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n-3 Polyunsaturated Fatty Acids Do Not Alter Immune And Inflammation Measures In Endurance Athletes

By: **David C. Nieman**, Dru A. Henson, **Steven R. McAnulty**, Fuxia Jin, and Kendra R. Maxwell

Abstract

The purpose of this study was to test the influence of 2.4 g/d fish oil n-3 polyunsaturated fatty acids (n-3 PUFA) over 6 wk on exercise performance, inflammation, and immune measures in 23 trained cyclists before and after a 3-d period of intense exercise. Participants were randomized to n-3 PUFA (n = 11; 2,000 mg eicosapentaenoic acid [EPA], 400 mg docosahexaenoic acid [DHA]) or placebo (n = 12) groups. They ingested supplements under double-blind methods for 6 wk before and during a 3-d period in which they cycled for 3 hr/d at ~57% Wmax with 10-km time trials inserted during the final 15 min of each 3-hr bout. Blood and saliva samples were collected before and after the 6-wk supplementation period, immediately after the 3-hr exercise bout on the third day, and 14 hr postexercise and analyzed for various immune-function and inflammation parameters. Supplementation with n-3 PUFA resulted in a significant increase in plasma EPA and DHA but had no effect on 10-km time-trial performance; preexercise outcome measures; exercise-induced increases in plasma cytokines, myeloperoxidase, blood total leukocytes, serum C-reactive protein, and creatine kinase; or the decrease in the salivary IgA:protein ratio. In conclusion, 6 wk supplementation with a large daily dose of n-3 PUFAs increased plasma EPA and DHA but had no effect on exercise performance or in countering measures of inflammation and immunity before or after a 3-d period of 9 hr of heavy exertion.

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Keywords: cytokines, leukocytes, salivary IgA, myeloperoxidase

Transient inflammation and immune dysfunction are part of the normal human response to prolonged and intense exercise and are characterized in part by production of inflammatory cytokines, acute-phase proteins, and oxidative stress from reactive oxygen species, as well as alterations in both innate and adaptive immunity (Nieman, 2007; Nieman et al., 2007; Toft et al., 2000).

At sufficiently high intakes, long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs), as found in oily fish and fish oils, decrease the production of inflammatory

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eicosanoids, cytokines, and reactive oxygen species and have immunomodulatory effects (Calder, 2008; Fritsche, 2006; Richard, Kefi, Barbe, Bausero, & Visioli, 2008). As reviewed by Calder (2006a, 2006b, 2007, 2008), long-chain n-3 PUFAs act both directly (e.g., by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism) and indirectly (e.g., by altering the expression of inflammatory genes through effects on transcription-factor activation). Epidemiological studies suggest an inverse relationship between C-reactive protein and a diet rich in marine products (Niu et al., 2006). Experimental studies indicate that n-3 PUFAs exert anti-inflammatory effects in part by decreasing cytokine release from a variety of cells and limiting leukocyte chemotaxis and the expression of adhesion molecules (Calder, 2008; De Caterina, Liao, & Libby, 2000). The eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) membrane composition of immune cells can be altered by long-term ingestion of n-3 PUFAs, thereby influencing phagocytosis, T-cell signaling, and antigen presentation capabilities (Calder, 2007).

