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Effects of supplemental long-chain omega-3 fatty acids and erythrocyte membrane fatty acid content on circulating inflammatory markers in a randomized controlled trial of healthy adults

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Abstract

The long-chain omega-3 polyunsaturated (n-3 PUFA), eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), may have anti-inflammatory effects. We evaluated the dose-response effect of EPA+DHA supplementation on circulating TNF- α , IL-6, and CRP and explored associations between red blood cell (RBC) membrane PUFA content and TNF- α , IL-6, and CRP. Young adults with low fish intake (n = 116) received one of five doses (0, 300, 600, 900, or 1,800 mg/d EPA+DHA) for 5 months. There were no significant effects of supplemental EPA+DHA on IL-6 or CRP; however, there was a marginal treatment effect for TNF- α (p < 0.08). At baseline, higher quartiles of RBC DHA were associated with lower TNF- α (p = 0.001); higher quartiles of arachidonic acid were associated with higher TNF- α (p = 0.005). EPA+DHA supplementation had no dose-response effect on TNF- α , IL-6, or CRP in healthy young adults; however, associations between inflammatory markers and RBC PUFA warrant further investigation.

Keywords

eicosapentaenoic acid (EPA); docosahexaenoic acid (DHA); docosapentaenoic acid (DPA); omega-3; inflammation; C-reactive protein (CRP)

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1. INTRODUCTION

Chronic inflammatory diseases are increasingly prevalent and typically managed by costly medical therapies that have significant side effects. The important role of diet in reducing low grade inflammation and thereby modulating the risk of chronic diseases, including cardiovascular disease (CVD), is supported by observational data [1]. In particular, the potential anti-inflammatory effect of increased dietary long-chain omega-3 (n-3) PUFA—specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—is demonstrated by mechanistic research [2] and population studies in which higher EPA +DHA intake is associated with lower levels of inflammation [3-6]. However, randomized controlled trials with n-3 PUFA supplementation have yielded mixed results in terms of both inflammatory markers and CVD outcomes [7-16].

Clinically evaluating the effects of n-3 PUFA interventions on inflammatory status is difficult due to considerable acute variations in inflammatory markers caused by factors unrelated to the study intervention. Blood concentrations of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6), as well as the acute phase reactant, C-reactive protein (CRP), are used as markers of systemic inflammatory status because they provide quantitative assessments of ongoing inflammatory processes [17, 18]. However, acute elevations in these markers can be caused by unavoidable stimuli in the daily lives of research participants, including unreported acute illness [19], changes in physical activity [20], and psychosocial stressors [21]. This poses a significant challenge for evaluating the effects of interventions by introducing extreme changes in inflammatory markers that are not due to treatment effects. Excluding these acutely elevated values according to pre-defined thresholds may enable more accurate assessment of the relationship between n-3 PUFA and chronic inflammation.

Another approach to evaluating links between PUFA and inflammation is utilizing blood biomarkers of fatty acid intake [22]. The EPA+DHA content of the RBC membrane is a useful biomarker of dietary n-3 PUFA intake as it reflects dietary intake over the course of the RBC lifespan (~120 d) and correlates with concentrations in tissues and other cells [23-25]. Inverse associations between RBC n-3 PUFA content and inflammatory marker concentrations have been reported [26-28]. However, the relationships between the content of individual n-3 and n-6 PUFA in RBC membranes and concentrations of circulating inflammatory markers is not completely understood. Additionally, whether changes in RBC PUFA relate to changes in inflammatory status has not been determined. Despite this, fish oil supplementation across a range of non-pharmacological doses (< 2 g/d) is commonly used in the U.S. for anti-inflammatory purposes [29, 30]. Understanding the links between supplemental n-3 PUFA intake, tissues levels, and markers of inflammation is important given inadequate EPA+DHA intake in the U.S. and the increasing burden of chronic inflammatory diseases.

We recently reported that EPA+DHA supplementation dose-dependently increased RBC EPA+DHA content and that additional factors, such as body weight and baseline RBC EPA +DHA, further explained variability in the RBC EPA+DHA response [31]. In the present study, we evaluated the dose-response effects of 0, 300, 600, 900, and 1800 mg/d EPA

+DHA on circulating markers of inflammation (TNF- α , IL-6, and CRP) in healthy young adults. We also explored the relationships between both the baseline content and post-supplementation changes in RBC n-3 and n-6 PUFA content with circulating inflammatory marker concentrations, and assessed the potential of n-3 PUFA supplementation to alter circulating white blood cell populations as an additional immunoregulatory effect [32].

