

Predicting the effects of supplemental EPA and DHA on the omega-3 index

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ABSTRACT

Background: Supplemental long-chain omega-3 (n-3) fatty acids (EPA and DHA) raise erythrocyte EPA + DHA [omega-3 index (O3I)] concentrations, but the magnitude or variability of this effect is unclear.

Objective: The purpose of this study was to model the effects of supplemental EPA + DHA on the O3I.

Methods: Deidentified data from 1422 individuals from 14 published n-3 intervention trials were included. Variables considered included dose, baseline O3I, sex, age, weight, height, chemical form [ethyl ester (EE) compared with triglyceride (TG)], and duration of treatment. The O3I was measured by the same method in all included studies. Variables were selected by stepwise regression using the Bayesian information criterion.

Results: Individuals supplemented with EPA + DHA ($n = 846$) took a mean \pm SD of 1983 ± 1297 mg/d, and the placebo controls ($n = 576$) took none. The mean duration of supplementation was 13.6 ± 6.0 wk. The O3I increased from $4.9\% \pm 1.7\%$ to $8.1\% \pm 2.7\%$ in the supplemented individuals ($P < 0.0001$). The final model included dose, baseline O3I, and chemical formulation type (EE or TG), and these explained 62% of the variance in response ($P < 0.0001$). The model predicted that the final O3I (and 95% CI) for a population like this, with a baseline concentration of 4.9%, given 850 mg/d of EPA + DHA EE would be $\sim 6.5\%$ (95% CI: 6.3%, 6.7%). Gram for gram, TG-based supplements increased the O3I by about 1 percentage point more than EE products.

Conclusions: Of the factors tested, only baseline O3I, dose, and chemical formulation were significant predictors of O3I response to supplementation. The model developed here can be used by

researchers to help estimate the O3I response to a given EPA + DHA dose and chemical form. *Am J Clin Nutr* 2019;110:1034–1040.

Keywords: n-3 fatty acids, omega-3 index, dietary supplements, EPA, DHA, statistical models

Introduction

The literature supporting the benefits of long-chain omega-3 (n-3) fatty acids (LC n-3 FAs; i.e., EPA and DHA) in cardiovascular disease (CVD) has been mixed (1). A 2018 meta-analysis concluded that the current evidence did not support a

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Abbreviations used: BIC, Bayesian information criterion; CHD, coronary heart disease; CVD, cardiovascular disease; EE, ethyl ester; FA, fatty acid; LC n-3 FA, long-chain n-3 fatty acid; O3I, omega-3 index; RCT, randomized controlled trial; TG, triglyceride.

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role for n-3 fatty acids (FAs) in CVD risk reduction (2), but in 3 major randomized trials reported in late 2018, n-3 FA treatment significantly reduced risk for vascular death (3), myocardial infarction (4), and major adverse cardiovascular events (5). The latter study was particularly compelling as it used 4 g EPA (as opposed to the usual 0.84 g EPA + DHA) in statin-treated patients and found a 25% risk reduction in CVD events. Hence, some recent data support a role for n-3 FAs in reducing risk for CVD.

One LC n-3 FA-based marker for which target concentrations have been proposed is the erythrocyte membrane EPA + DHA content [hereafter the omega-3 index (O3I)] (6). The O3I is EPA+DHA expressed as a weight percentage of total FAs. It has been shown to be a stable biomarker of dietary intake and a valid surrogate of tissue LC n-3 FAs (7). However, there is still large interperson variability in tissue incorporation as it relates to intake (8). The O3I was first proposed as a potential risk factor for coronary heart disease (CHD) death in 2004 (6), and subsequent cross-sectional as well as prospective studies have supported its clinical utility for that purpose (9, 10). Analysis of these studies shows that an O3I of 8% or more is protective against fatal CHD (11). The O3I is also inversely correlated with major depressive disorders and inflammatory biomarkers (12, 13).

