ORIGINAL RESEARCH

Determinants of Erythrocyte Omega-3 Fatty Acid Content in Response to Fish Oil Supplementation: A Dose–Response Randomized Controlled Trial

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***Background*—**The erythrocyte membrane content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which constitutes the omega-3 index (O3I), predicts cardiovascular disease mortality. The amount of EPA+DHA needed to achieve a target O3I is poorly defined, as are the determinants of the O3I response to a change in EPA+DHA intake. The objective of this study was to develop a predictive model of the O3I response to EPA+DHA supplementation in healthy adults, specifically identifying factors that determine the response.

***Methods and Results*—**A randomized, placebo-controlled, double-blind, parallel-group study was conducted in 115 healthy men and women. One of 5 doses (0, 300, 600, 900, 1800 mg) of EPA+DHA was given daily as placebo or fish oil supplements for ≈5 months. The O3I was measured at baseline and at the end of the study. There were no significant differences in the clinical characteristics between the groups at baseline. The O3I increased in a dose-dependent manner ($P<0.0001$), with the dose of EPA+DHA alone accounting for 68% (quadratic, $P<0.0001$) of the variability in the O3I response. Dose adjusted per unit body weight (g/kg) accounted for 70% (linear, $P<0.0001$). Additional factors that improved prediction of treatment response were baseline O3I, age, sex, and physical activity. Collectively, these explained 78% of the response variability ($P<0.0001$).

***Conclusions*—**Our findings validate the O3I as a biomarker of EPA+DHA consumption and identify additional factors, particularly body weight, that can be used to tailor EPA+DHA recommendations to achieve a target O3I. **(*J Am Heart Assoc*. 2013;2:e000513 doi: 10.1161/JAHA.113.000513)**

Key Words: blood cell • fatty acids • fish oil • metabolism • nutrition

The marine-derived omega-3 (n-3) fatty acids (FAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are recommended for reducing the risk of cardiovascular disease (CVD), especially sudden cardiac death.[1–4] However, a dietary reference intake for EPA+DHA has not been established.[5] Use of biomarker-based approaches has made it possible to study the association of different blood or tissues levels of EPA+DHA on important health benefits or outcomes, such as risk of CVD events. The omega-3 index (O3I), which is the sum of EPA+DHA content in red blood cell (RBC) membranes, is a biomarker of n-3 FA status[6,7] that is highly correlated with myocardial EPA+DHA content.[8,9] An O3I of ≥8% has been recommended as a cardioprotective level[7] on the basis of associations with reduced risk of primary cardiac arrest,[10] sudden cardiac death,[11] coronary atherosclerosis,[12] and acute coronary syndrome.[13,14] In studies of Americans not taking n-3 FA supplements, mean O3I values range from 4% to 5%.[15–18] In 2 larger observational studies of US adults that did not exclude supplement users, O3I values were somewhat higher, averaging 5.3%[19] and 5.6%.[20]

Because of limitations in the current evidence, dietary recommendations for achieving a target O3I cannot be made. Observational studies have confirmed that dietary or supplemental intake of EPA+DHA is associated with higher levels of the O3I,[13,15,21,22] and additional factors such as body weight and health status modify this relationship.[13,15,20–23] However, these studies lack precision and accuracy because of use of food frequency questionnaires and other dietary recall methods.[24]

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Similarly, results from past supplementation studies had limitations that restrict their usefulness for making O3I-based recommendations. Specifically, trials have been too short in duration,[16,25–27] have not examined a range of dietary doses,[16,25,28] had too few participants,[16,28,29] reported high variability in measurement results,[30] and/or reported higher than expected baseline O3I values.[30] Importantly, no prior supplementation studies analyzed the contribution of demographic and clinical factors to the O3I response to supplemental EPA+DHA.

Therefore, the objective of the present study was to model the O3I response to supplemental EPA+DHA intake within attainable dietary ranges and to identify factors that modify this response. We hypothesized that modeling the body-weight-adjusted dose of EPA+DHA as a predictor would yield a more precise estimation of response to treatment than dose alone and that additional factors would also influence the O3I response to supplementation. This information is important for making EPA+DHA recommendations to achieve a target O3I for CVD risk reduction.

Methods

Participants

Healthy, young men and women (20 to 45 years of age) who reported low or no habitual consumption of oily fish (eg, salmon, tuna, and herring; <4 servings per month) and not taking n-3 FA supplements or consuming n-3 FA supplemented foods were recruited. All race and ethnic groups were eligible. Exclusion criteria included serious medical conditions, history of diabetes, or smoking; chronic anti-inflammatory medications; consumption of n-3 FA supplements and n-3 FA-supplemented foods in the past 3 months; pregnant, nursing, or planning a pregnancy; planning to change dietary habits; and body mass index (BMI) <20 or >30 kg/m^2.

Potential subjects were screened initially via telephone interview to determine if they met the following criteria: age, self-determined health status, weight, fish intake, and willingness to participate in the study and adhere to all aspects of the study protocol. Individuals who met the telephone screening criteria were scheduled for additional screening at the Penn State Clinical Research Center. After written informed consent was obtained, study participants were comprehensively evaluated via an examination that included anthropometric measurements, biochemical assessment for traditional CVD risk factors, complete blood count and standard chemistry panel to rule out the presence of serious illness, medical history, and physical examination. The medical history included questions regarding the participants' self-reported exercise and physical activity habits, which were used to assign participants to 1 of 5 physical activity levels (none, little to no exercise; light, 1 to 3 days/week; moderate, 3 to 5 days/week; heavy, 6 to 7 days/week; very heavy, twice per day). The Harris–Benedict equation was used to estimate individual daily calorie requirements based on body weight, height, age, and reported physical activity. The study protocol was approved by the Institutional Review Board at the Pennsylvania State University and registered on ClinicalTrials.gov (NCT01078909). All procedures followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, revised in 2000.

Intervention

This was a randomized, placebo-controlled, double-blind, parallel-group study. Participants (n=125) were randomized to 1 of 5 doses (0, 300, 600, 900, 1800 mg) of EPA+DHA given daily as fish oil supplements (Nordic Naturals) for 5 months, the approximate time it takes for membrane FA composition to reach a new steady state.[29] A computer-generated randomization scheme was developed in advance. The randomization scheme was stratified by sex and age and used a balanced block size of 5 to ensure even distribution among treatment groups. Using this randomization scheme, eligible participants were assigned to blinded treatments at the baseline visit by the study coordinator. Treatments were matched to a coded alpha identifier with a sealed envelope containing the code break for treatment; bottles were labeled A, B, C, D, or E by the manufacturer corresponding to different treatments (ie, A=300 mg, B=1800 mg, C=900 mg, D=0 mg, and E=600 mg). All researchers, clinicians, and participants were blinded to treatment assignment. The head nurse of the Clinical Research Center kept the envelope sealed until completion of the study. Investigators were unblinded after analyses of between-group treatment effects were performed.

