Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men1–3

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ABSTRACT

Background: Increasing intakes of long-chain n–3 polyunsaturated fatty acids (PUFAs) can decrease markers of immunity. However, dose- and age-related responses have not been identified.

Objective: The objective was to determine the effects of different amounts of eicosapentaenoic acid (EPA) on innate immune outcomes in young and older males.

Design: In a controlled, double-blind study, healthy young and older men consumed 1 of 4 supplements provided as capsules: placebo (corn oil) or different amounts of an oil providing 1.35, 2.7, or 4.05 g EPA/d for 12 wk. Blood samples were collected at baseline and after 12 wk.

Results: EPA was incorporated in a linear dose-response fashion into plasma and mononuclear cell (MNC) phospholipids; incorporation was greater in the older men. EPA treatment did not alter neutrophil or monocyte phagocytosis, monocyte respiratory burst, or the production of inflammatory cytokines by MNCs in the young or older men. EPA treatment caused a dose-dependent decrease in neutrophil respiratory burst only in the older men. Increased incorporation of EPA into plasma or MNC phospholipids was associated with decreased production of prostaglandin E2 by MNCs from both young and older men.

Conclusions: Older subjects incorporate EPA into plasma and MNC phospholipids more readily than do younger subjects. Other than prostaglandin E2 production, innate immune responses in young subjects are not affected by an EPA intake of ≤4.05 g/d. Older subjects are more sensitive to the immunologic effects of EPA, and the neutrophil respiratory burst is lower at higher EPA intakes.

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KEY WORDS Fish oil, n–3 polyunsaturated fatty acids, innate immunity, monocyte, neutrophil, cytokine, phagocytosis, respiratory burst

INTRODUCTION

Inflammatory cells such as neutrophils, monocytes, and macrophages form part of the innate immune response that is involved in early host defense against invading bacteria (1). Inflammatory cells recognize bacteria in a nonspecific way and destroy them by phagocytosis or via the production of superoxide and related reactive oxygen species in the respiratory burst (1). Bacterial cell wall components, such as lipopolysaccharide, stimulate the production of eicosanoids [eg, prostaglandin E2 (PGE2)] and cytokines [eg, tumor necrosis factor (TNF) and interleukin (IL) 1 and IL-6] by monocytes and macrophages. These inflammatory cytokines provide a link between inflammatory cells and specific immunity because they can stimulate T and B lymphocytes (1).

There is continuing interest in the effects of long-chain n–3 polyunsaturated fatty acids (PUFAs) on human immune function and inflammatory processes (see 2–4 for reviews). Fish oil has been reported to decrease the expression of intercellular adhesion molecule 1 (ICAM-1; CD54) on the surface of monocytes (5), superoxide production by neutrophils (6–8) and monocytes (9), and the production of TNF-α, IL-1β, and IL-6 by lipopolysaccharide-stimulated mononuclear cells (MNCs) (10–15). Many of the previous studies were not controlled (5, 7–12, 14, 15) or used very high doses (between 2.7 and 8.6 g/d) of long chain n–3 PUFAs (6–10, 12–14). Most often, the idea that long-chain n–3 PUFAs diminish inflammatory cell functions is interpreted in a favorable way, with the conclusion that they are antiinflammatory and thus will be beneficial to health (2–4). However, because these cells are among the cellular components of the immune system, a reduction in their activity could also compromise host defense. It is important to ensure that there is no adverse immunologic effect of increased consumption of these PUFAs because increased intakes of PUFAs have been recommended (16–19). There is also a need to identify dose-dependent effects and to ascertain whether different effects occur in different subgroups of the population so that better recommendations can be made. Therefore, the current study investigated the effects of 3 intakes of long-chain n–3 PUFAs, mainly in the form of eicosapentaenoic acid (EPA; 20:5n–3), on innate immunity in young and older men.

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SUBJECTS AND METHODS

Materials

Phosphate-buffered saline tablets were obtained from Unipath Ltd, Basingstoke, United Kingdom. Histopaque, HEPES-buffered RPMI medium, glutamine, antibiotics (penicillin and streptomycin), Escherichia coli 0111:B4 lipopolysaccharide, boron trifluoride, butylated hydroxytoluene, formaldehyde, solvents, and standard chemicals were purchased from Sigma Chemical Co Ltd, Poole, United Kingdom. Fluorescein isothiocyanate-labeled mouse anti-human CD14, R phycocyanin–labeled mouse anti-human CD54 and R phycocyanin–labeled mouse anti-human metal binding activator 1 (MAC-1; CD11b) were purchased from Serotec Ltd, Kidlington, United Kingdom.

Subjects and study design

Ethical permission for all procedures involving human volunteers was obtained from the relevant ethical committees. Healthy young (18–42 y) and older (53–70 y) men were invited to participate in the study. All subjects completed a health and lifestyle questionnaire before entering the study. Volunteers were excluded if they took any prescribed medication; were vegetarian; smoked 10 cigarettes/d; drank >10 units of alcohol/ wk; had a body mass index (BMI; in kg/m²) >32; or consumed >1 portion of oily fish/ wk. One hundred young men and 69 older men were enrolled in the study; 93 young men and 62 older men completed the study. Reasons for drop-out were as follows: inconvenience (n = 4 young and 4 older men), noncompliance with capsule consumption (n = 1 young man), car accident (n = 1 young man), diarrhea (n = 1 young man), constipation (n = 2 older men), myocardial infarction (n = 1 older men). Drop-outs were distributed across all treatment groups. Data are reported for those subjects who completed the study.

Subjects were randomly allocated in a double-blind fashion to 1 of 4 treatment groups: placebo, low EPA, moderate EPA, and high EPA. Baseline characteristics of the subjects in each of the treatment groups are shown in Table 1. There was an effect of age [P < 0.001 for BMI and diastolic blood pressure and P = 0.044 for systolic blood pressure; two-factor analysis of variance (ANOVA)] but no effect of treatment group and no treatment group × age interaction for the characteristics shown in Table 1. The older subjects had a significantly higher BMI and blood pressure than did the young subjects (Table 1).