Currently, there are no guidelines to help researchers determine the LC n-3 FA dose needed to attain a specific O3I or to guide clinicians in recommending LC n-3 FA doses for individual patients. Several factors are known to alter an individual's O3I response to dose, the most important of which, after the dose itself, appear to be body weight and baseline O3I (14, 15). Other potential factors that could affect response to dose are sex (14, 16), age (14, 17), the chemical formulation of EPA and DHA (18, 19), genetic factors (20), smoking (21), dietary fat content of the accompanying meal (22, 23), and possibly even choline intake (24).

In a prior, single-center study, Flock et al. (14) developed equations describing the dose-response relationship between supplemental EPA + DHA and the O3I. In the present study, we used individual-level data from Flock et al. (14) and 13 other intervention studies in which the O3I was measured pre- and postdosing to determine which factors affect the response of the O3I to supplementation with LC n-3 FAs. Using basic demographic data along with baseline O3I values, we set out to build a regression model to predict O3I responses to dose.

Methods

Sample

Data from 1422 individuals from 14 published intervention studies (14, 16, 25–36) in which EPA + DHA dietary supplements or prescription products were given were used to predict changes in the O3I (Table 1). All investigators provided data on EPA and DHA dose, duration of supplementation, pre- and post-O3I (both determined using the same laboratory method) values from both placebo-treated and LC n-3 FA-treated subjects, and standard demographic factors. Individual study sample sizes ranged from 10 to 453, and a variety of participant types was included. All studies were approved by local human subjects

research committees, and details can be found in each cited publication.

Statistical analysis

We used paired *t* tests to compare the effects of treatment on the O3I from baseline to end of treatment. Mixed-effects models were used to predict changes in concentrations adjusting for age, sex, race, BMI, and weight, and a random-effects term for each study was fit separately for individuals taking supplements compared with placebo. A significance level of 0.05 was used for these analyses. All statistical analyses were performed in R, version 3.5 (www.r-project.org).

To build a predictive model, we used the stepwise Bayesian information criterion (BIC) to identify the best-fitting and most parsimonious model (38). We considered 8 predictor variables for absolute change in the O3I: EPA + DHA dose (in mg/d, including both linear and quadratic terms), baseline O3I (including both linear and quadratic terms), sex, age, BMI (including both linear and quadratic terms), race (Asian, black, Hispanic, other, white), duration of treatment (weeks, including both linear and quadratic terms), and type of chemical formulation [ethyl ester (EE) or triglyceride (TG)]. We also considered 3 potential statistical interaction terms: baseline O3I and dose, dose and duration, and formulation type and dose. Other factors that are known to affect the O3I, such as whether the product was taken with a meal or not (23), were not collected in these studies.

We performed the stepwise BIC approach in conjunction with a 10-fold cross-validation. Specifically, we randomly broke the entire data set into 10 equally sized, mutually exclusive subsets, each containing ~90% of the data ($n \approx 1280$; the discovery data sets). Each discovery data set then corresponded directly to a validation data set (i.e., the remaining 10% of the data, $n \approx 142$ each). The stepwise BIC model-fitting approach was performed on each of the 10 discovery data sets and validated on the corresponding validation data set. The consensus final model predictor variables and associated β estimates were obtained by averaging across the estimates from the 10 validation data sets. R^2 was used to evaluate overall model predictive ability. Furthermore, the unique contribution of each term in the model to the overall R^2 was evaluated by looking at change in R^2 resulting from adding the term to the model.

Results

Subject characteristics and overall change in the O3I

The average age, body weight, and BMI of the participants was 56 years, 83 kg, and 28 kg/m², respectively (Table 1). The cohort was 74% male and 91% white. Treatment groups took a mean of 1983 mg/d supplemental EPA + DHA (range: 254–4400 mg/d), and placebo groups took 0 mg/d for a mean of 13.6 ± 6.0 wk (range: 4–24 wk). Compliance was reported in 7 of the 14 studies and averaged 95%. The O3I increased from 4.9% ± 1.7% to 8.1% ± 2.7% in the supplemented individuals ($n = 846$; $P < 0.0001$; a 65% increase) and remained unchanged in the placebo groups (from 5.0% ± 1.7% to 5.1% ± 1.7%; $n = 576$; $P = 0.20$).