The intervention was designed to provide doses of EPA+DHA that could be achievable by consumption of oily fish. For example, a single serving (100 g) of light canned tuna provides ≈270 mg of EPA+DHA, whereas the same serving size of wild Atlantic salmon provides ≈1840 mg of EPA+DHA.[31]

All participants were instructed to consume (with food) 6 identical capsules per day containing either placebo or fish oil that collectively delivered the target dose of EPA+DHA in triglyceride form. Analysis of the fish oil capsules verified that they contained 20% EPA (20:5, n-3), 13% DHA (22:6, n-3), 17% palmitic acid (16:0), 14% oleic acid (18:1, n-9), 8% palmitoleic acid (16:1, n-7), 8% myristic acid (14:0), 4% stearic acid (18:0), 4% eicosadienoic acid (20:2, n-6), and small amounts of other fatty acids. The soybean-oil placebo capsules contained 53% linoleic acid (18:2, n-6), 23% oleic acid, 10% palmitic acid, 6% alpha-linolenic acid (18:3, n-3), 4% stearic acid (18:0), and small amounts of other FAs. The total amount of FAs provided per day by the capsules in each regimen is presented in Table 1.

Table 1. Fatty Acid Composition (mg/daily dose) of the Study Interventions

	EPA+DHA, mg/day				
Fatty Acid	0	300	600	900	1800
Myristic, 14:0	11	79	147	215	420
Palmitic, 16:0	563	629	694	760	957
Stearic, 18:0	238	242	246	251	264
Palmitoleic, 16:1 n-7	8	83	159	235	461
Oleic, 18:1 n-9	1269	1187	1104	1021	773
Linoleic, 18:2 n-6	2918	2454	1991	1527	136
Linoelaidic, 18:2 n-6 *trans*	33	44	55	66	99
Eicosadienoic, 20:2 n-6	3	35	68	100	196
α-Linolenic, 18:3 n-3	351	300	249	198	46
Arachidonic, 20:4 n-6	2	11	20	29	55
EPA, 20:5 n-3	9	191	374	556	1103
Docosapentaenoic, 22:5 n-3	1	20	40	59	118
DHA, 22:6 n-3	6	121	237	352	698
Total EPA+DHA	15	312	610	908	1801

Values were calculated from independent analysis of fatty acid composition (only fatty acids detected in ≥1% of total fatty acids for either activate treatment or placebo capsules are shown). DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

All participants were instructed to maintain their weight and activity level and their usual (limited) consumption of fatty fish as well as their nonconsumption of off-study fish oil capsules during the course of the study. The participants were supplied with log sheets and contacted monthly to ensure compliance and to discuss any difficulties with taking the capsules. Also, participants reported back to the Clinical Research Center after 8 weeks to return log sheets and remaining containers and to receive new supplies.

Blood Sample Collection

Whole-blood samples were collected by vein puncture in the fasting state (12 hours with nothing but water, 48 hours without alcohol, and 2 hours without vigorous exercise) before and after the intervention. A general chemistry profile was obtained as was a complete blood count using fresh blood samples (Chem 24 panel; Quest Diagnostics). Whole blood was centrifuged at 1500*g* for 15 minutes at 4°C. Except for end points that required unfrozen specimens, samples were stored at −80°C until they were analyzed.

Serum parameters

Total cholesterol and triglycerides (TGs) were measured by enzymatic analysis (Quest Diagnostics; coefficient of variation [CV] <2% for both). High-density lipoprotein cholesterol (HDL-C) was estimated according to the modified heparin-manganese procedure (CV <2%). The Friedewald equation[32] was used to calculate low-density lipoprotein cholesterol (LDL-C=total cholesterol−[HDL+TG/5]).

Liver enzymes were measured as part of a general chemistry battery of blood tests (Chem 24 panel; Quest Diagnostics). Serum high-sensitivity C-reactive protein was measured by latex-enhanced immunonephelometry (Quest Diagnostics; assay CV <8%).

RBC fatty acid analysis

Red blood cells were isolated from blood samples drawn into heparin-containing tubes. RBC FA composition was analyzed by gas chromatography with flame ionization detection as previously described.[7] Briefly, unwashed packed RBCs were directly methylated with boron trifluoride and hexane at 100°C for 10 minutes. The FA methyl esters thus generated were analyzed using a GC2010 gas chromatograph (Shimadzu Corporation) equipped with an SP2560 fused-silica capillary column (Supelco, Bellefonte, PA). Fatty acids were identified by comparison with a standard mixture of FAs characteristic of RBCs (GLC 727; NuCheck Prep), which also was used to determine individual FA response factors. FA composition was expressed as a percentage of total identified FAs (CV <3.7%). High and low O3I controls were included in every analytical run.

Statistical Analysis

All statistical analyses were performed using Minitab (version 16.2; Minitab). Differences between treatment groups were