All subjects consumed nine 1-g capsules/d for 12 wk. The capsules were provided by Pronova Biocare AS, Lysaker, Norway. The placebo group consumed nine 1-g corn oil capsules/d that provided ≈5 g linoleic acid (18:2n-6) daily in addition to habitual consumption. Subjects in the low-EPA group consumed nine 1-g capsule/d that provided 6 g corn oil and 3 g EPA-rich oil daily. Subjects in the moderate-EPA group consumed nine 1-g capsule/d that provided 3 g corn oil and 6 g EPA-rich oil daily. Subjects in the high-EPA group consumed nine 1-g capsule/d that provided 9 g EPA-rich oil. The capsules were provided to the subjects in small plastic pots, each containing the daily allocation (ie, 9 capsules), with instructions to take 3 capsules 3 times daily.

The EPA-rich oil (EPAX 4510TG) contained 45% EPA and 10% DHA and 0.1 g/d, respectively, in the placebo, low-EPA, moderate-EPA, and high-EPA groups. Habitual intakes of linoleic acid and EPA were not measured but would be expected to be in the range of 8–16 (x̄ = 12.5) (20) and <0.2 g/d (17, 19), respectively. All capsules contained 3.6 mg α-tocopherol. Thus, all subjects consumed an additional 32 mg α-tocopherol/d from the capsules [average habitual intake would be expected to be in the range 5–20 (x̄ = 10) mg/d (21)]. Compliance was assessed by counting the returned capsules.

Blood was collected immediately before the interventions began and at 12 wk. Heparinized evacuated tubes were used for blood collection. Blood samples were collected between 0700 and 1000 after the subjects had fasted for ≥10 h. Plasma was stored at −70 °C until analyzed.

Preparation of MNCs

Blood was layered onto Histopaque (density: 1.077 g/L; ratio of blood to Histopaque: 1:1; Sigma-Aldrich, St Louis, MO) and centrifuged for 15 min at 800 × g at 20 °C. The peripheral MNCs were collected from the interface and washed once with RPMI

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**TABLE 1**

Characteristics of the young and older treatment groups at baseline

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of subjects</th>
<th>Age (y)</th>
<th>BMI (kg/m²)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Diastolic blood pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Older</td>
<td>Young</td>
<td>Older</td>
<td>Young</td>
</tr>
<tr>
<td>Placebo</td>
<td>24</td>
<td>16</td>
<td>25.3 ± 1.3</td>
<td>61.3 ± 1.2</td>
<td>23.8 ± 0.6</td>
</tr>
<tr>
<td>Low EPA</td>
<td>23</td>
<td>16</td>
<td>24.0 ± 1.1</td>
<td>60.9 ± 1.8</td>
<td>25.1 ± 0.7</td>
</tr>
<tr>
<td>Moderate EPA</td>
<td>23</td>
<td>15</td>
<td>24.6 ± 1.3</td>
<td>60.7 ± 1.6</td>
<td>23.4 ± 0.4</td>
</tr>
<tr>
<td>High EPA</td>
<td>23</td>
<td>15</td>
<td>23.6 ± 1.2</td>
<td>59.7 ± 1.5</td>
<td>24.1 ± 0.6</td>
</tr>
</tbody>
</table>

1 EPA, eicosapentaenoic acid. There was a significant effect of age for BMI (P < 0.001), diastolic blood pressure (P < 0.001), and systolic blood pressure (P = 0.044) but no effect of treatment group and no age × treatment group interaction (2-factor ANOVA).

2 x ± SEM (all such values)
medium containing 0.75 mmol/L glucose and antibiotics (penicillin and streptomycin) (culture medium). After being resuspended in 4-mL culture medium, the cells were layered onto 4-mL Histopaque, recentrifuged (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 Z1 Cell Counter (Coulter Electronics, Luton, United Kingdom).

**Analysis of adhesion molecule expression on monocytes**

Whole blood (100 μL) was incubated with 10 μL fluorescently labeled anti-CD14/anti-CD54 (to distinguish monocytes as CD14+ and to determine the expression of CD54 on monocytes) and anti-CD14/anti-CD11b (to distinguish monocytes as CD14+ and to determine the expression of CD11b on monocytes) for 30 min at 4 °C. Erythrocytes were then lysed by using 2 mL Cell Lysing Buffer and leukocytes were washed with Cell Wash and then fixed with 0.2 mL Cell Fix solution. Fixed leukocytes were analyzed in a FACSCalibur flow cytometer (Becton Dickinson, Oxford, United Kingdom). Fluorescence data were collected on 2 × 10⁶ cells and analyzed with the use of CELLQUEST software (Becton Dickinson).

**Analysis of plasma and MNC phospholipid fatty acid composition**

Lipid was extracted from plasma or MNCs with chloroform: methanol (2:1, by vol), and phospholipids were isolated by thin-layer chromatography with a mixture of hexane:diethyl ether: acetic acid (90:30:1, by 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 and analyzed by gas chromatography as described elsewhere (22).

**Measurement of phagocytic activity and respiratory burst**

Phagocytosis and respiratory burst by neutrophils and monocytes were determined with the use of PHAGOTEST and BURSTTEST kits, respectively. Before the kits were used, 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 preheated water bath at 37 °C for 10 min with opsonized fluorescein isothiocyanate-labeled E. coli (20 μL). The reaction was stopped by adding ice-cold quenching solution (100 μL). For measurement of respiratory burst, aliquots (100 μL) of blood were incubated in a preheated water bath at 37 °C for 10 min with opsonized E. coli or with phorbol myristyl acetate (PMA) or with 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 respiratory burst incubations, erythrocytes were lysed, leukocytes were fixed, and DNA was stained according to the manufacturer’s instructions. Cell preparations were then analyzed by flow cytometry in a Becton Dickinson FACSCalibur flow cytometer. Fluorescence data were collected on 2 × 10⁶ cells and analyzed by using CELLQUEST software. Neutrophils and monocytes were identified by forward and side scatter. The percentage of neutrophils or monocytes engaging in phagocytosis or respiratory burst (% positive) was determined.

