints would also argue against an

impaired capacity to form arachidonic acid as an explanation for the decreased disease activity with the vegetarian diet treatment.

Several studies have found that relatively high intakes (> 2 g/d) of 20:5*n*-3 and 22:6*n*-3 lead to mild symptomatic improvement in RA (Kremer *et al.* 1987). In the present study the proportions of 20:5*n*-3 and 22:6*n*-3 fell during the vegan diet even though the proportion of 18:3*n*-3 was unchanged in the plasma phospholipids. This is in agreement with previous studies (Sanders *et al.* 1978) that reported lower proportions of 20:5*n*-3 and 22:6*n*-3 in vegans compared with omnivores. Despite the absence of 20:5*n*-3 and 22:6*n*-3 fatty acids in the vegan diet, disease activity was reduced in the patients. Neither were there significant differences in the plasma phospholipid concentrations of *n*-3 fatty acids between responders and non-responders.

A significant reduction in TBARS in the diet group compared with the control group agrees with findings from patients with fibromyalgia who fasted and used a vegetarian diet for 3 weeks (Høstmark *et al.* 1993). It is possible that the reduced malonaldehyde measured during the vegetarian diets could be an indicator of reduced eicosanoid formation. However, since there was no significant difference in the concentration of TBARS between the diet responder group and the diet non-responder group this may reflect a high intake of antioxidants, such as α-tocopherol and β-carotene, and/or a low fat intake (Table 1) with the vegetarian diets, rather than a reduced production following reduced disease activity.

In conclusion, the alterations of the fatty acid profiles in plasma phospholipids as a result of a vegan and a lactovegetarian diet were extensive in patients with RA. However, the clinical improvement could not be attributed to the changes in plasma phospholipid fatty acids.

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