Inflammation, Vol. 22, No. 4, 1998

EFFECTS OF DIETARY FISH OIL AND SOY PHOSPHATIDYLCHOLINE ON NEUTROPHIL FATTY ACID COMPOSITION, SUPEROXIDE RELEASE, AND ADHESION

PATRIZIA GUARINI,[1] PAOLO BELLAVITE,[2] DOMENICO BIASI,[1] ANTONIO CARLETTO,[1] SIMONETTA GALVANI,[1] PAOLA CARAMASCHI,[1] LISA M. BAMBARA,[1] and ROBERTO CORROCHER[1]

[1]*Institute of Medical Pathology*
[2]*Clinical Chemistry and Microscopy*
University of Verona, Ospedale Policlinico
Verona, Italy

Abstract—Fifty-seven healthy volunteers matched for sex and age were subdivided in 3 groups and their usual Western diets were supplemented according to three different protocols: group 1, fish oil supplement (20 ml/day); group 2, soybean phosphatidylcholine (PC) (25 g/day) and group 3, no supplementation (control group). After 2 weeks several important modifications of neutrophil fatty acid composition were observed: fish oil induced a significant decrease of linoleic (LA) and arachidonic acid (AA) and a significant increase of eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), while soy PC induced significant increases of LA, total polyunsaturated fatty acid (PUFA) and PUFA/SFA ratio. Neutrophil superoxide generation and adhesion were not modified by fish oil diet, on the contrary a slight but significant increase of $O_2^{\cdot-}$ production in response to fMLP was measured after soy PC diet. Our study confirms the possibility of changing neutrophil fatty acid composition in vivo by dietary means, but also suggests that the manipulation of cell functions, like superoxide anion generation and adhesion, is not easily and directly achieved by controlling membrane lipid environment.

INTRODUCTION

Several studies have demonstrated that dietary supplementation with $\omega 3$ PUFA results in reduction of chemotactic migration in neutrophils (1–3) modification of superoxide anion generation, either increased or decreased (4, 5), reduction in vitro (3) or in animal model (6), or increase (7) of neutrophil adhesion, reduction

0360-3997/98/0800-0381$15.00/0

of LTB_4 synthesis (3, 8), and concomitant increase of LTB_5 synthesis (2, 8) and modification in the synthesis of cytokines in monocytes (9). Very scanty are the data concerning the effect of dietetic intervention with $\omega 6$ fatty acids (linoleic acid) on the functions above described. In 1992 Jannace et al. (10) studied 8 normal subjects who supplemented their usual diet for 3 days with 27 g/day of soy PC and found that, after supplementation, neutrophil phagocytosis and killing increased several folds and arachidonic acid concentration and release augmented as well.

These data and observations prompted us to study the effect of dietary supplementation with $\omega 3$ fatty acids (cod liver oil) and $\omega 6$ fatty acids (soy phosphatidylcholine) on the respiratory burst and adhesion of neutrophils and on the fatty acid composition of these cells, in three groups of normal subjects, of which two followed the dietary modification and one served as control maintaining their usual diet.

Neutrophils were isolated from peripheral blood, then divided in two aliquots, one of which was used for the determination of fatty acids, the other was used for superoxide generation measurements and adhesion assay. Superoxide production and adhesion were measured on resting cells and on cells stimulated by different agonists (fMLP, PMA and zymosan).

MATERIAL AND METHODS

Subjects. Fifty seven healthy volunteers (28 males, 29 females; mean age 32 ± 6.5, range 18–50) participated to the trial. A dietician carefully interviewed the volunteers at the time they donated blood to ensure that the individuals were continuing their usual dietary habits while taking the supplementation. To monitor the diets, the subjects were asked to keep a 7 day dietary record during the experimental period. The subjects continued their normal activities during the whole study. The subjects were randomly assigned to one of these two dietary interventions for two weeks: 20 ml/day cod liver oil (diet 1), 25 g/day soy phosphatidylcholine (diet 2) or to a control group (diet 0). Eighteen subjects were assigned to diet 1, twenty-one to diet 2 and eighteen served as control group, since they followed their usual diet. At baseline and after the 2 weeks of dietary intervention the following parameters were measured in neutrophils: superoxide production and adhesion and fatty acid composition.

