Title

Omega-3 fatty acids, subclinical atherosclerosis, and cardiovascular events: Implications for primary prevention

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Author contributions

All authors contributed to the conception and design of the work, interpretation of data, revising the work critically for important intellectual content. ZA and AA performed the statistical analysis. AA drafted the manuscript with input from all authors. All authors approved the final version of the manuscript.

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Declaration of interests

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Omega-3 fatty acids, subclinical atherosclerosis, and cardiovascular events: Implications for primary prevention

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Abstract

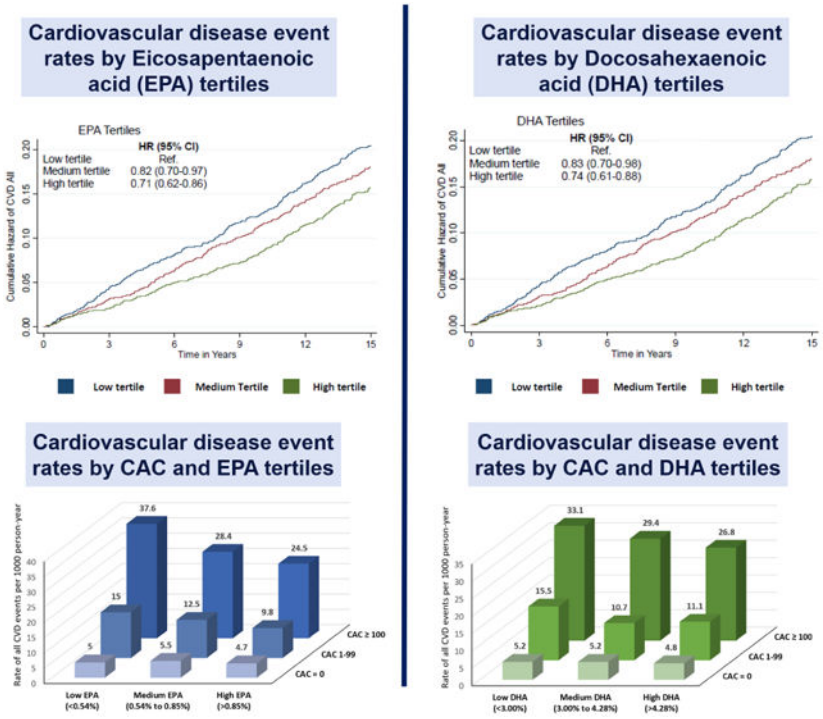
Background and aims: High-dose eicosapentaenoic acid (EPA) therapy was beneficial in high-risk patients without clinical cardiovascular disease (CVD). Whether higher plasma levels of EPA and docosahexaenoic acid (DHA) have similar benefits in those without subclinical CVD is unclear. We aim to evaluate the interplay between plasma omega-3 fatty acids and coronary artery calcium (CAC) in relation to CVD events.

Methods: We examined 6568 participants from the Multi-Ethnic Study of Atherosclerosis (MESA) with plasma EPA and DHA levels and CAC measured at baseline. The primary outcome was incident CVD events (myocardial infarction, angina, cardiac arrest, stroke, CVD death). Hazard ratios for the primary outcome were adjusted for potential confounder using Cox regression.

Results: Mean±SD age was 62.1±10.2 years and 52.9% were females. The median follow-up time was 15.6 years. Higher $\log_e$(EPA) (adjusted hazard ratio, aHR=0.83; 95% CI, 0.74-0.94) and loge(DHA) (aHR=0.79; 95% CI, 0.66-0.96) were independently associated with fewer CVD events. The difference in absolute CVD event rates between lowest vs. highest EPA tertile increased at higher CAC levels. The adjusted HR for highest vs. lowest EPA tertile within CAC=0 was 1.02 (95% CI, 0.72-1.46), CAC=1-99 was 0.71 (95% CI, 0.51-0.99), and CAC≥100 was 0.67 (95% CI, 0.52-0.84). A similar association was seen in tertiles of DHA by CAC category.

Conclusions: In an ethnically diverse population free of clinical CVD, higher plasma omega-3 fatty acid levels were associated with fewer long-term CVD events. The absolute decrease in CVD events with higher omega-3 fatty acid levels was more apparent at higher CAC scores.

Graphical Abstract



Keywords

Eicosapentaenoic acid; docosahexaenoic acid; coronary artery calcium; cardiovascular disease; primary prevention

1. Introduction

Higher blood levels of omega-3 fatty acids are associated with fewer cardiovascular events, as shown in observational studies.[1] However, clinical trials show inconsistent results for the role of omega-3 fatty acid supplementation in primary atherosclerotic cardiovascular disease (CVD) prevention.[2] Two possible explanations for the inconsistent trial results are that trials of omega-3 fatty acids differ in the achieved blood levels of omega-3 fatty acids and the risk profile of the populations enrolled.