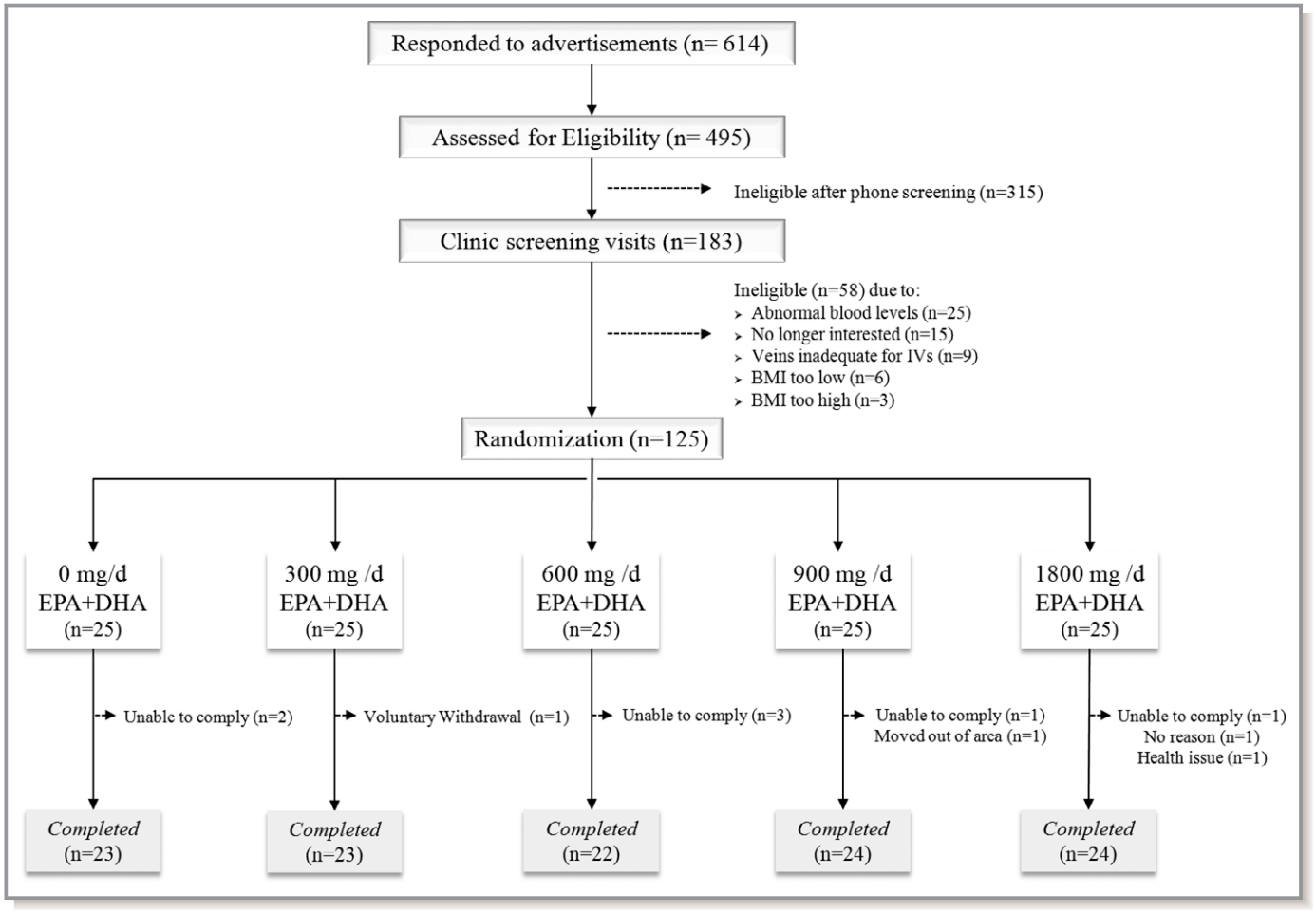

Figure 1. Schematic of subject flow and reasons for exclusion. BMI indicates body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

tested by analysis of variance using a general linear model. Baseline values were included as a covariate. Tukey-adjusted *P* values were used for post hoc comparisons among the 5 groups. Adjusted $P<0.05$ was considered significant. Continuous data are reported as the mean±SEM. For descriptive purposes, categorical data are presented as frequencies and percentages. Fit statistics were assessed for continuous variables to identify any outliers (±3 SD) and for normality. Nonnormally distributed data are reported as median and interquartile range (IQR).

Regression modeling was performed using the Assistant menu-based tool for regression. Univariate regression models were used to determine the effects of each subject characteristic on the O3I. The Best Subsets Regression procedure was used to compare all possible models and identify the best-fitting models. Multivariable regression models were used to determine the effect of dose on O3I in conjunction with other predictors (eg, sex, age, body weight, physical activity, baseline O3I). Interactions terms between treatment and participant characteristics (ie, sex, age, body weight, and physical activity) also were tested to identify interindividual differences in O3I response to treatment. Final models were selected on the basis of optimized fit statistics (smallest Mallow's C_p) and explanatory power (largest adjusted R^2). Graphic representations were generated in Minitab as scatter plots for outcome versus predictor with regression lines. Change scores were calculated as the end-of-treatment value minus baseline value. Residual versus fit plots were examined to ensure homoscedasticity.

Results

The study design and flow of participants are shown in Figure 1. A total of 495 individuals were screened between September 2011 and March 2012; 125 participants who met the inclusion criteria were randomly assigned to a treatment group. All baseline measurements were completed between October 2011 and April 2012. Nine subjects withdrew from the study between baseline and the final point, which left 116 participants who completed the study. The reasons for

Table 2. Baseline Characteristics of the Subjects Who Completed the Study (n=115)

	EPA+DHA, mg/day				
	0	300	600	900	1800
N	23	23	21	24	24
Age, y	25.7±1.4	25.8±1.5	27.1±1.6	25.8±1.3	26.0±1.2
Male, n (%)	11 (48)	12 (55)	11 (48)	13 (54)	13 (54)
Race, n (%)					
White	19 (83)	18 (82)	17 (81)	17 (71)	22 (92)
Black	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Asian	2 (9)	4 (18)	4 (19)	3 (12)	2 (8)
Hispanic	1 (4)	0 (0)	0 (0)	4 (17)	0 (0)
Body mass index, kg/m^2	24.6±0.6	23.4±0.5	24.5±0.6	24.0±0.4	25.4±0.6
Blood pressure, mm Hg					
Systolic	111±3	111±2	114±2	112±2	116±2
Diastolic	75±2	72±2	76±2	74±1	76±1
Lipids and lipoproteins, mg/dL					
Total cholesterol	171.8±5.7	172.9±8.1	163.0±6.0	165.0±6.3	171.0±6.6
LDL-C	100.1±5.4	104.6±6.9	92.6±5.1	93.1±5.3	97.0±5.7
HDL-C	55.5±2.1	49.3±2.6	53.3±2.8	53.9±2.9	55.0±3.2
Triglycerides	81.7±6.6	97.3±5.6	84.2±7.7	89.9±5.7	94.6±7.7
Glucose, mg/dL	90.1±0.8	88.9±1.4	87.9±1.2	86.8±1.0	89.7±1.2
C-reactive protein,* mg/L	0.50 (0.2 to 2.0)	0.70 (0.2 to 1.4)	0.60 (0.2 to 0.7)	0.75 (0.3 to 2.7)	0.80 (0.2 to 1.5)
Erythrocyte n-3 fatty acid content, % by weight					
EPA	0.49±0.04	0.42±0.04	0.40±0.02	0.51±0.06	0.46±0.03
DHA	3.88±0.16	3.87±0.22	3.85±0.18	3.80±0.22	3.82±0.20
Omega-3 index	4.37±0.18	4.29±0.24	4.28±0.19	4.31±0.27	4.28±0.22

Data are mean±SEM unless otherwise noted. There were no significant differences between treatment groups for any of these metrics at baseline (group effect $P<0.05$). DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
*Median; interquartile range in parentheses.

withdrawing were: inability to comply with the intervention (5 subjects), no longer interested (2 subjects), moved (1 subject), and no reason given (1 subject). Returned capsule counts and log sheets were used to assess compliance.