**Measurement of cytokine production by lipopolysaccharide-stimulated MNC cultures**

MNCs (2 × 10⁶) were cultured for 48 h in culture medium and supplemented with 50 mL/L autologous plasma and 15 mg/L lipopolysaccharide; the final culture volume was 2 mL. This concentration of lipopolysaccharide and the incubation period of 48 h were selected on the basis of the results of preliminary experiments in which these conditions were found to result in maximal production of each of the cytokines being studied here (data not shown). At the end of the incubation, the plates were centrifuged and the culture medium was collected and frozen in aliquots. The concentrations of TNF-α, IL-1β, IL-6, and PGE₂ were measured by specific ELISAs. Limits of detection for these assays were 3 ng/L (TNF-α), 2 ng/L (IL-1β and IL-6), and 0.1 ng/mL (PGE₂) (data supplied by the manufacturer of the kits). The inter- and intraassay CVs were <10% for all cytokine ELISAs.

**Data presentation and statistical analysis**

Data are presented as means ± SEMs. Data for each treatment group at each time point and data for changes from baseline (ie, week 12 – week 0) in each treatment group were tested for normality by using the Kolmogorov-Smirnov test. Data that were not normally distributed were log transformed before statistical analysis. Baseline data and changes from baseline were analyzed by two-factor ANOVA with the use of age and treatment group as factors. When the interaction was significant, post hoc analysis of the effect of treatment group was performed by one-factor ANOVA with Tukey’s test, and post hoc analysis of the effect of age was determined with an unpaired Student’s t test. Comparisons between wk 12 and wk 0 were made by paired Student’s t test. Linear relations were determined as Spearman’s correlation coefficients (r) or as Pearson’s correlation coefficients (r) as appropriate. All statistical tests were performed by using SPSS version 11.0 (SPSS Inc, Chicago, IL), and a value of P < 0.05 indicated statistical significance.

**RESULTS**

**Fatty acid composition of plasma phospholipids**

At baseline there was a significant effect of age on the proportions of palmitic acid (16:0), di-homo-γ-linolenic acid (DGLA; 20:3n-6), EPA, DHA (P < 0.001 for all), and docosapentaenoic acid (DPA; 22:5n-3) (P = 0.01) in plasma phospholipids, but there was no effect of treatment group and no treatment group interaction (two-factor ANOVA). The young subjects had higher proportions of palmitic acid (29.6 ± 0.3 versus 26.9 ± 0.5% of total fatty acids) and lower proportions of DGLA, EPA, DPA, and DHA than did the older subjects (Table 2).

A two-factor ANOVA of the effects of age and treatment group on the enrichment of plasma phospholipids with fatty acids (expressed as a change in proportion, i.e., week 12 – week 0) showed significant effects of age on DGLA, EPA, DPA (P < 0.001), and arachidonic acid (20:4n-6; P = 0.021); significant effects of treatment group on linoleic acid, DGLA, arachidonic acid, EPA, DPA, and DHA (P < 0.001); and a significant age × treatment group interaction for DGLA (P < 0.001), EPA (P = 0.009), and DPA (P = 0.017).
TABLE 2
Fatty acid composition of plasma phospholipids in the young and older subjects at baseline and after 12 wks of supplementation

<table>
<thead>
<tr>
<th>Treatment group and time</th>
<th>Linoleic acid % by wt of total fatty acids</th>
<th>DGLA % by wt of total fatty acids</th>
<th>Arachidonic acid % by wt of total fatty acids</th>
<th>EPA % by wt of total fatty acids</th>
<th>DPA % by wt of total fatty acids</th>
<th>DHA % by wt of total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Young 21.7 ± 0.3 20.8 ± 0.8</td>
<td>Older 27 ± 0.1 29.0 ± 0.2</td>
<td>Young 9.0 ± 0.2 8.4 ± 0.5</td>
<td>Older 0.9 ± 0.1 1.5 ± 0.2</td>
<td>Young 0.9 ± 0.0 1.0 ± 0.1</td>
<td>Older 3.1 ± 0.2 5.0 ± 0.5</td>
</tr>
<tr>
<td>0 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 wk</td>
<td>21.9 ± 0.4 219 ± 0.7</td>
<td>26 ± 0.1 28.0 ± 0.2</td>
<td>93.3 ± 0.3 8.4 ± 0.5</td>
<td>1.0 ± 0.1 1.9 ± 0.3</td>
<td>1.0 ± 0.0 1.0 ± 0.1</td>
<td>3.4 ± 0.1 5.1 ± 0.5</td>
</tr>
<tr>
<td>12 – 0 wk</td>
<td>0.3 ± 0.4 0.1 ± 0.7</td>
<td>−0.1 ± 0.0 −0.1 ± 0.1</td>
<td>0.3 ± 0.2 −0.0 ± 0.2</td>
<td>0.0 ± 0.1 0.4 ± 0.3</td>
<td>0.0 ± 0.0 −0.0 ± 0.0</td>
<td>0.3 ± 0.2 0.1 ± 0.2</td>
</tr>
<tr>
<td>Low EPA</td>
<td>21.3 ± 0.6 208.0 ± 0.6</td>
<td>2.8 ± 0.1 3.3 ± 0.2</td>
<td>8.9 ± 0.4 9.3 ± 0.5</td>
<td>1.2 ± 0.1 1.5 ± 0.3</td>
<td>1.0 ± 0.0 1.2 ± 0.1</td>
<td>3.4 ± 0.2 4.8 ± 0.5</td>
</tr>
<tr>
<td>0 wk</td>
<td>20.2 ± 0.5 182.0 ± 0.6</td>
<td>2.1 ± 0.1 ± 0.5 ± 0.2</td>
<td>8.5 ± 0.4 8.0 ± 0.4</td>
<td>3.0 ± 0.2 2.5 ± 0.3</td>
<td>1.4 ± 0.1 1.2 ± 0.1</td>
<td>3.8 ± 0.2 5.7 ± 0.5</td>
</tr>
<tr>
<td>12 wk</td>
<td>−1.1 ± 0.4 −2.4 ± 0.5</td>
<td>−0.7 ± 0.2 −1.1 ± 0.2</td>
<td>−6.0 ± 0.4 −1.2 ± 0.3</td>
<td>1.8 ± 0.2 3.6 ± 0.4</td>
<td>0.4 ± 0.1 0.7 ± 0.1 ± 0.0</td>
<td>0.4 ± 0.2 0.8 ± 0.4</td>
</tr>
<tr>
<td>Moderate EPA</td>
<td>21.6 ± 0.5 21.5 ± 0.5</td>
<td>3.2 ± 0.2 3.2 ± 0.2</td>
<td>9.2 ± 0.4 9.1 ± 0.6</td>
<td>0.9 ± 0.1 1.3 ± 0.1</td>
<td>1.0 ± 0.0 1.0 ± 0.1</td>
<td>3.3 ± 0.1 4.3 ± 0.3</td>
</tr>
<tr>
<td>0 wk</td>
<td>17.7 ± 0.5 17.2 ± 0.7</td>
<td>1.9 ± 0.1 ± 0.3 ± 0.5</td>
<td>7.9 ± 0.3 7.6 ± 0.3</td>
<td>4.9 ± 0.4 7.3 ± 0.4 ± 0.5</td>
<td>1.8 ± 0.1 2.5 ± 0.7 ± 0.6</td>
<td>4.6 ± 0.2 5.7 ± 0.2</td>
</tr>
<tr>
<td>12 wk</td>
<td>−3.9 ± 0.5 −4.0 ± 0.5</td>
<td>−1.0 ± 0.1 −1.0 ± 0.1</td>
<td>−1.3 ± 0.3 −1.3 ± 0.3</td>
<td>1.4 ± 0.4 2.0 ± 0.5 ± 0.3</td>
<td>0.8 ± 0.1 1.4 ± 0.1 ± 0.0</td>
<td>1.3 ± 0.2 1.4 ± 0.4</td>
</tr>
<tr>
<td>12 – 0 wk</td>
<td>−4.3 ± 0.7 −5.5 ± 0.6</td>
<td>−0.8 ± 0.1 −2.1 ± 0.2 ± 0.3</td>
<td>−8.0 ± 0.3 −18.0 ± 0.3</td>
<td>4.7 ± 0.7 7.9 ± 0.4 ± 0.8</td>
<td>0.9 ± 0.1 1.4 ± 0.2 ± 0.3</td>
<td>1.4 ± 0.2 1.2 ± 0.2</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM. n = 24, 23, 23, and 23 for the young and n = 16, 16, 15, and 15 for the older subjects in the placebo, low-EPA, moderate-EPA, and high-EPA groups, respectively. DGLA, di-homo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid. At baseline there was a significant effect of age on the proportions of DGLA, EPA, DHA (P < 0.001 for all), and DPA (P = 0.01), but there was no effect of treatment group and no age x treatment group interaction (2-factor ANOVA). The young subjects had lower proportions of DGLA, EPA, DPA, and DHA. A 2-factor ANOVA showed a significant effect of age on the change in proportion of DGLA, EPA, DPA (P < 0.001 for all), and arachidonic acid (P = 0.02) and of treatment group on the change in proportion of linoleic acid, DGLA, arachidonic acid, EPA, DPA, and DHA (P < 0.001). There was a significant age x age x treatment group interaction for the change in DGLA (P < 0.001), EPA (P = 0.009), and DPA (P = 0.017).