Reagents. Zymosan, fMLP, PMA, and reference fatty acids were purchased from Sigma Chemical Company, St. Louis, Missouri; cytochrome *c* from Boehringer, Mannheim, Germany; purified serum bovine albumin and human albumin from Behring Institute, Marburg, Germany; Percoll was from Pharmacia, Uppsala, Sweden. Sterile 96-well microtitre plates with flat-bottomed wells (Linbro type) were from Flow Laboratories. The microplates were pre-coated with fetal bovine serum (Flow Laboratories) in order to abolish non-specific cell activation as described (11). Hanks balanced salt solution (without calcium and magnesium) (HBSS) and Dulbecco's phosphate-buffered saline (PBS) were from Gibco Ltd, (Paisley, Scotland); the composition of HBSS was 0.4 g/l KCl, 0.06 g/l KH_2PO_4, 8 g/l NaCl, 0.09 g/l Na_2HPO_4, 1 g/l glucose (pH 7.4); the composition of PBS was 0.2 g/l KCl, 0.2 g/l KH_2PO_4, 0.047 g/l $MgCl_2$, 8 g/l NaCl, 1.15 g/l Na_2HPO_4 (pH 7.4). Other materials and reagents were of the highest purity available. Zymosan was opsonized with a pool of

normal human sera and serum tested zymosan was stored in aliquots at −20°C. In order to avoid contamination, a possible cause of artifactual activation or priming of the cells, sterile apyrogenic solutions and disposable plasticware were used in all experiments, which were carried out, whenever possible, under a laminar flow hood. Reagents were prepared using pyrogen-free water or 0.9% NaCl solutions.

Cell Preparation. Neutrophils were obtained from blood of normal volunteers by centrifugation over discontinuous Percoll gradients (12). EDTA-anticoagulated blood samples were centrifuged over Percoll gradients for 20 min at 1800 rpm with a Sorvall T6000B centrifuge. Neutrophils were recovered as a broad band at the 73%–62% interface, divided into two aliquots, one of which was used for the determination of fatty acid, the other was used for the measurement of superoxide production and adhesion. Cells were diluted with 1 volume of PBS, and centrifuged for 10 min at 1200 rpm. The pellet was usually slightly contaminated by erythrocytes, that were lysed by a brief hypotonic shock: cells were resuspended in 5 ml of 0.2% NaCl for 20 s, then the isotonicity was restored by addition of 5 ml of 1.6% NaCl and the right pH was restored by addition of 2 ml of PBS. After hypotonic lysis of contaminating erythrocytes and two washings with PBS, the cells (>95% neutrophils, >99% viable as judged by trypan blue exclusion test) were finally suspended in HBSS, containing 0.2% human serum albumin (H-A) and kept at room temperature until use. A few minutes before use, 100x concentrated solutions of $CaCl_2$ and $MgSO_4$ were added to the cell suspensions at final concentration of 0.5 mM and 1 mM respectively.

Superoxide Measurement and Adhesion. A microplate assay of $O_2^{\cdot-}$ production and adhesion was performed according to previously reported procedures (11, 13), with the following modifications. The assay medium was H-A supplemented with 0.5 mM $CaCl_2$ and 1 mM $MgSO_4$ (H-ACM). The microplate wells were supplemented with 25 μl of 0.6 mM cytochrome *c* and with either 25 μl of the stimulant dissolved in H-ACM (fMLP, PMA or zymosan) at the final concentration of 5 $\times$ 10^{-7} M, 10 ng/ml and 0.5 mg/ml respectively or with 25 μl of H-ACM (resting assays). Assays were currently done in triplicate for each experimental condition. The plate was brought to 37°C, and 50 μl of the neutrophil suspension (2 $\times$ 10^5 cells), pre-warmed at 37°C, were added to each well. The plate was then incubated for the indicated time in a humidified thermostat at 37°C. When required, the plate was rapidly transferred into a microplate reader (Reader 400, SLT Labs Instruments) and the reduction of cytochrome *c* was measured at 550 nm using 540 nm as reference wavelength, and using 0.037 optical density units as standard for 1 nmol of reduced cytochrome *c* (11).

For adhesion measurements, after 40 min of incubation the plates were transferred to an automatic washer (Easy Washer 2, SLT Labs Instruments) and subjected to two washing cycles with PBS at room temperature. Adherent cells were quantitated by measuring the membrane enzyme acid phosphatase and the percentage of adhesion was calculated on the basis of a standard curve obtained with known numbers of neutrophils from the same subject (11).