The final study population was young, healthy, normal-weight adults with a low O3I status. Study participants were predominantly white and non-Hispanic. Among study completers, compliance was high (mean, 97%; range, 85% to 100%). One participant with a very high O3I at baseline (ie, 8.3%) was identified as an outlier and excluded from the analysis to ensure the sample was representative of individuals who consumed low n-3 FA, our target study population. Baseline characteristics of the 115 participants are shown in Table 2.

There were no significant differences between groups at baseline with respect to participant characteristics as well as lipids and lipoproteins, liver enzymes, glucose, and high-sensitivity C-reactive protein (Table 2). Erythrocyte FA content

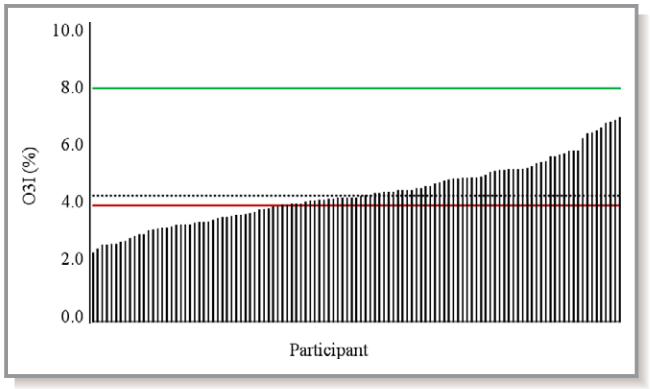

Figure 2. Distribution of the percentage of red blood cell (RBC) EPA+DHA values (omega-3 index [O3I]) in the study population at baseline (n=115). Lines at 8% and 4% indicate proposed low- and high-risk horizons, respectively, and the dotted line at 4.3% is the population average. DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

was similar between groups. The mean O3I at study entry (±SEM) was 4.3±0.1%, with a range of 2.3% to 6.8% (Figure 2). On average, women had a higher O3I than men ($P<0.001$). Body weight, BMI, blood pressure, and heart rate did not change significantly during the study (not shown).

Serum Parameters

Total cholesterol, LDL-C, HDL-C, TGs, glucose, liver enzymes, high-sensitivity C-reactive protein, and additional measures of health status remained unchanged for all treatment groups (Table 3).

Erythrocyte Fatty Acids

EPA+DHA supplementation increased the O3I in a dose-dependent manner (Table 4, Figure 3). The increase in both EPA and DHA resulted in a significant increase in O3I of 121% (from 4.3% to 9.5%) for the 1800 mg/day dose, 75% for the 900 mg/day dose, 59% for the 600 mg/day dose, and 44% for the 300 mg/day dose (all $P<0.0001$ versus placebo). This effect was accompanied by a significant decrease in total n-6 FAs (Table 5). No change in O3I was observed for the placebo group from baseline. Participants taking 300 mg/day achieved a median O3I of 6.1% (IQR, 5.8% to 7.1%); however,

Table 3. Effects of Treatment on General Health Profile (n=115)

	EPA+DHA, mg/day					
Serum Measure	0 (n=23)	300 (n=23)	600 (n=21)	900 (n=24)	1800 (n=24)	*P* Value*
Sodium, mmol/L	139.5.1±0.3	139.6±0.3	139.4±0.3	139.3±3	139.4±0.3	0.95
Potassium, mmol/L	4.0±0.1	4.1±0.1	4.0±0.1	4.1±0.1	4.0±0.1	0.20
Chloride, mmol/L	105.1±0.4	105.0±0.4	105.4±0.4	104.9±0.4	105.3±0.4	0.90
Uric acid, μmol/L	309.6±8.0	300.8±8.0	301.7±8.4	296.2±7.8	299.2±8.0	0.81
Phosphorus, mmol/L	1.1±0.0	1.2±0.0	1.2±0.0	1.2±0.0	1.2±0.0	0.12
Calcium, mmol/L	2.3±0.0	2.3±0.0	2.3±0.0	2.3±0.0	2.3±0.0	0.94
AST, μkat/L	0.3±0.0	0.4±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.72
ALT, μkat/L	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.94
ALP, μkat/L	1.0±0.0[a]	0.9±0.0	0.9±0.0	0.9±0.0	0.9±0.0[b]	0.02
LD, μkat/L	2.5±0.1	2.7±0.1	2.4±0.1	2.6±0.1	2.4±0.1	0.45
Bilirubin (direct), μmol/L	2.8±0.2	2.6±0.2	3.1±0.3	2.7±0.2	2.8±0.2	0.76
Glucose, mmol/L	4.9±0.1	4.9±0.1	4.9±0.1	5.0±0.1	4.9±0.1	0.67
BUN, mmol/L	5.1±0.3	4.9±0.3	5.0±0.4	4.9±0.3	5.5±0.3	0.72
Creatinine, μmol/L	71.2±163.9	73.1±163.9	395.65±174.0	303.3±160.4	79.4±163.7	0.50
BUN/Creatinine ratio	17.0±0.7	14.9±0.8	15.9±0.8	16.3±0.7	16.7±0.7	0.35
Protein (total), g/L	67.6±0.8	67.8±0.8	66.4±0.8	68.1±0.8	67.6±0.8	0.65
Albumin, g/L	44.2±0.6	44.6±0.6	43.3±0.6	44.4±0.6	43.8±0.6	0.52
Globulin, g/L	23.4±0.3	23.2±0.4	23.1±0.4	23.8±0.4	23.7±0.4	0.65
A/G ratio	1.9±0.1	2.0±0.1	1.9±0.1	1.9±0.1	1.9±0.1	0.22
TC, mmol/L	4.3±0.1	4.4±0.1	4.4±0.1	4.3±0.1	4.3±0.1	0.84
LDL-C, mmol/L	2.5±0.1	2.6±0.1	2.6±0.1	2.5±0.1	2.6±0.1	0.74
HDL-C, mmol/L	1.3±0.0	1.4±0.0	1.3±0.1	1.4±0.0	1.3±0.0	0.14
Triglycerides, mmol/L	1.0±0.1	0.9±0.1	1.0±0.1	0.9±0.1	0.9±0.1	0.76
TC/HDL-C ratio	3.4±0.1	3.2±0.1	3.5±0.1	3.2±0.1	3.4±0.1	0.06
hs-CRP,[†] nmol/L	3.8 (1.9 to 12.4)	4.8 (1.9 to 11.4)	4.8 (2.9 to 10.5)	5.7 (3.8 to 14.8)	5.7 (1.9 to 11.4)	0.87
Iron (total), μmol/L	21.8±1.7	21.4±1.7	22.3±1.8	19.3±1.7	17.7±1.7	0.29

Data are least-square mean±SEM (all such values). Values with different superscript letters are significantly different, $P<0.05$ (Tukey-adjusted values from post hoc tests). A/G indicates albumin/globulin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LD, lactate dehydrogenase; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

*P values are for the main effect of treatment. Baseline values included as a covariate. Significance set at $P<0.002$ to account for multiple testing.