2. METHODS

2.1. Study design and intervention

Healthy young adults ($n = 125$) between the ages of 20-45 y and with BMI between 20-30 kg/m² reporting no or low habitual oily fish consumption (<4 servings per month) and not taking n-3 PUFA supplements were recruited. Additional exclusion criteria included use of n-3 PUFA supplemented foods in the past 3 months, history of diabetes, serious medical conditions, smoking, chronic anti-inflammatory medications, planning to change dietary habits, and pregnant, nursing, or planning a pregnancy. Details of the study recruitment and screening have been reported previously [31]. The study was approved annually by the Pennsylvania State University Institutional Review Board.

Each participant was randomly assigned to one of five doses (0, 300, 600, 900, 1800 mg) daily of EPA+DHA as fish oil supplements, using soybean oil as the placebo (Nordic Naturals, Watsonville, CA) for approximately 5 months. The intervention provided nutritionally-achievable doses of EPA+DHA in triglyceride form equivalent to that which could be obtained by consumption of oily fish. The fatty acid profile of the supplements has been described in detail [31]. The fish oil capsules contained approximately 20% EPA, 2% docosapentaenoic acid (DPA), and 13% DHA. Participants agreed to maintain their weight, activity level, usual (limited) fish consumption, and not take any other n-3 PUFA supplements during the study. Daily log sheets and monthly check-ins were completed to verify compliance. Participants returned to the Pennsylvania State University Clinical Research Center every 8 weeks to receive new supplies, return empty containers, and provide completed log sheets.

2.2. Blood sample collection

At the beginning of the study and after the treatment period, participants reported to the Clinical Research Center after a 12-h overnight fast to provide a blood sample by venipuncture. Whole blood was centrifuged at $1500 \times g$ for 15 min at 4°C. Serum was collected and stored at -80 °C until they were analyzed, except for the complete blood count (CBC), which was measured in EDTA anticoagulated whole blood samples using a Coulter LH 750 with VCS technology (Quest Diagnostics, Pittsburgh, PA). Red blood cells (RBC) were collected following separation from plasma by centrifugation and frozen at -80 °C until analyzed. Fatty acid analysis was performed as described previously [31].

2.3. Inflammatory marker concentrations

Serum concentrations of TNF- α and IL-6 were measured using high-sensitivity ELISA kits (R&D Systems, Minneapolis, MN) in duplicate (CV <10%). Serum high-sensitivity CRP

was measured by latex-enhanced immunonephelometry (Quest Diagnostics; assay CV < 8%).

2.4. Statistical analysis

Statistical analyses were performed using Minitab (version 16.2, Minitab, State College, PA). Analytes that were assayed in duplicate (i.e., TNF- α and IL-6) were averaged before analysis. Fit statistics were assessed for each variable to identify any outliers and to test assumptions of normality. Subjects with acute inflammation (i.e., baseline or endpoint TNF- α and IL-6 values >3.0 ng/L or CRP values >3.0 mg/L), were identified as outliers and secondarily removed from the analysis to ensure that analysis was performed on samples from the target population of non-inflamed adults [33, 34]. A natural log transformation was applied to baseline and endpoint values of IL-6 and CRP because of non-normal distribution (skew > 1), and further analysis of associations and treatment effects were performed on the transformed values.

Independent two-sample t tests were used to assess sex differences in baseline inflammatory marker concentrations. Associations between baseline inflammatory marker concentrations and BMI, body weight, blood pressure, and age were assessed using Pearson correlation tests.

General linear models were used to test the effects of treatment on inflammatory marker concentrations and CBC measures. Baseline values were included as covariates. We present 4 models for the effect of supplementation on TNF- α , IL-6, and CRP concentrations: unadjusted (model 1), adjusted for baseline value (model 2), adjusted for baseline value, sex, and age (model 3), and adjusted for baseline value, sex, age, blood pressure, and body weight (model 4). Tukey-adjusted p-values were used for *post-hoc* comparisons between treatment groups, with adjusted p < 0.05 considered significant.