Coronary artery calcium (CAC) is a specific marker of subclinical coronary atherosclerosis and a strong independent predictor of cardiovascular events.[3,4] Due to its ability to discriminate and reclassify CVD risk, CAC is endorsed by the AHA/ACC guidelines for consideration in the clinician-patient risk discussion for primary prevention measures when traditional risk estimates are uncertain.[5] To date, none of the clinical trials testing omega-3 fatty acids measured CAC or examined patients according to the presence or absence

of subclinical atherosclerosis. As a result, whether the benefit of higher blood levels of omega-3 fatty acids extends to those with or without subclinical CVD remains unknown.

In this study, we sought to examine the interplay between plasma omega-3 fatty acids and CAC in relation to long-term CVD outcomes in the Multi-Ethnic Study of Atherosclerosis (MESA). We hypothesized that in those without clinical CVD, plasma omega-3 fatty acid levels predict long-term CVD events, and subclinical CVD measured by CAC modifies the association such that individuals with the highest CAC exhibit the greatest benefit from higher plasma omega-3 fatty acids.

2. Patients and methods

2.1 Data availability

Access to data from MESA may be requested by qualified researchers trained in human subject confidentiality protocols by contacting the MESA Data Coordinating Center at chsccweb@u.washington.edu.

2.2 Study population

MESA was designed to study the characteristics and risk factors of subclinical CVD and predictors of disease progression to overt CVD. Full details on MESA study design were published elsewhere.[6] In brief, 6814 men and women free of clinically overt CVD from 4 self-identified ethnic groups were recruited from 6 field centers between 2000 and 2002. After enrollment, participants were followed prospectively with regular annual contact to identify new medical diagnoses and hospital admissions. The present analysis used data obtained from the baseline visit with follow-up data for clinical events through the end of calendar year 2017. MESA was approved by the institutional review committees of each enrollment center. All participants provided a written informed consent at the time of study enrollment.

Supplemental Figure 1 is a flow chart depicting the study population. We considered all MESA participants for inclusion for this analysis. All participants underwent baseline CAC measurement. We excluded 241 patients with missing plasma omega-3 fatty acid measurements. We also excluded 5 participants who had CVD events prior to the completion of the baseline visit.

2.3 Baseline clinical evaluation

During the baseline visit (Visit 1) of the study, all MESA participants completed a detailed baseline assessment including detailed history using standardized questionnaires and physical examination. Standardized systolic and diastolic blood pressure measurements and assessment of body mass index were obtained. Fasting blood samples were collected from all participants and stored according to study protocol.[6]

2.4 Omega-3 fatty acid measurements

Plasma omega-3 fatty acids were measured in the majority of MESA participants. We focused on levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

expressed as a percentage of total plasma fatty acids. Individual fatty acids were quantified using EDTA stored plasma collected from participants during Visit 1. Extraction was performed using a chloroform/methanol extraction method.[7] Thin-layer chromatography was used to separate phospholipids fatty acids from free fatty acids, triglycerides and cholesterol esters. After harvesting the phospholipid fatty acid component, individual phospholipid fatty acids were then detected using gas chromatography flame ionization after the fatty acids were derived to methyl esters.[7] The limit of detection for each fatty acid was 0.03%. The inter-assay coefficients of variation were 7.6% for EPA and 8.5% for DHA.

2.5 CAC measurements

Full details on the methods used for CAC measurement in MESA were previously published.[8] In brief, CAC was measured at Visit 1 using cardiac-gated computed tomography. Images were calibrated using phantoms with known calcium density. CAC scores were obtained from two sequential scans within a 2-minute breathing hold in each participant. Images were interpreted at the MESA CT reading center (Lundquist Institute at Harbor-University of California Los Angeles Medical Center, Torrance, CA) and CAC scores were calculated using the Agatston method.

2.6 Definition and ascertainment of events

The primary outcome for this analysis was incident CVD events defined as a composite of incident myocardial infarction, angina, cardiac arrest, stroke or transient ischemic attack, or CVD death. The secondary outcome was incidence of hard CVD events defined as a composite of incident myocardial infarction, cardiac arrest, stroke, coronary heart disease or stroke death. All events were independently adjudicated by two physicians through the MESA Coordinating Center. Data on the primary and secondary outcomes were analyzed as time-to-first event.

2.7 Statistical analysis

Plasma EPA and DHA were studied individually and then in combination (EPA+DHA) by adding together the percentages of EPA and DHA of total plasma fatty acids. Participants were categorized into tertiles of plasma EPA, DHA, or EPA+DHA levels. Participants were also categorized into three clinically relevant CAC categories: CAC score of zero, 1 to 99, and ≥100.

Categorical variables were summarized by their frequency and percentage and compared using Chi-square testing or Fisher’s exact when appropriate. Continuous variables were summarized using means and standard deviations or medians and quartiles when appropriate. Analysis of variance (ANOVA) and the Kruskal-Wallis test were used to compare continuous variables across more than two categories.

