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An omega-3 fatty acid plasma index ≥4% prevents progression of coronary artery plaque in patients with coronary artery disease on statin treatment

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HIGHLIGHTS

- An omega-3 index < 3.43% predicts coronary plaque progression despite statin therapy.
- An omega-3 index ≥4% prevents coronary plaque progression in nondiabetics on statin.
- An omega-3 index ≥4% prevents progression of all coronary plaque subtypes.
- EPA and DHA increased plasma levels variably from 1.85% to 13.02%.
- Omega-3 fatty acid plasma level predicts benefit better than treatment assignment.

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ABSTRACT

Background and aims: Higher blood levels of the omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been associated with fewer cardiovascular events and lower mortality in prospective studies. Our aim was to determine a target level of EPA and DHA to prevent progression of coronary artery plaque.

Methods: 218 subjects with stable coronary artery disease on statins were randomized to high-dose EPA and DHA (3.36 g daily) or no omega-3 for 30 months. Coronary plaque volume was measured by coronary computed tomographic angiography. Plasma phospholipid levels of EPA, DHA and total fatty acids were measured by gas chromatography mass spectrometry. The omega-3 fatty acid index was calculated as EPA + DHA/total fatty acid.

Results: Mean (SD) age was 62.9 (7.8) years; mean (SD) LDL-C level 78.6 (27.3) mg/dL and median triglyceride level 122 mg/dL. Subjects assigned to EPA and DHA had increased plasma EPA and DHA levels variably from 1.85% to 13.02%. Plasma omega-3 fatty acid index ≥4% prevented progression of fibrous, noncalcified, calcified and total plaque in nondiabetic subjects whereas those in the lowest quartile (< 3.43%) had significant progression of fibrous, calcified and total plaque. No difference was observed in diabetic subjects.

Conclusions: EPA and DHA added to statins prevented coronary plaque progression in nondiabetic subjects with mean LDL-C < 80 mg/dL, when an omega-3 index ≥4% was achieved. Low omega-3 index < 3.43% identified nondiabetic subjects at risk of coronary plaque progression despite statin therapy. These findings highlight the importance of measuring plasma levels of omega-3 fatty acids early and at trial conclusion. Targeting an omega-3 index ≥4% maximizes cardiovascular benefit.

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1. Introduction

A high level of residual risk of atherosclerotic plaque progression and cardiovascular disease (CVD) events remains despite achieving low-density-lipoprotein cholesterol (LDL-C) levels ≤ 70 mg/dL with statin treatment [1–3]. Therefore, additional modalities to reduce residual risk are needed. Omega-3 fatty acids are long-chain, polyunsaturated fatty acids and include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Prospective epidemiologic studies have shown that plasma levels of omega-3 fatty acids predict CVD and outcomes. In the observational Multi-Ethnic Study of Atherosclerosis, the highest quartiles of plasma EPA and DHA levels were associated with a hazard ratio of 0.49 and 0.39, respectively, for incident CVD compared with the lowest quartile [4]. In the Cardiovascular Health Study, a prospective cohort study of 2692 adults free of coronary artery disease at baseline, high plasma levels of EPA and DHA were associated with a 27% reduction in total mortality due to fewer cardiovascular deaths (hazard ratio, 0.73; 95% confidence interval [CI], 0.61–0.86, p for trend ≤0.008) [5]. In a pooled analysis of 19 international prospective or retrospective studies of 45,637 individuals for primary prevention of coronary heart disease, in continuous (per 1-SD increase) multivariable-adjusted analyses, the highest quintile of EPA was associated with a 29% reduction in nonfatal myocardial infarction (relative risk, 0.71; 95% CI, 0.56–0.90) and highest quintile of DHA was associated with a 23% reduction in fatal coronary heart disease (relative risk, 0.77; 95% CI, 0.64–0.89) [6]. Furthermore, in 2500 participants from the Framingham Heart Study already on a statin, the erythrocyte omega-3 fatty acid index was better than total cholesterol in predicting development of coronary heart disease, stroke and total mortality [7]. These epidemiologic findings support a beneficial relationship between EPA and DHA levels and health outcomes.

