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# Relation between the fatty acid composition of peripheral blood mononuclear cells and measures of immune cell function in healthy, free-living subjects aged 25–72 y[1–3]

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## ABSTRACT

**Background:** There is little information about the relation between the fatty acid composition of human immune cells and the function of those cells over the habitual range of fatty acid intakes.

**Objective:** The objective of the study was to determine the relation between the fatty acid composition of human peripheral blood mononuclear cell (PBMC) phospholipids and the functions of human immune cells.

**Design:** One hundred fifty healthy adult subjects provided a fasting blood sample. The phagocytic and oxidative burst activities of monocytes and neutrophils were measured in whole blood. PBMCs were isolated and used to measure lymphocyte proliferation in response to the T cell mitogen concanavalin A and the production of cytokines in response to concanavalin A or bacterial lipopolysaccharide. The fatty acid composition of plasma and PBMC phospholipids was determined.

**Results:** Wide variations in fatty acid composition of PBMC phospholipids and immune cell functions were identified among the subjects. The proportions of total polyunsaturated fatty acids (PUFAs), of total n−6 and n−3 PUFAs, and of several individual PUFAs in PBMC phospholipids were positively correlated with phagocytosis by neutrophils and monocytes, neutrophil oxidative burst, lymphocyte proliferation, and interferon γ production. The ratios of saturated fatty acids to PUFAs and of n−6 to n−3 PUFAs were negatively correlated with these same immune functions. The relation of PBMC fatty acid composition to monocyte oxidative burst was the reverse of its relation to monocyte phagocytosis and neutrophil oxidative burst.

**Conclusion:** Variations in the fatty acid composition of PBMC phospholipids account for some of the variability in immune cell functions among healthy adults. *Am J Clin Nutr* 2003;77:1278–86.

**KEY WORDS** Fatty acid, immunity, lymphocytes, monocytes, neutrophils, cytokines, phagocytosis, oxidative burst

## INTRODUCTION

Many studies have found that fatty acids have immunologic effects. These effects were most clearly identified in animal experiments, which showed that n−3 polyunsaturated fatty acids (PUFAs) are especially potent, although other fatty acids were also reported to have various effects (1–4). In humans, the literature is more limited, but it suggests that certain fatty acids, particularly n−3 PUFAs, have immunomodulatory actions (1–4). For example, markedly increased consumption of α-linolenic acid (ALA; 18:3n−3) reduced the production of interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) by blood monocytes (5) and reduced the proliferation of blood lymphocytes (6). Supplementation of the diet of healthy human volunteers with fish oil, which markedly increased the intake of long-chain n−3 PUFAs; resulted in decreased lymphocyte proliferation (7–10); decreased production of IL-2, interferon γ (IFN-γ), IL-1, IL-6, and TNF (5, 7–9, 11, 12); and decreased oxidative burst by neutrophils (13–15) and monocytes (16). Thus, in agreement with animal studies, several studies in healthy humans showed that substantially increasing the dietary intake of n−3 PUFAs results in decreased immune cell function. Although the amounts of n−3 PUFAs provided in these studies are greatly in excess of habitual intakes of these fatty acids in Western countries such as the United Kingdom (17), variations in the habitual intake of these and other fatty acids could contribute to the variation in immune function observed among human subjects.

It has been established that there are significant relations between the increased dietary intake of certain fatty acids, especially n−3 PUFAs, and their content in immune cell phospholipids (5, 10, 11, 18–23). Very little is known, however, about the relations between habitual fatty acid intake, the fatty acid composition of immune cells, and immune cell function, and therefore we set out to investigate these relations in healthy, free-living adults. This study was part of an investigation of the effects of n−3 PUFAs on human health-related outcomes [known as the Ministry of Agriculture, Fisheries and Food (MAFF)-LINK AFQ111] and represents the baseline data from subjects involved in that study.

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**TABLE 1**
Characteristics of the subjects[1]

| | Median | Minimum | Maximum |
|---|---|---|---|
| Age (y) | | | |
| Men | 55.5 | 26 | 71 |
| Women | 56.0 | 27 | 70 |
| BMI (kg/m²) | | | |
| Men | 27.2 | 20.7 | 34.0 |
| Women | 24.5[2] | 18.3 | 33.2 |

[1] $n$ = 88 men, 62 women.
[2] Significantly different from men, $P < 0.001$ (Mann-Whitney $U$ test).

## SUBJECTS AND METHODS

### Materials

Tablets of phosphate-buffered saline (PBS) were obtained from Unipath Ltd (Basingstoke, United Kingdom). Histopaque, HEPES-buffered RPMI medium, glutamine, antibiotics (penicillin and streptomycin), concanavalin A (Con A), *Escherichia coli* 0111:B4 lipopolysaccharide (LPS), phorbol 12-myristate 13-acetate (PMA), boron trifluoride, butylated hydroxytoluene, formaldehyde, solvents, and standard chemicals were purchased from Sigma Chemical Co Ltd (Poole, United Kingdom). Kits for measurement of phagocytosis and oxidative burst in whole blood (PHAGOTEST and BURSTTEST, respectively) were purchased from Becton Dickinson (Oxford, United Kingdom). [$^3$H]Thymidine was purchased from Amersham International Ltd (Amersham, United Kingdom). Cytokine EASIA enzyme-linked immunosorbent assay kits were obtained from BioSource International (Nivelles, Belgium).

### Subjects and study design

Ethical permission for all procedures involving human volunteers was obtained from the University of Reading Ethics and Research Committee and the West Berkshire Health Authority Ethics Committee. Moderately hyperlipidemic but otherwise healthy adults aged 25–72 y were invited to participate in the study. Moderate hyperlipidemia was defined as a fasting total cholesterol concentration between 4.6 and 8.0 mmol/L and a fasting triacylglycerol concentration between 0.8 and 3.2 mmol/L. All volunteers completed a questionnaire on health and lifestyle before entering the study. Volunteers were excluded if they were taking any prescribed hypolipidemic or antiinflammatory medication; had been diagnosed as having cardiovascular disease, diabetes, liver or endocrine dysfunction, or chronic inflammatory disease; were pregnant or lactating; were vegetarian; consumed fish oil, evening primrose oil, or vitamin supplements; smoked > 15 cigarettes/d; exercised strenuously > 3 times/wk; had a body mass index (BMI; in kg/m²) < 18 or > 34; consumed > 2 portions of oily fish/wk; or were nonconsumers of margarine. A total of 150 subjects (88 men, 62 women) were recruited to the study. Subject characteristics are shown in **Table 1**.