TABLE 1 Summary of 14 studies using EPA + DHA supplements¹

Study (first author)	Study design ²	Subject characteristics	Formulation type	n-3 FA doses, ³ mg	Duration, wk	n-3 Treated, n	Sex, % male	Age, y	Weight, kg	BMI, kg/m ²	Mean	
											Baseline O3I, %	Final O3I, %
Carney (26)	Parallel	Coronary heart disease patients	EE	0, 1680	10	56	68	58.69	99.86	33.26	4.6	7.5
Dewell (35)	Parallel	Metabolic syndrome	TG	0, 1200 0, 3600	8	31	67	50.83	86.86	29.53	5.0	10.3
Flock (14)	Parallel	Healthy adults	TG	0, 300 0, 600	20	93	52	26.09	71.76	24.52	4.3	6.3
Gidding (33)	Crossover	Hypertriglyceridemic adolescents	EE	0, 1800 0, 3360	8	39	69	13.77	88.13	31.20	4.5	7.0
Gronon (28)	Parallel	Peripheral artery disease patients	TG	0, 4400	4	35	97	68.54	84.31	27.85	4.3	7.6
Harris (32)	Pre-post	Healthy premenopausal women	TG	485	16	12	0	42.83	68.21	25.47	4.3	9.5
Harris (34)	Parallel	Heart failure patients	EE	0, 850	12	225	77	67.19	75.33	26.81	4.2	8.1
Hedengran (31)	Parallel	Hypertriglyceridemia	EE/TG	0, 2285 (TG), 0, 3306 (EE)	8	71	79	61.97	88.21	28.60	4.9	9.0
Heydari (30)	Parallel	Myocardial infarction patients	EE	0, 3360	24	129	81	59.19	86.97	29.06	4.7	6.7
Larson (27)	Pre-post	Healthy adults	EE	3400	4	10	40	36.30	75.61	24.74	6.7	11.1
Newman (29)	Pre-post	Hypertriglyceridemia	EE	3400	8	15	80	56.73	90.45	30.72	6.6	10.8
Sarter (16)	Pre-post	Healthy vegan subjects	TG	254	16	47	47	50.94	67.97	22.81	5.7	9.4
Shearer (25)	Parallel	Metabolic syndrome	EE	0, 3400	16	30	60	47.60	97.99	32.19	5.4	11.4
Skulas-Ray (36, 37)	Crossover	Hypertriglyceridemia	EE	0, 850 0, 3400	8	53 ⁵	89	44.57	93.70	29.24	4.4	6.4
Overall total or mean ± SD				1983 ± 1297	13.6 ± 6.0	846	74	56.3 ± 17.1	83.0 ± 18.6	28.2 ± 5.3	4.9 ± 1.7	8.1 ± 2.7

¹EE, ethyl ester; FA, fatty acid; O3I, omega-3 index; TG, triglyceride.

²Parallel: 2 groups given either long-chain (LC) n-3 FA supplements or placebo; crossover: 1 group given n-3 and placebo during sequential period; pre-post: no control group, LC n-3 FA treatment group.

³All doses refer to EPA + DHA combined unless otherwise noted, and the total dose is only for those taking EPA/DHA. "0" refers to the placebo group. When multiple doses are investigated, values for baseline and final O3I are provided separately for each dose group, while other study characteristics are provided in aggregate.

⁴Baseline O3I includes data from placebo and n-3 FA-treated subjects, whereas Final O3I is just for the n-3 FA-treated groups.

⁵Treated n for this study is larger than the reported n because some subjects were counted up to 3 times, once for each time they underwent a treatment.

