Cytometry (Communications in Clinical Cytometry) 34:17–21 (1998)

Platelet Fatty Acids and Peripheral Blood Lymphocyte Subsets in an Institutionalized Elderly Population

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The associations between platelet fatty acids and peripheral blood lymphocyte subsets were studied in 78 institutionalized elderly individuals (27 men and 51 women), aged 67 to 100. Platelet fatty acids were assessed by gas chromatography, and peripheral blood lymphocyte subsets were quantitated by immunophenotyping using flow cytometry. It was found that women had a higher number of total T-cells (CD3), T-helper (CD3+4+) cells, and B-cells (CD19). However, no gender differences were observed in the percentages of lymphocyte subsets. In elderly men, after adjusting for age and fatty acid intake, the platelet concentration of ω-3 polyunsaturated fatty acids was positively related to the percentage of CD3 and CD3+4+ bearing lymphocytes ($r_s = 0.59$, $P < 0.05$; and $r_s = 0.55$, $P < 0.05$, respectively), and the concentration of total saturated fatty acids was also positively associated with the percentage of B (CD19) cells ($r_s = 0.50$, $P < 0.05$). However, similar relationships were not observed in elderly women. No significant associations were found between *trans* fatty acids and any of the lymphocyte subsets in the study population. These findings suggest that fatty acids may be related to immune function. Any effects may be important in the host immune defence, especially in elderly individuals. Cytometry (Comm. Clin. Cytometry) 34:17–21, 1998.

Key terms: fatty acids; *trans*-fatty acids; lymphocytes; immune function; institutionalized elderly

Ageing is accompanied by a decline in physiological reserves and an increase in morbidity due to chronic degenerative and neoplastic diseases. These changes are, in part, nutritionally related and immune mediated (1, 2). The effects of ageing, combined with genetic endowment and environmental influences, such as nutritional factors, contribute to immune dysfunction in elderly individuals (3–5).

Changes in the fatty acid composition of both plasma and platelet-lipids occur with ageing (6). It is suggested that diet and metabolism may contribute to these changes (7). In an elderly institutionalised Japanese population, different fatty acids are associated with age in different ways (8).

Immunomodulatory functions of fatty acids have been demonstrated in many studies. Both human and animal studies have shown beneficial effects of ω-3 polyunsaturated fatty acids (PUFAs) on the inflammatory response of autoimmune diseases, such as rheumatoid arthritis (9). In vitro studies have shown that PUFAs suppress human peripheral blood lymphocyte proliferation and interleukin-2 (IL-2) production (10). The inhibition of proliferation caused by eicosapentaenoic acid led to a reduction in IL-2 concentration. Dietary supplementation with α-linolenic acid has been shown to suppress the proliferation of peripheral blood mononuclear cells stimulated by both phytohemagglutinin and concanavalin A (11).

Limited data are available on the relationships between fatty acids and immunocompetence in the elderly. Rasmussen et al. (12) recently reported negative correlations between basal natural killer cell (NK-cell) activity, and total PUFAs, total ω-6 PUFAs, and linoleic acid. Significant inverse relationships were also found between interferon (IF)-α-stimulated NK cells and the three groups of fatty acids, and between IL-2-stimulated NK cells and PUFAs.

In the present cross-sectional study, platelet fatty acids and peripheral blood lymphocyte subsets were measured

Contract grant sponsor: Meat Research Corporation, Australia.

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Received 20 November 1996; Accepted 3 November 1997

Table 1
Proportion of Subjects Who Had Been Diagnosed With the Selected Disease or Medical Conditions

	% Subject		
	Total (n = 78)	Men (n = 27)	Women (n = 51)
Cardiovascular	76.9	81.5	74.5
Respiratory	21.8	33.3	15.7
Bone	56.4	59.3	54.9
Digestive	39.7	40.7	39.2
Blood disorders	15.4	29.6	7.8
Cognitive impairment	53.8	51.9	54.9
Skin disorders	26.9	33.3	23.5
Sight and hearing	38.5	25.9	45.1
Endocrine disorders	20.5	22.2	19.6
Liver/kidney disorders	21.8	18.5	23.5
Reproductive disorders	25.6	14.8	31.4
Cancer	15.4	22.2	11.8
Systemic	15.4	22.2	11.8

in an institutionalized elderly population. The relationships between various fatty acids and lymphocyte subsets were examined.

