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Cross-sectional study of the combined associations of dietary and supplemental eicosapentaenoic acid + docosahexaenoic acid on Omega-3 Index

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ABSTRACT

Studies have linked an Omega-3 Index (O3I), which measures eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) in red blood cell membranes, of ≥8% with improved health. Previous studies found that the American Heart Association (AHA) recommendation of 1-2 seafood meals per week does not achieve an O3I ≥8% even with an EPA + DHA supplement; however, these studies did not assess the frequency or amount of supplemental intake. Among participants in a predominantly US and Canadian cohort with high nutrient supplement use, we hypothesized that those adhering to the AHA guidelines would not have an average O3I ≥8% but that those taking a daily supplement would. Fish consumption and EPA + DHA supplement use were reported by 1795 participants; 985 also completed a blood spot test for O3I. A majority (71%) consumed <2 servings per week of fatty fish, and 61% took an EPA + DHA supplement. The amount of EPA + DHA for 1 serving (based on the product label) significantly differed among the >400 supplement products (50-3570 mg). O3I was ≥8.0% in 19% of participants. Among non–supplement takers, 3% of those consuming 1 fish serving per week and 17% consuming ≥2 achieved an O3I ≥8.0%. Among those consuming ≥2 fish servings per week, only those also taking an average of 1100 mg/d of supplemental EPA + DHA had a median O3I ≥8.0%. Based on the relationship between supplemental EPA + DHA intake and O3I for non–fish eaters (R^2 = 0.40, P < .0001), an average of ~1300 mg/d of EPA + DHA achieved an O3I of 8.0%. This study suggests that following the AHA guidelines does not produce an O3I ≥8% nor does taking 1 serving per day of most omega-3 supplements.

Abbreviations: AHA, American Heart Association; ALA, α-linolenic acid; CI, confidence interval; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IQR, interquartile range; HHS, Health and Human Services; O3I, Omega-3 Index; USDA, United States Department of Agriculture.

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1. Introduction

Dietary intake of long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been linked to numerous cardiovascular health benefits such as reducing triglycerides [1-4], lowering blood pressure [5,6], raising high-density lipoprotein cholesterol [4], and reducing inflammation [7-9]. Although cause and effect relationships remain to be established, epidemiologic studies have shown a higher consumption of EPA and DHA (or higher blood levels) to be associated with a reduced risk of anxiety and depression [9-11], cancer [12,13], asthma [14], Alzheimer disease [15], type 1 diabetes [16], multiple sclerosis [17], cardiovascular disease [18-22], and total mortality [22-24]. Children of women who consume EPA and DHA during pregnancy and nursing have better neurological development and other health outcomes [25,26]. Evidence also suggests that intake of EPA and DHA can help treat symptoms of dry eye disease [27], lupus [28], and rheumatoid arthritis [29].

The main food source of EPA and DHA is fatty fish such as salmon, tuna, herring, and mackerel. Although α-linolenic acid (ALA), another omega-3 fatty acid found in plant oils such as flaxseed and soybean, can be converted to EPA and DHA, the average conversion rate is very low, making direct consumption of EPA and DHA the only way to significantly increase total body levels [30]. Additionally, there are hundreds of dietary supplements available to the public that contain EPA and DHA. Formulations for these supplements vary widely in the amount of EPA and DHA as well as in the type of oil and form of omega-3 fatty acid (eg, ethyl ester, triglyceride). Consumer behaviors for supplement users, including types of products used and patterns of use, are relatively unknown.

An individual's EPA + DHA status can be assessed by measuring the amount of EPA + DHA in the membranes of red blood cells, expressed as a percentage of the total fatty acids [18]. This measurement, called the *Omega-3 Index* (O3I), reflects intake of EPA + DHA via food and dietary supplements over approximately the previous 4 months [18]. O3I has been shown to be a reliable steady-state measurement of omega-3 status, whereas plasma and serum omega-3 measurements can be heavily influenced by an individual's most recent meal [31].

Numerous studies point to an O3I of 8% or greater as optimal for health and an index of less than 4% as deficient, particularly as it relates to cardiovascular disease [18-24]. Specifically, Harris et al found that, with regard to coronary heart disease mortality, an O3I ≥8% was associated with the greatest cardioprotection and an O3I ≤4% was associated with the least [18]. A meta-analysis of 10 cohorts found that the risk for fatal coronary heart disease was approximately 35% lower for those with an O3I of 8.3% vs an O3I of 4.2%, the medians of the highest and lowest quintile [19]. Additionally, 3 studies found a significantly lower risk of death from any cause with higher O3I: in the Framingham Offspring Study, there was a 34% lower risk for an O3I of 7.8% vs 3.7%, the medians of the highest and lowest quintile [22]; in the Women's Health Initiative Memory Study, there was a 31% lower risk for an O3I of >8% vs <4% [23]; and in a German study, there was a 22% lower risk for those with an O3I of >6.3% vs <5.2% [24].

The National Academy of Medicine (formerly Institute of Medicine) has not established a Dietary Reference Intake for EPA or DHA; however, there is an established Adequate Intake for ALA, which cannot be manufactured in the body. Up to 10% of that Adequate Intake, which equates to 110-160 mg/d for adults, can be comprised of EPA and DHA [32]. A 2018 Science Advisory from the American Heart Association (AHA) concluded that individuals should consume 1 to 2 seafood meals per week, especially species higher in long-chain omega-3 fatty acids, to lower the risk of cardiovascular diseases [33]. The 2015-2020 Dietary Guidelines for Americans by the United States Department of Agriculture (USDA) and Health and Human Services (HHS) recommendation for the general population for cardiovascular health is at least 2 servings, approximately 8 oz, of a variety of seafood per week, which provide an average of 250 mg/d of EPA and DHA [34]. Previous studies found that eating fish twice a week does not produce a median O3I ≥8% even with an EPA + DHA supplement [35,36]; however, these studies only captured whether participants used an EPA + DHA supplement but not the frequency of use or amount of EPA + DHA in the supplements.