2 Significantly different from baseline, P ≤ 0.002 (paired Student’s t test).

3 Significantly different from placebo, P ≤ 0.002 (one-factor ANOVA with post hoc Tukey’s test).

4 Significantly different from the young subjects, P ≤ 0.037 (unpaired Student’s t test).

5 Significantly different from low EPA, P < 0.05 (one-factor ANOVA with post hoc Tukey’s test).

6 Significantly different from moderate EPA, P ≤ 0.006 (one-factor ANOVA with post hoc Tukey’s test).
Plasma phospholipid EPA and DPA were significantly higher after supplementation in the 3 groups who received the EPA-rich oil than in the placebo group in both the young and older subjects \((P < 0.001\) for both, one-factor ANOVA; Table 2). Supplementation with the EPA-rich oil resulted in a significant increase in EPA and DPA in plasma phospholipids at all 3 doses in both young and older subjects \((P < 0.05, \text{paired Student's} \ t\text{test}; \text{Table 2})\). The increase in the EPA content of plasma phospholipids was related to the dose of EPA provided by the capsules \((\rho = 0.74\) for young and \(\rho = 0.882\) for older subjects; \(P < 0.001\) for both) and was greater in the older subjects than in the young subjects at each EPA dose (Table 2). The changes in DPA were also greater in the older than in the younger subjects at each supplementation dose (Table 2). The increase in the \(n-3\) long-chain PUFAs in plasma phospholipids was mirrored by a dose-dependent decrease in the proportion of the \(n-6\) PUFA DGLA (Table 2).

At baseline there was a significant effect of age \((P < 0.001)\) but not of treatment group on the ratio of arachidonic acid to EPA in plasma phospholipids, and there was no age \(\times\) treatment group interaction. The ratio was significantly higher in the young than in the older subjects. There was a significant effect of treatment group \((P < 0.001)\) but not of age on the change in the ratio of arachidonic acid to EPA, and there was no age \(\times\) treatment group interaction. The ratio was significantly lower at the end of supplementation than at baseline in the subjects who consumed EPA (Figure 1). A comparison of marginal means indicated that the ratio of arachidonic acid to EPA in plasma phospholipids was significantly decreased in the groups who consumed low, moderate, or high amounts of EPA \((P < 0.001\) for all, paired Student’s \(t\) test). Furthermore, at week 12, the ratio was significantly lower in the EPA groups than in the placebo group \((P < 0.001, \text{one-factor ANOVA})\) but was not different between the low-, moderate-, and high-EPA groups \((P > 0.13, \text{one-factor ANOVA})\).

### Fatty acid composition of MNC phospholipids

At baseline there was a significant effect of age on the proportions of palmitic acid \((P = 0.024)\), EPA \((P = 0.031)\), and DHA \((P = 0.002)\) in MNC phospholipids, but there was no significant effect of treatment group and no significant age \(\times\) treatment group interaction. The young subjects had higher proportions of palmitic acid \((21.3 \pm 0.5\%\) compared with \(19.0 \pm 0.8\%\) of total fatty acids) and lower proportions of EPA and DHA than did the older subjects (Table 3).