Fatty Acid Determination. Analysis of fatty acids was performed on aliquots of neutrophil preparations containing 10^7 cells, by extraction of total lipids with 4.5 ml of isopropanol/chloroform (11/7, v/v) added with 0.45 mM 2,6-di-tert-*p*-cresol (BHT) as antioxidant. A gas chromatographic method (Hewlett Packard 5890 chromatograph, Hewlett Packard, Palo Alto, California) was employed as previously described (14), based on fatty acid direct transesterification technique (15). Analysis was performed in duplicate on each sample. Peak identification and quantification were made with reference fatty acids commercially available. As internal standard, heptadecanoic acid (C17:0) was used. The heights of the peaks were measured and the quantification was performed with the aid of a PC Vectra QS/16S equipped with the software HP-3365 ChemStation (Hewlett Packard, Palo Alto, California) working in Microsoft Windows 3.0. Fatty acid composition data were expressed as mg/100 mg fatty acid methyl esters. The mean percentage recovery was 90.6 ± 2.7 (range 85–94%).

Data Analysis. Statistical analysis was performed on a PC Apple Macintosh SE/30 equipped

with the software Systat 5.0 working in Windows 3.0 (Microsoft). Values are expressed as mean ± *SD* and were compared using the paired Student's *t* test; the correlation between different parameters was studied using the Pearson correlation coefficient.

RESULTS

In Table 1 is shown the estimated mean baseline intake of nutrients in the three groups of subjects undergoing the study. No significant differences were found among the three groups.

The composition of fish oil and soy phosphatidylcholine is given in Table 2; subjects of group diet 1 assumed 1.3 g/day of EPA and 1.8 g/day of DHA, diet 2 supplied 1.5 g/day of LA.

The analysis of variance (ANOVA) demonstrated (data not shown) that in the pre-treatment period there was no significant difference in age, neutrophil fatty acid composition, superoxide production and adhesion (fMLP, PMA and zymosan stimulated) among the three groups of subjects undergoing the trial. The neutrophil fatty acid composition before and after fish oil supplementation is shown in Table 3. A significant increase of EPA and DHA incorporation in the membranes was observed and it was accompanied by an increase of total ω3-PUFA and a significant reduction in the levels of LA, AA and total ω6-PUFA: the ω3/ω6 ratio was consequently highly increased. Noteworthy, the level of total PUFA and the P/S ratio were unchanged. Fish oil treatment did not modify either superoxide generation and cell adhesion in response to the different agonists used (Table 4).

Supplementation of diet with soy phosphatidylcholine resulted in several notable modifications of neutrophil fatty acid composition (Table 5). Oleic acid (C18:1ω9) decreased, as well as eicosaenoic acid (C20:1ω9) and the total MUFA

Table 1. Estimated Mean Daily Intakes of Nutrients of the Three Groups of Subjects at Baseline (from 7 Day Food Record)

Nutrients	Control Group	Fish Oil Group	Soy PC Group
Energy (kj)	8542 ± 1651	8312 ± 1548	8491 ± 1709
Proteins (g)	82 ± 21	88 ± 29	79 ± 17
Carbohydrates (g)	277 ± 58	251 ± 65	287 ± 72
Total fat (g)	74 ± 17	72 ± 18	68 ± 15
Saturated fat (g)	19 ± 7	21 ± 5	19 ± 9
Polyunsaturated fat (g)	6 ± 2	8 ± 3	7 ± 2
Monounsaturated fat (g)	16 ± 5	16 ± 9	17 ± 4
Cholesterol (mg)	247 ± 99	235 ± 112	241 ± 95
Fiber (g)	23 ± 12	26 ± 10	21 ± 11

Table 2. Fatty Acid Composition of Fish Oil and Soy Phosphatidylcholine (PC). Subjects Were Treated for 2 Weeks with Either 20 ml/day of Fish Oil or 25 g/day of Soy PC