GrassrootsHealth, a nonprofit public health research organization, runs a long-term prospective cohort study that collects demographic and health information from voluntary participants residing around the world, many of whom take nutrient supplements. This study also collects extensive information about vitamin D and omega-3 supplements used by participants, including specific products used, amount taken, and frequency of use. The objective of this study was to assess fish consumption, EPA + DHA supplement use behaviors, and O3I measurements using a cross-sectional analysis of participants in the GrassrootsHealth study. Based on previous studies which found that the current AHA and USDA/HHS guidelines do not produce an O3I ≥8% [35-37], we hypothesized that participants reporting adherence to the current AHA or USDA/HHS guidelines would not have an average O3I at or above 8% but that those taking a daily EPA + DHA supplement would. Additionally, the relationship between intake of supplemental EPA + DHA and O3I among participants not consuming fatty fish was estimated to provide guidance about intake requirements to achieve an O3I that is optimal for health (≥8%). Finally, a subanalysis was done to determine if a difference in the relationship between EPA + DHA intake and O3I by source of supplemental EPA and DHA could be quantified.

2. Methods and materials

2.1. Study population

The GrassrootsHealth cohort study was initiated in 2008, and participation included completing online health questionnaires and submitting home blood spot 25(OH)D tests which were purchased by participants. Completing questionnaires and 25(OH)D tests was suggested to occur every 6 months for 5 years with the purpose of determining the association between 25(OH)D and health outcomes; however, participants were able to choose their own frequency and length of participation. Participants were also free to choose their own dietary and

supplement regimens and target nutrient concentrations; more than 90% reported taking at least 1 nutrient supplement. Participants in the study were recruited via the Internet, seminars hosted by GrassrootsHealth, and other in-person events. There were no exclusion criteria for enrollment. Participants represent a wide range of ages, nationalities, and health statuses. Additional information about the GrassrootsHealth cohort has been previously reported elsewhere [38].

In 2017, data collection was expanded to include information on omega-3 intake as well as optional O3I testing in addition to 25(OH)D testing. The study population for the analysis presented here included all participants in the GrassrootsHealth cohort study 18 years and older who provided information on omega-3 dietary and supplement intake, regardless of their participation in the optional O3I testing (Fig. 1). All participants provided informed consent, and this research study was approved by the Western Institutional Review Board (Olympia, WA, USA).

2.2. *Omega-3 intake assessment*

Data for the present study include information collected via online questionnaires between May 2017 and August 2018. During this time, participants reported their typical dietary intake of fatty fish by answering the question, "How many meals containing fatty/oily fish do you normally have per week, such as salmon, mackerel, herring, sardines or tuna?" Answer options were 0, 1, 2, 3, 4, more than 4, and I don't know. Participants also reported information about their use of any supplements containing omega-3 fatty acids in the prior 6 months. Data on omega-3 supplement use (up to 3 products) included brand and product name, amount when taken, frequency of use, and duration of use. Supplement formulation information, including type of supplement and amount of EPA and DHA, was extracted from the National Institute of Health's Dietary Supplement Label Database [39] or from online supplement stores. Supplemental EPA + DHA intake was determined by adding the EPA and DHA amount for 1 serving (as indicated on the product label), adjusted for frequency of use and amount taken, for all reported supplements with omega-3s. For example, if the participant indicated they took 1 capsule every day but the amount for 1 serving on the product label was 2 capsules, the amount of EPA + DHA in 1 serving of the supplement was divided in half to calculate the participant's supplemental EPA + DHA intake (a similar calculation would be performed if 2 capsules were taken every other day). As EPA + DHA amounts differ significantly between fish species and preparation style, calculating the exact amount of EPA +DHA from the diet was not possible because of the lack of information about the specific types of fish meals consumed. Participants also reported demographic information such as age, sex, and country of residence as well as information on health status and conditions on their online questionnaires.

2.3. *O3I testing*

Participants in the GrassrootsHealth cohort study were informed of the optional O3I testing via e-mail, social media, and in-person events. Participants purchased the home blood spot O3I tests along with 25(OH)D tests which were then delivered through the mail. Enclosed with the O3I blood spot test cards were an alcohol swab, gauze, bandage, a return envelope, and instructions. Participants were instructed to

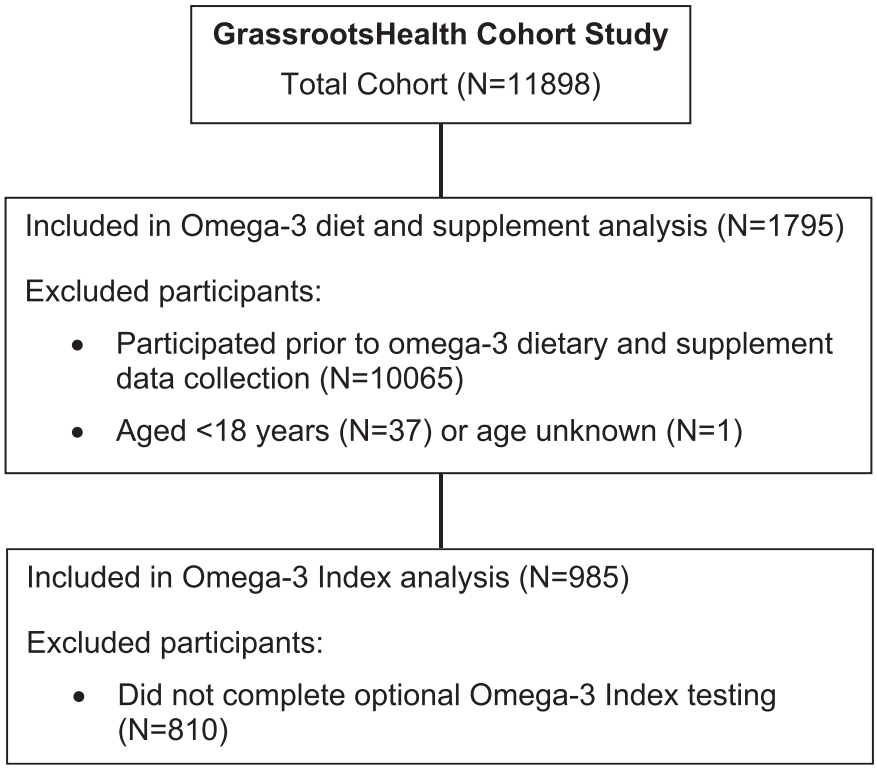

Fig. 1 – Diagram illustrating the selection of participants for analysis.

wash their hands and swab the area where the blood spot would be obtained with the alcohol swab. A self-loaded lancet was used by the participant to prick a fingertip and drop 2 to 4 drops of blood on the test card. After letting the test card dry for 30 minutes or longer, the participant was directed to mail the test card in the provided envelope to GrassrootsHealth where the test card was processed for shipment to OmegaQuant Analytics (Sioux Falls, SD, USA) for analysis. Direct methylation followed by quantification by capillary gas chromatography with flame ionization detection was used to analyze the dried blood spots and determine the EPA + DHA content [40]. Red blood cell membrane EPA + DHA (ie, the O3I) was calculated from the dried blood spot EPA + DHA value using a regression equation derived by comparing values in ~100 samples ($r = 0.96$, $P < .0001$) [40]. The interassay coefficient of variation was <5% [40]. Participants received the results of their test via the Internet using a personalized account at GrassrootsHealth with a secure password.