Baseline RBC membrane content of n-3 PUFA (alpha-linolenic acid [ALA], EPA, DPA, DHA) and n-6 PUFA (linoleic acid [LA], arachidonic acid [AA]) were assessed as quartiles and compared with circulating inflammatory marker concentrations using ANOVA. Tukey-adjusted p-values were used for *post hoc* comparisons between quartiles. Multiple regression models, with baseline inflammatory markers as the response variable and RBC PUFA as predictor variables, also were conducted to test for the combined effects of RBC PUFA on baseline inflammatory marker concentrations and to guard against spurious findings in the models that examined the relationships between baseline inflammatory marker concentrations and single RBC PUFA quartiles. Changes in RBC membrane content were compared with changes in inflammatory marker concentrations using Pearson correlation tests. Change scores were calculated as the end of supplementation value minus baseline value. Scatterplots were generated to illustrate exploratory analyses of continuous relationships between baseline RBC PUFA content and inflammatory marker concentrations with Pearson correlation coefficients and unadjusted p-values reported for each comparison.

3. RESULTS

3.1. Participant characteristics

The study design and flow of participants have been reported previously [31]. Of the 125 participants, nine withdrew from the study, leaving 116 participants who completed the study (mean compliance: 97%, range 85-100%). Two additional participants were removed from the analysis due to having either very high RBC EPA+DHA content at study entry (>8%) or an underlying health condition (leukopenia). Elevated baseline TNF- α (n=4), IL-6 (n=2), or CRP (n=12) data were excluded in order to limit our analyses to healthy non-inflamed adults and remove statistical outliers. Remaining subjects with elevated endpoint TNF- α (n=0), IL-6 (n=3), or CRP (n=4) were excluded from the treatment effect and change score analyses.

There were no significant differences between the treatment groups at baseline with respect to inflammatory marker concentrations and white blood cell populations (Table 1), RBC and platelet measures (Supplemental Table 1), or n-3 and n-6 PUFA content of RBC membranes [31]. The mean RBC EPA+DHA content at study entry ($\pm$ SEM) was $4.3 \pm 0.1\%$. Men had higher baseline TNF- α concentrations compared with women (1.5 ± 0.1 ng/L vs 1.2 ± 0.1 ng/L, $p < 0.001$), whereas women had higher CRP concentrations than men (1.1 ± 0.1 mg/L vs 0.6 ± 0.1 mg/L, $p = 0.001$). Individuals with higher baseline TNF- α also had higher body weight, diastolic blood pressure, and systolic blood pressure ($p = 0.001$ for all), whereas CRP was associated with higher BMI ($p = 0.009$) (Supplemental Table 2). There were no significant associations between age and baseline inflammatory marker concentrations.

3.2. Effect of supplementation on inflammatory markers

Following supplementation, there were no significant differences between treatment groups with respect to IL-6 or CRP concentrations (Table 2); however, a marginally significant treatment effect was observed for TNF- α in the adjusted models ($p < 0.08$). The 1,800 mg/d group experienced a 10% reduction in TNF- α concentration from baseline; however, no group was significantly different from placebo (Table 2).

There were no significant treatment effects on CBC measures with the exception of lymphocyte concentrations (Supplemental Table 3). The 1,800 mg/d group had a 17% higher lymphocyte concentration following supplementation compared to the placebo (Tukey-adjusted $p = 0.02$; Figure 1).

3.3. Associations between RBC content of PUFA and circulating inflammatory markers

Baseline inflammatory marker concentrations were compared between quartiles of n-3 and n-6 PUFA content in RBC membranes (Table 3). There were no significant differences in any inflammatory marker concentrations across quartiles of ALA or EPA content. Higher quartiles of DHA content were associated with lower TNF- α concentrations ($p = 0.001$), whereas higher quartiles of AA content were associated with higher TNF- α concentrations ($p = 0.005$) (Table 3; Figure 2). We also found that higher (highest vs. lowest quartiles) LA levels were associated with 29% lower IL-6 concentrations (Table 3; Figure 3).

Furthermore, participants in the lowest DPA quartile had approximately twice the CRP

concentrations of participants in all other DPA quartiles. These findings were consistent with the multivariable regression models (Supplemental Table 4).

Changes in inflammatory marker concentrations were compared with changes in n-3 and n-6 PUFA content in RBC membranes following supplementation (Supplemental Table 5). No significant associations were observed between the change in inflammatory marker concentrations versus change in n-3 or n-6 PUFA content. However, we found that changes in lymphocyte concentrations were positively associated with changes in EPA and DPA content following supplementation ($p = 0.001$ and $p = 0.008$, respectively; Supplemental Figure 1).