### Assessment of habitual nutrient intakes

Subjects completed a previously validated 180-question food-frequency questionnaire (24). Habitual nutrient intakes were determined with the use of FOODBASE software, version 1.3 (Institute of Brain Chemistry, London).

### Preparation of PBMCs

Blood samples were collected into heparin-containing evacuated tubes between 0800 and 1000 after a fast of ≥ 10 h. The blood was layered onto Histopaque (density: 1.077 g/L; ratio of blood to Histopaque: 1:1) and centrifuged for 15 min at 800 × $g$ at 20 °C. Peripheral blood mononuclear cells (PBMCs; a mixture of monocytes and lymphocytes) were collected from the interphase and washed once with RPMI medium containing 0.75 mmol glutamine/L and antibiotics (penicillin and streptomycin; culture medium). After resuspension in 4 mL culture medium, the cells were layered onto 4 mL Histopaque. They were centrifuged once more (15 min, 800 × $g$, 20 °C) to achieve a lower degree of erythrocyte contamination, washed with culture medium, and finally resuspended and counted on a Coulter cell counter (Z1; Coulter Electronics, Luton, United Kingdom).

### Analysis of plasma and PBMC phospholipid fatty acid composition

Lipid was extracted from plasma and PBMCs with chloroform:methanol (2:1, vol:vol), and the phospholipids were isolated by thin-layer chromatography with the use of a mixture of hexane:diethyl ether:acetic acid (90:30:1, vol:vol:vol) as the elution phase. Fatty acid methyl esters were prepared by incubation with 140 g boron trifluoride/L in methanol at 80 °C for 60 min. Fatty acid methyl esters were isolated by solvent extraction, dried, and separated by gas chromatography in a gas chromatograph (model 6890; Hewlett-Packard, Avondale, PA) fitted with a 30-m × 0.32-mm BPX70 capillary column with a film thickness of 0.25 μm. Helium at 1.0 mL/min was used as the carrier gas and the split-splitless injector was used with a split-to-splitless ratio of 20:1. Injector and detector temperatures were 275 °C. The column oven temperature was maintained at 170 °C for 12 min after sample injection and was programmed to increase from 170 to 210 °C at a rate of 5 °C/min before being maintained at 210 °C for 15 min. The separation was recorded with the use of HP GC CHEM STATION software (Hewlett-Packard). Fatty acid methyl esters were identified by comparison with standards run previously.

### Measurement of phagocytic activity and oxidative burst

Phagocytosis and oxidative burst by neutrophils and monocytes were determined with the use of the PHAGOTEST and BURSTTEST kits, respectively. Before use, blood was cooled on ice for 10 min and then mixed by vortex for 5 s. For measurement of phagocytosis, aliquots (100 μL) of blood were then incubated on ice (control) or in a heated water bath at 37 °C for 10 min with opsonized fluorescein isothiocyanate–labeled *E. coli* (20 μL). The reaction was stopped by the addition of ice-cold quenching solution (100 μL). For measurement of oxidative burst, aliquots (100 μL) of blood were incubated in a heated water bath at 37 °C for 10 min with opsonized *E. coli*, PMA, or washing solution as control (20 μL in each case). After incubation, a solution (20 μL) containing the fluorogenic substrate dihydrorhodamine 123 was added, and the samples were incubated for an additional 10 min at 37 °C.

At the completion of phagocytosis and oxidative burst incubations, erythrocytes were lysed, leukocytes were fixed, and the DNA was stained according to the manufacturer's instructions. Cell preparations were then analyzed by flow cytometry in a flow cytometer (FACSCalibur, Becton Dickinson). Fluorescence data were collected on 2 × 10$^4$ cells and analyzed with the use of CELLQUEST software. Neutrophils and monocytes were identified by forward and side scatter. Both the percentage of neutrophils or monocytes engaging in phagocytosis or oxidative burst (% active cells) and the median fluorescence intensity (MFI; a

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measure of the extent of phagocytosis or oxidative burst per leukocyte) were determined.

### Measurement of lymphocyte proliferation in PBMC cultures

PBMCs ($2 \times 10^5$) were cultured in culture medium supplemented with 50 mL autologous plasma/L and Con A at final concentrations of 5, 15, 25, 50, and 75 mg/L; the final volume of the culture was 200 μL, and all cultures were performed in triplicate. Proliferation was measured as the incorporation of [$^3$H] thymidine over the final 18 h of a 66-h culture period. Thymidine incorporation values (cpm/well) for the triplicate cultures were averaged (CV was always < 10% and usually < 5%).

### Measurement of the production of cytokines by PBMC cultures

PBMCs ($2 \times 10^6$) were cultured for 24 h in culture medium supplemented with 50 mL autologous plasma/L and either 25 mg Con A/L or 15 mg lipopolysaccharide (LPS)/L; the final culture volume was 2 mL. At the end of the incubation, the plates were centrifuged and the culture medium was collected and frozen in aliquots. The concentrations of cytokines were measured by specific EASIA enzyme-linked immunosorbent assays. TNF-α, IL-1β, and IL-6 were measured in the supernatant fluids of cells stimulated with LPS, and IL-2, IFN-γ, and IL-4 were measured in the supernatant fluids of cells stimulated with Con A. Limits of detection for these assays were 3 ng/L for TNF-α; 2 ng/L for IL-1β, IL-6, and IL-4; 100 U/L for IL-2; and 30 IU/L for IFN-γ (data supplied by the manufacturer of each kit). The interassay and intraassay CVs were < 10% for all cytokine enzyme-linked immunosorbent assays.