Thus, n-3 PUFAs exert anti-inflammatory and immunomodulatory effects and may act as nutritional countermeasures to exercise-induced inflammation and immune dysfunction for athletes. Simopoulos (2007) has reasoned that most athletes need 1–2 g marine n-3 PUFAs per day to counter excessive oxygen-radical formation, inflammation, and trauma from high-intensity exercise and the high n-6 PUFA levels of the Western diet. Few human studies, however, have been conducted to assess the anti-inflammatory and immunomodulatory influence of n-3 PUFAs in an exercise context, and results are mixed. An early study suggested that 3 weeks supplementation with 1.75 g EPA and 1.05 g DHA per day lowered plasma levels of acute-phase proteins before exercise and altered their pattern of change after exercise (Ernst, Saradeth, & Achhammer, 1991). Other studies showed an enhancement in T-cell function and a decrease in production of tumor-necrosis factor alpha with 6 weeks of 1.8 g/day n-3 PUFA supplementation by elite swimmers (Andrade, Ribeiro, Bozza, Costa Rosa, & do Carmo, 2007) and an improvement in postexercise lung function and a decrease in sputum cytokine concentrations with 3 weeks ingestion of 5.2 g/day n-3 PUFAs by asthmatic patients with a history of exercise-induced bronchoconstriction (Mickleborough, Lindley, Ionescu, & Fly, 2006; Mickleborough, Murray, Ionescu, & Lindley, 2003). In contrast, Toft et al. (2000) showed that 6 weeks supplementation with 3.6 g n-3 PUFA (53% EPA, 31% DHA) did not counter increases in tumor-necrosis factor alpha, IL-6, or blood neutrophil counts during the 3 hr after a marathon competition.

Limited data suggest that n-3 PUFA supplementation may improve exercise performance by increasing stroke volume and cardiac output, decreasing systemic vascular resistance, and improving parasympathetic–sympathetic balance (Ninio, Hill, Howe, Buckley, & Saint, 2008; Walser & Stebbins, 2008). One study reported a trend for improvement in exercise time to fatigue (after a 75-min exercise preload) after 4 g/day n-3 PUFA supplementation for 1 month (Huffman, Altena, Mawhinney, & Thomas, 2004), whereas 2.64 g/day n-3 PUFA intake by soccer athletes for 10 weeks had no effect on maximal aerobic and anaerobic power or running performance (Raastad, Høstmark, & Strømme, 1997).

Using a randomized, double blinded, placebo-controlled research design, we tested the influence of 2.4 g/day n-3 PUFA over 6 weeks on exercise performance, inflammation, and immune measures in 23 trained cyclists before and after a 3-day period of intense exercise. This research design allowed us to measure the chronic

effect of n-3 PUFA supplementation on inflammation and immune function after an overnight fast in cyclists during normal training and as a countermeasure to acute exercise-induced changes in these parameters.

Methods

Participants

Twenty-three trained cyclists were recruited and tested as experimental participants through local and college cycling clubs. Written informed consent was obtained from each participant, and the experimental procedures were approved by the institutional review board of Appalachian State University.

Research Design

Within 1 week before the start of supplementation, participants reported to the university's human performance laboratory for orientation and measurement of cardiorespiratory fitness. VO_{2max} was determined using a graded maximal protocol (25-W increase every 2 min, starting at 150 W) with the participants using their own bicycles on CompuTrainer Pro Model 8001 trainers (RacerMate, Seattle, WA). Oxygen uptake and ventilation were measured using the MedGraphics CPX metabolic system (MedGraphics Corp., St. Paul, MN). Heart rate was measured using a chest heart-rate monitor (Polar Electro Inc., Woodbury, NY). Basic demographic and training data were obtained through a questionnaire. Body composition was assessed by hydrostatic weighing using an electronic load-cell system (Exertech, Dresbach, MN). Estimated residual volume was measured using Goldman and Buskirk's (1961) equation, and Siri's (1961) equation.

Participants agreed to avoid using large-dose vitamin/mineral supplements (above 100% of recommended dietary allowances), herbs, and medications known to affect immune function during the course of the study. During orientation, a dietitian instructed the participants to follow a diet moderate in carbohydrate (using a food list) the weekend before and during the 3-day intensified exercise period. We have shown in previous studies that participants average 55–60% of energy from carbohydrates using this food list and that randomized groups do not differ in total energy intake (Nieman et al., 2007). At 12:00–12:30 p.m. before each 3-hr cycling bout, participants ingested a standardized liquid meal (Boost Plus, Mead Johnson Nutritionals, Evansville, IN) with an energy level of 63 kJ/kg (15 kcal/kg). Boost Plus is a nutritionally complete, high-energy oral supplement with an energy density of 6.4 kJ/ml (1.52 kcal/ml) and 16% of energy as protein, 34% as fat, and 50% as carbohydrate. In quantities of 1,000 ml, Boost Plus exceeds daily-value recommendations for all major vitamins and minerals. No other food and beverage (other than water) was consumed from this meal until the end of the cycling bout. Participants ingested 0.5–1 L water per hour of cycling.