A two-factor ANOVA of the effects of age and treatment group on the enrichment of MNC phospholipids with fatty acids (expressed as change in the proportion \(\text{ie wk 12 - wk 0}\)) showed significant effects of age on the change in DGLA \((P = 0.004)\), arachidonic acid \((P = 0.005)\), EPA \((P = 0.012)\), and DHA \((P < 0.001)\) and of treatment group on the change in EPA and DPA \((P < 0.001\) for both). There was a significant age \(\times\) treatment group interaction on change in the proportion of EPA in MNC phospholipids \((P = 0.048)\). The increase in the proportion of EPA in MNC phospholipids was greater with increasing doses of EPA and was greater in the older than in the younger subjects (Table 3). The increase in the EPA content of MNC phospholipids was related to the dose of EPA provided by the capsules \((\rho = 0.727\) for young and \(\rho = 0.809\) for older subjects, \(P < 0.001\) for both). Across all subjects and treatments there was a highly significant correlation between the EPA contents of plasma and MNC phospholipids \((r = 0.899, r = 0.898,\) and \(r = 0.903\) for all, young, and older subjects, respectively; \(P < 0.001\) for all). Furthermore, across all subjects and treatments there was a highly significant correlation between the changes in the EPA contents of plasma and MNC phospholipids \((r = 0.824, r = 0.827, r = 0.811\) for all, young and older subjects, respectively; \(P < 0.001\) for all). The change in the DPA content of MNC phospholipids was related to EPA dose \((\rho = 0.706\) for the young and \(\rho = 0.54\) for the older subjects, \(P < 0.001\) for both).

At baseline there was a significant effect of age \((P = 0.005)\) but not of treatment group on the ratio of arachidonic acid to EPA in MNC phospholipids, and there was no age \(\times\) treatment group interaction. The ratio was significantly higher in the young than in the older subjects. There was a significant effect of treatment group \((P < 0.001)\) but not of age on the change in the ratio of arachidonic acid to EPA, and there was no age \(\times\) treatment group interaction. The ratio was significantly lower in the subjects who consumed EPA at the end of supplementation than at baseline (Figure 2). A comparison of marginal means indicated that the ratio of arachidonic acid to EPA in MNC phospholipids was significantly lower at the end of supplementation than at baseline in the subjects who consumed EPA \((P < 0.001, \text{paired Student’s} \ t\text{test})\). Furthermore, at week 12, the ratio was significantly lower in the groups that had received EPA than in the placebo group \((P < 0.001, \text{one-factor ANOVA})\) but was not significantly different between the low-, moderate-, and high-EPA groups \((P > 0.31, \text{one-factor ANOVA})\).
TABLE 3
Fatty acid composition of peripheral blood mononuclear cell (MNC) phospholipids in the young and older subjects at baseline and after 12 wk of supplementation

<table>
<thead>
<tr>
<th>Treatment group and time</th>
<th>DGLA</th>
<th>Arachidonic acid</th>
<th>EPA</th>
<th>DPA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Older</td>
<td>Young</td>
<td>Older</td>
<td>Young</td>
</tr>
<tr>
<td>Placebo</td>
<td>0 wk</td>
<td>2.0 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>18.4 ± 0.6</td>
<td>18.7 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>12 wk</td>
<td>1.8 ± 0.1</td>
<td>1.9 ± 0.3</td>
<td>16.7 ± 0.7</td>
<td>15.2 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>12 – 0 wk</td>
<td>−0.2 ± 0.1</td>
<td>−0.3 ± 0.4</td>
<td>−1.7 ± 0.7</td>
<td>−3.4 ± 2.2</td>
</tr>
<tr>
<td>Low EPA</td>
<td>0 wk</td>
<td>2.2 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>18.6 ± 0.7</td>
<td>18.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>12 wk</td>
<td>2.0 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>16.4 ± 0.5</td>
<td>14.1 ± 2.2</td>
</tr>
<tr>
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<td>12 – 0 wk</td>
<td>−0.2 ± 0.1</td>
<td>−0.6 ± 0.2</td>
<td>−1.9 ± 0.5</td>
<td>−4.2 ± 1.9</td>
</tr>
<tr>
<td>Moderate EPA</td>
<td>0 wk</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>17.4 ± 0.8</td>
<td>18.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>12 wk</td>
<td>1.7 ± 0.1</td>
<td>1.4 ± 0.2</td>
<td>14.6 ± 0.5</td>
<td>14.0 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>12 – 0 wk</td>
<td>−0.2 ± 0.1</td>
<td>−0.6 ± 0.2</td>
<td>−2.8 ± 0.9</td>
<td>−4.8 ± 1.9</td>
</tr>
<tr>
<td>High EPA</td>
<td>0 wk</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.3</td>
<td>17.5 ± 0.7</td>
<td>18.6 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>12 wk</td>
<td>1.7 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>15.1 ± 0.5</td>
<td>13.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>12 – 0 wk</td>
<td>−0.2 ± 0.1</td>
<td>−0.9 ± 0.2</td>
<td>−2.3 ± 0.9</td>
<td>−6.0 ± 1.5</td>
</tr>
</tbody>
</table>

1 All values are x ± SEM. n = 24, 23, 23, and 23 for the young and n = 16, 16, 15, and 15 for the older subjects in the placebo, low-EPA, moderate-EPA, and high-EPA groups. DGLA, di-homo-y-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; E, eicosapentaenoic acid. At baseline there was a significant effect of age on the proportions of EPA (P = 0.031) and DHA (P = 0.002), but there was no significant effect of treatment group and no significant age × treatment group interaction (2-factor ANOVA). The young subjects had lower proportions of EPA and DHA than did the older subjects. A 2-factor ANOVA showed a significant effect of age on the change in DGLA (P = 0.004), arachidonic acid (P = 0.005), EPA (P = 0.012), and DHA (P = <0.001) and of treatment group on change in EPA and DPA (P < 0.001 for both). There was a significant age × treatment group interaction for change in the proportion of EPA (P = 0.048).

2 Significantly different from baseline, P ≤ 0.012 (paired Student’s t test).
3 Significantly different from placebo, P ≤ 0.042 (one-factor ANOVA with post hoc Tukey’s test).
4 Significantly different from low EPA, P ≤ 0.006 (one-factor ANOVA with post hoc Tukey’s test).
5 Significantly different from moderate EPA, P ≤ 0.043 (one-factor ANOVA with post hoc Tukey’s test).
6 Significantly different from the young subjects, P ≤ 0.028 (unpaired Student’s t test).