SUBJECTS AND METHODS

Subjects

Seventy-eight elderly individuals (27 men and 51 women), aged 67 to 100, residing at Kingston Centre, Melbourne, Australia, were recruited. Considering that elderly women outlive their male counterparts, we were unable to obtain age- and gender-matched subjects. The women were significantly older than men (mean age ± SD, 83.9 ± 7.4, and 78.6 ± 6.3 years, $P = 0.002$).

No attempt was made to select subjects on the basis of ethnicity, body habitus, and weight change, or other features of general health status or medication. The diseases and medical conditions of study subjects are listed in Table 1. The study was approved by the Human Ethics Committees of Monash Medical Centre and Kingston Centre.

Estimation of Food and Nutrient Intake

Information on food intake was obtained using a 7-day weighed food diary. Seven consecutive days were recorded so each day of the week was represented. A software package (DIET/1, Xylis) with an Australian food composition database (NUTTAB), was used to calculate daily intake of nutrients including saturated (SFAs), monounsaturated (MUFAs) and PUFAs.

Platelet Fatty Acids Analysis

Collection and preparation of platelets. Ten milliliters of EDTA-anticoagulated blood was used for platelet harvesting. The tubes of blood were centrifuged at 110*g* for 15 min and the platelet-rich plasma was removed, then recentrifuged at 2,000*g* for 10 min at room temperature. The plasma was removed and the platelets were washed with 0.9% sodium chloride solution containing 1 g EDTA/L. The platelet pellets were then frozen at −70°C until extraction and methylation.

Extraction and methylation of the platelet fatty acids. A modification of the one-step method described by Lepage and Roy (13) was performed. Samples were mixed with 2 ml of 4:1 methanol-toluene containing butylated hydroxy-toluene as antioxidant. Two hundred microliters of acetyl chloride was added slowly with continuous mixing, then samples were placed into an oven at 100°C for an hour. The tubes were cooled under running water, 5 ml of potassium carbonate was added, and the tubes were mixed and then centrifuged at 2,000*g* for 10 min at room temperature. The upper toluene phase was removed and then dried under nitrogen gas.

Immediately after extraction and methylation of the fatty acids, the methylated fatty acids were dissolved in 50 μL of chloroform for injection. A Shimadzu GC-9A gas chromatograph was used with a flame ionization detector and a Shimadzu Chromatopac C-R3A integrator (Shimadzu Corporation, Kyoto, Japan). Two 50-m fused silica (BP × 70, 0.25 μm) columns were joined to make a total column length of 100 m (SGE, Victoria, Australia). The methyl ester peaks were identified with standards obtained from Nu Chek Prep, Inc (Elysian, MN) and Alltech Associates Inc. (Deerfield, IL). Temperature programming was used for fatty acid determination. The starting temperature of 175°C was raised (after 35 min) at 2.5°C/min until 220°C was reached, then increased (after 17 min) at 2.5°C/min until 240°C was reached. The temperature was then held constant for 24 min. The coefficients of variation (CVs) in determining the percentages of the individual fatty acids varied depending on the concentration of the fatty acid in the sample. For major components (more than 5%), the CVs ranged from 7.1 to 11.2%. For trace fatty acids (0.1 to 1%), the CVs ranged from 8.1 to 18.4%.