The cyclists were randomized to n-3 PUFA or a placebo group. Under double-blind procedures, they ingested four soft-gel capsules per day (two in the morning on an empty stomach at 7:00–8:00 a.m., two at 6:00–8:00 p.m. before the evening meal) for 6 weeks before and during a 3-day period of intensified exercise. The soft-gel capsules were prepared by Cooper Concepts (The Cooper Aerobics Center, Dallas, TX). The n-3 PUFA capsules contained a high-potency fish-oil concentrate from anchovy and sardines, with soybean oil, natural flavors, tocopherols, canola oil, and citric acid, and provided 2,000 mg EPA and 400 mg DHA (in four soft gels). The

placebo capsules were identical in appearance and contained the same ingredients but without the fish-oil concentrate.

Before and after 6 weeks of supplementation, participants provided blood and saliva samples at 8:00 a.m. after an overnight fast, having avoided exercise training for at least 12 hr and before having ingested the morning dose of supplements. They then came to the laboratory for three consecutive days and cycled from 3:00 to 6:00 p.m. at ~57% W_{max}. During the test sessions, experimental participants cycled using their own bicycles on CompuTrainer Pro Model 8001 trainers with the exercise load set at ~57% W_{max}, as in a previous study conducted in our laboratory (Nieman et al., 2007). Metabolic measurements were made every 30 min of cycling using the MedGraphics CPX metabolic system to verify workload. Time trials over a 10-km distance were inserted at the end of each cycling bout (thus three total), with distances and workload monitored using CompuTrainer MultiRider software (version 3.0, RacerMate, Seattle, WA). Blood and saliva samples were obtained immediately after completion of the third 3-hr exercise bout (6:00 p.m.) and then again 14 hr later (8:00 a.m., overnight fasted, and having avoided supplements since 6:00–8:00 p.m. the previous day).

Blood Samples, C-Reactive Protein, Creatine Kinase

Blood samples were drawn from an antecubital vein with participants in a seated position. Routine complete blood counts were performed by our clinical hematology laboratory using a Coulter STKS instrument (Coulter Electronics, Hialeah, FL), and the samples provided hemoglobin and hematocrit for determination of plasma volume change using the method of Dill and Costill (1974). Other blood samples were centrifuged in sodium heparin or EDTA tubes, and plasma was aliquotted and then stored at –80 °C before plasma cytokine analysis. Serum C-reactive protein and creatine kinase were measured in a clinical laboratory using an LX-20 clinical analyzer (Beckman, Brea, CA).

Plasma EPA and DHA

After addition of 500 μg butylated hydroxytoluene and 20 μg of heptadecanoic acid methyl ester (NU-Chek Prep, Inc., Elysian, MN) internal standards to 100 μL thawed plasma, lipids were extracted and methyl esters were formed after the mixture was added to 2 ml of methanolic 5% HCl and incubated at 80 °C for 2 hr in an OLS200 Shaking Waterbath (Grant Instruments Ltd., Shepreth, Cambridgeshire, England; Takemoto et al., 2003). The samples were cooled to room temperature on completion of the incubation, and the methyl esters were extracted twice with 2 ml of n-hexane. The top layer of the supernatant was combined and dried with N-EVAP116 nitrogen evaporator (Organomation Associates, Inc., Berline, MA). The dried extract was then reconstituted in 200 μl of n-hexane supplemented with 0.05% butylated hydroxytoluene, of which 1 μl was injected into an HP 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a 5975B Inert XL MSD mass spectrometer detector. A DB-WAX GC column (30 m × 320 μm × 0.25 μm) from J&W Scientific (Agilent Technologies) was used to separate the methyl esters of the extracted fatty acids. Fatty-acid concentrations were calculated in relation to the heptadecanoic acid methyl ester internal standard peak. The concentration of each phospholipid fatty acid was expressed as a concentration (μg/ml plasma).