### Statistical analysis

Nutrient intake, plasma and PBMC fatty acid composition, lymphocyte proliferation, and cytokine production were determined for all subjects ($n$ = 88 men, 62 women). For logistical reasons, phagocytosis and oxidative burst were determined for a subset of subjects ($n$ = 55 men, 40 women). Data were tested for normality with the use of the Kolmogorov-Smirnov test. Data for the fatty acid composition of plasma and PBMC phospholipids were normally distributed and are expressed as means ± SEMs; the 10th and 90th percentile values are also shown. Fatty acid composition data for males and females were compared with the use of the unpaired Student's $t$ test. Some data for nutrient intakes and for immune cell functions were not normally distributed, and thus all nutrient-intake and immune-cell-function data are expressed as median and 10th and 90th percentile values. Nutrient-intake and immune-cell-function data for the men and the women were compared with the use of the Mann-Whitney $U$ test. Linear relations between the intakes of various fatty acids (g/d) and the proportions of those fatty acids in PBMC phospholipids functions were determined as partial correlation coefficients ($r$) with control for fat intake as grams per day and as percentage of energy. Linear relations between the proportions of fatty acids in plasma and PBMC phospholipids were determined as Pearson's correlation coefficients ($r$). Linear relations between age and immune cell functions, between BMI and immune cell functions, and between the proportions of fatty acids in PBMC phospholipids and immune cell functions were determined as Spearman's rank-order correlation coefficients (ρ). Proportions of fatty acids in PBMC phospholipids that showed significant correlations with immune cell functions were included in stepwise multiple regression analyses, and independent relations between the proportions of fatty acids in PBMC phospholipids and immune cell functions were determined. All statistical analyses were performed with the use of SPSS software, version 10.1 (SPSS Inc, Chicago), and a value of $P < 0.05$ was taken to indicate significance.

## RESULTS

### Subject characteristics and habitual nutrient intake

There were no differences between the men and the women with respect to age (Table 1). However, BMI was significantly ($P < 0.001$) lower for women than for men. Of the women, over one-half ($n$ = 34) were postmenopausal and not taking any type of hormone replacement therapy (HRT), whereas the remaining women were either postmenopausal and taking HRT ($n$ = 12), premenopausal and either taking ($n$ = 1) or not taking ($n$ = 13) the contraceptive pill, or of unspecified estrous status ($n$ = 2). Thirteen subjects (9%) were smokers (1–15 cigarettes/d).

There was a wide range of energy intakes among the subjects (**Table 2**). The men consumed an average of 16% more energy than did the women ($P = 0.037$; Table 2). There was wide variation in the intakes of the various macronutrients among the subjects (Table 2). On average, carbohydrate contributed ≈47% to energy intake, fat contributed ≈34%, and protein contributed ≈17% (Table 2). The contributions of carbohydrate, fat, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and PUFAs to energy intake did not differ between the sexes (Table 2). However, alcohol intake was significantly higher in the men than in the women ($P = 0.001$; Table 2), whereas the proportion of energy derived from protein was significantly higher in the women than in the men ($P = 0.037$; Table 2). Absolute intakes (ie, g/d) of the individual and total MUFAs and of n−3 PUFAs did not differ between the men and the women, except for that of docosapentaenoic acid (DPA; 22:5n−3), which was significantly higher in the men ($P = 0.039$; Table 2). Intakes of linoleic acid (LA; 18:2n−6) and arachidonic acid (AA; 20:4n−6) were significantly higher in the men than in the women ($P$ = 0.020 and 0.044, respectively), and this was reflected in a significant difference in the intake of total n−6 PUFAs ($P = 0.019$; Table 2).

### Fatty acid composition of plasma and PBMC phospholipids

There was wide variation in the proportions of certain fatty acids in plasma and PBMC phospholipids (**Table 3**). The proportions of the various fatty acids did not differ significantly between the sexes, except for LA in PBMC phospholipids, the proportion of which was significantly ($P = 0.018$) higher in the women.

The proportions of oleic acid, LA, ALA, AA, eicosapentaenoic acid (EPA; 20:5n−3), DPA, and docosahexaenoic acid (DHA; 22:6n−3) in PBMC phospholipids were not significantly correlated with habitual intakes of those fatty acids. The habitual intakes of total n−6 PUFAs or of LA were not correlated with the proportion of AA in PBMC phospholipids. There were no significant relations between the total n−3 PUFA or ALA intake and the proportion of EPA, DPA, or DHA in PBMC phospholipids.

The proportions of oleic acid, AA, and DHA in plasma phospholipids significantly correlated positively with the proportions of those fatty acids in PBMC phospholipids ($P < 0.001$, 0.017, and 0.001, respectively). The proportions of LA, ALA, EPA, and

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**TABLE 2**
Habitual energy and nutrient intakes of the subjects[1]