Event rates were calculated using the Kaplan-Meier method. Hazard ratios and 95% confidence intervals for the association of omega-3 fatty acid tertiles or CAC category with the primary or secondary outcomes were calculated using Cox proportional-hazard regression models. When omega-3 fatty acid levels were included in the models as a continuous variable, natural log-transformed values were used to improve the normality

of the variable. Graded hazard ratio curves were estimated between plasma omega-3 fatty acid levels and the primary and secondary outcomes using multivariable adjusted modeling with median omega-3 fatty acid values as the reference.

Regression models were adjusted for age, sex, race, statin use, aspirin use, hypertension, diabetes, smoking, family history of coronary heart disease, antihypertensive medication use, body mass index, triglyceride-to-total cholesterol ratio, and omega-3 fatty acid supplements. To compare between omega-3 fatty acids and their association with events, results were expressed per standard deviation of natural log-transformed omega-3 fatty acids. In an exploratory analysis, associations with events were estimated with both natural log-transformed EPA and DHA included in a regression model.

To test whether CAC modifies the association between omega-3 fatty acids and cardiac outcomes we examined the association between omega-3 fatty acid tertiles and outcomes stratified by CAC category (interaction on the additive scale) and tested CAC interaction terms for each of the omega-3 fatty acids (interaction on a multiplicative scale). Key analyses were repeated by sex and race subgroups. A 2-sided *P* value of less than 0.05 was considered statistically significant. All analyses were performed using Stata Statistical Software version 15 (StataCorp LLC, TX).

3. Results

Among the 6568 participants included in this analysis, the mean±SD age was 62.1±10.2 years and 52.9% were women. A total of 817 (12.5%) participants had a history of diabetes mellitus, the median triglyceride level was 111 mg/dL (IQR, 78-161) and 14.8% were on statin therapy.

The median EPA level was 0.67% of total plasma fatty acids (IQR, 0.49-0.99), and the median DHA level was 3.59% of total plasma fatty acids (IQR, 2.72-4.69). The correlation between EPA and DHA levels was strong and statistically significant in the overall sample and by sex and race subgroups (Supplemental Table 1). The range of EPA values (expressed as % of total fatty acids) in EPA tertiles were <0.54%, 0.54% to 0.85%, and >0.85%; DHA values in DHA tertiles were <3.00%, 3.00% to 4.28%, and >4.28%; EPA+DHA values in EPA+DHA tertiles were <3.60%, 3.60% to 5.12%, and >5.12%. Table 1 and Supplemental Table 2 show the baseline characteristics of participants by tertiles of EPA and DHA, respectively.

With regards to CAC distribution, 3294 (50.2%) had a CAC of zero, 1745 (26.6%) had CAC between 1 and 99, and 1529 (23.3%) had CAC ≥100. A CAC of zero was present in 49.6%, 51.2% and 49.7% of those within the low, medium and high EPA tertile groups, and in 49.4%, 50.3%, and 50.8% of those within the low, medium and high DHA tertile groups, respectively. There were no significant differences in EPA, DHA or EPA+DHA levels by baseline CAC category (Supplemental Figure 2).

During a median follow-up period of 15.6 years, 1004 (15.3%) CVD events occurred. A total of 769 (76.6%) CVD events occurred in those with any CAC and 492 (49.0%) CVD events occurred in those with CAC ≥100. CAC category significantly predicted long-term

CVD events (Supplemental Table 3). Compared to those with CAC of zero, those with a CAC of 1 to 99 had a 2.5-fold increase and those with CAC ≥100 had a 6-fold increase in the hazard of developing CVD. These associations remained strong and statistically significant after adjusting for potential confounders. Similar findings were noted with hard CVD events. The cumulative hazard of CVD and hard CVD events by CAC category is seen in Supplemental Figure 3.

A total of 378 (37.7%) CVD events occurred in those with the lowest EPA tertile (event rate, 13.6 per 1000 person-year; 95% CI, 12.3-15.0) compared to 293 (29.2%) CVD events in those with the highest EPA tertile (event rate, 10.1 per 1000 person-year; 95% CI, 9.0-11.3). Similar results were seen for DHA tertiles where 374 (37.3%) of CVD events occurred in the lowest DHA tertile (event rate, 13.3 per 1000 person-year; 95% CI, 12.0-14.7) compared to 305 (30.4%) CVD events in those with the highest DHA tertile (event rate, 10.5 per 1000 person-year; 95% CI, 9.5-11.8). The cumulative incidence of CVD events by EPA and DHA tertiles are seen in Figure 1 (cumulative incidence of hard CVD events seen in Supplemental Figure 4).