Immunophenotyping

Venous blood samples were obtained in EDTA-treated tubes early in the morning. Blood specimens were set up on the same day of collection. One hundred microliters of whole blood was placed in a Wasserman tube. The following FITC and PE conjugated antibody combinations were used: G1+G1 (negative control by isotope matched), CD45+CD14, CD3+CD4, CD3+CD8, CD2+CD19 (B cell); CD56 (NK cell) was run alone (Coulter, Hialeah, FL). Antibody was added and then incubated for 60 min at room temperature. Cells were lysed and fixed by using the 35-s cycle of QPREP machine (Coulter). The specimens were then stored in the dark overnight at 4°C. On the next day, cell suspensions were spun at 1,200 rpm for 5 min. The supernatant was removed and the cells were resuspended in 300 μL PBS/1% BSA/0.01% NaAz, and then 300 μL of 2% paraformaldehyde was added. Enumeration of cells was done using an EPICS 752 flow cytometer (Coulter) with at least 5,000 cells counted in the lymphocyte gate. Total lymphocyte and white blood cell counts used to calculate the absolute counts of lymphocytes were obtained from the routine full blood examination tests done by the Department of Haematology at Monash Medical Centre.

Table 2
Platelet Fatty Acid Profiles of the Subjects[a]

Fatty acid (% of total fatty acids)	Men (n = 27)	Women (n = 51)
Total saturated fatty acids (%)	40.62 ± 1.48	40.38 ± 1.90
Palmitic (C16:0)	17.97 ± 1.56	18.01 ± 2.10
Stearic (C18:0)	17.98 ± 1.37	17.68 ± 1.17
Arachidic (C20:0)	1.30 ± 0.15	1.31 ± 0.16
Behenic (C22:0)	2.34 ± 0.35	2.36 ± 0.44
Total monounsaturated fatty acids (%)	14.94 ± 1.06	14.79 ± 1.21
Palmitoleic (C16:1 ω7)	0.42 ± 0.18	0.44 ± 0.18
Oleic (C18:1 ω-9)	12.92 ± 0.86	12.81 ± 1.07
Eicosanoic (C20:1 ω-9)	0.42 ± 0.07	0.43 ± 0.08
Total polyunsaturated (ω-6) fatty acids (%)	27.92 ± 1.80	27.94 ± 1.57
Linoleic (C18:2)[b]	5.70 ± 0.99	6.24 ± 1.00
Homo-γ-linolenic (C20:3)	1.07 ± 0.17	1.25 ± 0.26**
Arachidonic (C20:4)	19.39 ± 1.40	18.90 ± 1.43
Docosatetraenoic (C22:4)	1.77 ± 0.33	1.56 ± 0.34
Total polyunsaturated (ω-3) fatty acids (%)	2.46 ± 0.35	2.56 ± 0.48
α-Linolenic (C18:3)	0.10 ± 0.03	0.11 ± 0.04
Eicosapentaenoic (C20:5)	0.26 ± 0.07	0.31 ± 0.12
Docosapentaenoic (C22:5)	1.13 ± 0.21	1.17 ± 0.25
Docosahexaenoic (C22:6)	0.96 ± 0.19	0.98 ± 0.27
Total trans fatty acids (%)	1.43 ± 0.51	1.53 ± 0.47
Palmitelaidic (C16:1 ω-7)	0.21 ± 0.03	0.21 ± 0.04
Elaidic (C18:1 ω-9)	0.45 ± 0.13	0.51 ± 0.14
Vaccenic (C18:1 ω-7)	0.56 ± 0.09	0.57 ± 0.11
Linoelaidic (C18:2 ω6)[c]	0.21 ± 0.48	0.24 ± 0.44

[a]Values in mean ± SD.
[b]*cis-cis*, it did not include *cis-trans* or *trans-cis*.
[c]*trans-trans* only.
*$P < 0.05$, **$P < 0.01$, significantly different from men, after adjusting for fatty acid intake.

Table 3
Peripheral Blood Lymphocyte Subsets of the Subjects[a]

Lymphocyte subsets	n	Men	n	Women
Absolute counts (×10⁶/L)				
CD2 cells	26	1,204 ± 371	46	1,418 ± 515
CD3 cells	27	945 ± 347	48	1,204 ± 463*
CD3+4+ cells	27	603 ± 222	48	795 ± 341*
CD3+8+ cells	26	302 ± 176	44	381 ± 256
CD19 cells	26	115 ± 70	47	149 ± 89
CD56 cells	22	194 ± 139	36	179 ± 94
Percentage of total lymphocytes				
CD2 cells	26	78.7 ± 9.3	46	78.9 ± 13.5
CD3 cells	27	62.6 ± 14.2	48	67.4 ± 9.8
CD3+4+ cells	27	40.2 ± 11.8	48	44.6 ± 10.9
CD3+8+ cells	26	20.1 ± 8.9	44	21.2 ± 11.1
CD19 cells	26	7.2 ± 3.6	47	8.5 ± 3.7
CD56 cells	22	13.5 ± 6.5	36	11.5 ± 5.4

[a]Values in mean ± SD.
*$P < 0.05$, **$P < 0.01$, significantly different from men, after adjusting for age.