2.4. *Statistical analyses*

Participant demographic characteristics and fatty fish consumption were summarized with frequencies and medians (along with interquartile range [IQR]). The percent of participants using a supplement with EPA and/or DHA (marine/algal oil) was calculated. The proportion using each EPA + DHA supplement type (liquid, capsule, etc) and oil type (fish, krill, etc) was presented, as was information about patterns of supplement use, including frequency of use and servings per day. The numbers of brands and products (distinguished by brand and formulation differences such as oil types and amounts, EPA or DHA amount, supplement type, serving size, and co-nutrients) were reported. For analyses, supplements with fish oil or fish oil concentrate were combined using the term *fish oil*. This included all types of fish oil regardless of chemical form (ie, triglyceride or ethyl ester) because the specific type could not be definitively determined for all products; cod liver oil remained as a distinct group. The EPA + DHA content in reported supplement products was summarized, and the median (IQR) intake of EPA + DHA from supplements was calculated. For participants who completed a blood spot test, the proportion of participants with O3I measurements in the following categories was calculated: <4.0%, 4.0%-5.9%, 6.0%-7.9%, and ≥8.0%. Median (IQR) O3I and percent of participants with O3I ≥8.0% were compared for EPA + DHA supplement users vs nonusers and by fish intake, separately and combined (Mann-Whitney and Kruskal-Wallis tests were used for median O3I; χ^2 and Cochran-Mantel-Haenszel tests were used for percent O3I ≥8.0%). To detect an absolute difference of 1% in O3I between any 2 groups (80% power, $\alpha = .05$, 2-tailed test), a minimum of 50 participants per group was calculated based on the average standard deviation in O3I measurements (1.8%) from a previous similar study [35].

To determine the amount of EPA + DHA needed for 50% of the population to achieve an O3I of 8.0%, the relationship between supplemental EPA + DHA intake and O3I among those reporting no fatty fish intake was estimated with a linear regression model for participants with known supplemental EPA + DHA intake of up to 2000 mg/d for at least 4 months because O3I measures intake for the previous ~4 months (only 5% used >2000 mg/d). Only participants who reported no fatty fish intake were used to ensure that there were no major nonsupplemental sources of EPA + DHA input. A sensitivity analysis was conducted to assess the relationship between roughly estimated EPA + DHA intake from both fatty fish and supplements using participants with all amounts of fish intake. To calculate the approximate EPA + DHA from diet and supplements, 1250 mg of EPA + DHA (the average amount provided by 4 oz of wild Coho salmon, sardines, Bluefin tuna, and Albacore tuna [41]), divided by 7 (ie, 179 mg), was added to the daily amount of EPA + DHA from supplements for each reported weekly number of servings of fatty fish. Additionally, the relationship between EPA + DHA intake and O3I by source of supplemental EPA and DHA (fish oil vs krill oil) was estimated to determine if there was a difference between the two. The latter comparison included participants with all reported fish intakes because intakes did not differ between the fish oil supplement and krill oil supplement groups ($P > .10$). Linear regression assumptions, including residual distribution normality, homoscedasticity, linearity, and influential outliers, were assessed and confirmed for all models. Only participants using supplement products that could be specifically identified in the National Institute of Health's Dietary Supplement Label Database [39] or in online supplement stores were included in analyses of the relationship between supplemental EPA + DHA intake and O3I. Fewer than 3% of reported products could not be identified because of insufficient product label information (eg, participant could not remember the exact product, or just brand name was reported). If more than 1 questionnaire or blood spot test was completed, the most recent was used for analysis. Statistical analyses were performed using the R software (www.r-project.org).

3. Results

In total, 1795 participants completed the health questionnaire, and 985 submitted a sample for omega-3 testing. The characteristics of this cohort are found in Table 1. Participants resided in 27 countries worldwide (95% in the United States or Canada) and were predominately middle-aged and non-Hispanic white. Nearly three quarters (71%) consumed less than 2 servings of fatty fish per week. Participants who completed an O3I test had a higher median age and a higher proportion of males and lipid-lowering medication (statins, fibrates, or nicotinic acid) users compared to those who did not complete an O3I test; race/ethnicity, weight, body mass index, smoking status, alcohol use, typical exercise, disease diagnoses, and fatty fish consumption did not differ.

Almost two thirds (61%) of participants took a supplement with EPA and/or DHA (marine/algal oil) in the prior 6 months, and approximately one third (37%) did not. Less than 2% of participants did not provide enough data, or the nutrition label was inadequate, to determine whether their omega-3 supplement contained EPA + DHA. Among EPA + DHA supplement users, 14% reported taking 2 or more different EPA + DHA supplements. A vast majority (87%) took a softgel or capsule, 15% took a liquid supplement, and 1% took a gummy or other type of supplement. Approximately half (57%) took an EPA + DHA supplement every day, 28% took it 4-6 d/wk, 11% took it

Table 1 – Demographic characteristics of GrassrootsHealth cohort

Characteristic	All participants (n = 1795)	Participants with O3I (n = 985)	Participants without O3I (n = 810)	P value [a]
Sex				<.0001
Male	715 (40%)	433 (44%)	282 (35%)	
Female	1080 (60%)	552 (56%)	528 (65%)	
Age (y)	59 (49-67)	61 (52-68)	56 (44-65)	<.0001
Race/ethnicity [b]				.22
Non-Hispanic white	1654 (93%)	911 (94%)	743 (92%)	
Hispanic	38 (2%)	18 (2%)	20 (2%)	
Asian/Pacific Islander	36 (2%)	17 (2%)	19 (2%)	
African American	18 (1%)	6 (1%)	12 (1%)	
Multiple/other	27 (2%)	17 (2%)	10 (1%)	
Weight (kg)	70 (60-82)	71 (60-82)	69 (60-81)	.21
Body mass index	24 (22-27)	24 (22-27)	24 (22-27)	.73
Smoking status [b]				.48
Current smoker	50 (3%)	25 (3%)	25 (3%)	
Never or former smoker	1738 (97%)	957 (97%)	781 (97%)	
Alcohol use in the prior 6 mo [b]				.97
Used alcohol	1297 (73%)	712 (73%)	585 (73%)	
Did not use alcohol	489 (27%)	268 (27%)	221 (27%)	
Typical exercise [b]				.33
None or mild	881 (49%)	474 (48%)	407 (51%)	
Moderate or strenuous	904 (51%)	507 (52%)	397 (49%)	
Disease diagnoses [c]				
History of cardiovascular disease	49 (3%)	31 (3%)	18 (2%)	.23
History of nonskin cancer	188 (10%)	108 (11%)	80 (10%)	.45
Diabetes	58 (3%)	35 (4%)	23 (3%)	.39
Medication				.03
Use of lipid-lowering medication [d]	98 (5%)	64 (6%)	34 (4%)	
Fatty fish consumption [b]				.26
0 serving/wk	632 (36%)	359 (37%)	273 (35%)	
1 serving/wk	617 (35%)	324 (34%)	293 (37%)	
2 or more servings/wk	500 (29%)	281 (29%)	219 (28%)	