When examined as a continuous variable, higher EPA levels were significantly associated with fewer CVD events (HR for loge-transformed EPA, 0.82; 95% CI, 0.74-0.91). Likewise, higher levels of DHA (HR for loge-transformed DHA, 0.84; 95% CI, 0.72-0.99) and EPA+DHA (HR for loge-transformed EPA+DHA, 0.81; 95% CI, 0.69-0.94) were significantly associated with fewer CVD events. The inverse association between omega-3 fatty acid levels and CVD events remained statistically significant after adjusting for potential confounders (Table 2) and when natural $\log_e$-transformed omega-3 fatty acid measurements were indexed to their respective standard deviations (Supplemental Table 4). Similar findings were noted with hard CVD events. In an exploratory analysis, when $\log_e$-transformed EPA and DHA levels were both included in an adjusted regression model, only $\log_e$-transformed EPA levels predicted CVD events but neither $\log_e$-transformed EPA nor DHA levels predicted hard CVD events (Supplemental Table 5). Supplemental Figure 5 illustrates the EPA and DHA graded hazard ratio curves for CVD and hard CVD events.

CVD event rates were compared in omega-3 fatty acid tertiles stratified by CAC categories (Figure 2 and Supplemental Table 6). Those with CAC ≥100 and the lowest EPA tertile had the highest event rates (37.6 events per 1000 person-year; 95% CI, 32.6-43.4), while those with CAC of zero and highest EPA had the least CVD event rates (4.7 events per 1000 person-year; 95% CI 3.7-5.9). Those with in the highest EPA tertile and CAC ≥100 had a higher CVD event rate (24.5 events per 1000 person-year; 95% CI, 20.8-28.9) compared to those with low EPA and CAC of zero (5.0 events per 1000 person-year; 95% CI, 4.0-6.3). CVD event rates in those with CAC of zero remained low regardless of EPA tertile. Similar findings were noted when examining events in DHA or EPA+DHA tertiles stratified by CAC category and when examining hard CVD event rates.

Table 3 shows the multivariable-adjusted hazard ratios for CVD and hard CVD events for EPA and DHA tertiles stratified by CAC category. In patients with CAC of zero, the hazard of CVD events in the highest EPA tertile was not significantly different compared to the lowest tertile (adjusted HR, 1.02; 95% CI, 0.72-1.46). However, when CAC was 1 to 99,

the hazard of CVD events was lower by 29% in the highest EPA tertile compared to the lowest tertile (adjusted HR, 0.71; 95% CI, 0.51-0.99). The relative hazard reduction in CVD events with the highest EPA tertile was more apparent compared to the lowest EPA tertile when CAC was ≥100 (adjusted HR, 0.67; 95% CI, 0.52-0.84). Similarly, the relative hazard reduction in CVD events with higher DHA or EPA+DHA tertiles was more apparent at higher CAC scores. The strength of associations between omega-3 fatty acids tertiles and events were consistent when studying hard CVD events; however, the estimated 95% CI's were wider due to a fewer hard-CVD events, compared with all CVD events.

None of the multiplicative interaction terms for CAC by EPA or DHA tertile were statistically significant for events. Supplemental Table 4 shows the association between omega-3 fatty acids indexed to their standard deviations and CVD events stratified by CAC category. Higher omega-3 fatty acids continued to be significantly associated with lower CVD events after adjusting for CAC, demographic characteristics, comorbid conditions and medication use (Supplemental Table 7).

The main findings of this analysis are consistent numerically across sex categories (Supplemental Tables 8 and 9). Subgroup analysis by race suggests a stronger inverse association between DHA levels and CVD events (adjusted HR, 0.57; 95% CI, 0.38-0.87) as well as hard CVD events (adjusted HR, 0.56; 95% CI, 0.34-0.90) in Black participants compared to other race groups. However, small sample size within some comparison strata limited statistical power especially with hard CVD events. The main findings were consistent after adjusting for the use of omega-3 fatty acid supplements (Supplemental Table 10) or excluding those on omega-3 fatty acid supplements (Supplemental Table 11).

4. Discussion

In the current study, we examined the interplay between plasma omega-3 fatty acids and CAC in relation to long-term CVD events in MESA. In an ethnically diverse population free of clinical CVD, higher plasma omega-3 fatty acid levels were associated with lower long-term CVD events, independent of traditional risk factors. The absolute decrease in long-term events associated with higher plasma omega-3 fatty acids was dependent upon the presence and degree of subclinical atherosclerosis measured by CAC. In participants with CAC of zero, event rates were low and not significantly different across levels of plasma omega-3 fatty acids. However, in those with higher CAC, the absolute decrease in CVD events with higher omega-3 fatty acid levels was significant especially at higher CAC levels (Graphical Abstract).

4.1 Plasma omega-3 fatty acids predict long-term CVD risk

Evidence from clinical trials supports an inverse association between omega-3 fatty acid intake and incident coronary heart disease.[9,10] Due to individual variability in omega-3 fatty acid bioavailability, recent studies have focused on the effect of blood levels of omega-3 fatty acids on CVD risk.[11] In a pooled analysis including 45,637 participants, higher EPA levels predicted lower nonfatal myocardial infarction, and higher DHA predicted lower fatal coronary heart disease.[1] Interestingly, neither EPA nor DHA predicted total coronary heart disease and EPA did not predict fatal coronary heart disease.