[†]Median with interquartile range in parentheses.

Table 4. Effects of Treatment on Omega-3 Index (n=115)

Omega-3 Index	0 (n=23)	300 (n=23)	600 (n=21)	900 (n=24)	1800 (n=24)	*P* Value*
	mg/day					
Baseline	4.38±0.22	4.29±0.22	4.28±0.23	4.31±0.22	4.28±0.22	0.998
Post†	4.35±0.23[a]	6.19±0.23[b]	6.82±0.24[bc]	7.53±0.22[c]	9.49±0.22[d]	<0.0001
Change†	0.04±0.23[a]	1.88±0.23[b]	2.51±0.24[bc]	3.22±0.22[c]	5.19±0.22[d]	<0.0001

Data are least-square mean±SEM. Values with different superscript letters are significantly different, $P<0.05$ (Tukey-adjusted values from post hoc tests).
**P* values are for the main effect of treatment.
†Baseline values included as a covariate.

no participant assigned to a dose ≤600 mg/day achieved an O3I of 8% (Figure 3). Participants taking 900 mg/day achieved a median O3I of 7.6% (IQR, 6.6% to 8.3%), whereas the 1800 mg/day group achieved a median O3I of 9.9% (IQR, 8.9% to 10.5%).

Regression Modeling

Univariate models of O3I response to treatment

Regression modeling was used to assess the relationship between supplemental EPA+DHA intake and O3I. A linear regression model demonstrated that the change in O3I was

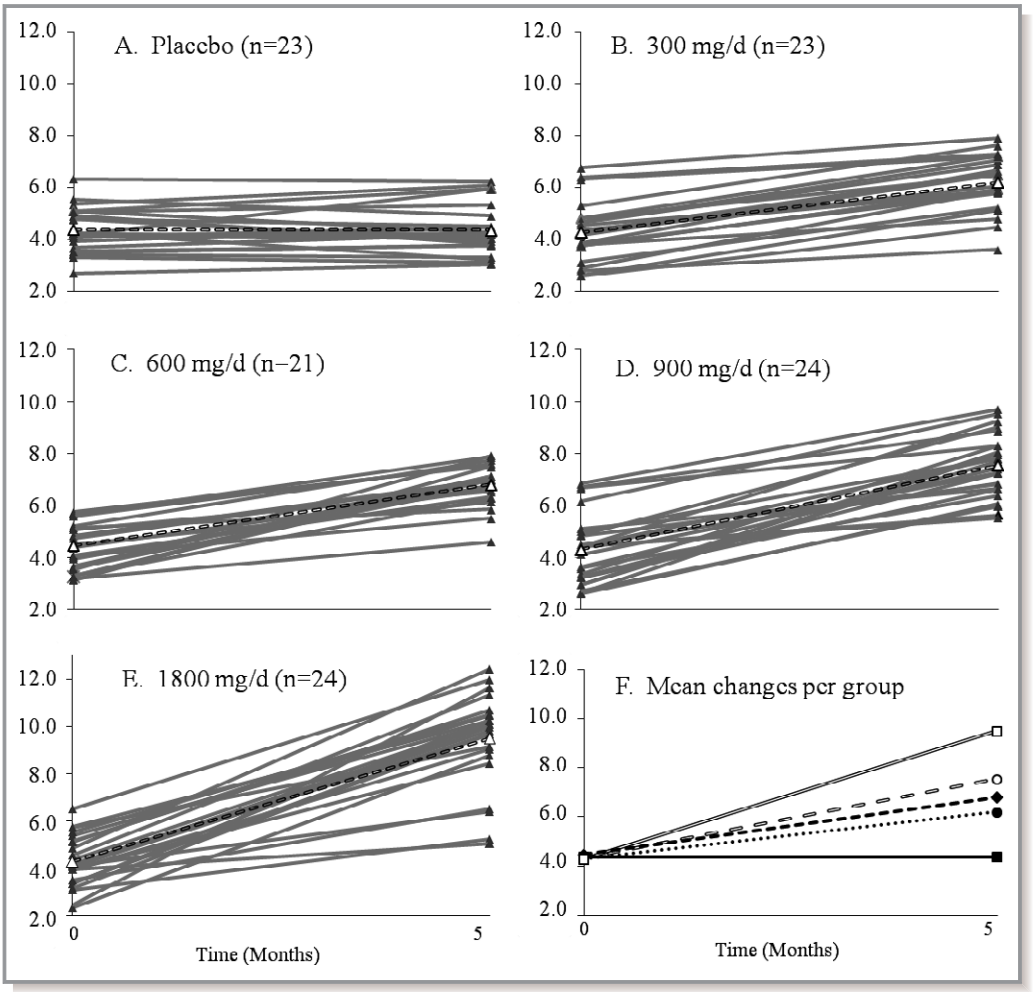

Figure 3. Changes in the omega-3 index (O3I) before and after healthy adults were supplemented for 5 months with either 0 (A; n=23), 300 (B; n=23), 600 (C; n=21), 900 (D; n=24), or 1800 (E; n=24) mg/day of EPA+DHA. A through E, Each participant is denoted by a solid line with a black triangle (▲). The mean change per group is denoted as a dashed line with a white triangle (△). F, Mean changes for each supplement group of 0 (■), 300 (●), 600 (♦), 900 (○), and 1800 (□) mg/day of EPA+DHA. DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

Table 5. Effects of Treatment on Erythrocyte Fatty Acid Profile (n=115)