Although two randomized clinical trials with low-dose (1 g) omega-3 fatty acid supplementation showed benefit on CVD events [8–10], several subsequent trials with 1 g in the statin-era reported no benefit [11–15]. In contrast, with a higher dose of omega-3 fatty acid, the Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) reported a 25% reduction in the primary endpoint of a composite of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization or unstable angina in statin-treated subjects with a median triglyceride level of 216.5 mg/dL in those randomized to 4 g of ethyl eicosapentaenoate, a derivative of EPA, compared to a mineral oil placebo [16]. We previously reported that statin-treated subjects with stable coronary artery disease and normal triglyceride levels (median 122 mg/dL) randomized to high-dose (3.36 g daily) EPA and DHA and adherent to therapy had no change in coronary artery plaque progression compared to 5% progression in those not receiving EPA and DHA (p = 0.018) [17]. Herein, we report the results of the clinical trial based on plasma levels of EPA and DHA achieved. Our goal was to determine a target plasma level of omega-3 fatty acid associated with the prevention of coronary artery plaque progression.

2. Patients and methods

2.1. Study design

We analyzed data from the Slowing HEART diSease with lifestyle and omega-3 fatty acids (HEARTS) trial, a randomized, controlled, parallel clinical trial conducted at Beth Israel Deaconess Medical Center (BIDMC), Boston, MA. The study design has been described previously [17]. All subjects signed an informed consent, and the protocol was approved by the BIDMC Institutional Review Board. The results of the primary end point of the trial, namely, the effect of omega-3 fatty acid supplementation on progression of coronary artery plaque at 30 months of follow-up, have been reported [17].

2.2. Participants and study intervention

The trial included subjects aged 21–80 years who had stable coronary artery disease as defined previously [17]. Subjects were randomized to either open-label, omega-3 ethyl esters (Lovaza), 465 mg EPA and 375 mg DHA per capsule, four capsules daily for a total daily dose of 1.86 g EPA and 1.5 g DHA or no Lovaza (control) for 30 months. Statins and aspirin were recommended to all subjects. Subjects were instructed not to take over-the-counter omega-3 fatty acid supplementation. During each visit, a detailed medication list was obtained to detect the use of over-the-counter omega-3 fatty acids.

2.3. Outcomes and data collection

At baseline and 6-month intervals, a detailed history, physical examination, height, weight, waist measurement and blood pressure measurement were obtained. Blood samples were collected after a 12-h fast. Lipid panel, chemical profile, glucose, hemoglobin A1c (HbA1c), white blood cell count (WBC) and absolute neutrophil, lymphocyte, monocyte and platelet counts were measured at Quest Diagnostics (Cambridge, MA). High-sensitivity C-reactive protein (hs-CRP) was measured by immunoturbimetric assays using an automated, standardized, high throughput method with coefficient of variation below 5% at Boston Heart Diagnostics (Framingham, MA).

2.4. Measurement of fatty acid levels

Fatty acid profiles (EPA, DHA, arachidonic acid [AA] and total fatty acids) of total plasma phospholipids were assayed after plasma serum lipid extraction and separation of phospholipids by thin layer chromatography using gas liquid chromatography under contract by Nutrasource Diagnostics (Guelph, Canada) at Boston Heart Diagnostics as previously described [18,19]. The omega-3 fatty acid index was calculated as the percentage of EPA and DHA of total fatty acid level.

2.5. Image acquisition, reconstruction and coronary plaque analysis

Prospective electrocardiogram gated imaging was performed at BIDMC at baseline and 30-month follow-up using a 320-row detector scanner (Aquilion ONE, Toshiba Medical Systems, Otawara, Japan). The protocol for performance of coronary computed tomographic angiography, plaque identification and quantification has been previously published [17,20,21]. Semiautomated software (SUREPlaque, version 6.3.2, Vital Images, Minnetonka, MN, USA) was used to reconstruct 3-dimensional coronary computed tomography angiographic images for coronary segment plaque volume analysis [22–25]. Image acquisition details and representative images are shown in Supplement 2 of reference 21. Segments with prior revascularization or significant calcification causing calcium-bloom artifact were excluded. To ensure measurement of the same segment at 30-month follow-up, focal calcification or branches served as fiducial markers. Independent plaque analysis was performed by two readers blinded to treatment assignment. Hounsfield unit (HU) densities were used to define plaques as fatty (−100 to 49 HU), fibrous (50–150 HU) and calcified plaque > 150 HU [17]. Noncalcified plaque was measured as the sum of fatty and fibrous plaque. The intra-observer and inter-observer agreement indexes were 0.99 and 0.98, respectively, showing excellent correlation between readings [17]. Plaque volume was indexed to the length of the vessel examined and expressed in mm^3/mm.