Plasma Cytokine and Myeloperoxidase Measurements

Enzyme-linked immunosorbent assays were used, in accordance with the manufacturer protocol, to measure total plasma concentrations of IL-1 receptor antagonist (IL-1RA), interleukin-6 (IL-6 and IL-6 high sensitivity), interleukin-8 (IL-8), and myeloperoxidase (R&D Systems, Minneapolis, MN). All samples and provided standards were analyzed in duplicate. High-sensitivity kits were used to analyze preexercise and recovery IL-6. The minimum detectable concentrations of IL-1ra, IL-6, IL-6 (high sensitivity), IL-8, and myeloperoxidase were <2.2pg/ml, <0.7 pg/ml, <0.039 pg/ml, <3.5 pg/ml, and 0.100 ng/ml, respectively. Preexercise and postexercise samples were analyzed on the same assay plate to decrease interkit assay variability. Data were analyzed with SOFTmax software (Molecular Devices, Sunnyvale, CA).

Saliva was collected and stored, and volume and total protein were measured as previously described (Nieman et al., 2007). Salivary IgA concentrations were measured using a quantitative indirect competitive immunoassay provided by Salimetrics, LLC (State College, PA). All samples and standards were analyzed in duplicate. The minimum detectable concentration for the assay was <2.5 μg/ml. The data are expressed as concentration of sIgA relative to total protein concentration (μg/mg).

Statistical Analysis

Data are expressed as $M \pm SE$. Data in Table 1[ID]TBL1[/ID] were compared between groups using Student's t tests. All other data (Tables 2[ID]TBL2[/ID] and 3[ID]TBL3[/ID], Figures 1[ID]FIG1[/ID] and 2[ID]FIG2[/ID]) were analyzed using a 2 (groups) × 4 (time points) repeated-measures ANOVA. When Box's M suggested that the assumptions necessary for the univariate approach were not tenable, the multivariate approach to repeated-measures ANOVA was used (Pillai's trace). When interaction effects were below or equal to a p value of .05, change data were calculated (presupplementation to one of the three other time points) and compared between groups using Student's t tests, with significance set at $p \leq .05$. This data analysis allowed group comparisons for three different effects: 6 weeks chronic supplementation (pre- and postsupplementation, 8:00 a.m., overnight fasted), acute postexercise changes, and 14 hr postexercise recovery.

Results

Participant characteristics and performance data for the 23 cyclists randomized to placebo (n = 10 men, 2 women) and n-3 PUFA (n = 9 men, 2 women) and completing all phases of the study are summarized in Table 1. Outcome data for male and female cyclists did not differ significantly and are presented together for each group. During the 3-hr cycling bouts, temperature averaged 22.4 ± 0.06 °C, and relative humidity, 35.8% ± 1.6%.

No significant differences were found between groups for age, body composition, or maximal performance measures (Table 1). Participants (all combined) had averaged 211 ± 37.0 km/week of cycling during the previous year and performed 0.96 ± 0.05 hr training per day during the 6-week supplementation period. Thus, the 3-day intensified exercise period (9 hr exercise) represented a 2.1-fold increase in training duration per bout at this time of the year (late winter) and a substantial increase overall in exercise workload and intensity. Participants in all

groups were able to maintain a mean power output of 56.8% ± 0.2% W_{max} at an oxygen consumption of ~65% VO_{2max} during the exercise bouts (Table 1). Mean 10-km time-trial duration (average of three trials conducted during the last portion of each 3-hr bout) did not differ between groups (Table 1). Plasma volume change did not differ between groups postexercise on the third day and averaged less than 2% because of ingestion of 0.5–1.0 L water per hour of exercise (data not shown).