Statistical Analyses

The Statistic Analysis System (SAS Institute, Cary, NC) package was used to perform statistical analyses. Results are expressed as mean ± standard deviation (SD). Gender comparison controlling for age was made using an analysis of variance (ANOVA). Spearman's rank correlation coefficients (r_s) were used to determine the degree and direction of association between two variables. Significance level was set at 5%.

RESULTS

Mean concentrations of various fatty acids, as a percentage of the total fatty acids in platelets are presented in Table 2. After controlling for daily intake of SFAs, MUFAs, and PUFAs, men and women did not differ in terms of platelet concentration of total SFAs, MUFAs, and PUFAs (ω-3 and ω-6) or *trans* fatty acids. Women, however, had a significantly higher percentage of homo-γ-linolenic acid (C20:3 ω-6) compared to men.

Table 3 shows the age-adjusted absolute counts and percentages of peripheral blood lymphocyte subsets of the study population. Women were found to have a higher absolute count of CD3- and CD3+4+-bearing lymphocytes than men. Gender differences, however, were not observed in these lymphocyte subsets when expressed as percentages.

Associations between platelet fatty acids and lymphocyte subsets are shown in Table 4. In men, the concentration of total ω-3 PUFAs was positively related to the percentage of CD3 cells and CD3+4+ cells ($r_s = 0.54$ and 0.51, $P < 0.05$ in both cases) after adjusting for fatty acid intake. These relationships became slightly stronger after further adjusting for age ($r_s = 0.59$ and 0.55, $P < 0.05$, respectively). Furthermore, the concentration of total SFAs was positively associated with the percentage of CD19 cells ($r_s = 0.50$, $P < 0.05$) for men. In contrast, no significant relationships were observed in women.

DISCUSSION

Gender Differences in Immune Function

Women are known to have better humoral immunocapabilities than men (14). Gender difference has also been noted in cell-mediated immunity (15, 16). It was observed in this cross-sectional study that women had a higher absolute count of CD3 and CD3+4+ cells compared to men. It has been proposed that the gender difference in immune function may be due to the effects of sex hormones (17). Although all the female subjects were postmenopausal and not on hormone replacement therapy, they may have benefited from the residual effect(s) of estrogen and progesterone.

Platelet Fatty Acids and Their Associations With Immune Function

A major role of fatty acids in the body is as structural components of membranes. Fatty acids measured in platelets, and expressed as a relative percentage of total fatty acids, may provide a guide to membrane fatty acid composition, in addition to the intake of these fatty acids (18). Fatty acid intake and dietary fats have numerous important

Table 4
Spearman's Correlation Coefficients of the Associations Between Platelet Fatty Acids and Peripheral Blood Lymphocyte Subsets (in percent) in the Institutionalized Elderly, After Adjusting for Daily Intakes of Saturated, Monounsaturated, and Polyunsaturated Fatty Acids[a]

Fatty acids (% of total fatty acids)	CD3	CD3+4+	CD3+8+	CD19	CD56
Men					
Saturated fatty acids	−0.41	−0.22	−0.03	0.43 (0.50)*	0.20
Monounsaturated fatty acids	−0.01	−0.10	0.27	−0.20	−0.04
ω-6 Polyunsaturated fatty acids	0.32	0.28	−0.04	0.10	−0.34
ω-3 Polyunsaturated fatty acids	0.54* (0.59*)	0.51* (0.55*)	−0.24	−0.12	−0.30
Trans fatty acids	0.31	0.35	−0.07	−0.19	−0.23
Women					
Saturated fatty acids	−0.18	−0.15	−0.23	−0.03	−0.05
Monounsaturated fatty acids	−0.22	0.07	−0.19	0.21	−0.02
ω-6 Polyunsaturated fatty acids	0.08	0.01	0.13	0.03	0.05
ω-3 Polyunsaturated fatty acids	0.08	0.22	0.11	−0.30	0.14
Trans fatty acids	0.20	0.04	0.19	0.05	−0.27