Values are n (%) for categorical variables and medians (IQR) for continuous variables.
[a] Categorical variables were compared for those with and without an O3I test using χ^2 and Cochran-Mantel-Haenszel tests; continuous variables were compared using the Mann-Whitney test.
[b] Twenty-two had missing race/ethnicity data, 7 had missing smoking data, 9 had missing alcohol data, 10 had missing exercise data, and 46 did not know their typical fish consumption.
[c] Cardiovascular disease included heart attack, stroke, and angina pectoris; diabetes included both type 1 and type 2.
[d] Lipid-lowering medication included statins, fibrates, and nicotinic acid.

2-3 d/wk, 2% took it once per week or less, and 2% took it inconsistently. Almost one third (29%) took 1 serving (as indicated on the product label) on average per day, 38% took less, and 33% took more. Fig. 2 shows the proportion of participants taking each EPA + DHA supplemental oil type. The most commonly used oil types were fish oil (54%) and krill oil (43%). The median intake of EPA + DHA from these supplements was 403 mg/d (IQR: 155-1078).

Among the 1092 EPA + DHA supplement users, 213 different brands and 409 different supplement products were used (Supplemental Table S1). There was significant variability in the amount of EPA + DHA per single serving (as indicated on the product label) among EPA + DHA supplement products; the amount in supplements containing fish oil as the only source of EPA + DHA ranged from 50 to 3570 mg (median: 840 mg, IQR: 500-1200), and the amount in supplements with krill oil as the only source ranged from 70 to 410 mg (median: 182, IQR: 94-240). The most popular fish oil supplement had 1100 mg of EPA + DHA, more than 7 times the amount in the most popular krill oil supplement, which contained 155 mg of EPA + DHA per serving. Thirty EPA + DHA products (7%) did not provide the amount of EPA and/or DHA on their supplement label.

Among participants who did an O3I test (n = 985), 8% had an O3I <4.0%, 44% had an O3I 4.0%-5.9%, 28% had an O3I 6.0%-7.9%, and 19% had an O3I ≥8.0%. Table 2 presents median (IQR) O3I and percent of participants achieving an O3I ≥8.0% by EPA + DHA supplement use and fish intake. The O3I was higher for EPA + DHA supplement users vs nonusers and for those consuming more fatty fish. Fig. 3 shows the median (IQR) O3I, and Fig. 4 shows the percent of participants achieving an O3I ≥8.0% for both fatty fish consumption and EPA + DHA supplement use (nonusers compared to those who used a supplement at or above vs below the approximate median of 400 mg/d). Median O3I for non–supplement users was 4.8% for those consuming 1 serving of fatty fish per week and 5.7% for those consuming 2 or more servings. A median O3I ≥8.0% was only achieved by fish consumers also taking an average of 1100 mg/d of supplemental EPA + DHA.

The relationship between supplemental EPA + DHA intake (all supplement oil types combined) by O3I for participants

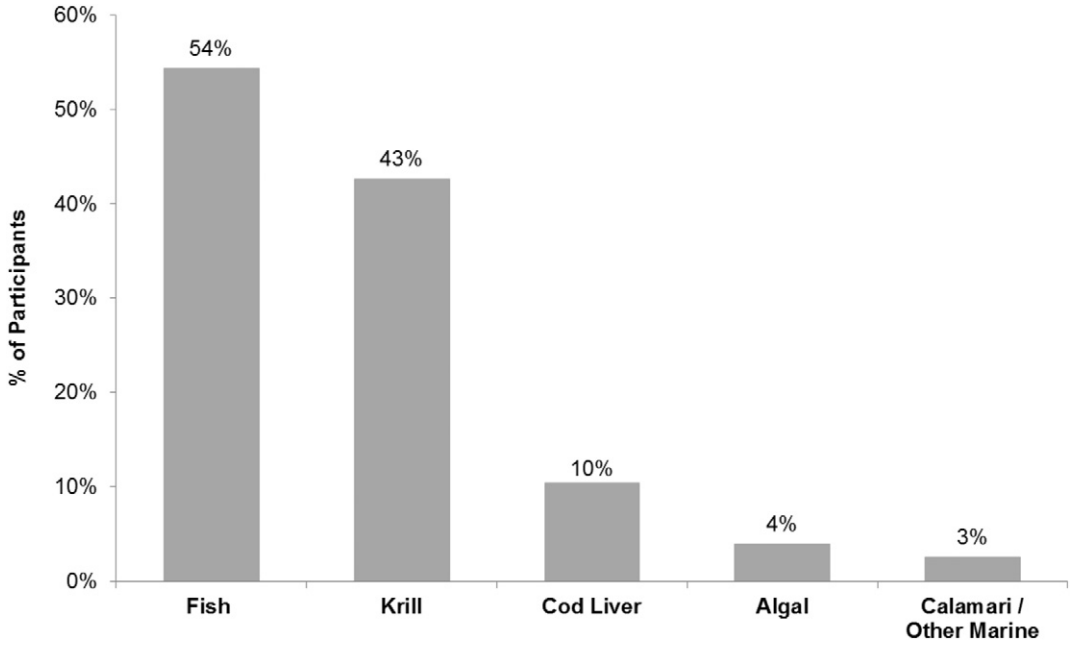

Fig. 2 – Percent of participants using each supplement oil type among EPA + DHA supplement users (n = 1092). Those taking more than 1 supplement type were counted in more than 1 category; hence, the total is 114%.

who had been supplementing for at least 4 months (up to 2000 mg/d) with no fatty fish intake (to ensure no other major nonsupplemental sources of EPA + DHA input) is plotted in Fig. 5. For 50% of the population to achieve an O3I of 8.0%, 1280 mg/d of supplemental EPA + DHA was needed. The lower line of the 80% probability band indicates the amounts required to ensure 90% of participants achieve a given O3I. Approximately 1900 mg/d of supplemental EPA + DHA was needed for 90% of the population to achieve an O3I of 8.0%. A sensitivity analysis (Supplemental Fig. S1), which assessed the relationship between roughly estimated EPA + DHA intake from both fatty fish and supplements, was conducted using participants with all amounts of fish intake. This analysis revealed similar results (~1350 mg/d of EPA + DHA for 50% of the population to achieve an O3I of 8.0%; ~2000 mg/d of EPA + DHA for 90% of the population).