After 6 weeks of ingesting n-3 PUFA supplements at 7:00–8:00 a.m. and 6:00–8:00 p.m. each day, participants had overnight-fasted plasma EPA and DHA levels were 311% and 40%, respectively, above placebo levels, which remained significantly elevated immediately and 14 hr postexercise (Figures 1 and 2). Tables 2 and 3 show that the pattern of change over time for total blood leukocytes, serum C-reactive protein, serum creatine kinase, the ratio of salivary IgA to protein (sIgA:P), plasma myeloperoxidase, plasma IL-1ra, IL-6, and IL-8 did not differ significantly between n-3 PUFA and placebo groups. Significant exercise-induced changes (all participants combined) were measured for total blood leukocytes (81%), C-reactive protein (115%), creatine kinase (52%), sIgA:P (–39%), myeloperoxidase (72%), IL-8 (50%), IL-6 (664%), and IL-1ra (101%).

Discussion

Despite 6 weeks ingestion of 2.4 g/day marine n-3 PUFA supplements by trained cyclists and a significant increase in their plasma EPA and DHA, no group differences in inflammation or immune outcomes relative to placebo were measured before or after a 3-day period of intensified exercise. In addition, n-3 PUFA supplementation had no effect on 10-km time-trial performance.

Most of the outcome measures included in this study increased after the third day of exercise, reflecting the acute but normal inflammatory response of prolonged and intensive exertion. Despite the well-established anti-inflammatory effects of n-3 PUFAs (Calder 2006b, 2008) and the promotion of EPA and DHA supplements for endurance athletes as anti-inflammatory countermeasures to intensive exercise (Simopoulos, 2007), the trained cyclists in this study experienced no measurable anti-inflammatory effects from n-3 PUFA relative to placebo. These findings are in agreement with those of Toft et al. (2000), who showed no effects of 6 weeks supplementation with 3.6 g n-3 PUFA (53% EPA and 31% DHA) on muscle damage, race performance, blood neutrophil counts, or plasma cytokine levels in 10 runners competing in the Copenhagen Marathon, compared with 10 marathoners not using n-3 PUFA supplements. In that study, EPA increased and arachidonic acid decreased in blood mononuclear cells, and the authors reasoned that the 6-week dosing regimen was not only adequate but that larger doses for a longer time period would be unlikely to have any effects on exercise-induced cytokine production. Although Toft et al.'s study did not include a placebo control, use double-blinded methods, or include a presupplementation blood measure, our results using a stronger research design extend and confirm this conclusion.

Other exercise-related n-3 PUFA-supplementation studies are difficult to apply to our research design and do not provide strong evidence of anti-inflammatory effects in trained participants (Andrade et al., 2007; Ernst et al., 1991). Large-dose n-3 PUFA supplementation for 3 weeks appears to be effective in reducing inflammation and exercise-induced bronchospasm symptoms in asthmatic patients (Mickleborough et al., 2006). These findings are in concert with the growing realization that EPA and DHA supplementation is more likely to exert anti-

inflammatory and immunomodulatory effects in diseased patients or in those at high risk for disease than in healthy individuals(Kew et al., 2003; Kew et al., 2004; Sijben & Calder, 2007; Yusof, Miles, & Calder, 2008). Although unstudied, untrained participants may experience greater anti-inflammatory effects from n-3 PUFA supplementation after exercise than trained participants who have developed endogenous anti-inflammatory defenses (Flynn & McFarlin, 2006).

The proposed improvement in exercise performance with n-3 PUFA supplementation was not supported in our study. Walser and Stebbins (2008) reported increased stroke volume, cardiac output, and oxygen delivery and decreased systemic vascular resistance in untrained participants performing 20 min of cycle-ergometer exercise after 6 weeks of 5 g/day EPA and DHA supplementation. Ninio et al. (2008) demonstrated that 12 weeks supplementation with 1.92 g/day EPA and DHA reduced resting and submaximal-exercise heart rates and increased parasympathetic activity in sedentary, overweight adults. Evidence showing that these results extend to improved endurance performance in aerobically trained participants, however, is lacking (Huffman et al., 2004; Raastad et al., 1997; Toft et al., 2000). For example, 2.64 g/day EPA and DHA supplementation over 10 weeks by male soccer athletes did not improve maximal aerobic power, anaerobic power, and running performance relative to a placebo group despite increases in plasma EPA and DHA of 175% and 40%, respectively (Raastad et al.).