Expression of CD54 and CD11b on monocytes

At baseline there was a significant effect of age on the proportion of monocytes expressing CD54 or CD11b (P < 0.001 for both), but there was no effect of treatment group and no age × treatment group interaction for either adhesion molecule. The proportion of monocytes expressing these molecules was lower in the young than in the older subjects (86.9 ± 1.5% compared with 99.4 ± 0.3% and 88.5 ± 1.4% compared with 99.0 ± 0.5% for CD54 and CD11b, respectively).

A two-factor ANOVA of the effects of age and treatment group on the change in the proportion of monocytes expressing CD54 or CD11b showed no significant effects of age or treatment group and no age × treatment group interaction.

Phagocytosis by neutrophils and monocytes

At baseline there was a significant effect of age on the percentage of monocytes, but not of neutrophils, engaging in the phagocytosis of E. coli (P < 0.001), but there was no effect of treatment group and no age × treatment group interaction. The percentage of monocytes phagocytosing E. coli was higher in the older than in the younger subjects (Table 4). A two-factor ANOVA of the effects of age and treatment group on the change in neutrophil phagocytosis from baseline showed that there was a significant effect of age (P < 0.001) but not of treatment group and no interaction between these factors. There were no significant effects of age or treatment group and no interaction between these factors for monocyte phagocytosis.

Respiratory burst by neutrophils and monocytes

At baseline there were significant effects of age (P < 0.001) and treatment group (P = 0.016) on the percentage of neutrophils engaging in respiratory burst in response to E. coli, and there was a significant age × treatment group interaction (P = 0.021). There was a significant effect of age (P < 0.001) but not of treatment group and there was no age × treatment group interaction for neutrophil respiratory burst in response to PMA. The percentage of neutrophils with a respiratory burst response to either E. coli or PMA was significantly higher in the young than in the older subjects (Table 5).

At baseline there were significant effects of age (P = 0.004) and treatment group (P = 0.038) but no interaction between these factors for the percentage of monocytes engaging in respiratory burst in response to PMA. The percentage of monocytes with a respiratory burst response to PMA was higher in the young than in the older subjects (Table 5). There were no significant effects of age or treatment group and no age × treatment group interaction for monocyte respiratory burst in response to E. coli.
A two-factor analysis of covariance (using the value at week 0 as the covariate) of the effects of age and treatment group on the change in neutrophil respiratory burst to E. coli from baseline showed significant effects of treatment group ($P = 0.004$) and age ($P < 0.001$) and a significant interaction between them ($P = 0.022$). The effect of treatment group occurred in the older subjects (Table 5), in whom an increasing EPA dose resulted in a decreased response ($\rho = -0.42, P = 0.013$; Figure 3), such that at the highest EPA dose the change in respiratory burst was significantly different from that in the placebo group ($P = 0.046$; one-way ANOVA with post hoc Tukey’s test).

A two-factor ANOVA of the effects of age and treatment group on the change in neutrophil respiratory burst to PMA from baseline showed significant effects of treatment group ($P = 0.003$) and age ($P < 0.001$) and a significant interaction between them ($P = 0.004$). There was a significant effect of age ($P < 0.001$) but not of treatment group and no significant interaction between these factors for change in monocyte respiratory burst to E. coli or PMA.

**Cytokine production by lipopolysaccharide-stimulated MNCs**

At baseline there was a significant effect of age ($P < 0.001$ for both) but not of treatment group and no age $\times$ treatment group interaction for the production of TNF-$\alpha$ and IL-6 by lipopolysaccharide-stimulated MNCs. The production of both of these inflammatory cytokines was greater in the older than in the young subjects (Table 6). At baseline there were no significant effects of age or treatment group and no age $\times$ treatment group interaction for the production of IL-1$\beta$ by lipopolysaccharide-stimulated MNCs, although the effect of age tended toward significance ($P = 0.082$).

### Table 4

Phagocytosis of *Escherichia coli* by neutrophils or monocytes in the young and older subjects at baseline and after 12 wk of supplementation$^7$

<table>
<thead>
<tr>
<th>Treatment group and time</th>
<th>Neutrophils</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Older</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 wk</td>
<td>95.2 ± 0.4</td>
<td>93.5 ± 2.2</td>
</tr>
<tr>
<td>12 wk</td>
<td>95.5 ± 0.4</td>
<td>86.4 ± 2.5</td>
</tr>
<tr>
<td>12 – 0 wk</td>
<td>0.2 ± 0.7</td>
<td>−7.1 ± 2.9</td>
</tr>
<tr>
<td>Low EPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 wk</td>
<td>95.5 ± 0.9</td>
<td>93.6 ± 2.0</td>
</tr>
<tr>
<td>12 wk</td>
<td>94.2 ± 1.1</td>
<td>88.9 ± 2.2</td>
</tr>
<tr>
<td>12 – 0 wk</td>
<td>−1.3 ± 1.3</td>
<td>−4.7 ± 3.2</td>
</tr>
<tr>
<td>Moderate EPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 wk</td>
<td>94.1 ± 0.7</td>
<td>90.3 ± 2.8</td>
</tr>
<tr>
<td>12 wk</td>
<td>92.9 ± 1.5</td>
<td>87.6 ± 1.2</td>
</tr>
<tr>
<td>12 – 0 wk</td>
<td>−1.2 ± 1.7</td>
<td>−2.7 ± 2.5</td>
</tr>
<tr>
<td>High EPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 wk</td>
<td>94.1 ± 1.2</td>
<td>94.5 ± 1.8</td>
</tr>
<tr>
<td>12 wk</td>
<td>94.7 ± 0.7</td>
<td>83.0 ± 2.5</td>
</tr>
<tr>
<td>12 – 0 wk</td>
<td>0.6 ± 1.3</td>
<td>−11.5 ± 3.7</td>
</tr>
</tbody>
</table>

$^7$ All values are $\bar{x} \pm$ SEM. $n = 24, 23, 23$, and 23 for the young and $n = 16, 16, 15$, and 15 for the older subjects in the placebo, low-EPA, moderate-EPA, and high-EPA groups, respectively. EPA, eicosapentaenoic acid. At baseline there was a significant effect of age on the percentage of monocytes, but not of neutrophils, engaging in phagocytosis ($P < 0.001$), but there was no effect of treatment group and no age $\times$ treatment group interaction (2-factor ANOVA). The percentage of monocytes phagocytosing *E. coli* was higher in the older than in the young subjects. A 2-factor ANOVA showed that there was a significant effect of age ($P < 0.001$) on the change in neutrophil phagocytosis, but there was no effect of treatment group and no age $\times$ treatment group interaction. There was no significant effect of age or treatment group and no significant interaction between these factors for the change in monocyte phagocytosis.
A two-factor ANOVA of the effects of age and treatment group on the change in cytokine production from baseline showed a significant effect of age on the production of TNF-α (P = 0.012) but no effect of treatment group, no age × treatment group interaction, no effects of age or treatment group, and no significant age × treatment group interaction for the production of IL-1β or IL-6.