2.6. Statistical analysis

Categorical variables were expressed as counts and percentages and were compared with either Chi-square or Fisher's exact tests. Normality tests were conducted using the Shapiro-Wilk test. Continuous variables were reported as the mean and standard deviation (SD) for normally

Table 1
Baseline characteristics stratified by omega-3 index group and diabetes status.

Characteristics	Omega-3 Index at 30 months		*p* value	Nondiabetics	Diabetics	*p* value
	< 4%	≥4%				
	(n = 85)	(n = 133)		(n = 154)	(n = 64)	
Control, No. (%)	70 (82.4)	29 (21.8)	< 0.001	69 (44.8%)	30 (46.9%)	0.780
Lovaza, No. (%)	15 (17.6)	104 (78.2)		85 (55.2%)	34 (53.1%)	
Age, mean (SD), y	62.1 (7.7)	63.5 (7.8)	0.219	62.8 (8.1)	63.2 (7.2)	0.782
Male, No. (%)	72 (84.7)	110 (82.7)	0.698	131 (85.1%)	51 (79.7%)	0.330
Inclusion criteria (may have more than one), No. (%)						
History of MI	41 (48.2)	58 (43.6)	0.503	70 (45.5%)	29 (45.3%)	0.985
PTCA	51 (60.0)	83 (62.4)	0.722	92 (59.7%)	42 (65.6%)	0.416
CABG	28 (32.9)	26 (19.5)	0.025	39 (25.3%)	15 (23.4%)	0.769
Cardiovascular risk factors, No. (%)						
Hypertension	75 (88.2)	103 (77.4)	0.045	122 (79.2%)	56 (87.5%)	0.150
Diabetes	30 (35.3)	34 (25.6)	0.124	0 (0.0%)	64 (100.0%)	NA
Anthropometric and Blood Pressure, mean (SD)						
Weight, kg	90.6 (14.6)	91.0 (13.3)	0.834	89.2 (12.6)	94.7 (15.8)	0.008
Body mass index, kg/m^2	30.7 (3.7)	30.3 (3.5)	0.479	30.0 (3.4)	31.5 (3.7)	0.004
Waist circumference, cm	106.7 (10.2)	105.7 (10.2)	0.502	104.7 (9.5)	109.6 (11.1)	0.001
Systolic BP, mmHg	122.5 (13.8)	124.3 (14.8)	0.369	122.9 (13.7)	125.2 (16.0)	0.290
Diastolic BP, mmHg	72.1 (9.1)	73.3 (10.1)	0.379	73.6 (9.3)	71.10 (10.5)	0.088
Complete blood count, mean (SD)						
WBC, 10^9 cells/L	6.7 (1.8)	6.5 (1.6)	0.260	6.6 (1.7)	6.5 (1.6)	0.885
Monocytes, cells/μL	545.3 (186.2)	515.0 (158.7)	0.199	529.7 (162.7)	519.9 (188.2)	0.701
Neutrophils, cells/μL	4325.2 (1499.4)	4061.2 (1439.8)	0.195	4206.1 (1571.6)	4063.2 (1177.1)	0.513
Lymphocytes, cells/μL	1631.2 (544.6)	1689.2 (632.0)	0.487	1625.7 (562.4)	1764.9 (673.1)	0.118
Platelets, cells/μL	194.9 (46.2)	187.7 (53.4)	0.308	188.1 (50.6)	196.1 (51.3)	0.293
Lipids, mean (SD)						
Total cholesterol, mg/dL	151.3 (39.7)	154.3 (33.7)	0.547	153.7 (35.4)	151.9 (38.0)	0.746
Triglyceride, mg/dL	120.0 [79.0, 165]	119.5 [81.3, 177.3]	0.908	119.0 [78.0, 163.0]	126.0 [85.5189.5]	0.429
HDL-C, mg/dL	45.8 (14.8)	48.7 (15.0)	0.172	48.7 (15.4)	44.9 (13.7)	0.091
LDL-C, mg/dL	78.0 (29.7)	78.9 (25.8)	0.822	78.7 (26.4)	78.1 (29.6)	0.885
Biochemical profile, mean (SD)						
Glucose, mg/dL	109.7 (35.2)	103.3 (27.0)	0.160	93.2 (10.4)	136.0 (40.5)	< 0.001
HbA1c, %	6.3 (1.1)	6.1 (.8)	0.151	5.8 (0.3)	7.1 (1.1)	< 0.001
Medication, No. (%)						
Statin	78 (91.8)	130 (97.7)	0.050	149 (96.8%)	59 (92.2%)	0.142
Aspirin	80 (94.1)	129 (97.0)	0.316	148 (96.1%)	61 (95.3%)	0.789
ACE-I	50 (58.8)	68 (51.1)	0.266	82 (53.2%)	36 (56.3%)	0.685
ARB	16 (18.8)	26 (19.5)	0.895	22 (14.3%)	20 (31.3%)	0.004
Hydrochlorothiazide	17 (20.0)	22 (16.5)	0.516	28 (18.2%)	11 (17.2%)	0.862
Furosemide	9 (10.6)	10 (7.5)	0.433	8 (5.2%)	11 (17.2%)	0.004
Calcium channel blocker	15 (17.6)	33 (24.8)	0.213	34 (22.1%)	14 (21.9%)	0.974
Beta blockers	61 (71.8)	95 (71.4)	0.957	112 (72.7%)	44 (68.8%)	0.553

ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; CABG, coronary artery bypass grafting; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; PCI, percutaneous coronary intervention; WBC, white blood cell count.
Values expressed as mean (SD) except triglyceride which is median [interquartile range].
To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129.

distributed variables or median and interquartile range [IQR] for non-normally distributed variables. Continuous variables were compared using unpaired (between group comparisons) and paired t-tests (within group comparisons) for normally distributed variables or the Mann-Whitney-U test (between group comparisons) and Wilcoxon signed rank test (within group comparisons) for non-normally distributed variables. Those with an omega-3 index < 4% were compared to those with an index ≥4%. The sample was then divided into quartiles of the plasma omega-3 fatty acid index, levels of EPA, DHA and EPA plus DHA and the EPA to AA ratio. The percent change in plaque volume in various quartiles was compared to the lowest quartile using the Mann-Whitney *U* test. All analyses were stratified by diabetes status. A 2-sided *p* value ≤ 0.05 was considered statistically significant. Data analyses were performed using SPSS 20.0 (IBM Corp. Armonk, NY).

3. Results

A total of 218 subjects had plasma levels of fatty acids and coronary plaque measured at baseline and 30-month follow-up. As shown in Table 1, hypertension and coronary artery bypass graft (CABG) surgery were significantly less prevalent, and statin use was higher at baseline in those with an omega-3 index ≥4% compared to < 4% at 30 months. Diabetic subjects had a higher body mass index and waist circumference, higher levels of glucose and HbA1c and were more likely to receive an angiotensin receptor blocker or furosemide compared to nondiabetic subjects (Table 1). Therefore, we stratified results for change in coronary plaque by diabetes status. As shown in Table 2 for the total group, those with an omega-3 fatty acid index ≥4% had significant prevention of progression of fibrous plaque compared to those with an omega-3 index < 4% ($p = 0.011$).

When stratified by diabetes status, the nondiabetic subjects with an omega-3 index ≥4% had significant prevention of progression of fibrous, noncalcified, calcified and total plaque volume compared to those with an omega-3 index < 4% (Table 2). In contrast, no difference in plaque change was observed for those with diabetes.

We next examined plaque change stratified by quartiles of the plasma omega-3 fatty acid measures to estimate a more precise cutpoint for benefit. Those in the highest quartile of the omega-3 fatty acid index

Table 2
Percent change in plaque volume stratified by omega-3 fatty acid index of 4% at 30 months.