4. DISCUSSION

The primary objective of this study was to determine the effect of supplemental EPA+DHA, across a range of non-pharmacological doses, on circulating inflammatory marker concentrations in healthy adults after 5 months of supplementation. There were no significant effects of EPA+DHA supplementation on IL-6 or CRP concentrations. However, a marginally significant treatment effect was observed for TNF- α due to reductions from baseline following 1,800 mg/d EPA+DHA. More research is needed to evaluate whether TNF- α concentrations may be modestly reduced by high doses of EPA+DHA (i.e., 1.8 g/d) administered for long periods of time. Although most clinical studies of n-3 PUFA supplementation have not demonstrated reductions in TNF- α , patients with congestive heart failure may experience a benefit [35-37]. Analytical approaches that account for acute elevations in TNF- α may improve the sensitivity of future clinical studies in both healthy individuals and those with specific inflammatory conditions, thereby helping to clarify the relationship between n-3 PUFA supplementation and TNF- α .

We also examined the relationship between RBC n-3 and n-6 PUFA content and inflammatory marker concentrations before and after supplementation to determine whether this provided insight into a more direct biological relationship between tissue PUFA and inflammation. Incorporation of dietary PUFA into membrane phospholipids can affect cell signaling and lipid mediator production [32]. However, the change in RBC n-3 and n-6 PUFA content was not related to changes in TNF- α , IL-6, or CRP concentrations following supplementation. This may be due to the absence of changes in inflammatory markers following the fish oil intervention. Our results may also suggest that RBC fatty acid content, although an important biomarker, may not play a direct role in regulating inflammatory signaling.

Of note is that there were several baseline associations between RBC n-3 PUFA content and inflammatory markers, which supports the modest anti-inflammatory effect of increasing n-3 PUFA intake in healthy adults with low habitual n-3 PUFA intake, suggesting that small differences in dietary n-3 PUFA, especially in sub-optimal ranges, may influence the development of low-grade inflammation. We found that higher RBC content of DHA was associated with lower TNF- α at baseline, while individuals with the lowest DPA content had the highest baseline CRP. Whether low RBC DHA and/or DPA content is a cause or effect of elevated inflammatory markers is unclear; however, this relationship between RBC DHA

content and TNF- α lends support to the potential ability of fish oil supplementation to reduce TNF- α suggested by the trend observed in response to 1,800 mg/d supplementation. Dietary and supplemental sources of n-3 PUFA typically contain both EPA and DHA, but their accumulation and retention in tissues varies [38]. The fact that RBC membranes are more responsive to DHA may explain why only RBC DHA content was significantly related to TNF- α [31].

Clinical research on the individual roles of EPA, DPA, and DHA is relatively scarce, and the links between these individual fatty acids and inflammation remain unclear [39]. Inconsistent findings in observational studies of n-3 PUFA and inflammatory markers are likely due to differences in which fatty acid pool was assessed (e.g. plasma vs. RBC membrane), variability in subject population (e.g., age, sex, BMI, etc.) [40, 41], and genetic differences that influence PUFA metabolism and responses to supplementation [42]. Therefore, additional research is needed to prospectively assess the individual effects of EPA vs. DHA on markers of inflammation before any definitive conclusions can be made about their differential effects. Moreover, the relationship between DPA—which is typically found in animal products— and inflammation needs to be investigated further.

In the present study, the association between n-6 PUFA and inflammatory marker concentrations also varied depending upon the specific fatty acid. Individuals with the highest RBC content of AA had higher TNF- α at baseline compared to those with lower AA content. This may reflect the ability of AA to generate eicosanoids that promote pro-inflammatory cytokine production [43]. In contrast, individuals with the highest LA content had lower baseline IL-6 concentrations compared to those with the lowest LA content. Upon further investigation, we found that baseline LA and AA content were significantly inversely correlated (Supplemental Figure 2). This is particularly interesting since it has been proposed that dietary intake of LA is pro-inflammatory, and thus increases CVD risk, largely based on the fact that LA is a precursor of AA [44, 45]. However, the potential for dietary and tissue LA to influence inflammation remains unclear [46], and additional studies examining the differences between LA and AA in modulating inflammation are needed.

Exploratory analyses revealed several potential relationships between baseline inflammatory marker concentrations and participant characteristics. Being male and have higher body weight was associated with higher TNF- α concentrations, whereas being female and having higher BMI were associated with higher CRP. Gender effects remained significant after accounting for differences in bodyweight and BMI (data not shown), which has been previously reported by others [47-53]. Sex-specific differences in body fat distribution [50, 54] and hormones may account for these differences [50, 55, 56].