**PGE₂ production by lipopolysaccharide-stimulated MNCs**

At baseline there were no significant effects of age or treatment group and no age × treatment group interaction for the production of PGE₂ by lipopolysaccharide-stimulated MNCs (Table 6). A two-factor ANOVA of the effects of age and treatment group on the change in PGE₂ production from baseline showed no significant effects of age or treatment group or interaction between these factors.

Across both time points and across all EPA doses, there was a significant positive relation between PGE₂ production by lipopolysaccharide-stimulated MNCs and MNC phospholipid arachidonic acid content (r = 0.176, 0.237, and 0.27 for all, young, and older subjects, respectively; P = 0.006, 0.001, and 0.001, respectively) and a significant negative relation between PGE₂ production by lipopolysaccharide-stimulated MNCs and MNC phospholipid EPA content (r = −0.273, −0.341, and −0.23 for all, young, and older subjects, respectively; P < 0.001, < 0.001, and 0.046, respectively). There was a significant
positive relation between PGE₂ production and the ratio of arachidonic acid to EPA in MNC phospholipids ($r = 0.22$, $0.276$, and $0.166$ for all, young, and older subjects, respectively; $P = 0.001$, $< 0.001$, and $0.037$, respectively). There was a significant negative relation between the change in PGE₂ production and the change in MNC phospholipid EPA content ($r = -0.225$, $-0.237$, and $-0.174$ for all, young, and older subjects, respectively; $P = 0.014$, $0.024$, and $0.049$, respectively).

**DISCUSSION**

This study identified that free-living, healthy men aged 53–70 years have higher contents of EPA, DPA, and DHA in plasma phospholipids and of EPA and DHA in MNC phospholipids than do younger men. To our knowledge this is the first time that such an age-dependent difference in the long-chain n-3 PUFA contents of plasma or cell lipids has been reported. However, Bolton-Smith et al. (23) reported a significant age-dependent increase in the long-chain n-3 PUFA content of adipose tissue from both males and females. One possible explanation for our observed differences in long-chain n-3 PUFA contents between the young and older men may have been that their diets were different, particularly with respect to long-chain n-3 PUFAs or to their precursor $\alpha$-linolenic acid. The current study, dietary intake data were not collected. However, in the absence of significant fish consumption or supplementation with fish oils, it seems that higher intakes of long-chain-3 PUFAs in the older subjects in this study was unlikely. The latest dietary survey data available for adults in the United Kingdom, gathered in 2000–2001, indicate that there is no significant change in total n-3 PUFA intake, the bulk of which (>85%) would be in the form of $\alpha$-linolenic acid, with age (20). Thus, it seems unlikely that the older subjects had higher intakes of $\alpha$-linolenic acid than did the young subjects in the current study; thus, this can be ruled out as a cause of the higher long-chain-3 PUFA contents observed in the older subjects. The capacity to convert $\alpha$-linolenic acid to EPA and DPA, which is very low in males (24), appears to decline with age (25), and the conversion of DPA to DHA is negligible in men (24–27). Thus, the higher long-chain n-3 PUFA contents of plasma and MNC phospholipids in the older men than in the young men are not likely a result of increased synthesis from the precursor $\alpha$-linolenic acid. An alternative explanation may be differences in n-6 PUFA intakes. However, the latest dietary survey data available for adults in the United Kingdom (20) indicate that there is no significant change in average n-6 PUFA intakes or in the distribution of intakes with age. Because the differences in long-chain n-3 PUFA contents of phospholipids between the young and the older subjects appeared not to relate to differences in dietary intakes of the preformed long-chain fatty acids, their precursor, or n-6 PUFAs or to synthesis from the precursor, some difference in the way that the body handles dietary long-chain n-3 PUFA contents may occur with age. Another possibility is that aging is associated with increased utilization of n-6 PUFAs, resulting in a relatively greater pool of n-3 PUFAs remaining for incorporation into phospholipids.

It was observed that the enrichment of plasma and MNC phospholipids with long-chain n-3 PUFAs was significantly greater in the older than in the young men when an EPA-rich oil was consumed. It seems unlikely that a decrease in n-6 PUFA consumption among older subjects during the study would be a contributory factor to this, because the increase in EPA was
observed even in the low-EPA group, who consumed an additional 3.3 g linoleic acid/d from the capsules. The observation of greater enrichment of plasma phospholipids with EPA in the older men is consistent with the findings of Meydani et al (28), who showed greater incorporation of EPA (and DHA) into total plasma lipids in the older than in young women, who consumed 2.4 g EPA + DHA/d. However, to our knowledge, this is the first time that such an age-dependent difference in the ability to incorporate long-chain n-3 PUFAs into cell phospholipids has been reported. The observation of a greater incorporation of these fatty acids in the older than in the young subjects is consistent with the observation that the long-chain n-3 PUFAs contents of plasma and MNC phospholipids were higher in the older subjects at study entry. Furthermore, because the supplemental intakes of EPA and DHA were much greater than the habitual intakes of these fatty acids (habitual intakes of long-chain n-3 PUFAs in adults in the United Kingdom, in the absence of fish-oil supplementation, are <0.25 g/d; 17, 19), the finding of a higher incorporation of these fatty acids in the older subjects is consistent with the suggestion of a difference in body handling of these fatty acids with aging. This difference may relate to the changes in body composition that occur with aging with a decrease in energy expenditure (29–32) and in lean tissue mass (33–37). These factors would mean that a lower proportion of dietary n-3 PUFA would be oxidized, thus leaving a greater proportion for incorporation into phospholipid pools, as observed in the current study, and into adipose tissue, as observed by Bolton-Smith et al (23).