Our analysis extends these findings by suggesting a favorable association between EPA and DHA and long-term CVD events in an ethnically diverse population. We demonstrate that higher plasma levels of omega-3 fatty acids were significant predictors of lower CVD events independent of traditional risk factors. Our results support emphasizing intake of fish and other nutrient sources that increase plasma omega-3 fatty acid levels as part of a healthy dietary prescription.[5] Moreover, these results suggest that measuring omega-3 fatty acid levels may be useful in risk stratifying patients without clinical CVD.

We found a stronger inverse association between plasma DHA levels and long-term CVD events among Black participants compared with other racial groups. Prior observational studies have shown similar findings.[1] In the Vitamin D and Omega-3 Trial (VITAL) trial, EPA and DHA supplementation in Black participants was associated with a 77% reduction in myocardial infarction (HR, 0.23; 95% CI, 0.11-0.47) while other racial/ethnic groups had smaller reductions (p-interaction by race = 0.001).[12] These racial differences may be due to genetic variations affecting omega-3 fatty acid metabolism.[13] Black individuals have higher levels of inflammatory biomarkers [14] and may have greater benefit from the anti-inflammatory effects of omega-3 fatty acids.[15] The observed racial differences in our study require further confirmation.

4.2 The additive interaction between omega-3 fatty acids and CAC on events

The presence and extent of subclinical atherosclerosis varies in a population free of clinical CVD.[16] We show that in those with elevated CAC, higher EPA and DHA levels were strongly beneficial. However, significant residual risk persists within the highest EPA or DHA tertiles when CAC is present. Therefore, even when omega-3 fatty acid levels were high, patients with elevated CAC are in need of intensive CVD risk reduction through optimal lifestyle changes and guideline-directed medical therapy when appropriate. A CAC of zero identifies a population at low risk of long-term CVD events regardless of plasma omega-3 fatty acid levels. These findings may have important implications if omega-3 fatty acid therapies are to be considered in primary prevention.

4.3 Randomized controlled trials of omega-3 fatty acids show conflicting outcomes

Trials of omega-3 fatty acids report conflicting outcomes with most trials reporting no or little benefit in patients without clinical CVD.[17] In both the A Study of Cardiovascular Events in Diabetes (ASCEND) and VITAL trials, EPA and DHA supplementation (840 mg/day) had no benefit compared with placebo in patients without clinical CVD.[12,18] The Outcomes Study to Assess STatin Residual Risk Reduction With EpaNova in HiGh CV Risk PatienTs With Hypertriglyceridemia (STRENGTH) trial showed no benefit with high-dose EPA and DHA (4 g/day) on CVD outcomes in patients with and without clinical CVD.[19]

By contrast, the Japan EPA Lipid Intervention Study (JELIS) enrolled patients with and without clinical CVD and demonstrated 19% reduction in major coronary events with of open-label EPA (1.8 g/day) compared to no EPA.[20] The Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) trial demonstrated a significant 25% reduction in ischemic events in patients with hypertriglyceridemia randomized to high-dose EPA (4 g/day) compared with placebo.[21] Achieved EPA levels

were higher in JELIS (166 μg/mL) and REDUCE-IT (144 μg/mL) than in other trials. Moreover, in both JELIS and REDUCE-IT, EPA levels correlated with CVD benefit.[22,23] REDUCE-IT also enrolled patients with more risk factors for subclinical CVD with diabetes, hypertriglyceridemia and a third CVD risk factor present in all of the primary prevention cohort.[21] As a result, subjects without clinical CVD enrolled in REDUCE-IT and randomized to the control arm had more frequent events (13.6%) than their counterparts in STRENGTH (2.8%), JELIS (1.7%) and other primary prevention trials (3.2% in VITAL, 9.2% in ASCEND).[12,18,20].

4.4 Baseline risk and blood omega-3 fatty acids may explain conflicting trial results

When the results of clinical trials are taken together in light of our findings, the presence and degree of subclinical atherosclerosis in clinical trial participants without overt CVD may have determined the absolute change in event rates seen with higher levels of omega-3 fatty acids. Since subclinical atherosclerosis measured by CAC has been shown to identify patients at higher risk for events more effectively than other imaging tests or blood biomarkers,[4,24] future trials of omega-3 fatty acids for primary CVD prevention should consider enrolling high-risk patients based on CAC to enrich for CVD events.

When interpreting the results of primary CVD prevention trials using omega-3 fatty acids, our findings highlight the importance of considering differences in achieved omega-3 fatty acid levels as an independent predictor of future CVD events. Differences in achieved blood levels may be due to variations in the dose of omega-3 fatty acids used in clinical trials or the variability in omega-3 fatty acid metabolism between study subjects.[11] Taken together, future primary CVD prevention trials using omega-3 fatty acids should consider measuring plasma omega-3 fatty acids as a strategy to confirm adequate supplementation and account for between-individual differences in omega-3 fatty acid metabolism.