Fish Oil			Soy Phosphatidyl Choline		
Fatty acid	mg/ml	%	Fatty acid	mg/g	%
C12:0	0.33	0.07	C12:0	0.02	0.01
C14:0	25.14	5.33	C14:0	0.31	0.23
C16:0	74.25	15.74	C16:0	32.68	23.76
C16:1w7	42.55	9.02	C16:1w7	0.35	0.25
C18:0	19.74	4.18	C18:0	7.46	5.42
C18:1w9	98.99	20.99	C18:1w9	17.73	12.89
C18:2w6	10.97	2.33	C18:2w6	59.80	43.48
C18:3w3	5.00	1.06	C18:3w3	17.93	13.04
C18:4w3	21.02	4.46	C18:4w3	0.03	0.02
C20:0	0.48	0.10	C20:0	0.57	0.41
C20:1w9	5.45	1.16	C20:1w9	0.24	0.17
C20:2w6	1.78	0.38	C20:2w6	0.30	0.22
C20:4w6	3.33	0.71	C20:4w6	0.04	0.03
C20:5w3	67.07	14.22	C20:5w3	—	—
C22:0	0.37	0.08	C22:0	—	—
C22:1w9	5.89	1.25	C22:1w9	0.07	0.05
C24:0	0.30	0.06	C24:0	—	—
C22:6w3	89.05	18.88	C22:6w3	—	—
SFA	120.61	25.57	SFA	41.04	29.84
MUFA	152.88	32.41	MUFA	18.39	13.37
PUFA	198.22	42.02	PUFA	78.10	56.79
UFA	351.10	74.43	UFA	96.49	70.16
w3	182.14	38.61	w3	17.96	13.06
w6	16.08	3.41	w6	60.14	43.73
P/S	1.64	1.64	P/S	1.90	1.90
w3/w6	11.33	11.33	w3/w6	0.30	0.30

significantly diminished; linoleic acid significantly increased while arachidonic remained unchanged; the total amount of $\omega 6$ significantly increased (before 23.15 ± 1.46, after 24.61 ± 1.59, $P < 0.001$) as well as total PUFA (before 24.83 ± 1.27, after 26.33 ± 1.43, $P < 0.001$); as a consequence the P/S ratio increased.

After soy phosphatidylcholine supplementation fMLP-induced superoxide generation increased significantly (Table 6, before 5.62 ± 2.79, after 6.43 ± 2.8 nmoles/10^6 cells, $P = 0.016$), while adhesion was not modified by the dietary intervention. No correlation could be demonstrated between superoxide production and any of the fatty acid (either single or classes) measured. In the group of control subjects who did not modify diet, neutrophil fatty acid composition, superoxide generation and adhesion were totally unchanged after 2 weeks.

Table 3. Fatty Acid Composition of Neutrophils Before and After Fish Oil Supplementation (20 ml/day). Fatty Acids Were Expressed as mg/100 mg of Fatty Acid Methyl Esters

Fatty Acid	*N*	Before	After	% variation	p[a]
C12:0	18	0.16 ± 0.08	0.14 ± 0.07	—	ns
C14:0	18	0.57 ± 0.13	0.56 ± 0.09	—	ns
C16:0	18	20.56 ± 0.86	20.73 ± 1.60	—	ns
C18:0	18	19.60 ± 1.11	19.66 ± 1.15	—	ns
C20:0	18	0.43 ± 0.22	0.32 ± 0.21	—	ns
C22:0	18	1.38 ± 0.22	1.31 ± 0.18	—	ns
C24:0	18	2.04 ± 0.33	2.11 ± 0.47	—	ns
SFA	18	44.74 ± 1.66	44.84 ± 1.97	—	ns
C18:1ω9	18	27.93 ± 2.06	28.22 ± 1.79	—	ns
C20:1ω9	18	0.98 ± 0.14	1.01 ± 0.15	—	ns
C22:1ω9	18	0.94 ± 1.20	0.50 ± 0.38	—	ns
MUFA	18	29.84 ± 1.76	29.72 ± 1.81	—	ns
C18:2ω6	18	8.89 ± 1.93	8.19 ± 1.75	−7.87	0.001
C20:2ω6	18	1.00 ± 0.37	0.94 ± 0.35	—	ns
C20:4ω6	18	13.16 ± 1.80	11.66 ± 1.71	−11.4	0.001
ω6	18	23.04 ± 2.02	20.78 ± 1.96	−9.8	0.001
C18:3ω3	18	0.04 ± 0.09	0.11 ± 0.37	—	ns
C18:4ω3	18	0.27 ± 0.56	0.18 ± 0.42	—	ns
C20:5ω3	18	0.46 ± 0.36	1.83 ± 0.79	298	0.001
C22:6ω3	18	0.85 ± 0.45	1.81 ± 0.54	113	0.001
ω3	18	1.61 ± 0.92	3.92 ± 1.43	143	0.001
PUFA	18	24.66 ± 1.53	24.70 ± 1.57	—	ns
UFA	18	54.50 ± 1.38	54.42 ± 1.83	—	ns
ω3/ω6	18	0.07 ± 0.04	0.19 ± 0.07	171	0.001
P/S	18	0.55 ± 0.05	0.55 ± 0.05	—	ns