The ability of MNCs to produce PGE2 was closely related to the arachidonic acid and EPA contents of the cell phospholipids and to the ratio between these 2 fatty acids. Thus, the principal mechanism underlying the change in PGE2 production that accompanied increased consumption of long-chain n-3 PUFAs (10, 11, 13) appears to relate to the altered availability of substrate (ie, arachidonic acid). However, the current study clearly indicated that a substantial increase in the EPA content of MNC phospholipids (4-fold) and a corresponding decrease in arachidonic acid (≈25%) is likely required to affect PGE2 production. The current study indicates that such changes in MNC fatty acid composition, and subsequently in PGE2 production, can only be achieved by substantially increased intakes of EPA (certainly >1.35 g/d). This conclusion is consistent with the amounts of EPA provided in earlier studies that showed decreased PGE2 production by lipopolysaccharide-stimulated MNCs after fish-oil supplementation of the diet (10, 11, 13).

Markedly increased intakes of long-chain n-3 PUFAs, mainly EPA, did not affect any neutrophil or monocyte functions in the young men, apart from a trend toward decreased PGE2 production with increasing EPA intake. The lack of effects of the EPA-rich oil on neutrophil and monocyte phagocytosis and respiratory burst and on inflammatory cytokine production are consistent with several other studies in young male subjects that studied the effects of intakes of long-chain n-3 PUFAs of 0.3–3.2 g/d (21, 38–41). However, the current study extended these previous studies by using intakes of EPA ≤ 4.05 g/d. Several open studies report that fish-oil supplementation of the diet of young or middle-aged subjects decreases superoxide production by neutrophils (7, 8) and monocytes (9) and the production of TNF-α, IL-1β, and IL-6 by lipopolysaccharide-stimulated MNCs (10–12, 14, 15). The lack of a control group in these studies makes the findings difficult to interpret, because changes in the outcomes reported may occur over time. The only controlled studies of fish oil conducted in young subjects that showed effects on the outcomes considered here are those of Thompson et al (6) for neutrophil respiratory burst and of Caughey et al (13) for TNF-α and IL-1β production by lipopolysaccharide-stimulated MNCs. The reasons why the findings of the current study are different from those of these earlier studies might relate to technical differences such as different stimuli used for respiratory burst and differences in the precise nature of sample in which cytokine measurements were made. One other difference between the studies is that the oil capsules used in the current study contained a greater amount of α-tocopherol than did the capsules used in many previous studies. This might have protected the cells examined from the deleterious inhibitory effects of lipid peroxidation. Whatever the reason for the differences from some earlier studies, the data from the current study indicate that young men can substantially increase their intakes of long-chain n-3 PUFAs, especially EPA, perhaps as a strategy to decrease their cardiovascular disease risk (16–19, 42, 43), without any significant compromise of innate immunity.

Markedly increased intakes of long-chain n-3 PUFAs, mainly as EPA, by older men did not affect neutrophil or monocyte phagocytosis, monocyte respiratory burst, or the production of inflammatory cytokines. These observations are consistent with those of an earlier study in older men and women that consumed a lower intake of long-chain n-3 PUFAs than used in the present study (1.1 g/d; 44). The current study extends that study by using intakes of EPA ≤ 4.05 g/d. However, neutrophil respiratory burst, particularly in response to E. coli, was decreased in a dose-dependent manner, becoming significantly impaired at the 2 higher EPA doses used. Thies et al (44) reported no effect of 1.1 g long-chain n-3 PUFAs/d on the neutrophil respiratory burst in response to E. coli. The current study confirmed the lack of a significant effect of long-chain n-3 PUFAs at 1.65 g/d on this outcome. Because inappropriate or excessive superoxide production is a characteristic of inflammatory conditions and is damaging to host tissues (45, 46), the effect of higher intakes of long-chain n-3 PUFAs seen in this study may have a beneficial antiinflammatory effect. However, superoxide production is also a key component of the innate immune response to bacteria; therefore, the impaired activity seen at the higher intakes of long-chain n-3 PUFAs may indicate a weakened ability to deal with bacterial infections. Nevertheless, these data indicate that older men could substantially increase their intake of long-chain n-3 PUFAs, perhaps as a strategy to decrease their cardiovascular disease risk (16–19, 42, 43), without any significant compromise of innate immunity.

To our knowledge this is the first controlled study to compare the effects of long-chain n-3 PUFAs on immunologic outcomes in young and older subjects. One important finding was that older men incorporate more EPA into MNC (and plasma) phospholipids than do young men. A second important finding was that young men did not appear to suffer any impairment of innate immune functions even when they consumed 4.05 g EPA + 0.9 g DHA/d. A third important finding was that most innate immune functions remained unaffected in older men, even when they consumed this high intake of long-chain n-3 PUFAs. However, at this intake, neutrophil superoxide production in response to E. coli may be impaired by up to 20%. Whether this is a clinically
relevant impairment, either in terms of a genuine antiinflammatory effect or an impairment of host defense, is not clear. Whether this effect is clinically relevant or not, the current study identified an intake of long-chain n–3 PUFAs (1.35 g EPA + 0.3 g DHA/d) that is substantially greater than the habitual intake, but that does not have any detrimental effects on innate immune functions in healthy young or older men.

Several important conclusions can be made from this study. First, older men may handle dietary long-chain n–3 PUFAs in a different way than do young men. Second, increasing the intake of long-chain n–3 PUFAs to 1.65 g/d in men with a habitual intake of <0.25 g/d does not affect innate immune function. Third, an EPA + DHA intake of 1.65 g/d is below the threshold required to exert antiinflammatory effects in healthy men. Finally, impairment of some innate immune functions can occur in older, but not in young, men at high long-chain n–3 PUFA intakes. Thus, consumption of n–3 PUFAs could be significantly increased without inducing adverse effects on the innate immune response.

EAM, KWJW, and PCC designed the study. DR, EAM, and TB recruited the subjects and carried out the intervention, KWJW and PCC supervised the intervention and the data collection. DR, EAM, TB, SJW, and CER collected the data. EAM and PCC analyzed the data. EAM and PCC wrote the manuscript with help from all other authors. None of the authors had any financial or personal interest in any company or organization sponsoring or involved in the research.

REFERENCES