Fatty Acid	Common Name	EPA+DHA, mg/day					*P* Value*
		0 (n=23)	300 (n=23)	600 (n=21)	900 (n=24)	1800 (n=24)	
Saturated fatty acids							
C14:0	Myristic	0.37±0.02	0.37±0.02	0.37±0.02	0.34±0.02	0.39±0.02	0.53
C16:0	Palmitic	21.82±0.20	21.85±0.20	22.13±0.20	21.97±0.20	22.08±0.20	0.76
C18:0	Stearic	17.19±0.16	17.37±0.16	17.23±0.17	17.36±0.16	17.43±0.16	0.82
C20:0	Arachidic	0.19±0.01	0.17±0.01	0.18±0.01	0.18±0.01	0.17±0.01	0.71
C22:0	Behenic	0.15±0.01	0.16±0.01	0.15±0.01	0.16±0.01	0.16±0.01	0.92
C24:0	Lignoceric	0.32±0.03	0.36±0.03	0.34±0.03	0.38±0.03	0.39±0.04	0.59
Monounsaturated fatty acids							
C16:1n7	Palmitoleic	0.33±0.02	0.28±0.02	0.27±0.02	0.27±0.02	0.25±0.02	0.11
C18:1n9	Oleic	13.52±0.15	13.32±0.15	13.51±0.16	13.21±0.15	13.03±0.15	0.12
C20:1n9	Gadoleic	0.25 ± 0.01^{a}	0.23±0.01	0.23±0.01	0.21 ± 0.01^{b}	0.21 ± 0.01^{b}	<0.001
C24:1n9	Nervonic	0.32±0.03	0.36±0.03	0.34±0.04	0.37±0.03	0.39±0.03	0.65
Trans fatty acids							
C16:1n7t	Palmitelaidic	0.15±0.01	0.15±0.01	0.16±0.01	0.15±0.01	0.16±0.01	0.14
C18:1t	Elaidic	1.02±0.04	0.95±0.04	0.95±0.04	0.96±0.04	0.95±0.04	0.56
C18:2n6t	Linoelaidic	0.33±0.01	0.31±0.01	0.29±0.01	0.31±0.01	0.29±0.01	0.38
n-6 Polyunsaturated fatty acids							
C18:2	Linoleic	13.68 ± 0.28^{a}	13.02±0.28	13.33 ± 0.29^{a}	12.81±0.27	12.05 ± 0.27^{b}	0.001
C18:3	γ-Linolenic	0.16 ± 0.01^{a}	0.14±0.01	0.14±0.01	0.14±0.01	0.12 ± 0.01^{b}	<0.001
C20:2	Eicosadienoic	0.38 ± 0.01^{a}	0.35 ± 0.01^{ab}	0.33 ± 0.01^{bc}	0.33 ± 0.01^{bc}	0.31 ± 0.01^{c}	<0.0001
C20:3	Dihomo-γ-linolenic	2.00 ± 0.05^{a}	1.82 ± 0.05^{ab}	1.73 ± 0.05^{b}	1.72 ± 0.05^{bc}	1.56 ± 0.05^{c}	<0.0001
C20:4	Arachidonic	16.10 ± 0.24^{a}	15.21 ± 0.24^{ab}	14.54 ± 0.25^{b}	14.43 ± 0.24^{bc}	13.58 ± 0.24^{c}	<0.0001
C22:4	Docosatetraenoic	4.10 ± 0.10^{a}	3.45 ± 0.10^{b}	3.05 ± 0.11^{bc}	2.90 ± 0.10^{cd}	2.58 ± 0.10^{d}	<0.0001
C22:5	Docosapentaenoic	0.76 ± 0.03^{a}	0.58 ± 0.03^{b}	0.52 ± 0.03^{bc}	0.50 ± 0.02^{cd}	0.42 ± 0.02^{cd}	<0.0001
n-3 Polyunsaturated fatty acids							
C18:3	α-Linolenic	0.20 ± 0.01^{a}	0.18±0.01	0.17±0.01	0.17±0.01	0.15 ± 0.01^{b}	0.001
C20:5	Eicosapenataenoic	0.47 ± 0.09^{a}	0.91 ± 0.09^{b}	1.23 ± 0.10^{bc}	1.44 ± 0.09^{c}	2.46 ± 0.09^{d}	<0.0001
C22:5	Docosapentatenoic	2.42 ± 0.09^{a}	3.13 ± 0.09^{b}	3.24 ± 0.09^{bc}	3.54 ± 0.08^{c}	3.90 ± 0.08^{d}	<0.0001
C22:6	Docosahexaenoic	3.87 ± 0.16^{a}	5.30 ± 0.16^{b}	5.60 ± 0.17^{bc}	6.06 ± 0.16^{c}	7.03 ± 0.16^{d}	<0.0001

Data are least-square mean±SEM (all such values). Values indicate erythrocyte content by percent weight. Different superscript letters are significantly different, $P<0.05$ (Tukey-adjusted values for post hoc tests). DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

*P values are for the main effect of treatment. Baseline values are included as a covariate. $P<0.002$ considered significant after accounting for multiple comparisons.

largely determined by the dose of EPA+DHA administered (R^2=65.4%, $P<0.0001$; Table 6). However, the Minitab's Assistant tool selected a quadratic fit to model the relationship between treatment dose and change in O3I (R^2=67.7%, $P<0.0001$; Figure 4). Baseline O3I and percent DHA in RBCs also independently predicted the change in O3I (R^2=4.9%, P=0.02; R^2=4.5%, P=0.02, respectively); thus, individuals with a low baseline O3I status experienced a greater percent rise in O3I as a result of the intervention. However, no other measured participant characteristics (ie, race, blood pressure, blood lipid levels, alcohol intake, meal frequency, or compliance) were significant independent predictors of the change in O3I in univariate models.

The dose of EPA+DHA adjusted per unit body weight (g/kg) also was a strong univariate predictor of change in O3I (R^2=69.8%, $P<0.0001$; Figure 5). Increasing the dose of EPA+DHA per unit body weight (grams of EPA+DHA per kilogram body weight) resulted in a greater O3I response; individuals with lower body weight and on higher doses experienced the greatest increase in O3I.

Table 6. Regression Models Predicting Change in Omega-3 Index (n=115)

Predictor	Coefficient±SE	*P* Value	R^2 (Adjusted R^2)
Univariate—linear			
Intercept	0.0065±0.0018	<0.0001	0.654 (0.651)
Treatment dose, g	0.0266±0.0018	<0.0001	
Univariate—Quadratic			
Intercept	0.0024±0.0022	0.274	0.677 (0.671)
Treatment dose, g	0.0435±0.0063	<0.0001	
Treatment dose squared	−0.0090±0.0032	0.006	
Univariate—Body weight adjusted			
Intercept	0.0056±0.0017	0.001	0.698 (0.695)
g/kg	2.0000±0.0124	<0.0001	
Multivariable model 1			
Intercept	0.0255±0.0042	<0.0001	0.754 (0.750)
g/kg	2.0092±0.1122	<0.0001	
Baseline O3I	−0.4653±0.0923	<0.0001	
Multivariable model 2*			
Intercept	0.0437±0.0054	<0.0001	0.779 (0.766)
g/kg	2.0042±0.1089	<0.0001	
Baseline O3I	−0.5796±0.1008	<0.0001	
Age	0.0003±0.0001	0.023	
Sex	−0.0035±0.0020	0.084	
PA	0.0005±0.0011	0.675	
PA×dose, g/kg	0.3236±0.1284	0.013	

O3I indicates omega-3 index; PA, physical activity; SE, standard error.
*Interaction terms centered prior to fitting regression model.