4.5 Linking omega-3 fatty acids, subclinical atherosclerosis, and CVD events

The mechanisms by which omega-3 fatty acids exert their benefits remain unknown. It is likely that the beneficial effects of omega-3 fatty acids work through multiple pathways.[25] Our finding that the association of omega-3 fatty acids is affected by the presence and degree of subclinical atherosclerosis (CAC) is hypothesis generating. Prior studies suggest a beneficial effect of omega-3 fatty acids on coronary plaque progression and stabilization.[26-28] Omega-3 fatty acids also have other potential non-plaque-mediated beneficial effects including significant reduction in blood triglycerides, antiplatelet aggregatory effects, improved arterial stiffness and heart rate variability, and improved physical function.[21,29-33]

4.6 Strengths and limitations

Our study has important strengths. First, our sample size is large, with a large number of events in a well-phenotyped multi-ethnic population. Second, we used plasma omega-3 fatty acids rather than the participant-reported intake of omega-3 fatty acids. Plasma omega-3 fatty acid measurement is more reliable and takes into account factors which may affect the bioavailability of dietary omega-3 fatty acids.[11] Furthermore, plasma omega-3 fatty acid levels have been shown to reflect long-term omega-3 fatty acid intake.[34]

Our study also has certain limitations. First, we could not fully differentiate the CVD effects of EPA from DHA due to their tight correlation. However, in an exploratory analysis adjusting for both EPA and DHA levels, our data suggest that plasma EPA level may be a better independent predictor of CVD events. The beneficial effects of omega-3 fatty acids on CVD has been proposed to be mainly due to EPA.[25] Second, we could not determine the effect of omega-3 fatty acid levels by CAC on events within groups according to diabetes, hypertriglyceridemia status due to small category sample size. Third, only 14.8% of participants in this study were on statin therapy at baseline and 4.1% were on omega-3 fatty acid supplements, limiting generalizability to these groups. Finally, due to the observational nature of this study, unmeasured or residual confounding cannot be excluded.

4.7 Conclusion

In an ethnically diverse population free of clinical CVD, we observed fewer long-term CVD events in those with higher plasma omega-3 fatty acids levels independent of measured confounders and subclinical atherosclerosis measured by CAC. The absolute decrease in long-term CVD events with higher omega-3 fatty acid levels was more apparent at higher CAC scores. This important interplay between omega-3 fatty acid levels and subclinical atherosclerosis in relation to CVD events offers a potential explanation for why clinical trials of omega-3 fatty acids yield inconsistent results for primary prevention. Future investigators should take these results into consideration when designing a primary CVD prevention trial of omega-3 fatty acids.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Higher plasma omega-3 fatty acids (EPA and/or DHA) significantly predict long-term CVD events independent of traditional CVD risk factors.
- When CAC is present, higher plasma omega-3 fatty acids are associated with lower CVD-events.
- Significant residual CVD risk exists in patients with elevated CAC even when plasma omega-3 fatty acid levels are high.

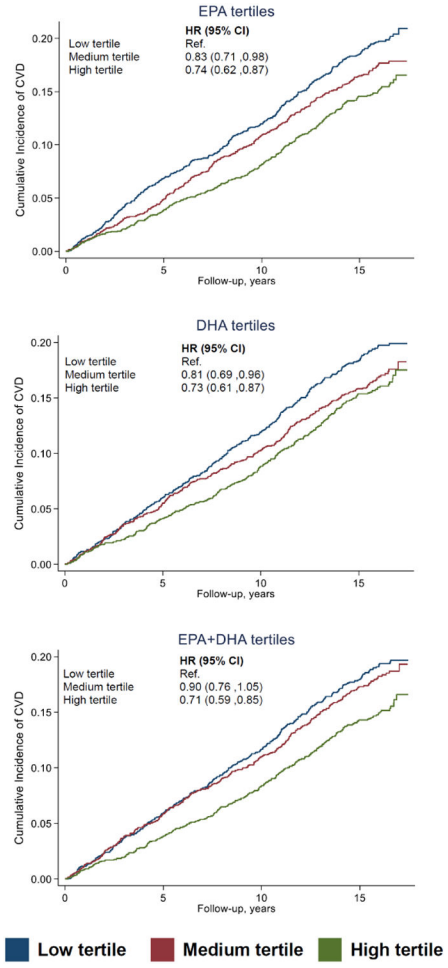

Figure 1. Cumulative hazard of cardiovascular disease events by EPA, DHA and EPA+DHA tertiles.

Hazard ratios were calculated using Cox regression models and are adjusted for demographic and cardiovascular risk factors and medication use.

EPA=eicosapentaenoic acid; DHA=docosahexaenoic acid.