Plaque subtype	Omega-3 Fatty Acid Index		*p* value
	< 4%	≥4%	
	Median [IQR]	Median [IQR]	
Total group	**n = 85**	**n = 133**	
Fatty	3.1 [-10.8, 15.8]	0.8 [-10.1, 16.5]	0.885
Fibrous	6.6 [-6.8, 25.4]	−0.4 [-11.9, 14.6]	0.011
Noncalcified	5.0 [-7.6, 20.7]	−1.4 [-9.7, 14.4]	0.078
Calcified	53.1 [4.2, 176.7]	47.4 [-0.4, 127.2]	0.418
Total	9.9 [-4.2, 28.2]	7.6 [-2.9, 18.8]	0.331
Nondiabetic subjects	**n = 55**	**n = 99**	
Fatty	5.3 [-8.6, 22.9]	0.8 [-10.5, 16.7]	0.508
Fibrous	10.3 [-2.0, 27.7]	−0.4 [-11.0, 14.7]	< 0.001
Noncalcified	10.9 [-3.9, 27.1]	−1.4 [-10.2, 14.4]	0.004
Calcified	96.3 [17.3, 258.2]	49.8 [-4.2, 128.9]	0.030
Total	17.6 [3.1, 37.2]	7.7 [-6.7, 19.0]	0.003
Diabetic subjects	**n = 30**	**n = 34**	
Fatty	−1.4 [-18.7, 8.6]	1.0 [-8.4, 16.6]	0.339
Fibrous	−0.8 [-15.7, 10.3]	−0.6 [-12.7, 14.7]	0.861
Noncalcified	−1.6 [-14.2, 11.0]	−0.9 [-9.3, 16.1]	0.501
Calcified	25.7 [-31.2, 78.6]	37.5 [15.3, 97.8]	0.148
Total	−3.4 [-15.2, 15.6]	6.2 [-2.5, 20.2]	0.082

at 30 months (Table 3) had a significantly lower percentage of diabetes and higher use of statin at baseline. Those in the highest quartile of EPA (Supplementary Table 1) were less likely to have history of CABG surgery and diabetes and more likely to have higher levels of total cholesterol and high-density lipoprotein cholesterol at baseline. Female sex was more prevalent and both total cholesterol and triglyceride levels were higher in those in the highest quartile of EPA and DHA (Table 4) and highest DHA quartile (Supplementary Table 2) at baseline. Those in the highest quartile of EPA to AA were less likely to have a history of hypertension (Supplementary Table 3). Of note, 12 and 3 subjects assigned to control were in the 3rd and 4th quartile of omega-3 fatty acid index, respectively; however, they were taking over-the-counter omega-3 fatty acids which could account for this finding (Table 3). Subjects assigned to Lovaza increased plasma EPA and DHA levels variably from 1.85% to 13.02%. Fifteen subjects assigned to Lovaza had an omega-3 fatty acid index < 4%. (Table 1). Of these, two stopped Lovaza early in the trial due to intolerance.

No significant difference in change in plaque subtypes was observed for the total group (Supplementary Table 4) or for diabetic subjects (Supplementary Table 5). In contrast, nondiabetic subjects in the 3rd quartile (5.41%–7.66%) and 4th quartile (≥7.67%) of omega-3 fatty acid index had significant prevention of progression of fibrous, noncalcified, calcified (3rd quartile compared to lowest) and total plaque compared to those in the lowest quartile (< 3.43%) who had significant within group progression at 30 months compared to baseline (Table 5A). Those in the 2nd quartile of the omega-3 fatty acid index (3.43%–5.40%) had significant prevention of progression of fibrous plaque and a trend for noncalcified and total plaque compared to the lowest quartile. Nondiabetic subjects in the highest quartile of EPA (Table 5B), EPA and DHA (Table 5D) and EPA to AA ratio (Table 5E) had significant prevention of progression of fibrous, noncalcified and total plaque compared to those in the lowest quartile. Compared to those in the lowest quartile, those in the 3rd quartile of EPA and DHA had significant prevention of fibrous, noncalcified and total plaque whereas those in the 3rd quartile of EPA alone had prevention of only fibrous plaque at a much lower level of significance ($p = 0.05$).

The benefit for EPA plus DHA was similar to that for the omega-3 index and stronger than EPA alone. Of note, there was no difference in plaque change by DHA quartile for the nondiabetic subjects (Table 5C).

Fig. 1 compares the median change in plaque volume in nondiabetic subjects with an omega-3 fatty acid index < 3.4% compared to ≥4%. Compared to those with an omega-3 fatty acid index < 3.4%, those with an index ≥4% had significantly lower median percent change of fibrous, noncalcified, calcified and total plaque volumes. The percent change in fatty plaque volume was nominally lower in the ≥4% group compared to < 3.4%, but the difference did not reach statistical significance.