[a] The paired Student *t* tests comparing values before and after diet supplementation from each subject are reported.

DISCUSSION

The present study was performed in order to compare the effects of two different dietetic interventions on two functions of neutrophils, i.e. the generation of superoxide anions and the extent of cell adhesion in response to suitable agonists.

Diet enrichment with fish oil results in profound modifications in neutrophil lipid composition: significant increase of EPA and DHA (ω3 PUFAs) and parallel decrease of levels of LA and AA (ω6 PUFAs). Consequently the ω3/ω6 ratio increases significantly, but noteworthy the total amount of PUFA and the

Table 4. Superoxide Production and Adhesion in Neutrophils Under Basal Condition and in Response to Different Stimuli, Before and After Fish Oil Supplementation (20 ml/day). Superoxide Production was Expressed as nmoles/10^6 Cells. Incubation of fMLP and PMA Stimulated Cells was Carried Out for 10 Min, Incubation of Zymosan Stimulated Cells for 40 Min. Adhesion was Expressed as Percentage of Adherent Cells and Incubation was 40 Min in All Conditions

	N	Before	After	% variation	p[a]
superox fMLP	17	5.87 ± 2.95	5.74 ± 3.20	—	ns
superox zym	17	7.42 ± 1.20	7.21 ± 0.83	—	ns
superox PMA	17	23.36 ± 4.76	21.24 ± 5.40	—	ns
adhesion fMLP	17	25.54 ± 8.65	29.61 ± 9.23	—	ns
adhesion zym	17	17.64 ± 4.80	19.41 ± 5.55	—	ns
adhesion PMA	17	51.93 ± 7.16	51.14 ± 9.05	—	ns

[a]The paired student *t* test comparing values before and after diet supplementation from each subject are reported.

P/S ratio remained unchanged, indicating that fish oil supplementation induces a substitution of PUFA $\omega 6$ with $\omega 3$, rather than a net enrichment of the cell with polyunsaturated fatty acids. The function parameters were not modified by fish oil diet.

Literature data on effects of dietary supplementation with $\omega 3$ fatty acids on leukocyte functions are not fully in agreement. Several studies have demonstrated that dietary supplementation with $\omega 3$ PUFA results in reduction of chemotactic migration in neutrophils (1–3), increased superoxide anion generation (5), reduction of neutrophil adhesion in vitro (3) or in animal model (6), reduction of LTB_4 synthesis (2, 8) and concomitant increase of LTB_5 synthesis (2, 3) and modification in the synthesis of cytokines in monocytes (9). On the other hand, several groups have demonstrated rather opposite effects of dietary supplementation with fish oil on neutrophil function: decreased superoxide anion generation in neutrophils (4), decreased respiratory burst in monocytes, but unchanged in neutrophils (16) and increased neutrophil adhesion (7).

Our results demonstrate that the significative modifications observed in the lipid pattern of neutrophils after fish oil diet are not accompanied by relevant changes in neutrophil $O_2^{\cdot -}$ generation and adhesion, in response to different agonist agents. Several hypotheses might explain this discrepancy: the duration of dietetic intervention was shorter in our study (2 weeks) compared to others, but we believe that this time is sufficient to enrich cell membrane with $\omega 3$ fatty acid, taking into account the mean life span of neutrophils; in fact, cell lipid composition was deeply modified after fish oil. Since the diet induced a complex series of modifications of fatty acids, it is also possible that the lack of effect on the in vitro leukocyte functions is due to a balance of inhibitory and stimulatory