| | Median | 10th Percentile | 90th Percentile |
|---|---|---|---|
| Energy (mJ/d) | | | |
| Men | 11.2 | 7.4 | 16.2 |
| Women | 9.4[2] | 6.9 | 12.2 |
| Protein (% of energy) | | | |
| Men | 15.8 | 13.7 | 19.0 |
| Women | 17.2[2] | 13.7 | 20.4 |
| Carbohydrate (% of energy) | | | |
| Men | 44.7 | 37.5 | 55.8 |
| Women | 48.2 | 38.8 | 55.5 |
| Alcohol (% of energy) | | | |
| Men | 3.4 | 0.5 | 11.3 |
| Women | 0.7[2] | 0.0 | 4.7 |
| Fat (% of energy) | | | |
| Men | 33.8 | 26.0 | 40.3 |
| Women | 32.6 | 26.1 | 40.4 |
| SFAs (% of energy) | | | |
| Men | 11.4 | 7.4 | 15.4 |
| Women | 11.8 | 8.2 | 16.0 |
| MUFAs (% of energy) | | | |
| Men | 10.6 | 7.9 | 13.0 |
| Women | 9.9 | 7.9 | 12.9 |
| PUFAs (% of energy) | | | |
| Men | 5.4 | 4.2 | 8.2 |
| Women | 5.1 | 3.6 | 7.9 |
| Oleic acid (g/d) | | | |
| Men | 25.6 | 15.4 | 42.4 |
| Women | 22.2 | 15.5 | 31.5 |
| Total n−6 PUFAs (g/d) | | | |
| Men | 13.7 | 7.5 | 24.7 |
| Women | 11.5[2] | 7.1 | 18.5 |
| Linoleic acid (g/d) | | | |
| Men | 13.2 | 7.2 | 24.1 |
| Women | 11.1[2] | 6.8 | 18.1 |
| AA (g/d) | | | |
| Men | 0.21 | 0.10 | 0.35 |
| Women | 0.17[2] | 0.05 | 0.31 |
| Total n−3 PUFAs (g/d) | | | |
| Men | 1.98 | 1.22 | 3.48 |
| Women | 1.67 | 1.14 | 2.92 |
| ALA (g/d) | | | |
| Men | 1.41 | 0.09 | 2.49 |
| Women | 1.25 | 0.10 | 1.89 |
| EPA (g/d) | | | |
| Men | 0.15 | 0.04 | 0.42 |
| Women | 0.11 | 0.06 | 0.39 |
| DPA (g/d) | | | |
| Men | 0.09 | 0.04 | 0.17 |
| Women | 0.08[2] | 0.02 | 0.15 |
| DHA (g/d) | | | |
| Men | 0.23 | 0.07 | 0.45 |
| Women | 0.17 | 0.09 | 0.42 |

[1] *n* = 88 men, 62 women. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Significantly different from men, $P < 0.05$ (Mann-Whitney *U* test).

DPA in plasma and PBMC phospholipids were not significantly associated, whereas the proportions of ALA and EPA in plasma phospholipids were significantly correlated positively with the proportion of DPA in PBMC phospholipids ($P = 0.001$ and $P = 0.031$, respectively).

## Immune function measurements

There was large variation among subjects in each of the immune functions examined (**Tables 4** and **5**). There were no significant relations between subject age or BMI and any of the immune cell functions measured. Nor were there any significant differences between the men and the women with respect to any of the immune cell functions measured.

## Relations between fatty acid composition of PBMC phospholipids and immune cell functions

A number of significant relations between the fatty acid composition of PBMC phospholipids and immune cell functions were identified.

### *Leukocyte phagocytosis*

Neutrophils and monocytes displayed the same pattern of relations between PBMC phospholipid fatty acid composition and the capacity of these leukocytes to take up *E. coli* by phagocytosis, expressed as MFI. For both cell types, MFI was significantly correlated negatively with the proportions of palmitic and oleic acids and with the ratios of SFA to PUFA and of n−6 to n−3 PUFAs in PBMC phospholipids (**Table 6**). The proportions of stearic acid, individual PUFAs (LA, ALA, AA, and DHA), total PUFAs, and total n−6 and total n−3 PUFAs were positively correlated with MFI (Table 6). There was no significant relation between the proportion of EPA in PBMC phospholipids and the MFI. With the use of multivariate analysis, the proportion of ALA in PBMC phospholipids was independently associated with the phagocytic activity of neutrophils ($r^2 = 0.329$, $P < 0.001$) and monocytes ($r^2 = 0.381$, $P < 0.001$). In addition, the proportion of DHA in PBMC phospholipids was independently associated with the phagocytic activity of monocytes ($r^2 = 0.451$, $P = 0.009$).

### *Leukocyte oxidative burst in response to* E. coli *or phorbol ester*

The relations between the fatty acid composition of PBMC phospholipids and the extent of oxidative burst in response to *E. coli* or PMA (expressed as MFI) differed between neutrophils and monocytes (Table 6). For neutrophils, these relations were similar to those observed for phagocytosis of *E. coli*: ie, significant positive correlations with the proportions of stearic acid, LA, AA, DHA, total PUFAs, total n−6 PUFAs, and total n−3 PUFAs and significant negative correlations with the proportion of oleic acid and with the ratios of SFA to PUFA and of n−6 to n−3 PUFAs (Table 6). However, for monocytes these relations were the opposite of those observed for phagocytosis of *E. coli*: ie, significant negative correlations with the proportions of stearic acid, LA, ALA, AA, DHA, total PUFAs, total n−6 PUFAs, and total n−3 PUFAs and significant positive correlations with the ratios of SFA to PUFA and of n−6 to n−3 PUFAs (Table 6). With the use of multivariate analysis, the proportions of ALA and AA were independently associated with the oxidative burst activity of neutrophils stimulated with *E. coli* ($r^2 = 0.519$, $P = 0.001$ and $r^2 = 0.426$, $P < 0.001$, respectively), whereas the proportion of AA was independently associated with the activity of neutrophils stimulated with PMA ($r^2 = 0.338$, $P < 0.001$). The proportion of ALA was independently associated with the oxidative burst activity of monocytes stimulated with *E. coli* ($r^2 = 0.204$, $P < 0.001$) or with PMA ($r^2 = 0.208$, $P < 0.001$).