Multivariable models predicting the change in the O3I

Various statistical models with increasing complexity were identified to model changes in the O3I. Adding baseline O3I as a predictor to the body-weight-adjusted model further explained the variability in O3I response (R^2=75.4%, P<0.0001; Table 6). Additional factors, including age, sex, and physical activity, also predicted change in the O3I (R^2=77.9%, P<0.0001; Table 6). Lower O3I status (P<0.0001) and older age (P=0.02) each predicted greater increases in O3I. The level of physical activity interacted with the effect of dose per unit of body weight on the O3I response; increased physical activity level predicted greater increases in O3I when included in the model of dose per body weight (P=0.01). Female sex tended to predict greater increases in O3I, although this was not statistically significant (P=0.08). Including these factors in the model, relative to the univariate model of dose per unit body weight, explained an additional 8.1% of the variability in O3I response.

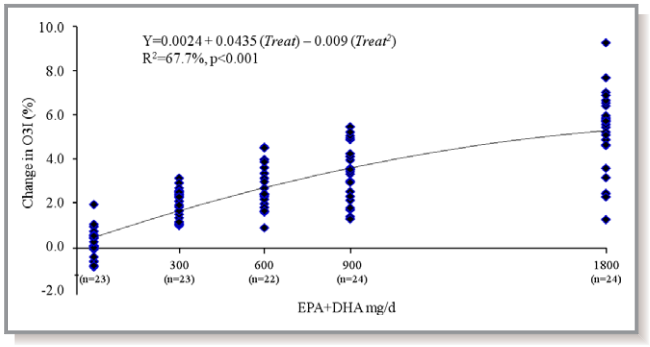

Figure 4. Treatment dose significantly predicted changes in omega-3 index (O3I; n=115). DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

Discussion

The present study modeled the effect of EPA+DHA supplementation and participant characteristics on the O3I

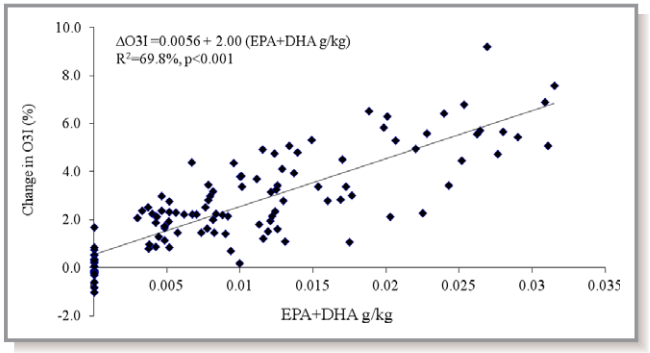

Figure 5. The amount of EPA+DHA in grams consumed per kilogram of body weight significantly predicted changes in omega-3 index (O3I; n = 115). DHA indicates docosahexaenoic acid; EPA, eicosapentaenoic acid.

response. We found that variations in dose explained 68% of the variability in the response; including body weight, baseline O3I, age, physical activity, and sex in the model explained an additional 10% of the variability in response (Table 6).

It is estimated that Americans consume <100 mg/day of EPA+DHA,[33] well below the current recommendations of 250 to 500 mg/day for healthy adults.[1–4] In the present study, participants had an average baseline O3I of 4.3%, which is consistent with previous studies of adults reporting low habitual fish intake.[19,25] Our results suggest that a healthy, normal-weight adult with low fish intake who increased his or her dietary intake by 250 to 500 mg/day of EPA+DHA would experience an increase in O3I values of about 1% to 2% (from 5.3% to 6.3%). Thus, our results demonstrate that increasing consumption of EPA+DHA to current recommended dietary intakes in people who consume very little (if any) oily fish would result in increased O3I levels associated with reduced acute coronary syndrome[13] and CVD mortality.[10,11]

Additional EPA+DHA intake beyond current recommendations is needed to achieve the higher target O3I values associated with the greatest reduction in CVD risk.[10,11,13] From our findings, we estimate that an average healthy adult with a low O3I (ie, 4.3%) would require at least 1 g/day of EPA+DHA for 5 months to achieve an O3I of 8% (Table 6). This dose has been shown to reduce all-cause mortality, cardiac death, and sudden death in post–myocardial infarction patients[34] and approximates the average intake in Japan, where CHD death rates are reduced relative to the rate in the United States.[35] Equations developed using data in our study also could be used to estimate EPA+DHA intake in research studies with greater accuracy and sensitivity than questionnaire-based dietary assessment methods.

The response to supplementation that we observed for the highest dose agrees with prior studies that administered EPA+DHA for up to 12 months.[16,29,30] On average, O3I increased from 4.3% to 9.5% (increase of 5.2% from baseline) with 1.8 g/day of EPA+DHA (60% EPA, 40% DHA) over a period of 5 months. In a recent study by Browning et al,[30] a similar increase (of 5 percentage points) was observed in older healthy adults supplemented with 1.9 g/day of EPA+DHA (46% EPA, 54% DHA) for 12 months. In comparison, Katan et al[29] reported healthy men supplemented with ≈2 g/day of EPA+DHA (85% EPA, 15% DHA) for 12 months increased the O3I by 4 percentage points, suggesting that, in addition to duration and dose, the relative proportions of EPA and DHA may influence O3I response. Katan et al[29] used a supplement that was almost completely EPA, which may explain the lower O3I response, considering turnover of DHA in RBC membranes is slower than that of EPA.[29,36,37] We used EPA+DHA supplements containing the same ratio of EPA to DHA contained in most over-the-counter fish oil supplements.[38] von Schacky et al[12] also used this ratio in a randomized, controlled trial of coronary heart disease patients consuming 3 g/day of EPA+DHA (62% EPA, 38% DHA) for 3 months followed by 1.5 g/day for 21 months. Patients in the fish oil group increased the O3I by 5.5 percentage points (from 3.4% to 8.9%), had less progression and more regression of coronary artery disease as measured by changes on coronary angiography compared with the placebo group.[12] Further research is needed to differentiate the specific effects of EPA and DHA on cardiovascular health before dietary recommendations can be made for EPA and DHA individually (or their ratio).[39]

Body weight explained additional variability in O3I response to EPA+DHA supplementation. Individuals with lower (versus higher) body weight tended to have a greater response to a given EPA+DHA intake. This suggests that EPA+DHA recommendations to achieve a target O3I may be most appropriately made on the basis of body weight, similar to current dietary protein requirements.[40] Using the body-weight-adjusted values (Table 6, Figure 5), it can be estimated that an individual weighing 75 kg would require about 1.2 g/day of EPA+DHA to increase his or her O3I from 4.3% to 8%; however, the requirement would only be 0.9 g/day if the same individual weighed 55 kg, or in contrast, 1.5 g/day for an individual weighing 95 kg, a range representing 3 to 5 typical fish oil capsules per day. Thus, accounting for individual differences in body weight could potentially improve precision for EPA+DHA recommendations.