Table 5. Fatty Acid Composition of Neutrophils Before and After Soy Phosphatidylcholine Supplementation (25 g/day). Fatty Acids Were Expressed as mg/100 mg of Fatty Acid Methyl Esters

Fatty Acid	*N*	Before	After	% variation	p[a]
C12:0	21	0.21 ± 0.18	0.12 ± 0.08	−42.86	0.043
C14:0	21	0.61 ± 0.20	0.50 ± 0.12	−18.03	0.027
C16:0	21	20.63 ± 1.31	20.79 ± 1.45	—	ns
C18:0	21	19.39 ± 1.11	19.61 ± 1.00	—	ns
C20:0	21	0.35 ± 0.21	0.35 ± 0.21	—	ns
C22:0	21	1.37 ± 0.23	1.37 ± 0.20	—	ns
C24:0	21	2.20 ± 0.54	2.30 ± 0.70	—	ns
SFA	21	44.76 ± 2.25	45.04 ± 2.06	—	ns
C18:1ω9	21	27.93 ± 1.97	26.55 ± 1.72	−4.94	0.001
C20:1ω9	21	0.95 ± 0.16	0.90 ± 0.17	−5.26	0.02
C22:1ω9	21	0.60 ± 0.68	0.43 ± 0.52	—	ns
MUFA	21	29.48 ± 2.16	27.88 ± 1.88	−5.43	0.001
C18:2ω6	21	8.14 ± 0.95	9.51 ± 1.01	16.83	0.001
C20:2ω6	21	0.83 ± 0.31	1.01 ± 0.39	21.69	0.001
C20:4ω6	21	14.18 ± 1.27	14.09 ± 1.40	—	ns
ω6	21	23.15 ± 1.46	24.61 ± 1.59	6.31	0.001
C18:3ω3	21	0.32 ± 0.07	0.05 ± 0.13	—	ns
C18:4ω3	21	0.12 ± 0.37	0.13 ± 0.33	—	ns
C20:5ω3	21	0.53 ± 0.36	0.55 ± 0.55	—	ns
C22:6ω3	21	1.00 ± 0.33	0.99 ± 0.53	—	ns
ω3	21	1.68 ± 0.62	1.71 ± 0.69	—	ns
PUFA	21	24.83 ± 1.27	26.33 ± 1.43	6.04	0.001
UFA	21	54.31 ± 2.09	54.21 ± 2.01	—	ns
ω3/s6	21	0.07 ± 0.03	0.07 ± 0.03	—	ns
P/S	21	0.55 ± 0.05	0.59 ± 0.05	7.27	0.003

[a] The paired Student *t* tests comparing values before and after diet supplementation from each subject are reported.

effects. In fact, it has been reported that in vitro, EPA and DHA are activators of neutrophil respiratory burst (4) and adhesion (7) and this could be an opposite effect with respect to the decrease of AA. If this is the case, the apparent discrepancies between different laboratories could be due to experimental differences that may affect such a balance.

Another possible interpretation of our findings is that, since the activation of NADPH oxidase, the enzyme responsible for $O_2^{\cdot -}$ generation, does not have absolute requirement of prostaglandin intermediates (17), changes in membrane fatty acids which are substrates for cyclooxygenase may not modify the respiratory burst through modification of biologically active eicosanoids. On the

Table 6. Superoxide Production and Adhesion in Neutrophils Under Basal Condition and in Response to Different Stimuli, Before and After Soy Phosphatidylcholine Supplementation (25 g/day). Superoxide Production was Expressed as nmoles/10^6 Cells. Incubation of fMLP and PMA Stimulated Cells was Carried Out for 10 Min, Incubation of Zymosan Stimulated Cells for 40 Min. Adhesion was Expressed as Percentage of Adherent Cells and Incubation was 40 Min in All Conditions

	N	Before	After	% variation	p[a]
superox fMLP	20	5.62 ± 2.79	6.43 ± 2.80	14.41	0.016
superox zym	18	8.32 ± 1.25	8.06 ± 0.83	—	ns
superox PMA	20	23.43 ± 5.15	22.19 ± 5.05	—	ns
adhesion fMLP	21	28.84 ± 8.07	29.93 ± 8.73	—	ns
adhesion zym	21	17.99 ± 6.57	19.73 ± 6.25	—	ns
adhesion PMA	21	49.98 ± 5.19	50.46 ± 6.84	—	ns