### *Lymphocyte proliferation in response to Con A*

The proportions of AA, DHA, total PUFAs, and total n−3 or n−6 PUFAs in PBMC phospholipids were positively correlated with the proliferation of lymphocytes in response to Con A

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**TABLE 3**
Fatty acid composition of peripheral blood mononuclear cell (PBMC) and plasma phospholipids[1]

| | PBMC | | | Plasma | | |
|---|---|---|---|---|---|---|
| | $\bar{x}$ ± SEM | 10th Percentile | 90th Percentile | $\bar{x}$ ± SEM | 10th Percentile | 90th Percentile |
| | *% of fatty acids by wt* | | | *% of fatty acids by wt* | | |
| Palmitic acid | | | | | | |
| Men | 16.9 ± 0.4 | 12.6 | 21.0 | 27.3 ± 0.3 | 24.4 | 30.5 |
| Women | 17.5 ± 0.8 | 11.7 | 22.0 | 27.6 ± 0.4 | 24.1 | 31.8 |
| Stearic acid | | | | | | |
| Men | 20.6 ± 0.4 | 15.9 | 25.4 | 16.2 ± 0.5 | 12.4 | 21.6 |
| Women | 21.1 ± 0.5 | 17.0 | 25.8 | 14.9 ± 0.5 | 8.9 | 21.2 |
| Oleic acid | | | | | | |
| Men | 17.1 ± 0.7 | 11.5 | 23.7 | 9.8 ± 0.4 | 5.2 | 13.4 |
| Women | 17.8 ± 0.6 | 12.5 | 23.4 | 10.4 ± 0.3 | 7.8 | 12.9 |
| Linoleic acid | | | | | | |
| Men | 8.8 ± 0.2 | 7.1 | 10.5 | 21.4 ± 0.5 | 18.3 | 26.0 |
| Women | 9.1 ± 0.2[2] | 7.3 | 10.9 | 22.2 ± 0.6 | 18.9 | 27.3 |
| ALA | | | | | | |
| Men | 0.27 ± 0.03 | 0.06 | 0.55 | 0.34 ± 0.02 | 0.20 | 0.53 |
| Women | 0.26 ± 0.03 | 0.05 | 0.59 | 0.33 ± 0.02 | 0.20 | 0.47 |
| AA | | | | | | |
| Men | 18.5 ± 0.5 | 13.5 | 23.7 | 8.9 ± 0.2 | 6.4 | 11.3 |
| Women | 18.0 ± 0.5 | 13.4 | 23.6 | 8.8 ± 0.3 | 6.2 | 11.3 |
| EPA | | | | | | |
| Men | 0.53 ± 0.03 | 0.26 | 0.88 | 1.14 ± 0.09 | 0.23 | 2.35 |
| Women | 0.54 ± 0.03 | 0.29 | 0.89 | 0.94 ± 0.08 | 0.27 | 1.64 |
| DHA | | | | | | |
| Men | 3.1 ± 0.1 | 1.9 | 4.6 | 3.9 ± 0.1 | 2.5 | 5.5 |
| Women | 2.7 ± 0.1 | 1.6 | 4.1 | 3.9 ± 0.2 | 2.4 | 5.4 |

[1] *n* = 88 men, 62 women. ALA, α-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
[2] Significantly different from men, $P < 0.05$ (Student's *t* test).

(**Table 7**). The proportions of palmitic and oleic acids and the ratios of SFAs to PUFAs and of n−6 to n−3 PUFAs were negatively correlated with lymphocyte proliferation (Table 7). There was no significant relation between the proportions of LA, ALA, and EPA in PBMC phospholipids and lymphocyte proliferation (Table 7). With the use of multivariate analysis, the proportion of palmitic acid was independently associated with lymphocyte proliferation ($r^2 = 0.071$; $P = 0.005$). The biological significance of this weak, although highly significant, relation is not clear.

### *Cytokine production after Con A stimulation of PBMCs*

The proportions of stearic acid, ALA, AA, total n−6 PUFAs, and total PUFAs in PBMC phospholipids were positively correlated with the production of IL-2, IFN-γ, and IL-4 in response to Con A (Table 7). In addition, the proportions of DHA and of total n−3 PUFAs in PBMC phospholipids were positively associated with IFN-γ production (Table 7). The proportions of palmitic and oleic acids and the ratios of SFAs to PUFAs and of n−6 to n−3 PUFAs were significantly correlated negatively with IFN-γ production (Table 7). The proportions of EPA and LA in PBMC phospholipids were not significantly related to the production of any of the cytokines measured. With the use of multivariate analysis, the proportion of ALA was independently associated with the production of IFN-γ and IL-4 ($r^2 = 0.30$, $P < 0.001$ and $r^2 = 0.227$, $P = 0.014$, respectively), the proportion of DHA was independently associated with IFN-γ production ($r^2 = 0.447$, $P = 0.002$), and the proportion of stearic acid was independently associated with the production of IFN-γ and IL-4 ($r^2 = 0.381$, $P < 0.001$ and $r^2 = 0.177$, $P < 0.001$, respectively).

### *Cytokine production after LPS stimulation of PBMCs*

There were no significant relations between the fatty acid composition of PBMC phospholipids and TNF-α production (Table 7). The proportions of stearic acid, LA, ALA, AA, total PUFAs, and total n−6 PUFAs in PBMC phospholipids were negatively correlated with IL-6 production in response to LPS (Table 7). In contrast, the proportion of oleic acid in PBMC phospholipids was positively correlated with IL-6 production (Table 7). Total n−3 PUFA and DHA contents in PBMC phospholipids were positively correlated with IL-1β production (Table 7). With the use of multivariate analysis, the proportions of stearic acid and AA were independently associated with the production of IL-6 ($r^2 = 0.198$, $P < 0.001$ and $r^2 = 0.252$, $P = 0.014$, respectively).

## DISCUSSION

There was significant interindividual variation in each of the immune cell functions measured in this study. A number of factors are thought to contribute to such variations (25). Several of these, such as acute exercise, chronic intensive exercise, heavy smoking, excessive alcohol consumption, use of certain medications, and the presence of infections, inflammatory disease, or cancer, can be excluded from the current study. Furthermore, age, sex, and BMI did not significantly influence any of the immune cell responses investigated here. One factor responsible for the age-related changes in immune function reported elsewhere is believed to be poor nutrient status (26). Therefore, the lack of influence of age on immune function may result from the fact that all of the subjects studied here were well nourished, as evidenced