TABLE 2 Statistical model summary¹

Characteristic	Value
Average R^2 (SD): discovery samples (10 samples of $n = 1280$ each)	0.59 (0.006)
Average R^2 (SD): validation samples (10 samples of $n = 142$ each)	0.62 (0.05)
Variables included and significant in validation sample (unique R^2) ²	<ul style="list-style-type: none"> • Baseline O3I (quadratic) (0.04) • Dose (mg/d) (quadratic) (0.16)³ • Type (0.01)
Model equation from validation samples ⁴	$\text{Change in O3I} = 2.60 + 0.921 * \text{TG} - 0.842 * \text{Baseline O3I}$ $+ 0.050 * \text{Baseline O3I}^2 + 0.0027 \text{ Dose}$ $- (4.1 \times 10^{-7}) \text{ Dose}^2$

¹O3I, omega-3 index; TG, triglyceride.

²Unique R^2 is calculated as the change in model R^2 obtained when dropping the term but keeping all other terms in the model.

³There is high covariation between dose and type because the type variable has 3 groups [placebo, ethyl ester (EE), and TG], and so Dose = 0 is the same as the placebo group. Baseline O3I values are entered as full integer, not as fraction or percentage. Dose is entered as mg/d. For type, enter "1" for TG forms and "0" for EE forms. O3I = RBC EPA + DHA as a percentage of total fatty acids.

⁴The SDs of the 6 model coefficients across the 10 validation samples are 0.40, 0.17, 0.012, 0.12, 0.0004, and 9×10^{-8} , respectively.

Modeling the O3I response to treatment

After development and validation following the 10-fold cross-validation approach, the model included baseline O3I and EPA + DHA dose, including quadratic terms for both variables, and the chemical form (type) of EPA + DHA. This model explained 62% of the variance. By comparing the R^2 values with and without each variable, we found that dose was uniquely responsible for 80% of the explanatory ability (R^2) followed by baseline O3I (15%) and type of supplement (5%). Explained variation was similar for discovery samples and validation samples. Model coefficients, terms, and R^2 (overall and unique contribution) values are in **Table 2**, with additional details on the model-building and cross-validation process in **Supplemental Tables 1 and 2**.

The predicted change in the O3I (adjusted using the model) was significantly higher for TG compared with EE forms (mean, +0.89%; 95% CI: 0.63%, 1.15%). The equation was rearranged to estimate the dose (separately for EE and TG forms) necessary to attain an O3I of 8% as a function of the baseline O3I (**Figure 1**; see **Supplemental Materials**, Results). To confirm that this model would also perform well for predicting the final O3I, we compared the predicted with the observed final O3I values (**Figure 2**). Observed values correlated with predicted values, with an R^2 of 0.69. To evaluate model robustness, we considered 1) weight (kg) instead of BMI, 2) dose/kg body weight instead of dose, and 3) the ratio of EPA to DHA in the supplement. In all cases, there was little effect on the final model (details not shown).

Discussion

In this report, we developed a model to predict the effects of LC n-3 FA supplementation on the O3I. The model had a relatively high R^2 value in both discovery and validation samples, as well as between observed and predicted O3I values. In order of importance, the major determinants of the O3I response were dose, the baseline O3I, and type of supplement. The response was not linear; therefore, some polynomial terms were needed to best predict the response.