Among those who used only fish oil supplements for at least 4 months, 43% achieved an O3I ≥8.0%, whereas among those who used only krill oil supplements for at least 4 months, only 4% achieved an O3I ≥8.0%. Fig. 6 shows a plot of supplemental EPA + DHA intake by O3I for those taking supplements with fish oil only and for those taking supplements with krill oil only for at least 4 months (up to 2000 mg/d). The best fit line indicates that 1240 mg/d of EPA + DHA from fish oil was needed for 50% of the population to achieve an O3I of 8.0%, without regard to fatty fish consumption which did not differ between groups. The data for krill oil supplement users only reached to 540 mg of EPA + DHA (the highest amount taken), which corresponded to an O3I of 6.6%.

Table 2 – O3I results by EPA + DHA supplement use and fatty fish intake

	O3I	P value [a]	O3I ≥ 8.0%	P value [b]
All participants with O3I (n = 985)	5.8% (4.9%-7.4%)		191 (19%)	
EPA + DHA supplement [c]		<.0001		<.0001
Users (n = 686)	6.3% (5.3%-8.0%)		172 (25%)	
Nonusers (n = 282)	4.9% (4.1%-5.7%)		19 (7%)	
Fatty fish consumption [d]		<.0001		<.0001
0 serving/wk (n = 359)	5.2% (4.3%-6.6%)		47 (13%)	
1 serving/wk (n = 324)	5.7% (5.0%-7.1%)		63 (19%)	
2 or more servings/wk (n = 281)	6.8% (5.6%-8.2%)		79 (28%)	

Values are medians (IQR) for O3I and n (%) for the percent of participants with O3I ≥8.0%.
[a] Median O3I was compared using Mann-Whitney and Kruskal-Wallis tests.
[b] Percent of participants with O3I ≥8.0% was compared using χ^2 and Cochran-Mantel-Haenszel tests.
[c] Seventeen participants who completed an O3I test did not provide enough data, or the nutrition label was inadequate, to determine whether their omega-3 supplement contained EPA + DHA and were excluded.
[d] Twenty-one participants who completed an O3I test did not know their typical fish intake and were excluded.

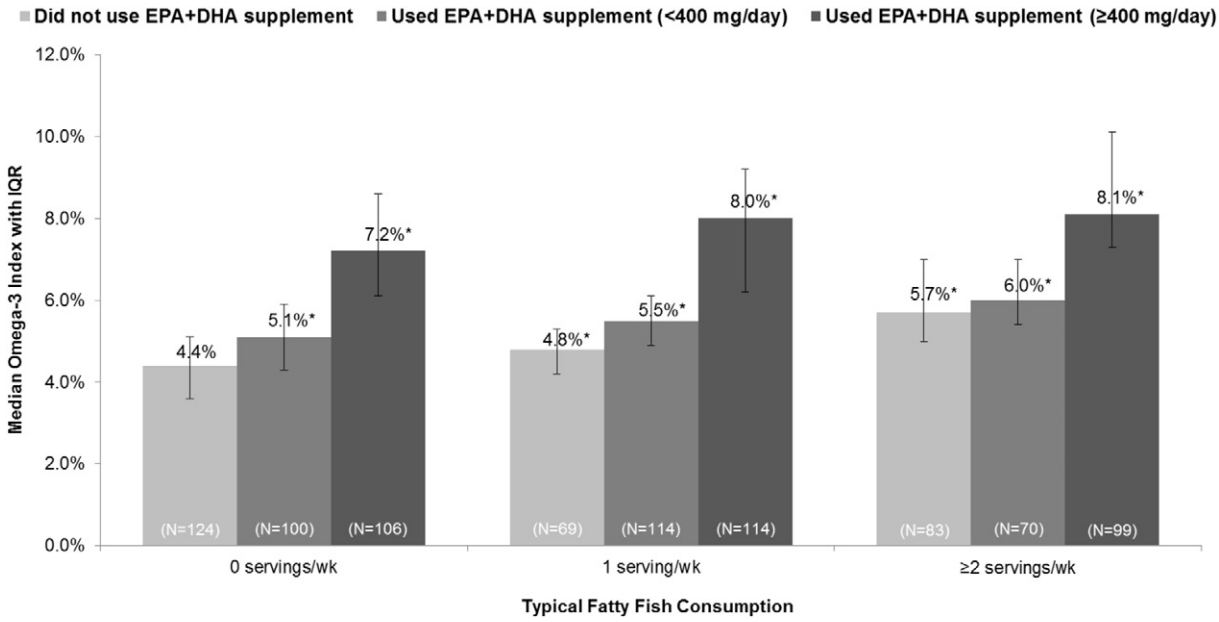

Fig. 3 – Median O3I by typical fatty fish intake and EPA + DHA supplement use. Error bars represent IQR, and asterisks (*) represent *P* values < .01 compared to those who did not use an EPA + DHA supplement and reported no fatty fish intake. Participants with unknown fish intake or EPA + DHA supplemental amount were excluded. Median supplemental EPA + DHA intake for those with <400 mg/d was 155 mg/d for all 3 fish consumption groups, and intake for those with ≥400 mg/d was 1000 mg/d for the group with 0 fish serving per week and 1100 mg/d for the groups with both 1 and ≥2 fish servings per week.