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**TABLE 4**
Phagocytic activity in response to *Escherichia coli* and oxidative burst activity in response to *E. coli* and phorbol 12-myristate 13-acetate (PMA)[1]

| Cell type and function | Median | 10th Percentile | 90th Percentile |
|---|---|---|---|
| Neutrophil, phagocytosis | | | |
| Percentage active cells (%)[2] | | | |
| Men | 74.0 | 45.1 | 92.9 |
| Women | 81.9 | 32.1 | 93.2 |
| MFI[2] | | | |
| Men | 1548 | 517 | 4848 |
| Women | 2308 | 539 | 4247 |
| Monocyte, phagocytosis | | | |
| Percentage active cells (%)[2] | | | |
| Men | 24.7 | 6.2 | 40.3 |
| Women | 23.0 | 3.3 | 38.3 |
| MFI[2] | | | |
| Men | 1138 | 459 | 3093 |
| Women | 1533 | 451 | 3460 |
| Neutrophil, oxidative burst | | | |
| Percentage active cells (%)[2] | | | |
| Men | 94.5 | 83.6 | 98.7 |
| Women | 94.2 | 80.8 | 98.7 |
| MFI[2] | | | |
| Men | 620 | 226 | 1321 |
| Women | 585 | 203 | 1365 |
| Neutrophil, oxidative burst | | | |
| Percentage active cells (%)[3] | | | |
| Men | 94.8 | 86.9 | 98.9 |
| Women | 95.5 | 71.4 | 99.6 |
| MFI[3] | | | |
| Men | 1445 | 511 | 2589 |
| Women | 1596 | 470 | 2864 |
| Monocyte, oxidative burst | | | |
| Percentage active cells (%)[2] | | | |
| Men | 54.0 | 7.1 | 84.5 |
| Women | 60.9 | 7.6 | 80.6 |
| MFI[2] | | | |
| Men | 173 | 77 | 873 |
| Women | 121 | 53 | 846 |
| Monocyte, oxidative burst | | | |
| Percentage active cells (%)[3] | | | |
| Men | 64.7 | 4.8 | 97.2 |
| Women | 76.5 | 2.1 | 96.4 |
| MFI[3] | | | |
| Men | 196 | 81 | 621 |
| Women | 173 | 88 | 602 |

[1] $n$ = 55 men, 40 women. MFI, median fluorescence intensity. There were no significant differences between the sexes.
[2] *E. coli* used as the stimulus.
[3] PMA used as the stimulus.

**TABLE 5**
Production of cytokines by peripheral blood mononuclear cells (PBMCs) and proliferation of lymphocytes[1]

| Cell type and function | Median | 10th Percentile | 90th Percentile |
|---|---|---|---|
| PBMCs, IL-2 production (kU/L) | | | |
| Men | 6.3 | 3.9 | 14.7 |
| Women | 6.2 | 2.7 | 18.1 |
| PBMCs, interferon γ production (kU/L) | | | |
| Men | 63.7 | 15.3 | 330.7 |
| Women | 94.0 | 8.6 | 294.7 |
| PBMCs, IL-4 production (ng/L) | | | |
| Men | 33.5 | 9.8 | 182.7 |
| Women | 26.8 | 5.7 | 130.0 |
| PBMCs, IL-6 production (ng/L) | | | |
| Men | 37 288 | 6517 | 65 495 |
| Women | 30 089 | 7225 | 67 728 |
| PBMCs, IL-1β production (ng/L) | | | |
| Men | 4205 | 1277 | 10 025 |
| Women | 4729 | 1608 | 11 499 |
| PBMCs, TNF-α production (ng/L) | | | |
| Men | 10 352 | 2660 | 19 693 |
| Women | 9371 | 3277 | 20 509 |
| Lymphocytes, proliferation (cpm/well) | | | |
| Men | 29 224 | 15 251 | 54 073 |
| Women | 31 347 | 5875 | 56 239 |

[1] $n$ = 88 men, 62 women. IL, interleukin; TNF-α, tumor necrosis factor α. There were no significant differences between the sexes.

by their BMI values and total energy intakes. In contrast with the lack of influence of age and sex, the fatty acid composition of immune cells appears to play a significant role in determining immune cell functions. Other factors that are likely to contribute to the interindividual variation in immune cell functions observed here include the status of other nutrients, particularly vitamins and minerals, and genetic variation, such as polymorphisms in the promoter regions of cytokine genes that control transcription of those genes.

Although the subjects involved in this study exhibited a range of habitual fatty acid intakes, the average energy, macronutrient, and fatty acid intakes were similar to those of the average diet in the United Kingdom (17, 27). The lack of significant relations between fatty acid intake and the proportions of particular fatty acids in PBMC phospholipids suggests either that the range of fatty acid intakes was insufficiently wide to influence the composition of the cells or that factors other than dietary intake are responsible for determining the fatty acid composition of immune cell phospholipids, at least over the range of intakes observed in these subjects. One other possible explanation is that the assessment of fatty acid intake was not sufficiently complete or accurate. The significant relations between the proportions of some fatty acids in plasma phospholipids and of those, or related, fatty acids in PBMC phospholipids suggests that one source of fatty acids for immune cells is circulating phospholipids. The composition of plasma phospholipids in the fasting state is determined largely by metabolic processes occurring in the liver, and, if the relations observed here are causal, this fact would indicate a role for the liver in determining the fatty acid composition of immune cells. Immune cells possess lipoprotein receptors (28–32) and can take up lipoproteins (33) and intact phospholipids (34). Thus, mechanisms exist whereby fatty acids from circulating phospholipids originating from the liver could be incorporated into immune cell phospholipids. In addition, immune cells can metabolize PUFAs (35, 36) and can modify the fatty acid composition of their plasma membrane according to different environmental conditions (37–39). One reason that the fatty acid composition of immune cell phospholipids does not closely reflect that of the diet may be the fact that the cells exert a significant level of control over their plasma membrane composition. Clearly, this situation does not prevail when intakes of certain PUFAs (eg, γ-linolenic acid, AA, EPA, and DHA) greatly exceed the habitual intakes observed here, because, in such conditions, significant changes occur in the fatty acid composition of human immune cell phospholipids (5, 10, 11,