We also found that diastolic and systolic blood pressures were positively associated with TNF- α . Several studies have demonstrated an association between elevated TNF- α and hypertension [57-62]. Although mechanistic evidence suggests that TNF- α can directly influence hypertension [63], elevated TNF- α in observational studies could be both a cause and effect of hypertension.

Unexpectedly, we found that 1,800 mg/d EPA+DHA increased circulating lymphocyte concentrations relative to the placebo group. Greater EPA+DHA content in the RBC membrane was also associated with increasing lymphocyte concentrations following supplementation. Although few studies that we are aware of have reported the effects of EPA+DHA on circulating leukocytes, an increase in the number of circulating lymphocytes in healthy adults (n=27) has also been found after 4 weeks of supplementation with fish oil (860 mg/d EPA+DHA) and borage oil (831 mg/d gamma-LA) [64]. While this is counter to some mechanistic research on *in vitro* cell proliferation, we speculate that it could relate to reduced expression of the cellular adhesion molecules L-selectin and soluble intercellular adhesion molecule-1 (ICAM-1, resulting in reduced lymphocyte adhesion to the endothelium [65, 66] and thus a greater proportion freely circulating in peripheral blood. Lipid mediators formed from n-3 PUFA, which have been shown to influence hematopoietic stem cell and myeloid progenitor cell differentiation, could also potentially explain this finding [67]. However, since we did not differentiate between types of lymphocytes we are unable to determine whether the observed increase in circulating lymphocytes can be attributed to a particular cell type (i.e. T cell, B cell, NK cell). Further clinical research is needed to assess the effects of supplemental n-3 PUFA intake on sub-types of circulating lymphocytes and cellular adhesion molecules.

4.1. Strengths and limitations

The present study had a number of strengths, including the placebo-controlled, double-blind study design comparing five treatment groups, large sample size, well-characterized study cohort, relatively longer duration of supplementation (5 months), and use of a validated biomarker of cell membrane fatty acid content. The study population had a baseline RBC EPA+DHA content that is representative of the average American not taking n-3 PUFA supplements. We instructed participants to reschedule visits if they were experiencing or recovering from any acute illness or injury and secondarily excluded from analysis any participants with elevated inflammatory markers. This analytical approach eliminated highly influential outliers and increased the sensitivity of models to detect effects in our target population of healthy adults. Moreover, the study also had a dropout rate of 7%, which is relatively low considering the duration of the study intervention.

The predominantly Caucasian, young, healthy participant population as well as the absence of dietary assessment are limitations. Population subgroups may have unique responses to supplemental EPA+DHA intake. Thus, future studies should consider identifying racial differences and genetic determinants of n-3 PUFA metabolism. Participants were instructed to limit their intake of n-3 PUFA from food, specifically fatty fish, and continue their habitual diets throughout the duration of the intervention. However, we did not monitor their dietary habits and therefore their fatty acid intake from other foods, as well as non-fatty acid dietary components, could have confounded our findings. Despite this, participants were required to maintain their habitual diets and, thus, dietary changes most likely did not affect the study results.

Lastly, although TNF- α , IL-6, and CRP are well-established circulating inflammatory markers, they provide little information regarding the origin of inflammation. Consequently,

we are unable to determine whether the relationships between n-3 and n-6 PUFA content of RBC membranes and these inflammatory markers reflect events originating in certain tissues (e.g., liver, adipose tissue, etc.). Omega-3 PUFA supplementation may also affect serum cytokines not measured in the present study (e.g., IL-2, IL-8, IL-10). Measuring tissue-specific inflammatory markers (e.g. resistin) as well as serum markers beyond TNF- α , IL-6, and CRP would provide greater insight.