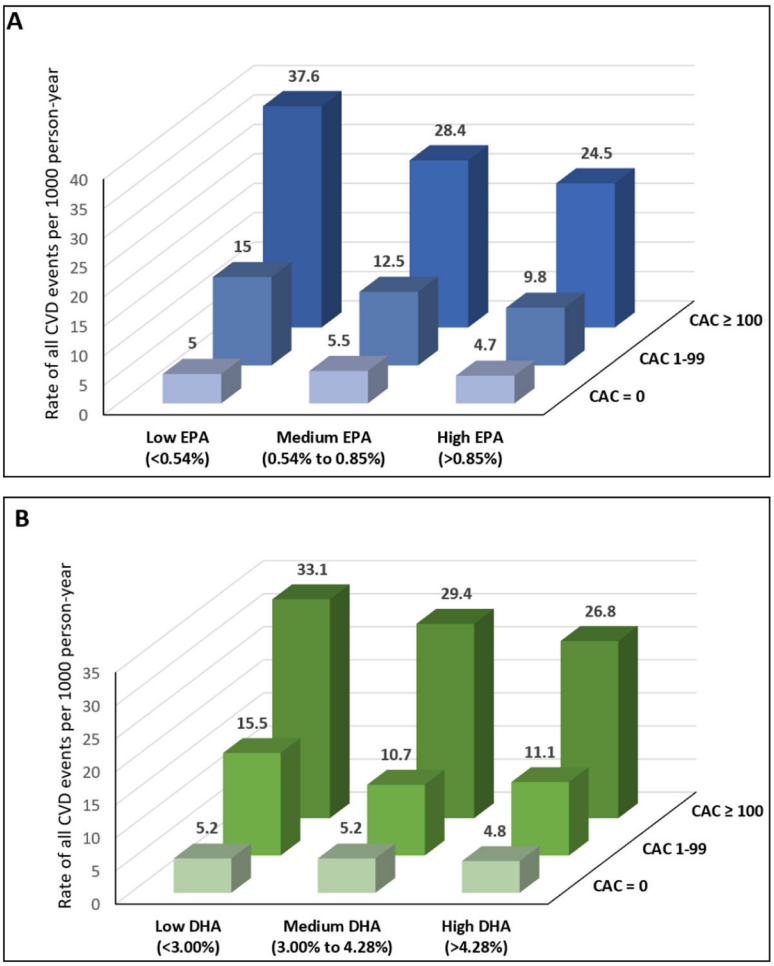

Figure 2. Cardiovascular event rates per 1000 person-year by CAC category and tertiles of EPA (A) and DHA (B).

CAC = coronary artery calcium score; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

Table 1.

Baseline characteristics by tertiles of plasma eicosapentaenoic acid

	Total n=6568	Low EPA tertile n=2197	Medium EPA tertile n=2183	High EPA tertile n=2188	*p* value
Age, mean (SD)	62.1 (10.2)	61.6 (10.5)	61.8 (10.2)	62.9 (10.0)	<0.001
Female sex, n (%)	3472 (52.9%)	1,103 (50.2%)	1,158 (53.1%)	1,211 (55.4%)	0.003
BMI, kg/m^2, mean (SD)	28.3 (5.5)	28.6 (5.6)	28.8 (5.6)	27.6 (5.2)	<0.001
Total cholesterol, mg/dL, mean (SD)	194.1 (35.7)	191.6 (37.4)	195.3 (35.2)	195.5 (34.2)	<0.001
Triglycerides, mg/dL, median (IQR)	111 (78, 161)	115 (80, 162)	115 (80, 169)	104 (73, 152)	<0.001
HDL-C, mg/dL, mean (SD)	51.0 (14.9)	48.6 (13.8)	50.9 (15.2)	53.5 (15.2)	<0.001
LDL-C, mg/dL, mean (SD)	117.1 (31.3)	116.3 (31.9)	117.5 (31.0)	117.6 (31.1)	0.321
Hypertension, n (%)	2,936 (44.7%)	934 (42.5%)	1,021 (46.8%)	981 (44.8%)	0.018
Diabetes, n (%)	817 (12.5%)	295 (13.5%)	276 (12.7%)	246 (11.3%)	0.081
Race, n (%)					<0.001
White	2531 (38.5%)	755 (34.4%)	887 (40.6%)	889 (40.6%)	
Chinese	794 (12.1%)	179 (8.2%)	194 (8.9%)	421 (19.2%)	
Black	1799 (27.4%)	517 (23.5%)	670 (30.7%)	612 (28.0%)	
Hispanic	1444 (22.0%)	746 (34.0%)	432 (19.8%)	266 (12.2%)	
Smoking status, n (%)					<0.001
Never	3294 (50.3%)	1059 (48.3%)	1051 (48.3%)	1184 (54.3%)	
Former	2395 (36.6%)	798 (36.4%)	808 (37.2%)	789 (36.2%)	
Current	858 (13.1%)	336 (15.3%)	315 (14.5%)	207 (9.5%)	
Medications, n (%)					
ACE-inhibitors	775 (11.8%)	271 (12.4%)	277 (12.7%)	227 (10.4%)	0.038
Beta-blockers	589 (9.0%)	174 (7.9%)	189 (8.7%)	226 (10.4%)	0.017
Any Lipid lowering therapy	1058 (16.1%)	240 (10.9%)	376 (17.3%)	442 (20.2%)	<0.001
Statins	968 (14.8%)	213 (9.7%)	349 (16.0%)	406 (18.6%)	<0.001
Other					
CAC, mean (SD)	143.0 (406.8)	141.0 (420.4)	147.8 (414.4)	140.1 (384.9)	0.742
Omega-3 fatty acid supplements	249 (4.0%)	38 (1.9%)	43 (2.2%)	168 (8.3%)	<0.001

EPA tertiles were defined according to the following values expressed as % EPA of total fatty acids: Low tertile (<0.54%), middle tertile (0.54% to 0.85%), high tertile (>0.85%).