Individuals with a higher baseline O3I experienced a lower O3I response to treatment. This finding is consistent with previous evidence demonstrating that individuals with higher EPA+DHA levels incorporate additional EPA+DHA at a slower rate than those with lower baseline levels.[16,27] We also found that the incorporation of EPA+DHA into RBC membranes increased in a dose-dependent and potentially saturable manner (Figure 4), suggesting that RBC membrane EPA+DHA concentrations are regulated to some degree and at some point reach a point of saturation.

Our multivariable model, which included body-weight-adjusted dose, baseline O3I, age, sex, and physical activity, accounted for more than three fourths of the variability in O3I response to supplementation (Table 6). Because smoking status has been shown previously to be inversely associated with O3I,[21,22,41] we excluded smokers from our study and therefore were unable to assess the effect of smoking on O3I response.

Age was a predictor of the change in O3I in the multivariable model. Older individuals experienced a greater increase in their O3I as a result of the intervention, although the effect size was small, and individuals in our study were relatively young overall (aged 20 to 45 years). Nonetheless, aging may cause alterations in n-3 FA metabolism. Vandel et al[42] reported that elderly adults (average age, 74 years old) given 1 g/day of EPA+DHA for 3 weeks experienced 42% higher DHA incorporation into plasma lipids than young adults (average age, 24 years old); EPA incorporation was similar for both groups. Reasons for these age-related differences in n-3 FA metabolism are not well understood, although the emerging link between low DHA status and cognitive decline in the elderly has prompted the conduction of clinical trials to examine the role of n-3 FAs in older populations.[43] Epidemiological studies frequently have reported an association between age and O3I[13,15,19,20,22,44–46]; however, more research is needed to understand the mechanisms as well as the relevance of age-related differences in response to EPA+DHA supplementation.

We also found that women on average had a higher O3I than men at study entry, with a strong trend for sex ($P<0.10$) to be a predictor of O3I responses in the multivariable model. The relationship between sex and O3I has been reported previously, although it is inconsistent and not well understood.[13,20–22,45,46] Body weight might be responsible, in part, for the variability in sex, because women tend to weigh less than men; however, this factor was accounted for in the model by adjusting the dose per unit body weight.

We were surprised to find that a physical activity and treatment interaction significantly predicted O3I response to treatment. Participants who were more physically active tended to experience greater increases in O3I as dose increased. This suggests that exercise may enhance incorporation of EPA+DHA in RBC membranes in individuals taking fish oil supplements, although a mechanism to explain this relationship is not immediately obvious. Such a hypothesis could readily be tested in a prospective trial.

The TG-lowering effects of supplemental n-3 FAs also have been well demonstrated, particularly in individuals with elevated TGs,[47] and an inverse relationship between O3I and serum TGs has been previously reported.[13] However, no changes in serum TG, LDL-C, or HDL-C concentrations were observed in the present study. The lack of TG-lowering effect was not unexpected because we enrolled a normotriglyceridemic population and the highest dose was still <2 g/day of EPA+DHA.[48]

Strengths and Limitations

Among the strengths of this study were the placebo-controlled, double-blind study design that compared 5 doses of EPA+DHA, a relatively large sample size, a low dropout rate (7%), an adequate duration of supplementation (≈5 months), and the use of validated analytical methods to determine biomarker response to treatment. Moreover, our statistical approach involved unbiased selection procedures to identify variables for optimized modeling of O3I responses to EPA+DHA supplementation.

Limitations include the predominantly white, young, healthy population studied as well as the lack of background dietary and other demographic/behavioral/physiologic/genetic data that might have allowed us to predict with greater power the changes in the O3I. A recent Framingham cross-sectional analysis found that pedigree (ie, ancestry) explained 24% of the variability in O3I,[20] whereas dietary EPA+DHA intake and fish oil supplement use explained 40% of the variability (dose or duration of supplement use was not considered). We did not examine genetic differences in the present study; our study was not designed to identify genetic predictors of the O3I response to supplementation. Nonetheless, genome-wide association studies in large populations given a fixed dose of EPA+DHA may help to identify genetic loci responsible for additional variability in the O3I response.[20,49]

The lack of information on levels of EPA+DHA in participants' background diets, as well as other nutrients in the habitual diet, could have affected the O3I response to supplemental EPA+DHA via effects on metabolism as well as uptake into cell membranes through effects on enzymatic and nonenzymatic oxidation.[50,51] For example, variations in choline intake can affect the rise in RBC n-3 FA levels with supplementation.[52] Therefore, obtaining dietary data prior to and during the intervention could be useful for future research. Body composition data also would be worthwhile to collect. Adipose tissue serves as a storage site for FAs in the form of TGs; therefore, additional research is needed to determine how the amount and location of body fat (ie, subcutaneous versus visceral adiposity) may affect the O3I response to EPA+DHA intake.

Conclusions

Marine-derived n-3 FA supplementation explained two thirds of the variability in response to RBC EPA+DHA content, and several factors beyond dose (ie, body weight, baseline O3I, age, physical activity, and sex) addeed more precision to the predictive model. These results can be used to estimate an

individual's required supplemental intake for achieving a target O3I to make biomarker-based dietary recommendations for EPA+DHA and, conversely, to estimate dietary intake based on levels of O3I (with or without including additional predictive factors identified herein). However, future studies are needed to assess how EPA and DHA individually or in different ratios affect O3I responses. Additional research also is needed to evaluate whether background diet and nutrients beyond EPA+DHA affect biomarker responses. Finally, research is needed to clarify the association between changes in the O3I and different disease states and health outcomes. In conclusion, our study has provided models that quantitatively demonstrate the relationship between dietary supplemental doses of EPA+DHA and biomarker responses, providing a useful tool for future research studies of marine n-3 FAs that, in aggregate, can inform evidence-based dietary recommendations.

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Disclosures

Financial support had no role in the design and conduct of the study and in the collection, analysis, and interpretation of the data. Dr Harris is a member of the scientific advisory boards of Omthera and Aker Biomarine and has been a consultant to Monsanto, Acasti, GlaxoSmithKline (GSK), and Amarin. He was on the speakers' bureau for Reliant and GSK. He is currently the president of OmegaQuant Analytics, LLC, and is a senior research scientist at Health Diagnostic Laboratory, Inc., 2 companies that offer blood omega-3 FA testing. None of the other authors have conflicts of interest to declare.

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