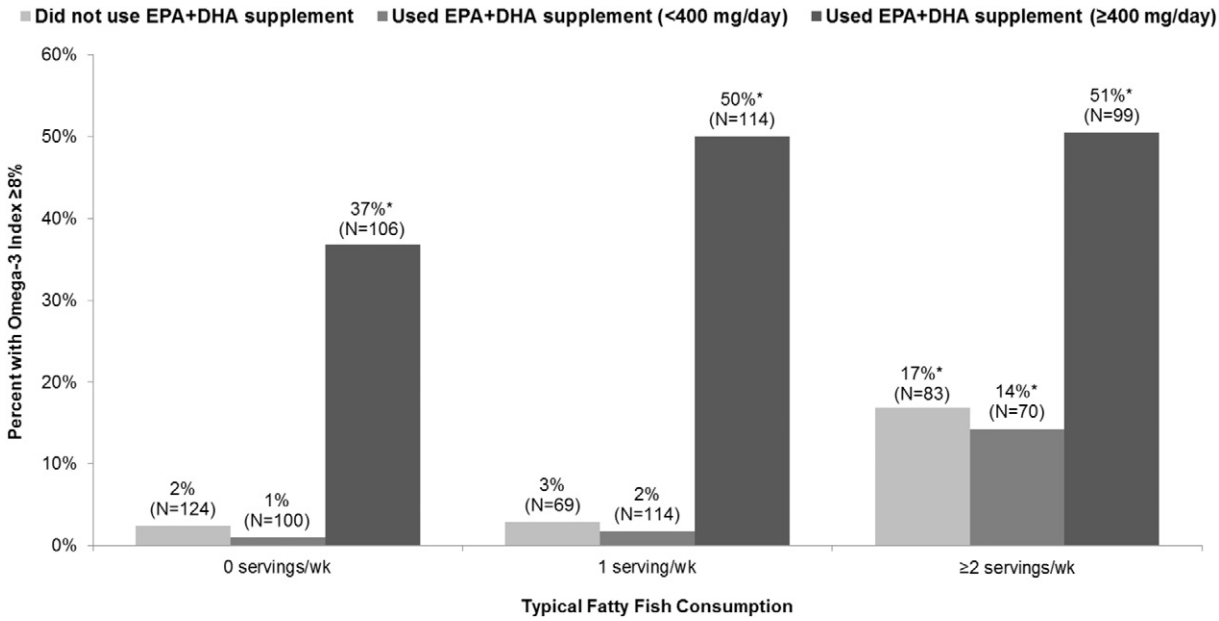

Fig. 4 – Percent of participants who achieved an O3I ≥8.0% by typical fatty fish intake and EPA + DHA supplement use. Asterisks (*) = *P* values < .01 compared to those who did not use an EPA + DHA supplement and reported no fatty fish intake. Participants with unknown fish intake or EPA + DHA supplemental amount were excluded. Median supplemental EPA + DHA intake for those with <400 mg/d was 155 mg/d for all 3 fish consumption groups, and intake for those with ≥400 mg/d was 1000 mg/d for the group with 0 fish serving per week and 1100 mg/d for the groups with both the 1 and ≥ 2 fish servings per week.

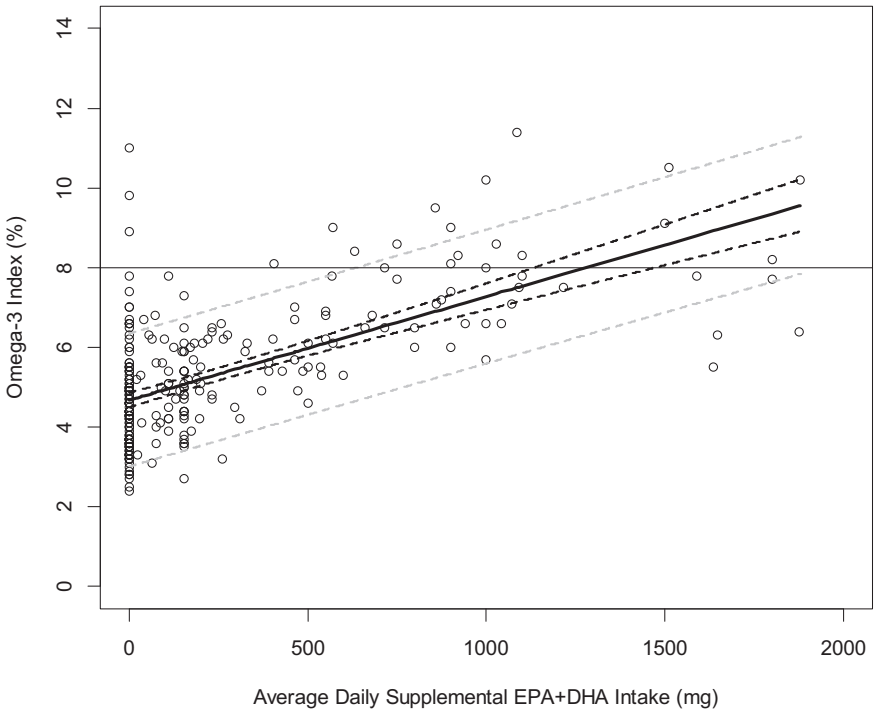

Fig. 5 – O3I as a function of daily EPA + DHA intake from supplements among those who reported no fatty fish intake (n = 265) with linear best fit line and 95% CI (inner dashed lines). Outer dashed lines represent 80% prediction band (90% of participants are above the lower line). Each dot represents the supplemental EPA + DHA intake amount and O3I of a single participant taking up to 2000 mg/d of supplemental EPA + DHA for at least 4 months. Non–supplement users and those taking omega-3 supplements without EPA or DHA = 0 mg/d. Model R^2 was 0.40 and $P < .0001$. Equation: O3I = 4.68 + 0.0026*(EPA + DHA in mg). The 95% CI for the coefficient (0.0026) was 0.0022-0.0030.

4. Discussion

This study provides an assessment of EPA + DHA supplement products and consumer behaviors, along with O3I measurements, of adult participants with supplementation practices of their own choosing. Participants in this prospective cohort study used a wide variety of EPA + DHA supplement products (approximately 1 for every 3 supplement users), most with either fish or krill oil. Although 61% of participants in this cohort reported taking an EPA + DHA supplement, only 19% achieved an O3I ≥8.0%. For participants not taking an EPA + DHA supplement but adhering to the current fish intake guidelines, only 3% of those consuming 1 serving of fatty fish per week and 17% of those consuming ≥2 achieved an O3I ≥8.0%. The median O3I among those consuming 2 or more servings of fatty fish per week only surpassed an O3I of 8.0% among those also taking an average of 1100 mg/d of supplemental EPA + DHA. This indicates that, for most people, the AHA and USDA/HHS fish intake guidelines do not produce an O3I in the optimal range for health, nor does simply taking an omega-3 supplement without regard for how much EPA and DHA is in the supplement. Although our hypothesis that participants reporting adherence to the current guidelines would not have an average O3I ≥8% was accepted, we rejected our hypothesis that those taking a daily EPA + DHA supplement would achieve this level because it depended on the amount of supplemental EPA + DHA taken per day.