4. Discussion

In the current study, several important new findings emerged. First, in the total group, those with an omega-3 fatty acid index ≥4% had significant prevention of progression of fibrous coronary plaque compared to an omega-3 fatty acid index < 4% ($p = 0.011$). When stratified by diabetes status, the results became more significant: nondiabetic subjects with an omega-3 fatty acid index ≥4% had significant prevention of progression of fibrous, noncalcified, calcified and total coronary plaque compared to those with an omega-3 fatty acid index < 4%. In contrast, no difference in plaque change was observed for those with diabetes. We next examined coronary plaque change by quartiles of omega-3 fatty acids to determine a more precise cutpoint for differences in plaque change. Nondiabetic subjects with an omega-3 fatty acid index < 3.43% had significant within group progression of fibrous, calcified and total plaque whereas those < 4% did not. This finding suggests that an omega-3 fatty acid index < 3.43% may identify subjects at risk for significant coronary plaque progression. Nondiabetic subjects in the 3.43% to < 5.41% quartile had significant prevention of fibrous, noncalcified and total plaque but not calcified plaque. Since those with an omega-3 index ≥4% had prevention of progression of all 4 plaque subtypes including calcified plaque, an omega-3 index ≥4% may be a reasonable target to achieve prevention of progression of all four coronary artery plaque subtypes. The amount of calcified plaque has been shown to be predictive of cardiovascular events [26,27]; therefore, the slowing of progression of calcified plaque with EPA and DHA may have benefit on cardiovascular events and should be examined further.

We previously reported that our primary endpoint, change in noncalcified plaque, was not significant in our intention-to-treat analysis for our total group of subjects [17]. Our current findings suggest that plasma level of omega-3 fatty acid achieved is a more important factor in determining outcome than treatment assignment and support the importance of measuring plasma levels of omega-3 fatty acids in randomized trials both initially, perhaps again early in the trial to detect response to supplementation, and at the trial conclusion. Fifteen of 119 subjects randomized to EPA and DHA supplementation in the current study had an omega-3 fatty acid index < 4%. Two of these subjects did not tolerate Lovaza and stopped early; thus, the reason for their omega-3 fatty acid index < 4%. The remaining 13 were compliant, a finding suggesting that EPA and DHA increased plasma levels to a variable degree. Variability in individual blood levels of omega-3 fatty acids achieved has also been reported in the Japan EPA Lipid Intervention Study (JELIS). In JELIS, those without clinical CVD randomized to 1.8 g EPA daily did not have a significant reduction in major coronary events compared to control [28]. However, when plasma levels of omega-3 fatty acids were measured, a significant reduction in major coronary events was observed in the subgroup who achieved a plasma EPA level > 133 μg/ml and EPA to arachidonic acid ratio > 0.75 [10,29]. Thirty-nine percent of subjects in JELIS did not achieve a plasma EPA level > 133 μg/ml in spite of taking 1.8 g/day of EPA [29]. In a 28-day dosing study with 4 g per day of omega-3 fatty acid, about 16% did not achieve an EPA to AA ratio > 0.75 [reviewed in 10]. Therefore, predicting an individual's response to a specific dose is difficult and this could account for the lack of benefit in some clinical trials. Further studies should examine reasons accounting for differences in individual response. If mechanisms can be identified, then interventions can be undertaken to maximize EPA and DHA levels.

Table 3
Baseline characteristics according to omega-3 fatty acid index quartiles at 30 months for total group.