In addition to dose and the baseline O3I, which have been previously reported to influence the O3I response to EPA + DHA

treatment (14, 15), supplement type also significantly affected the response. TG formulations produced an ~1 percentage point greater increase in the O3I for the same EPA + DHA dose than an EE formulation. Some (18, 19), but not all (31, 39, 40) studies have reported increased bioavailability of LC n-3 FA in the TG compared with EE form. There also appears to be a "food effect" for EPA + DHA (i.e., poorer absorption in the absence of a fatty meal), which is considerably more pronounced for the EE form than the TG (22, 23). Hence, a lower response to EE compared with TG forms on the O3I might be partly explained by this factor. Current evidence does not support a major effect of FA desaturase gene variants on the

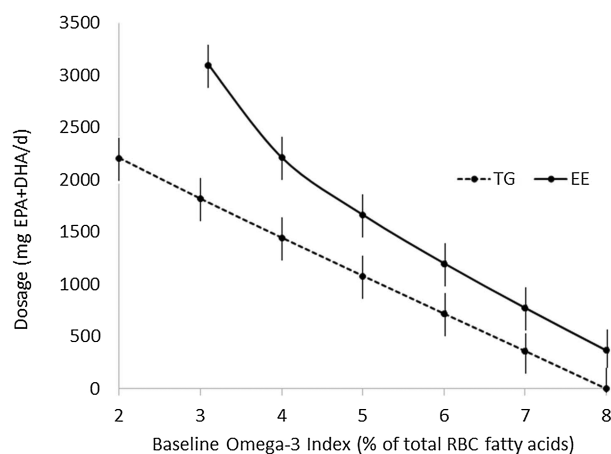


FIGURE 1 LC n-3 fatty acid doses needed to attain an RBC EPA + DHA concentration (omega-3 index, or O3I in the equations) of 8% in ~13 wk, as a function of the baseline O3I. The model equations as estimated using the 10-fold cross-validation approach described in the article with an n of 1422 were rearranged to solve for the necessary dosage to attain a postsupplementation O3I of 8%. The results are shown with separate lines for ethyl ester (EE) and triglyceride (TG) supplements. The error bars reflect the 95% CIs. The equation for TG is: $Dose = 3292.68293 -$

$$\frac{\sqrt{7.29 \times 10^{-6} + 1.64 \times 10^{-6}(-4.479 + 0.158 \text{Baseline O3I} + 0.05 \text{Baseline O3I}^2)}}{8.2 \times 10^{-7}}$$

The equation for EE is: $Dose = 3292.68293 -$

$$\frac{\sqrt{7.29 \times 10^{-6} + 1.64 \times 10^{-6}(-5.4 + 0.158 \text{Baseline O3I} + 0.05 \text{Baseline O3I}^2)}}{8.2 \times 10^{-7}}$$