4.2. Conclusions

Consumption of nutritionally-achievable doses of EPA+DHA for 5 months had no effect on circulating IL-6 or CRP concentrations in healthy young adults; however, 1,800 mg/d EPA +DHA may exert a modest anti-inflammatory effect as evidenced by a marginally significant reduction in TNF- α . Additional markers of inflammation, including cellular adhesion molecules, need to be examined to better understand the clinical effects of supplemental EPA+DHA intake on inflammation, as well as to clarify the observed increase in lymphocyte concentrations following 1,800 mg/d EPA+DHA. We also found that increased RBC DHA content was associated with lower TNF- α at baseline, whereas low DPA content was associated with higher CRP. In contrast, changes in RBC membrane content of n-3 or n-6 PUFA were not associated with changes in inflammatory marker concentrations following EPA+DHA supplementation, indicating a need for longer term studies and exploration of other biomarkers of fatty acid content (e.g., white blood cells) that may be more directly involved in the regulation of inflammation. Collectively, our findings indicate that EPA+DHA intake, over a range of nutritionally-achievable doses, had no effect on circulating TNF- α , IL-6, or CRP in healthy young adults after 5 months of supplementation. However, despite no relationship between changes in RBC PUFA content and inflammatory markers, the observed baseline associations warrant further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AA	arachidonic acid
ALA	alpha-linolenic acid
CRP	c-reactive protein
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
IL-6	interleukin-6
LA	linoleic acid
n-3	omega-3
n-6	omega-6
PUFA	polyunsaturated fatty acid
TNF-α	tumor necrosis factor alpha

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HIGHLIGHTS

- We evaluate the dose-response effect of EPA+DHA on serum inflammatory markers.
- We explore associations between RBC PUFA content and inflammatory markers.
- EPA+DHA supplementation had no effect on IL-6 or CRP concentrations.
- High doses of EPA+DHA (1.8 g/d) may reduce TNF- α concentrations.
- Higher RBC DHA was associated with lower TNF- α concentrations.

Figure 1. Changes in lymphocyte concentrations following 5 months of EPA+DHA supplementation. Values are means $\pm$ SEM (n=114). Values with different letters are significantly different, $p < 0.05$ (Tukey-adjusted values for post hoc tests).

Figure 2.

Scatterplots for baseline RBC membrane content of AA (A) and DHA (B) versus circulating TNF- α concentrations in healthy adults (n=110). Pearson correlation coefficients and unadjusted p-values are reported for each comparison. AA, arachidonic acid.

Figure 3.

Scatterplots for baseline RBC membrane content of LA (A) and AA (B) versus circulating IL-6 concentrations in healthy adults (n=112). Pearson correlation coefficients and unadjusted p-values are reported for each comparison. AA, arachidonic acid; LA, linoleic acid.

Table 1

Baseline white blood cell count and inflammatory marker concentrations

	EPA+DHA				
	0 mg/d (n=23)	300 mg/d (n=23)	600 mg/d (n=21)	900 mg/d (n=24)	1800 mg/d (n=23)
White blood cells, cells/ μ L ^a	5400 $\pm$ 280	5630 $\pm$ 180	6000 $\pm$ 320	5800 $\pm$ 220	5800 $\pm$ 240
Neutrophils	3080 $\pm$ 230	3130 $\pm$ 150	3380 $\pm$ 270	3230 $\pm$ 160	3240 $\pm$ 170
Lymphocytes	1800 $\pm$ 90	1910 $\pm$ 120	1870 $\pm$ 80	1940 $\pm$ 100	1880 $\pm$ 100
Monocytes	395 $\pm$ 25	421 $\pm$ 19	480 $\pm$ 29	429 $\pm$ 23	454 $\pm$ 27
Eosinophils	133 $\pm$ 16	154 $\pm$ 26	168 $\pm$ 29	168 $\pm$ 30	158 $\pm$ 26
Basophils	22.2 $\pm$ 2.0	23.0 $\pm$ 2.7	28.1 $\pm$ 3.8	29.6 $\pm$ 3.3	20.4 $\pm$ 2.4
Inflammatory markers					
TNF- α , ng/L ^b	1.4 (1.1, 1.6)	1.4 (1.3, 1.5)	1.3 (1.1, 1.5)	1.4 (1.2, 1.5)	1.5 (1.2, 1.6)
IL-6, ng/L ^c	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)	1.0 (0.8, 1.2)	0.9 (0.7, 1.1)	1.0 (0.8, 1.2)
CRP, mg/L ^d	0.5 (0.3, 0.8)	0.6 (0.4, 1.1)	0.5 (0.3, 0.6)	0.6 (0.4, 1.0)	0.6 (0.4, 0.9)

Groups were not significantly different for any of these metrics at baseline (p for group effect >0.05). CRP, C-reactive protein; IL-6, interleukin-6; TNF- α , tumor necrosis factor-alpha.

^a Values are means $\pm$ SEM. To convert white blood cells (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) from cells/ μ L to 10^9 /L, multiple by 0.001.