Approximately 1300 mg/d of supplemental EPA + DHA was needed for 50% of the population to achieve an O3I of 8.0% among participants with no fatty fish intake (ie, no other major nonsupplemental sources of EPA + DHA input). According to the USDA Nutrient Database [41], this amount is approximately equal to 4 servings per week (with a serving size of 4 oz) of salmon (Chinook and Atlantic: 1968-2433 mg), mackerel (Pacific and Jack: 2095 mg), herring (Atlantic and Pacific: 2283-2408 mg), or canned anchovies (2329 mg). This amount of EPA + DHA was found in a single serving (as indicated on the product label) of only 14% of EPA + DHA supplements used by participants (20% of fish oil supplements and 0% of krill oil supplements). To estimate the average supplemental EPA + DHA intake amount needed for fatty fish eaters to achieve an O3I of 8.0%, the daily supplemental EPA + DHA intake amount can be lowered by the amount of dietary EPA + DHA from fish based on the specific fish species and preparation style [41].

To increase O3I to 8% or higher, attention needs to be paid to the amount of EPA + DHA in foods and supplements. The amount of EPA + DHA in 1 serving (as indicated on the product label) significantly differed among the supplement products used by participants in this study (50 to 3570 mg), which was an unexpected finding. A common error discovered in participant reporting of label information was misreporting the oil amount in supplement products for the amount of total omega-3s or EPA + DHA, which suggests that they may think that they are getting more EPA + DHA than they actually are because the

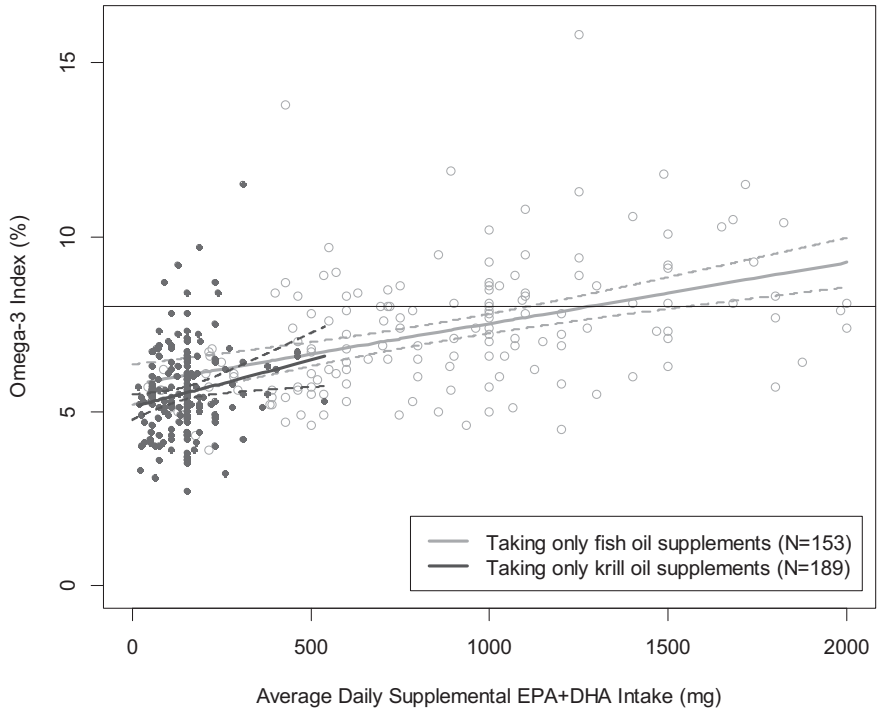

Fig. 6 – O3I as a function of daily EPA + DHA intake from supplements by oil type (fish vs krill) with linear best fit lines and 95% CIs (dashed lines). Light gray dots and line represent participants only taking fish oil supplements (n = 153), and dark gray dots and line represent participants only taking krill oil supplements (n = 189), up to 2000 mg/d for at least 4 months. Fish oil supplement model R^2 was 0.19 and $P < .0001$. Equation: O3I = 5.77 + 0.0018*(EPA + DHA in mg); 95% CI for the coefficient (0.0018) was 0.0012-0.0023. Krill oil supplement model R^2 was 0.03 and $P = .01$. Equation: O3I = 5.13 + 0.0027*(EPA + DHA in mg); 95% CI for the coefficient (0.0027) was 0.0006-0.0048.

amount of oil is higher than the amount of EPA + DHA. Furthermore, many foods and products advertising "omega-3s" contain only ALA, which contributes very little toward O3I [30]. Also, all seafood options do not provide the same amount of EPA + DHA. For example, shrimp and tilapia, which are 2 of the most popular types of seafood consumed in the United States, have less than 300 mg of EPA + DHA per serving, whereas fatty fish from cold waters can provide more than 1000 mg per serving [41].

Several other studies have also found that the current AHA and USDA/HHS guidelines of 2 servings of seafood per week do not adequately raise omega-3 status [35-37]. A similar study to this one found that the only group to achieve a median O3I ≥8% reported eating at least 3 servings of fatty fish per week and taking an EPA + DHA supplement [35]. Those findings are in general agreement with the results presented here. Additionally, randomized clinical trials to assess omega-3 supplement dose-response have found that amounts of EPA + DHA in a similar range to what was found in this study are needed to achieve an O3I of at least 8.0%. Flock et al provided 115 men and women with 1 of 5 doses of fish oil supplements (0, 300, 600, 900, and 1800 mg/d) for 5 months; only the group provided 1800 mg/d achieved a mean O3I ≥8.0% [42]. In another dose-response trial, 24 elite athletes were given either 760 or 1140 mg/d of supplemental EPA + DHA from fish oil for 4 months; neither group achieved a mean O3I ≥8.0% (the latter group achieved a mean O3I of 7.0%) [43]. There is a lack of data on the O3I response to krill oil supplementation in these dose ranges; however, the mean O3I achieved in a 12-week krill oil trial among 58 participants given the highest dose (800 mg/d of EPA + DHA) was 6.3% [1]. The slopes of the linear relationships between supplemental EPA + DHA intake and O3I in the present study (0.0018 for fish oil supplements and 0.0027 for krill oil supplements) were lower than those found in these clinical trials (0.0026 in the Flock et al study of fish oil supplements [42] and 0.0036 in the Berge et al study of krill oil supplements [1]). These differences may be due to the different range of intakes analyzed or study differences such as using a mix of a wide variety of commercially available supplements vs a single study supplement; however, it is likely that the slopes in the present study better represent what could be expected in the real world compared to a highly controlled clinical trial.