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**TABLE 6**
Spearman's correlation coefficients (ρ) between the fatty acid composition of peripheral blood mononuclear cell phospholipids and the phagocytic and oxidative burst activities of neutrophils and monocytes, expressed as median fluorescence intensity[1]

| | Phagocytosis | | Oxidative burst | | | |
|---|---|---|---|---|---|---|
| | | | Neutrophils | | Monocytes | |
| | Neutrophils | Monocytes | *E. coli* | PMA | *E. coli* | PMA |
| Palmitic acid | −0.396[2] | −0.420[2] | −0.164 | −0.144 | 0.032 | 0.120 |
| Stearic acid | 0.464[2] | 0.402[2] | 0.496[3] | 0.406[2] | −0.417[2] | −0.336[4] |
| Oleic acid | −0.523[3] | −0.495[2] | −0.279[2] | −0.279[2] | 0.146 | 0.104 |
| LA | 0.296[2] | 0.318[2] | −0.064 | −0.065 | −0.279[4] | −0.142 |
| ALA | 0.660[3] | 0.626[3] | 0.626[3] | 0.444[3] | −0.480[3] | −0.452[3] |
| AA | 0.503[3] | 0.523[3] | 0.555[3] | 0.451[3] | −0.314[2] | −0.236[4] |
| EPA | 0.029 | 0.020 | 0.054 | −0.036 | −0.139 | −0.103 |
| DHA | 0.528[3] | 0.543[3] | 0.397[2] | 0.325[2] | −0.367[2] | −0.263[4] |
| SFAs:PUFAs | −0.517[3] | −0.589[3] | −0.507[3] | −0.411[3] | 0.310[2] | 0.243[4] |
| Total PUFAs | 0.599[3] | 0.645[3] | 0.556[3] | 0.401[2] | −0.447[3] | −0.343[2] |
| Total n−6 | 0.584[3] | 0.610[3] | 0.511[3] | 0.379[2] | −0.397[2] | −0.286[4] |
| Total n−3 | 0.517[3] | 0.587[3] | 0.556[3] | 0.423[3] | −0.382[2] | −0.303[4] |
| n−6:n−3 PUFAs | −0.248[4] | −0.312[2] | −0.412[3] | −0.320[2] | 0.289[4] | 0.263[4] |

[1] PMA, phorbol 12-myristate 13-acetate; LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFAs, saturated fatty acids; PUFAs, polyunsaturated fatty acids.
[2] $P < 0.01$.
[3] $P < 0.001$.
[4] $P < 0.05$.

14, 16, 18–23), and these changes appear to be dose-dependent, at least as far as EPA and DHA are concerned (23).

The fatty acid compositions of the phospholipids of neutrophils, monocytes, T lymphocytes, and B lymphocytes taken from healthy human volunteers are very similar and have no major differences in the proportions of long chain n−6 or n−3 PUFAs (21). Therefore, it was assumed that factors affecting the fatty acid composition of immune cells would be similar for both PBMC and neutrophil phospholipids; in the current study, the fatty acid composition of PBMC phospholipids was determined. Variations in the fatty acid composition of PBMC phospholipids were shown to contribute significantly to variation in each of the immune cell functional responses measured, except for TNF-α production. There were a number of consistent patterns of relation between PBMC phospholipid fatty acid composition and immune cell function. There were negative correlations between the proportions of palmitic and oleic acids and the ratio of SFAs to PUFAs and the neutrophil and monocyte phagocytosis of *E. coli*, neutrophil oxidative burst in response to *E. coli* and PMA, lymphocyte proliferation, and production of IFN-γ by mitogen-stimulated lymphocytes. There were positive correlations between the proportions of stearic acid, individual PUFAs (most frequently ALA, AA, and DHA), total PUFAs, total n−6 PUFAs, and total PUFAs and each of these immune cell responses. The final consistent series of (negative) correlations was between the ratio of n−6 to n−3 PUFAs and the neutrophil and monocyte phagocytosis of *E. coli*, neutrophil oxidative

**TABLE 7**
Spearman's correlation coefficients (ρ) between the fatty acid composition of peripheral blood mononuclear cell phospholipids and cytokine production or lymphocyte proliferation, as measured by [$^3$H]thymidine incorporation[1]

| | Peak thymidine incorporation | Cytokine | | | | | |
|---|---|---|---|---|---|---|---|
| | | IL-2 | Interferon γ | IL-4 | TNF-α | IL-6 | IL-1β |
| Palmitic acid | −0.297[2] | 0.039 | −0.329[2] | −0.209[3] | 0.000 | 0.144 | −0.174 |
| Stearic acid | −0.109 | 0.200[3] | 0.427[4] | 0.527[4] | −0.109 | −0.482[4] | 0.162 |
| Oleic acid | −0.192[3] | −0.206[3] | −0.526[4] | −0.435[4] | 0.15 | 0.429[4] | −0.183 |
| LA | 0.042 | 0.065 | 0.066 | 0.155 | 0.084 | −0.311[2] | −0.019 |
| ALA | −0.001 | 0.262[2] | 0.590[4] | 0.660[4] | −0.06 | −0.487[4] | 0.177 |
| AA | 0.203[3] | 0.299[4] | 0.397[4] | 0.373[4] | −0.163 | −0.414[4] | 0.084 |
| EPA | 0.110 | −0.103 | 0.148 | 0.038 | −0.043 | −0.124 | 0.055 |
| DHA | 0.326[4] | −0.009 | 0.377[4] | 0.147 | 0.001 | −0.090 | 0.255[2] |
| SFAs:PUFAs | −0.375[4] | −0.108 | −0.304[2] | −0.140 | 0.063 | 0.176 | −0.130 |
| Total PUFAs | 0.326[4] | 0.233[3] | 0.491[4] | 0.363[4] | −0.121 | −0.365[4] | 0.182 |
| Total n−6 | 0.185[3] | 0.332[4] | 0.454[4] | 0.453[4] | −0.168 | −0.513[4] | 0.080 |
| Total n−3 | 0.424[4] | −0.006 | 0.421[4] | 0.135 | −0.006 | −0.007 | 0.209[3] |
| n−6:n−3 PUFAs | −0.365[4] | 0.062 | −0.265[2] | 0.039 | 0.006 | −0.180 | −0.154 |

[1] IL, interleukin; TNF-α, tumor necrosis factor α; LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFAs, saturated fatty acids; PUFAs, polyunsaturated fatty acids.
[2] $P < 0.01$.
[3] $P < 0.05$.
[4] $P < 0.001$.