^b N=110, means (95% CI).

^c N=112, geometric means (95% CI).

^d N=102, geometric means (95% CI).

Table 2

Effects of treatment on inflammatory marker concentrations

Inflammatory marker	0 mg/d	300 mg/d	600 mg/d	900 mg/d	1800 mg/d	P value
TNF- α , ng/L ^a						
<i>Model 1</i>	1.36 (1.20, 1.53)	1.40 (1.23,1.57)	1.38 (1.20, 1.56)	1.39 (1.23, 1.55)	1.32 (1.15, 1.49)	0.95
<i>Model 2</i>	1.38 (1.28, 1.47)	1.38 (1.29, 1.47)	1.43 (1.33, 1.52)	1.41 (1.32, 1.49)	1.26 (1.17, 1.35)	0.07
<i>Model 3</i>	1.38 (1.29, 1.47)	1.39 (1.30, 1.48)	1.42 (1.33, 1.51)	1.41 (1.32, 1.49)	1.26 (1.17, 1.35)	0.08
<i>Model 4</i>	1.39 (1.30, 1.47)	1.39 (1.30, 1.48)	1.42 (1.33, 1.52)	1.41 (1.33, 1.50)	1.24 (1.14, 1.34)	0.04
IL-6, ng/L ^b						
<i>Model 1</i>	0.96 (0.80, 1.15)	0.78 (0.65, 0.95)	1.05 (0.86, 1.27)	1.00 (0.82, 1.21)	1.01 (0.84, 1.21)	0.21
<i>Model 2</i>	0.97 (0.81, 1.15)	0.80 (0.67, 0.96)	1.03 (0.86, 1.24)	0.99 (0.84, 1.18)	0.98 (0.83, 1.17)	0.23
<i>Model 3</i>	0.97 (0.81, 1.15)	0.81 (0.67, 0.97)	1.03 (0.86, 1.23)	0.99 (0.84, 1.18)	0.98 (0.83, 1.17)	0.24
<i>Model 4</i>	0.98 (0.82, 1.16)	0.82 (0.68, 0.98)	1.03 (0.85, 1.23)	1.00 (0.85, 1.19)	0.95 (0.80, 1.13)	0.37
CRP, mg/L ^c						
<i>Model 1</i>	0.50 (0.34, 0.73)	0.42 (0.29, 0.63)	0.48 (0.33, 0.71)	0.47 (0.31, 0.70)	0.44 (0.30, 0.65)	0.97
<i>Model 2</i>	0.52 (0.40, 0.67)	0.41 (0.31, 0.53)	0.54 (0.41, 0.70)	0.45 (0.33, 0.60)	0.42 (0.31, 0.55)	0.45
<i>Model 3</i>	0.52 (0.40, 0.68)	0.41 (0.31, 0.54)	0.54 (0.41, 0.71)	0.45 (0.33, 0.60)	0.41 (0.31, 0.54)	0.42
<i>Model 4</i>	0.52 (0.40, 0.68)	0.44 (0.33, 0.58)	0.54 (0.41, 0.70)	0.47 (0.35, 0.64)	0.36 (0.27, 0.48)	0.30

Model 1 includes unadjusted endpoint values. Model 2 includes treatment adjusted for baseline value. Model 3 includes treatment adjusted for baseline value, sex, and age; Model 4 includes treatment adjusted for baseline value, sex, age, blood pressure, and body weight. P values are for the main effect of treatment. CRP, C-reactive protein; IL-6, interleukin-6; TNF- α , tumor necrosis factor-alpha.

^aN=110, least squares mean (95% CI).

^bN=109, geometric means (95% CI).

^cN=98, geometric means (95% CI).

Table 3

Inflammatory marker concentrations at baseline by quartiles of selected RBC fatty acids