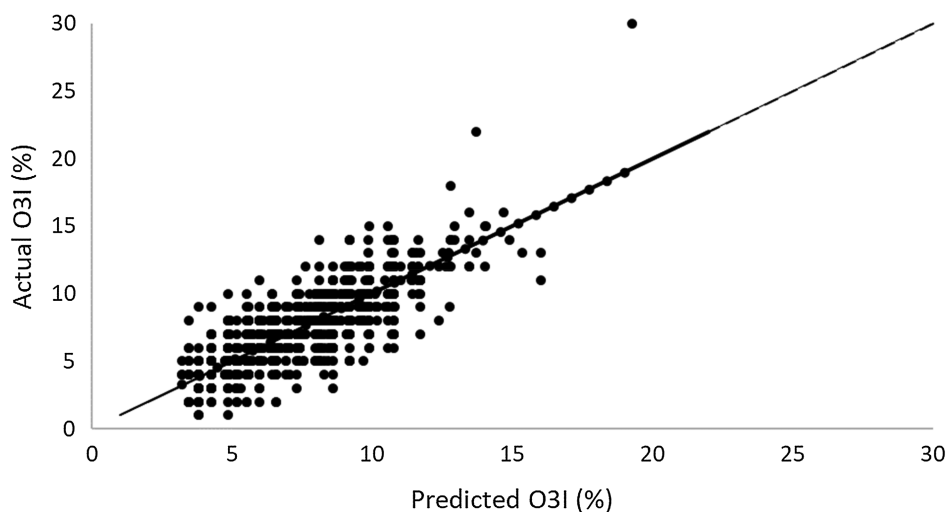


FIGURE 2 Predicted compared with observed RBC EPA + DHA concentration [omega-3 index (O3I)]. We used the model to calculate the predicted final O3I, which was then compared with the observed O3I values (see equation at end of paragraph). When using the model for the entire data set ($n = 1422$), observed values correlated with predicted values, with an R^2 of 0.69. Two lines are shown: the line $y = x$ (thin dashed line) illustrates the line of “perfect prediction,” and the heavy solid line represents the best-fit line of the predicted compared with observed O3I values (Actual O3I = $0.063 + 0.993 \times$ Predicted O3I). In the equation for the best-fit line, the intercept (0.063; SE = 0.13) is not significantly different from 0 (t test $P = 0.62$), and the slope (0.993; SE = 0.04) is not significantly different from 1 (t test $P = 0.69$). The predicted O3I = baseline O3I + the predicted change in O3I, which is: $2.60 + 0.921 \times 1$ [if triglyceride supplements were used; otherwise, $\times 0$] $- 0.842 \times$ baseline O3I [entered as an integer, not a fraction or percentage] $+ 0.05 \times$ (baseline O3I) $^2 + 0.0027 \times$ dose [mg EPA + DHA/d] $- 0.000000471 \times$ dose 2 .

RBC FA response to n-3 FA supplementation (41). Surprisingly, duration of supplementation did not enter the final model. This probably reflects the fact that a majority of the changes in the O3I occurred within 13–14 wk, which was the average duration in this set of studies. This does not mean, however, that a new steady state was established, since that takes somewhat longer to achieve (42, 43).

Potential research uses of the model

The model equation developed herein can be used to estimate the final O3I (and 95% CI) of a population given the LC n-3 FA dose and baseline O3I. As an example, a population with a baseline O3I of 4.9% (like this pooled cohort) that is given 840 mg EPA + DHA/d (e.g., 1 n-3 acid ethyl ester capsule) would achieve a mean O3I of $\sim 6.5\%$ (95% CI: 6.3%, 6.7%). Rearranging the equation, one can calculate the approximate EPA + DHA doses (TG forms) to achieve a mean O3I of 8% in 13 wk: 2200 mg/d for a baseline O3I of 2%, 1500 mg for a 4% baseline, and 750 mg for a 6% baseline (Figure 1). Using this example, we predict that the *minimum* dose (i.e., the lower bound of a 95% CI for the mean required dose) of EPA + DHA necessary to be 95% certain that the mean baseline O3I of 4% will increase to 8% (in 13 wk) is ~ 1750 g/d of TG or 2500 g/d of EE. The daily dose needed over a lifetime to achieve an O3I of 8% is likely much lower. For example, the average EPA + DHA intake in Japan is “only” 800–1000 mg/d, and the mean O3I in the Japanese is $>8\%$ (44).

A dose of about 2000 mg/d of EPA + DHA is much higher than current recommended intakes to reduce CVD risk. The 2015–2020 Dietary Guidelines for Americans recommend 8 oz of fish per week, which is said to be equivalent to about 250 mg/d EPA + DHA (45), and the American Heart Association

recommends 1–2 servings of “oily” fish per week (assumed to approximate 500 mg/d EPA + DHA) to reduce CVD risk (46, 47). Using the model and assuming the TG form, these EPA + DHA intakes would increase the O3I from 4% to $\sim 6\%$. According to National Health and Nutrition Examination Survey 2003–2008 data, 90% of Americans do not meet the 500-mg/d intake recommended by the Academy of Nutrition and Dietetics (48), and 80% do not meet the 250-mg/d goal (46).

Several large randomized controlled trials (RCTs) of n-3 FAs and CVD completed over the past decade have reported no effects, and these have been summarized multiple times in meta-analyses (2, 49). Only one of those trials directly measured the O3I and only in a subset of subjects. In that study, the average O3I increased from 4.8% to 6.7% after 3 mo. Only 23% of the treated patients reached an O3I $>8\%$ (34). The model developed herein predicted a final value of 6.5% for that study population.