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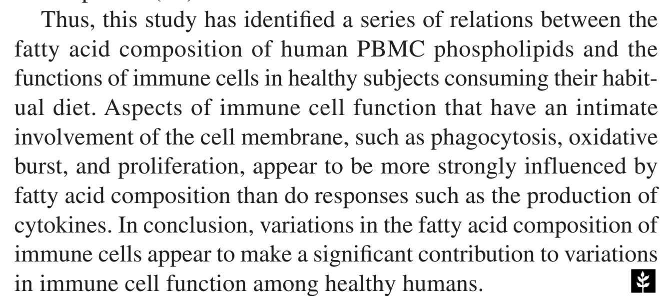
burst in response to *E. coli* and PMA, lymphocyte proliferation, and production of IFN-γ by mitogen-stimulated lymphocytes. Production of cytokines other than IFN-γ was less strongly related with the fatty acid composition of PBMC phospholipids, although there were some correlations with the production of IL-2, IL-4, and IL-1β that were similar to the other relations observed. There were few significant correlations between the proportion of LA in PBMC phospholipids and immune cell function, and there were no significant correlations involving EPA.

Taken together, these observations suggest that an increased content of SFAs, especially palmitic (but not stearic) acid, in immune cells decreases the functional activity of the cells, whereas an increased content of PUFAs increases their activity. Furthermore, whereas a greater content of either n−6 and n−3 PUFAs increases immune cell activity, n−3 PUFAs have a greater effect than do n−6 PUFAs: this is evident from the negative correlations between the ratio of n−6 to n−3 PUFAs in PBMC phospholipids and immune cell function. Multivariate analysis indicates that, of the n−6 PUFAs, AA is largely responsible for the enhancing effect, whereas, of the n−3 PUFAs, both ALA and DHA are responsible. However, having made these generalizations, we also note that correlations for monocyte oxidative burst activity and for IL-6 production were the opposite of those observed for the other immune cell functions.

The differential effect of SFAs and PUFAs indicates that the physical nature of the cell membrane may be an important determinant of immune cell function. This is likely to be particularly important for phagocytosis, lymphocyte proliferation, and some aspects of oxidative burst, each of which involves significant physical movement of the cell membrane. The opposite nature of the relations observed for neutrophil and monocyte oxidative burst suggests that the regulation of this process by fatty acid composition is altogether different between neutrophils and monocytes. This warrants further investigation.

The relations between PBMC phospholipid fatty acid composition and production of the cytokines by Con A-stimulated lymphocytes might reflect the importance of the fatty acid composition of membrane phospholipid in determining the generation of the signaling molecules in response to Con A stimulation that ultimately results in the activation of genes coding for the cytokines. The response of monocytes to LPS was less strongly related to the fatty acid composition of PBMC phospholipids: only the production of IL-6 was strongly related to PBMC phospholipid fatty acid composition, and the relations were the opposite of those observed for many other immune cell responses but similar to those observed for monocyte oxidative burst. These relations indicate that the ability of monocytes to produce IL-6 decreases as the total or n−6 PUFA contents of PBMC phospholipids increase. The reason that cytokine production by lymphocytes and monocytes is influenced in opposing ways by PBMC phospholipid fatty acid composition is unclear, but it most likely relates to the precise signaling pathways invoked in response to Con A in lymphocytes and to LPS in monocytes. It is important to note that the current study measured cytokine production at a single time point in culture and did not investigate the kinetics of cytokine production (or of the other immune cell functions reported).

Many of the relations between the fatty acid composition of PBMC phospholipids and the immune cell functions identified in the current study appear to contradict findings from studies in which volunteers have consumed greatly increased amounts of particular fatty acids such as ALA or long-chain n−3 PUFAs (5–16, 19). These studies reported decreased functional responses of lymphocytes, monocytes, and neutrophils that are greatly enriched in EPA and DHA (5–16, 19). In contrast, the current study indicates that the cellular content of EPA has little effect on immune cell function, at least over the range of EPA contents that arise from habitual fatty acid intakes, and that an increased content of DHA is associated with an increase in function (except for monocyte oxidative burst, which decreases). One explanation for this apparent discrepancy is that the relation between the proportion of certain PUFAs in immune cell phospholipids and the function of those cells (except for monocyte oxidative burst) is "bell shaped," as appears to be the case for the relation between the status of many nutrients and immune function (40). Thus, over the range of proportions of PUFAs in immune cell phospholipids that result from habitual intakes of those fatty acids (and perhaps at higher than habitual intakes), immune cell functions increase with increasing content because of the mechanisms described above (eg, alteration of the physical state of the plasma membrane). However, intakes of certain PUFAs (especially n−3 PUFAs) that greatly exceed habitual intakes will result in decreased immune cell functions through a number of mechanisms, including the generation of membranes that are too fluid (39, 41–43), effects on cell-signaling processes (20, 44, 45), alterations in eicosanoid profiles (5, 8, 11, 18, 20), the induction of lipid peroxidation (46), and the depletion of α-tocopherol (46).



Thus, this study has identified a series of relations between the fatty acid composition of human PBMC phospholipids and the functions of immune cells in healthy subjects consuming their habitual diet. Aspects of immune cell function that have an intimate involvement of the cell membrane, such as phagocytosis, oxidative burst, and proliferation, appear to be more strongly influenced by fatty acid composition than do responses such as the production of cytokines. In conclusion, variations in the fatty acid composition of immune cells appear to make a significant contribution to variations in immune cell function among healthy humans.

AMM, CMW, and PCC were involved in the study design. AMM and YEF were involved in subject recruitment under the supervision of CMW. SK, TB, AMM and YEF were involved in data collection under the supervision of CMW and PCC. SK and PCC performed the data analysis. SK and PCC wrote the manuscript, with contributions from all other authors. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

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