EPA = eicosapentaenoic acid; BMI = body mass index; HDL-C = high-density cholesterol; LDL-C = low-density cholesterol; ACE = angiotensin converting enzyme; CAC = coronary artery calcium score.

Table 2.

Association of omega-3 fatty acids with CVD and hard-CVD events

	Unadjusted		Adjusted [a]	
	Hazard Ratio (95% CI)	***p* value**	**Hazard Ratio (95% CI)**	***p* value**
All atherosclerotic cardiovascular disease events				
Log_e (EPA)	0.82 (0.74-0.91)	<0.001	0.83 (0.74-0.94)	0.002
Log_e (DHA)	0.84 (0.72-0.99)	0.032	0.79 (0.66-0.96)	0.017
Log_e (EPA+DHA)	0.81 (0.69-0.94)	0.006	0.78 (0.65-0.93)	0.007
Hard Atherosclerotic cardiovascular disease events				
Log_e (EPA)	0.81 (0.72-0.92)	0.001	0.85 (0.74-0.97)	0.020
Log_e (DHA)	0.82 (0.68-0.99)	0.034	0.78 (0.63-0.98)	0.031
Log_e (EPA+DHA)	0.79 (0.66-0.95)	0.010	0.78 (0.63-0.96)	0.022

[a]The multivariable model adjusted for age, sex, race, statin use, aspirin use, hypertension, diabetes, smoking, family history of coronary heart disease, anti-hypertensive medication use, body mass index and triglyceride to cholesterol ratio.

CVD = cardiovascular disease; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

Table 3.

Multivariable-adjusted hazard ratios of CVD and hard-CVD events for omega-3 fatty acid tertiles by coronary artery calcium category

	CAC=0 HR (95% CI)	CAC 1-99 HR (95% CI)	CAC ≥100 HR (95% CI)
All cardiovascular disease events			
EPA tertile			
Low	**Ref**	**Ref.**	**Ref.**
Medium	1.18 (0.84-1.65)	0.85 (0.62-1.15)	0.69 (0.55-0.87)
High	1.02 (0.72-1.46)	0.71 (0.51-0.99)	0.67 (0.52-0.84)
DHA tertile			
Low	**Ref**	**Ref.**	**Ref.**
Medium	1.05 (0.74-1.49)	0.67 (0.49-0.93)	0.81 (0.64-1.02)
High	0.96 (0.65-1.42)	0.68 (0.48-0.95)	0.73 (0.57-0.94)
EPA+DHA tertile			
Low	**Ref**	**Ref.**	**Ref.**
Medium	1.35 (0.95-1.92)	0.79 (0.58-1.08)	0.84 (0.66-1.05)
High	0.97 (0.65-1.44)	0.62 (0.44-0.88)	0.72 (0.56-0.92)
Hard cardiovascular disease events			
EPA tertile			
Low	**Ref**	**Ref**	**Ref**
Medium	1.11 (0.77-1.62)	0.80 (0.56-1.16)	0.75 (0.57-0.99)
High	1.07 (0.73-1.58)	0.69 (0.47-1.02)	0.76 (0.57-1.02)
DHA tertile			
Low	**Ref**	**Ref**	**Ref**
Medium	1.11 (0.76-1.63)	0.63 (0.43-0.92)	0.84 (0.63-1.11)
High	0.98 (0.64-1.51)	0.62 (0.42-0.93)	0.80 (0.59-1.09)
EPA+DHA tertile			
Low	**Ref**	**Ref**	**Ref**
Medium	1.38 (0.94-2.02)	0.71 (0.49-1.03)	0.83 (0.63-1.09)
High	0.95 (0.61-1.48)	0.55 (0.37-0.84)	0.77 (0.57-1.04)

EPA tertiles were defined according to the following values expressed as % EPA of total fatty acids: Low, <0.54%; middle, 0.54% to 0.85%; high >0.85%. DHA tertiles were defined according to the following values expressed as % DHA of total fatty acids: Low, <3.00%; middle 3.00% to 4.28%; high, >4.28%). EPA+DHA tertiles were defined according to the following values expressed as % EPA+DHA of total fatty acids: Low, <3.60%; medium, 3.60% to 5.12%; high, >5.12%. Multivariable model adjusted for age, sex, race, statin use, aspirin use, hypertension, diabetes, smoking, family history of coronary heart disease, anti-hypertensive medication use, body mass index and triglyceride to total cholesterol ratio.

CAC = coronary artery calcium score; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.