In 2018, the highest-dose RCT of LC n-3 FAs with clinical endpoints was reported. Bhatt et al. (50) used 4000 mg/d icosapent ethyl (EPA EE) in a cohort of >8000 statin-treated patients with hypertriglyceridemia. Major CVD events were reduced by 25%. Effects on the O3I were not reported in this study, and the equation derived here cannot be used to estimate the effects on the O3I because only EPA was provided. Pure EPA, while raising RBC EPA, also slightly lowers RBC DHA concentrations (51), so the net effect on the O3I is difficult to predict.

Strengths and limitations

A strength of the current study is that it included many diverse data sets, which increases the generalizability of the model since the studies included a variety of ages, dosages, chemical formulations, and study durations in both healthy and diseased

populations in 3 Western countries (United States, Italy, and Norway). Interstudy variability was minimized by use of the same laboratory method for the O3I. However, this analysis has several limitations and potential confounders, as well. For example, only one study directly (31) compared EE with TG LC n-3 FA forms, so the magnitude of the differences in the O3I response to these two forms should be considered tentative. Most studies used only one dosage compared with placebo, with only four studies (14, 31, 35, 36) investigating multiple doses. In addition, most of the cohorts were not racially diverse, with over 90% of subjects being white, likely explaining why race did not enter the final models. It is unknown how racial differences may affect the O3I response to dose. Similarly, because 74% of this aggregated cohort was male, the results may apply more robustly to men than women. Also, due to limitations in the range of baseline O3Is in these studies, the model works best for baseline values between ~2% and 6%. Hence, the predicted values are not as accurate for individuals who are already close to the target O3I of 8%. This exercise also does not address the question of how much EPA + DHA should be taken to maintain an O3I of 8% once it is achieved. As noted earlier, the mean duration of treatment was only 13.6 wk, and steady-state levels were probably not achieved. The ratio of EPA to DHA in a given supplement could affect RBC membrane incorporation of the sum (52), but the narrow range of EPA/DHA ratios of the supplements used in these studies caused this variable to not enter the final model. Finally, no studies using krill oil (LC n-3 FA present mostly in phospholipid forms) were available. A myriad of other possible factors also could influence absorption and membrane incorporation of LC n-3 FAs that were not considered in this analysis, including fasted or fed state, the amount of fat taken with the supplement, possible drug interactions, physical activity, other background dietary factors (e.g., alcohol intake), physiological state (e.g., pregnancy, lactation), genetic differences, and dyslipidemia.

Conclusions

In order for 95% of subjects (not just 50%) to achieve a desirable O3I from a baseline of ~4%, roughly 2000 g/d EPA + DHA (depending on the chemical form) would likely be required. As noted, the equation developed here can aid in predicting population O3I changes, but because of the large interindividual variability in the O3I response to EPA + DHA supplementation, it will likely be less useful in the clinical setting where direct testing of the O3I would be the preferred approach to assessing EPA + DHA status.

The authors' responsibilities were as follows—REW and KHJ: cowrote the first draft; REW, GCS, AB, and NLT: performed statistical modeling; WSH and NLT: extensively edited the manuscript; all other authors (who were the original authors of the studies included in the analysis) provided input on the final version; and all authors: approved the final manuscript. WSH is the founder and president of OmegaQuant Analytics, LLC, a commercial laboratory providing fatty acid analytical services for researchers, health care providers, and consumers. KHJ is an employee of and NLT is a biostatistical consultant for OmegaQuant. GCS has received honoraria from Amarin Corp. PMKE and WSH are members of the Scientific & Nutrition Advisory Council of the Seafood Nutrition Partnership. AB is an employee of the Global Organization for EPA and DHA Omega-3 s (GOED), a 501(c)6 not-for-profit trade association. None of the other authors have any conflicts to declare.

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