	Quartiles of RBC fatty acid content				P value
	Q1	Q2	Q3	Q4	
ALA, 18:3 n-3					
TNF- α , ng/L ¹	1.54 (1.37, 1.70)	1.38 (1.22, 1.55)	1.29 (1.12, 1.45)	1.33 (1.16, 1.49)	0.14
IL-6, ng/L ²	1.01 (0.84, 1.22)	0.99 (0.86, 1.13)	0.83 (0.67, 1.04)	0.94 (0.78, 1.12)	0.74
CRP, mg/L ³	0.43 (0.31, 0.60)	0.52 (0.39, 0.70)	0.60 (0.42, 0.87)	0.73 (0.50, 1.07)	0.15
EPA, 20:5 n-3					
TNF- α , ng/L	1.47 (1.31, 1.63)	1.32 (1.20, 1.44)	1.46 (1.30, 1.62)	1.27 (1.15, 1.39)	0.21
IL-6, ng/L	0.97 (0.79, 1.19)	0.94 (0.79, 1.12)	0.92 (0.77, 1.10)	0.93 (0.77, 1.11)	0.98
CRP, mg/L	0.66 (0.44, 0.99)	0.46 (0.35, 0.61)	0.67 (0.48, 0.93)	0.49 (0.34, 0.69)	0.26
DPA, 22:5 n-3					
TNF- α , ng/L	1.37 (1.20, 1.54)	1.32 (1.16, 1.49)	1.36 (1.20, 1.53)	1.47 (1.30, 1.64)	0.60
IL-6, ng/L	1.03 (0.83, 1.28)	0.85 (0.71, 1.01)	0.95 (0.80, 1.13)	0.94 (0.79, 1.11)	0.50
CRP, mg/L	1.05 (0.73, 1.53) ^a	0.40 (0.29, 0.55) ^b	0.43 (0.33, 0.57) ^b	0.53 (0.41, 0.71) ^b	<0.001
DHA, 22:6 n-3					
TNF- α , ng/L	1.55 (1.39, 1.71) ^a	1.48 (1.33, 1.63) ^a	1.36 (1.21, 1.51) ^{a,b}	1.13 (0.97, 1.29) ^b	0.001
IL-6, ng/L	0.91 (0.75, 1.09)	1.05 (0.89, 1.23)	0.97 (0.80, 1.18)	0.84 (0.69, 1.02)	0.34
CRP, mg/L	0.55 (0.40, 0.75)	0.57 (0.40, 0.81)	0.54 (0.38, 0.75)	0.59 (0.39, 0.89)	0.98
Omega-3 index (EPA+DHA)					
TNF- α , ng/L	1.56 (1.40, 1.72) ^a	1.49 (1.34, 1.65) ^a	1.33 (1.18, 1.49) ^{a,b}	1.13 (0.98, 1.29) ^b	0.001
IL-6, ng/L	0.92 (0.76, 1.10)	0.99 (0.82, 1.19)	1.02 (0.84, 1.22)	0.84 (0.70, 1.01)	0.46
CRP, mg/L	0.52 (0.37, 0.74)	0.68 (0.48, 0.96)	0.55 (0.39, 0.77)	0.51 (0.36, 0.72)	0.62
LA, 18:2 n-6					
TNF- α , ng/L	1.46 (1.29, 1.64)	1.45 (1.32, 1.58)	1.41 (1.27, 1.56)	1.20 (1.09, 1.32)	0.08
IL-6, ng/L	1.03 (0.86, 1.24) ^a	1.01 (0.84, 1.22) ^a	1.02 (0.85, 1.23) ^a	0.73 (0.63, 0.84) ^b	0.014
CRP, mg/L	0.55 (0.38, 0.80)	0.67 (0.47, 0.94)	0.55 (0.39, 0.78)	0.48 (0.35, 0.67)	0.60
AA, 20:4 n-6					
TNF- α , ng/L	1.18 (1.00, 1.33) ^a	1.36 (1.20, 1.52) ^{a,b}	1.39 (1.25, 1.53) ^{a,b}	1.59 (1.41, 1.77) ^b	0.005
IL-6, ng/L	0.79 (0.66, 0.94)	0.97 (0.80, 1.17)	0.91 (0.75, 1.10)	1.11 (0.95, 1.31)	0.06
CRP, mg/L	0.55 (0.39, 0.78)	0.57 (0.40, 0.81)	0.56 (0.40, 0.79)	0.56 (0.38, 0.81)	0.99

Quartiles are specific for the content of each fatty acid in RBC membranes reported as percent of total RBC fatty acids. P values are for trend across quartiles calculated using ANOVA. Values with different superscript letters are significantly different, $p < 0.05$ (Tukey-adjusted values for post hoc tests). AA, arachidonic acid; ALA, alpha-linolenic acid; CRP, C-reactive protein; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; LA, linoleic acid; n-3, omega-3; n-6, omega-6; Q, quartile; RBC, red blood cell.

¹ n=110, least squares means (95% CI).

² n=112, geometric means (95% CI).

³_{n=102}, geometric means (95% CI).