[a]Figures in parentheses are Spearman's correlation coefficients adjusted for age and fatty acid intake.
*$P < 0.05$, significantly different from zero.

influences on immune response (19). The effect of dietary fat on immunity may be mediated partly by alterations in cell membrane composition and fluidity, serum lipoproteins, or hormone status. Results from various studies indicate that a delicate balance may exist between the immunostimulatory, anti-inflammatory, and immunosuppressive actions of different fatty acids (20–22). An association of immunocompetence with fatty acid intakes, especially in men, has been previously reported in a free-living Anglo-Celtic population (23).

Platelet fatty acids may be used as an indicator of short-term intake of fatty acids (24). Results of the present study, as expected, showed that the male elderly who had a higher percentage of platelet ω-3 PUFAs tended to have a higher percentage of total T-cells, especially T-helper cells. Similarly, those with higher platelet SFAs had a higher percentage of B-lymphocytes.

The immunomodulatory effects of ω-3 PUFA have been shown in a number of clinical trials, where several inflammatory and autoimmune diseases have improved after dietary supplementation with fish oil or ω-3 PUFA. These studies have been summarized in details elsewhere (25). Meydani et al. (26) reported a reduction in the percentage of CD4+ and an increase in the percentage of CD8+ following the consumption of a low-fat low-cholesterol ω-3 PUFA-rich diet. These results conflict with the findings of the present study. Differences in study cohorts and study design may provide some explanations for these contradictory findings. One of the limitations in the current cross-sectional study was that the serum levels of other micronutrients that affect the immune system were not measured. On the other hand, dietary intervention studies usually include supplementation of other nutrients, which may have a significant impact on the immune system.

The beneficial effects of ω-3 PUFAs on immune responses reported elsewhere outweigh those of ω-6 PUFAs, by enhancing the production of the three-series prostaglandins and the five-series leukotrienes, which are less inflammatory than those of the two- and four-series. Furthermore, ω-3 PUFAs preserve cell-mediated immunity (19, 27). If the results of the present study reflect a biologically significant effect of ω-3 PUFAs on lymphocyte subsets, then it could be suggested that ω-3 PUFAs have a beneficial effect on the studied population of elderly men, insofar as inflammatory and infectious diseases are concerned.

The potential effects of the *cis-trans* configuration of PUFAs on health has been of great concern recently. Dietary intake data indicate the average Western diet contains approximately 5% *trans*-fatty acids (28). However, the age-related consumption of *trans*-fatty acids is not known. Recently, Ascherio et al. (29) reported that a high *trans*-fatty acid intake may contribute to the risk of myocardial infarction. Willett et al. (30) found a similar relationship with coronary heart disease risk. Nevertheless, results from various studies examining the effects of *trans*-fatty acids on health outcomes are contradictory. We found no significant associations of *trans*-fatty acids with lymphocyte subsets.

In conclusion, the results of this study provide evidence for an association of fatty acids, especially ω-3 PUFAs, with T-lymphocytes. Such a relationship may play an important role in the host defence mechanisms, especially for elderly individuals. This study also demonstrated gender differences in lymphocyte subsets, and the associations between platelet fatty acids and peripheral blood lymphocyte subsets in an institutionalized elderly population.

ACKNOWLEDGMENTS

This study was financially supported by the Meat Research Corporation (Australia). We were grateful to Ms. Victoria Funnell and Ms. Elizabeth Patterson for providing the opportunity and support needed to undertake the study at Kingston Centre, and to Mrs. Linda A. Walker for

her significant contribution to the recruitment and preparation of the field work. Finally, we are appreciative of the participants for their willing participation in this study.

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