[a] The paired Student *t* tests comparing values before and after diet supplementation from each subject are reported.

other hand, arachidonic acid is a major intermediate of the signal transduction pathways that lead to the activation of NADPH oxidase (18, 19); therefore its decrease after fish oil diet (Table 3) should be associated with a decrease of superoxide production. However, the negative results of our present experiment indicate that a 11% decrease of arachidonic acid content that may be achieved with our diet regimen, although significant, is not sufficient to affect the amount of free arachidonic acid that is available during cell activation. It is conceivable that the residual membrane arachidonic acid after the dietetic manipulation is far enough to allow the optimal enzymatic activity of neutrophil phospholipase A_2 and of the activation machinery.

The supplementation of diet with soy phosphatidylcholine elicited several modifications of neutrophil fatty acid composition: increased levels of LA, ω6 PUFAs, total PUFA and augmented P/S ratio. Such modifications in membrane lipid structure were accompanied by a little but significant increase in $O_2^{\cdot-}$ production after stimulus with fMLP, confirming the data regarding bactericidal activity in previous report (10). Differently, we could not demonstrate a significant increase of AA after this diet. In our opinion the increased oxidative metabolic response is conceivable also in presence of unchanged AA levels, since AA concentration is not considered a limiting factor in NADPH oxidase activation, process which requires extremely low amounts of this fatty acid. The reason by which only fMLP-stimulated $O_2^{\cdot-}$ release was increased by soy phosphatidylcholine diet remains to be investigated.

The relevance of correlations between eicosanoids, fatty acids and neutrophils in the pathophysiology of disease has been recently reviewed by Bates

(20), who stated that small alterations in structure can result in large changes in the neutrophil response, and that this would have important implications for further development and use of fatty acids for therapeutic purposes. Our data confirm the possibility of changing neutrophil fatty acid composition in vivo by dietary means, but also suggest that the manipulation of cell functions, like superoxide anion generation and adhesion, is not easily and directly achieved by controlling membrane lipid environment. Strategies to regulate neutrophil activity by diet needs to be further studied and clarified.

Acknowledgments—This work was supported by a grant 60% from Ministero Ricerca Scientifica e Tecnologica, from Public Health Department, Regione Veneto and by a grant from "Progetto Sanità" 1996–97, Fondazione Cassa di Risparmio di Verona, Vicenza, Belluno ed Ancona. The skillful technical assistance of Patrizia Pattini and Fabio Poli are gratefully acknowledged.

REFERENCES

1. FERRANTE, A., D. GOH, D. P. HARVEY, B. S. ROBINSON, C. S. HII, E. J. BATES, S. J. HARDY, D. W. JOHNSON, and A. POULOS. 1994. Neutrophil migration inhibitory properties of polyunsaturated fatty acids. *J. Clin. Invest.* **93:**1063–1070.
2. SPERLING, R. I., A. I. BENINCASO, C. T. KNOELL, J. K. LARKIN, K. F. AUSTEN, and D. R. ROBINSON. 1993. Dietary ω-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. *J. Clin. Invest.* **91:**651–666.
3. LEE, T. H., R. L. HOOVER, J. D. WILLIAMS, R. I. SPERLING, J. RAVALESE III, B. W. SPUR, D. R. ROBINSON, E. J. COREY, R. A. LEWIS, and K. F. AUSTEN. 1985. Effects of dietary enrichment with eicosapentaenoic acid and docosahexaenoic acid on in vitro neutrophil and monocyte leukotriene generation and neutrophil function. *N. Engl. J. Med.* **312:**1217–1224.
4. POULOS, A., B. S. ROBINSON, A. FERRANTE, D. P. HARVEY, S. J. HARDY, and A. W. MURRAY. 1991. Effect of 22–32 carbon n-3 polyunsaturated fatty acids on superoxide production in human neutrophils: synergism of docosahexaenoic acid with f-met-leu-phe and phorbol ester. *Immunology* **73:**102–108.
5. CHEN, L. Y., D. L. LAWSON, and J. L. MEHTA. 1994. Reduction in human neutrophil superoxide anion generation by n-3 polyunsaturated fatty acids: role of cyclooxygenase products and endothelium-derived relaxing factor. *Thromb. Res.* **76:**317–322.
6. LEHR, H. A., C. HÜBNER, B. FINCKH, D. NOLTE, U. BEISIEGEL, A. KOHLSCHÜTTER, and K. MESSMER. 1991. Dietary fish oil reduces leukocyte/endothelium interaction following systemic administration of oxidatively modified low density lipoprotein. *Circulation* **84:**1725–1731.
7. BATES, E. J., A. FERRANTE, D. P. HARVEY, and A. POULOS. 1993. Polyunsaturated fatty acids increase neutrophil adherence and integrin receptor expression. *J. Leukot. Biol.* **53:**420–426.
8. VON SCHACKY, C., R. KIEFL, A. J. MARCUS, M. J. BROEKMAN, and W. E. KAMINSKI. 1993. Dietary n-3 fatty acids accelerate catabolism of leukotriene B4 in human granulocytes. *Biochim. Biophys. Acta* **1166:**20–24.
9. ENDRES, S., R. GHORBANI, V. E. KELLEY, K. GEORGILIS, G. LONNEMANN, J. W. M. VAN DER MEER, J. G. CANNON, T. S. ROGERS, M. S. KLEMPNER, P. C. WEBER, E. J. SCHAEFER, S. M. WOLFF, and C. A. DINARELLO, 1989. The effect 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.
10. JANNACE, P. W., R. H. LERMAN, J. I. SANTOS, and J. J. VITALE. 1992. Effects of oral soy