It is important to point out that there was a large amount of variability in the blood levels associated with any specific supplemental EPA + DHA intake amount among participants not consuming fatty fish. For example, Fig. 5 shows that the range of response with 1000 mg/d was 5.7%-10.2%. Also, less than half of the variance (40%) in O3I was explained by supplemental EPA + DHA intake. Because this plot shows only those with no fatty fish intake, the primary food source of EPA + DHA, other factors such as age, sex, weight, smoking, alcohol intake, and medication use could explain this variation. Also, evidence shows that the omega-3 chemical

form (eg, ethyl ester, triglyceride) has differing effects on O3I [44]. Furthermore, there is some evidence that the proportion of EPA and DHA in supplements and a high intake of linoleic acid (an omega-6 fatty acid) may influence the O3I [45,46]. Similar interindividual variability has been observed for other nutrients such as vitamin D [38]. Rather than relying on the average intake amount, individuals should measure their O3I and determine a personalized dose based on lifestyle and fish consumption that will allow them to achieve a desired O3I. This also highlights the importance of measuring O3I during clinical trials rather than only assessing effect based on assigned intake.

The most common supplement types used in this cohort, fish and krill oil, both contain EPA and DHA, but there are important differences between the two that warrant consideration. Krill oil naturally contains astaxanthin [47], an antioxidant, and being at the bottom of the food chain, krill may have fewer contaminants such as mercury compared to large species of fish [48]. There is also evidence that EPA + DHA from krill oil phospholipids may be more effective at crossing the blood brain barrier and, at equivalent doses, have a greater impact on brain function due to its phospholipid form than fish oil [49,50]. Additionally, krill oil does not need to be consumed with a fatty meal because it consists of phospholipids which aid absorption [51]. Although the same is true of triglyceride-based fish oils, fish oil supplements in the ethyl ester form do require a fatty meal for maximum absorption [52]. Although some studies suggest that krill oil may be ~1.4-1.7 times more bioavailable than fish oil [53-55], krill oil supplements typically contain about 80% less EPA +DHA per serving and are in general more costly than fish oil supplements. Consideration may be given to taking both a krill and fish oil supplement (either as a combination supplement or 2 separate supplements) to achieve both the aforementioned benefits of krill oil and to more affordably achieve an O3I of at least 8% for optimal health.

The strengths of this analysis include the detailed data collection of the omega-3 supplement use among participants, including specific products and use patterns in the previous 6 months. Self-reported product label information and pictures of products were used to identify the exact products in the National Institute of Health's Dietary Supplement Label Database [39] or in online supplement stores to validate self-reported data and extract additional information about ingredients. More than 97% of the EPA + DHA supplement products reported were identified and validated. Also, using a population with a wide range of EPA + DHA supplemental intake amounts and O3I values allowed for a comprehensive, real-world assessment of supplement use, fatty fish intake, and the relationship between supplemental EPA + DHA intake and O3I.

Limitations of the analysis include the use of self-reported EPA + DHA supplement use and fatty fish consumption, which may have resulted in an inaccurate estimate of actual intake. Because only ~10% of participants consumed 3 or more servings of fatty fish per week, we were not able to assess O3I among individuals with higher fish intakes. Also, there was not an answer option for an average of less than 1 serving of fatty fish per week but more than 0 serving per week (such as 1 serving per month or 1 serving every other week); it is unknown if these participants selected either 0 or 1 serving per week. We also did not collect enough data about fish intake, such as fish species and preparation style, to be able to determine the exact amount of EPA + DHA from the diet. Also, because we were not able to definitively determine the type of fish oil (such as triglyceride and ethyl ester) for all products, all fish oil supplements were combined into the same group for analyses of the relationship between supplemental EPA + DHA intake and O3I. Therefore, we were not able to estimate and compare the relationship between supplemental EPA + DHA and O3I for specific EPA + DHA chemical forms in "fish oils." Nor were we able to determine if a difference in the relationship between supplemental EPA + DHA intake and O3I existed between krill oil and fish oil supplements because of the lack of overlap in EPA + DHA amount between the krill oil and fish oil supplements used by participants in this study. Additionally, individual response to a change in supplemental omega-3 intake could not be assessed because of the cross-sectional nature of the data. Because participants in this cohort are self-selected and have disposable income available to pay for vitamin D and O3I testing and many take omega-3 supplements (61% in the GrassrootsHealth cohort vs 8% in the general US population [56]), results regarding amount and patterns of EPA + DHA supplement use are not representative of the general population. Also, the majority of participants were non-Hispanic white and resided in the United States or Canada; therefore, results may not be generalizable to other ethnicities or to those residing in other countries with different fish consumption behaviors.

Although there is certainly benefit to achieving an O3I between 4% and 8% [57,58], evidence suggests that achieving an O3I of at least 8% is associated with greater risk reduction, especially for heart disease [18-24]. This study supports existing evidence that eating 1 or 2 servings of fatty fish per week does not by itself produce an O3I at or above 8% for most people. From a public health perspective, ~1300 mg/d of EPA + DHA may be needed for 50% of the population to achieve an O3I of 8%. Close to 2000 mg/d may be needed to ensure that 90% of the population achieves this target level. Higher intakes of fish (3 or more servings per week, which are typical in Japan where O3I levels average ~8% [59]) may also be key to raising the O3I in the general population. Recommendations regarding fish consumption and/or EPA and DHA intake for cardiovascular health, such as those from the AHA and USDA/HHS, should reflect at least the average amount needed to achieve optimal physiological status. Furthermore, given the extreme variation in EPA + DHA between seafood choices [41], recommendations should specify fatty or oily fish which have the highest EPA + DHA content rather than seafood in general. These viewpoints have been previously argued in the commentary by Kuller regarding the current AHA guidelines [60]. Furthermore, given the large amount of variability in levels among individuals reportedly taking the same supplemental EPA + DHA amount, individuals should measure their O3I and determine a personalized intake amount that is adequate for them to achieve a desired O3I rather than rely on a one-size-fits-all intake amount. Educating the general population about the importance of fish type, amount of EPA + DHA in omega-3 supplements, and O3I testing is important for those desiring to achieve an omega-3 status associated with disease prevention.

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