In conclusion, 2.4 g/day EPA and DHA supplementation for 6 weeks by trained cyclists did not improve 10-km time-trial performance or alter measures of inflammation and immunity before and after 3 days of prolonged and intensive exercise when compared with placebo. These results do not support the recommendation by Simopoulos (2007) that most endurance athletes ingest 1–2 g/day EPA and DHA to counter the trauma and inflammation induced by high-intensity exercise.

Acknowledgments

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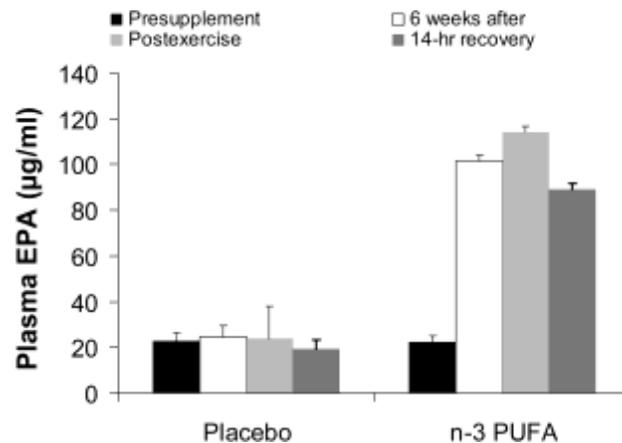
Figure 1 — Plasma eicosapentaenoic acid (EPA) before and after 6 weeks supplementation, immediately postexercise after 3 days cycling (3 hr/day), and 14 hr postexercise. PUFA = polyunsaturated fatty acids. Group × Time interaction $p < .001$. *$p < .05$, change from presupplementation relative to placebo.
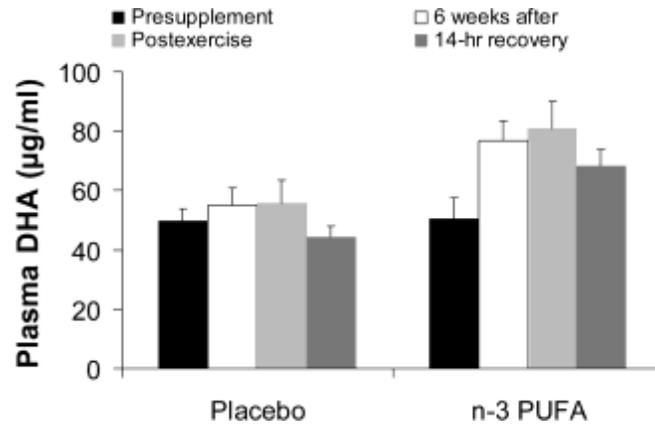
Figure 2 — Plasma docosahexaenoic acid (DHA) before and after 6 weeks supplementation and immediately and 14 hr postexercise. PUFA = polyunsaturated fatty acids. Group × Time interaction $p = .009$. *$p < .05$, change from presupplementation relative to placebo.