phosphatidylcholine on phagocytosis, arachidonate concentrations, and killing by human polymorphonuclear leukocytes. *Am. J. Clin. Nutr.* **56**:599–603.

11. BELLAVITE, P., S. CHIRUMBOLO, C. MANSOLDO, G. ANDINI, and P. DRI. 1992. A simultaneous assay for oxidative metabolism and adhesion of human neutrophils. Evidence for correlations and dissociations of the two responses. *J. Leukot. Biol.* **51**:329–335.
12. METCALF, J. A., J. I. GALLIN, W. M. NAUSEEF, and R. K. ROOT. editors. 1986. *In:* Laboratory Manual of Neutrophil Function. Raven Press; New York.
13. BELLAVITE, P., S. CHIRUMBOLO, G. LIPPI, P. GUZZO, and C. SANTONASTASO. 1993. Homologous priming in chemotactic peptide stimulated neutrophils. *Cell Biochem. Func.* **11**:93–100.
14. GIRELLI, D., M. AZZINI, P. GUARINI, M. T. TREVISAN, A. LUPO, P. BERNICH, G. PANZETTA, and R. CORROCHER. 1992. Red blood cells and platelet membrane fatty acids in non-dyalized and dyalized uremics. *Clin. Chim. Acta.* **211**:155–166.
15. LEPAGE, G., and C. C. ROY, 1986. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **27**:114–120.
16. SIRTORI, C. R., E. GATTI, E. TREMOLI, C. GALLI, G. GIANFRANCESCHI, G. FRANCESCHINI, S. COLLI, P. MADERNA, F. MARANGONI, P. PEREGO, and E. STRAGLIOTTO. 1992. Olive oil, corn oil, and n-3 fatty acids differently affect lipids, lipoproteins, platelets, and superoxide formation in type II hypercholesterolemia. *Am. J. Clin. Nutr.* **56**:113–122.
17. BELLAVITE, P. 1988. The superoxide-forming enzymatic system of phagocytes. *Free Rad. Biol. Med.* **4**:225–261.
18. BELLAVITE, P., P. GUARINI, D. BIASI, A. CARLETTO, M. T. TREVISAN, P. CARAMASCHI, L. M. BAMBARA, and R. CORROCHER. 1995. Correlations between the intensity of fMLP-dependent respiratory burst and cellular fatty acid composition in human neutrophils. *Br. J. Haematol.* **89**:271–276.
19. RUBINEK, T. and R. LEVY. 1993. Arachidonic acid increases the activity of the assembled NADPH oxidase in cytoplasmic membranes and endosomes. *Biochim. Biophys. Acta.* **1176**:51–58.
20. BATES, E. J. 1995. Eicosanoids, fatty acids and neutrophils: their relevance to the pathophysiology of disease. *Prost. Leukot. Essen. Fatty Acids* **53**:75–86.