Table 1   Participant Characteristics and Performance Measures, $M \pm SE$

Variable	Placebo ($n = 12$)	n-3 PUFA ($n = 11$)	p
Baseline measures			
age (years)	26.9 ± 2.8	24.1 ± 2.4	.460
body mass (kg)	67.4 ± 3.8	68.9 ± 3.0	.761
VO_{2peak} (ml · kg^{-1} · min^{-1})	58.8 ± 3.1	62.9 ± 3.8	.408
power$_{max}$ (W)	251 ± 18.5	284 ± 13.9	.172
HR$_{max}$ (beats/min)	185 ± 2.8	188 ± 3.0	.362
body composition (% fat)	16.4 ± 2.7	13.7 ± 1.8	.431
Performance measures			
mean power (W)	143 ± 10.3	162 ± 8.1	.164
power (%W$_{max}$)	56.8 ± 0.2	56.8 ± 0.2	.822
mean HR (beats/min)	144 ± 3.4	141 ± 2.2	.322
HR (%HR$_{max}$)	78.2 ± 1.1	74.8 ± 1.4	.069
mean VO_2 (ml/min)	$2,589 \pm 180$	$2,725 \pm 170$	.590
VO_2 (%VO_{2max})	66.3 ± 3.0	64.2 ± 3.2	.642
10-km time trials (min)	13.7 ± 2.2	12.7 ± 0.3	.171

Note. PUFA = polyunsaturated fatty acids; VO_2 = volume of oxygen consumption; HR = heart rate. Performance measures represent the mean of 3 exercise sessions.

Table 2   Plasma Measures Before and After 6 Weeks Supplementation and Immediately and 14 hr Postexercise

Variable	Presuppl	Postsuppl	Postexerc	14 hr postexerc	p
Blood leukocytes (10^9/L)					.331
placebo	5.83 ± 0.28	5.85 ± 0.33	9.94 ± 0.70	6.23 ± 0.41	
n-3 PUFA	6.24 ± 0.24	5.90 ± 0.23	11.24 ± 1.13	5.97 ± 0.19	
C-reactive protein (mg/L)					.758
placebo	2.09 ± 0.74	1.44 ± 0.49	3.86 ± 0.91	3.20 ± 0.77	
n-3 PUFA	2.10 ± 0.42	2.11 ± 0.66	3.78 ± 0.94	3.51 ± 0.90	
Creatine kinase (ukat/L)					.196
placebo	143 ± 38.8	141 ± 19.7	248 ± 79.6	177 ± 40.2	
n-3 PUFA	230 ± 60.9	224 ± 51.8	207 ± 30.5	149 ± 21.5	
Salivary IgA:protein (µg/mg)					.436
placebo	80.8 ± 17.7	83.6 ± 18.2	34.7 ± 6.0	116.9 ± 12.0	
n-3 PUFA	56.8 ± 8.3	56.9 ± 11.0	36.7 ± 7.8	87.7 ± 13.0	
Myeloperoxidase (ng/ml)					.631
placebo	109 ± 11.8	103 ± 10.2	156 ± 16.8	78.7 ± 5.9	
n-3 PUFA	121 ± 16.8	96.0 ± 10.7	174 ± 32.2	111 ± 19.0	

Note. PUFA = polyunsaturated fatty acids. *p* represents Group (2) × Time (4) interaction. Significant time effects were shown for all variables listed ($p < .025$[AUQ1]).

Table 3   Plasma Cytokine Levels Before and After 6 Weeks Supplementation and Immediately and 14 hr Postexercise

Variable	Presuppl	Postsuppl	Postexerc	14 hr postexerc	p
IL-1ra (pg/ml)					.710
placebo	275 ± 30.4	225 ± 22.8	467 ± 118	232 ± 22.5	
n-3 PUFA	259 ± 32.2	235 ± 28.6	538 ± 166	298 ± 56.9	
IL-6 (pg/ml)					.699
placebo	1.07 ± 0.11	1.14 ± 0.19	9.13 ± 1.38	1.36 ± 0.24	
n-3 PUFA	1.09 ± 0.21	1.19 ± 0.26	7.01 ± 1.65	1.44 ± 0.28	
IL-8 (pg/ml)					.742
placebo	19.1 ± 4.0	18.0 ± 3.8	23.5 ± 4.2	19.1 ± 4.2	
n-3 PUFA	19.9 ± 3.1	20.5 ± 3.2	22.6 ± 3.9	19.3 ± 2.9	

Note. *p* value represents Group (2) × Time (4) interaction. Significant time effects were shown for all variables listed ($p < .05$).