Characteristics	1st Quartile < 3.15% (n = 54)	2nd Quartile 3.15–4.96% (n = 55)	3rd Quartile 4.97–7.65% (n = 54)	4th Quartile ≥7.66% (n = 55)	*p* for trend
	No. (%)	No. (%)	No. (%)	No. (%)	
Control, No. (%)	46 (85.2)	38 (69.1)	12 (22.2)	3 (5.5)	< 0.001
Lovaza, No. (%)	8 (14.8)	17 (30.9)	42 (77.8)	52 (94.5)	
Age, mean (SD), y	61.3 (7.8)	62.9 (8.7)	63.6 (7.4)	64.0 (7.1)	0.063
Male, No. (%)	45 (83.3)	46 (83.6)	53 (98.1)	38 (69.1)	0.203
Inclusion criteria (may have more than one), No. (%)					
History of MI	26 (48.1)	27 (49.1)	22 (40.7)	24 (43.6)	0.471
History of PCI	31 (57.4)	36 (65.5)	39 (72.2)	28 (50.9)	0.655
History of CABG	14 (25.9)	20 (36.4)	9 (16.7)	11 (20.0)	0.152
Cardiovascular risk factors, No. (%)					
Hypertension	47 (87.0)	46 (83.6)	45 (83.3)	40 (72.7)	0.065
Diabetes	22 (40.7)	17 (30.9)	11 (20.4)	14 (25.5)	0.042
Anthropometric and blood pressure, mean (SD)					
Weight, kg	90.2 (13.1)	90.4 (15.6)	92.9 (12.4)	89.9 (13.9)	0.862
Body mass index, kg/m^2	30.6 (3.5)	30.3 (3.5)	30.2 (3.8)	30.7 (3.5)	0.959
Waist circumference, cm	106.9 (9.9)	105.1 (9.7)	106.4 (10.1)	106.1 (11.2)	0.843
Systolic BP, mmHg	123.0 (15.0)	123.2 (12.4)	126.1 (12.2)	122.0 (17.4)	0.973
Diastolic BP, mmHg	72.1 (9.8)	72.1 (7.7)	74.8 (9.1)	72.4 (11.7)	0.522
Complete blood count, mean (SD)					
WBC, 10^9 cells/L	6.7 (1.9)	6.4 (1.6)	6.7 (1.8)	6.4 (1.4)	0.610
Monocytes, cells/μL	566.6 (191.8)	488.5 (163.1)	524.4 (143.5)	528.3 (174.3)	0.453
Neutrophils, cells/μL	4254.3 (1579.0)	4149.2 (1455.0)	4342.8 (1691.3)	3915.1 (1075.4)	0.351
Lymphocytes, cells/μL	1683.9 (590.6)	1538.2 (404.5)	1657.8 (692.8)	1786.6 (657.1)	0.234
Platelets, cells/μL	193.8 (51.8)	196.5 (50.1)	168.3 (50.2)	203.0 (45.2)	0.993
Lipids, mean (SD)					
Total cholesterol, mg/dL	156.0 (44.1)	148.3 (31.2)	145.2 (26.4)	163.2 (38.4)	0.397
Triglyceride, mg/dL	133.0 [91.0, 171.0]	114.0 [74.0, 143.0]	118.5 [84.0, 178.0]	119.5 [82.0, 169.0]	0.783
HDL-C, mg/dL	45.4 (15.7)	48.3 (14.9)	43.8 (12.8)	52.8 (15.1)	0.052
LDL-C, mg/dL	81.1 (33.4)	74.9 (23.2)	74.2 (19.6)	84.2 (30.4)	0.619
Biochemical profile, mean (SD)					
Glucose, mg/dL	110.8 (37.7)	106.6 (29.6)	99.7 (17.5)	106.1 (33.4)	0.267
HbA1c, %	6.4 (1.2)	6.1 (0.8)	6.0 (0.6)	6.2 (0.9)	0.155
Medication, No. (%)					
Statin	48 (88.9)	54 (98.2)	52 (96.3)	54 (98.2)	0.042
Aspirin	51 (94.4)	53 (96.4)	54 (100.0)	51 (92.7)	0.890
ACE-I	32 (59.3)	32 (58.2)	29 (53.7)	25 (45.5)	0.129
ARB	7 (13.0)	13 (23.6)	10 (18.5)	12 (21.8)	0.375
Hydrochlorothiazide	11 (20.4)	9 (16.4)	10 (18.5)	9 (16.4)	0.674
Furosemide	6 (11.1)	4 (7.3)	4 (7.4)	5 (9.1)	0.735
Calcium channel blocker	7 (13.0)	13 (23.6)	14 (25.9)	14 (25.5)	0.116
Beta blockers	41 (75.9)	38 (69.1)	40 (74.1)	37 (67.3)	0.445

ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; CABG, coronary artery bypass grafting; HbA1c, hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; MI, myocardial infarction; PCI, percutaneous coronary intervention; WBC, white blood cell count.
Values expressed as mean (SD) except triglyceride which is median [interquartile range].
To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129.

In the VITamin D and OmegA-3 Trial (VITAL), subjects randomized to 1 g omega-3 fatty acid had no benefit on a composite of myocardial infarction, stroke or cardiovascular death [14]. However, several secondary endpoints were significantly reduced. Total myocardial infarction was reduced 28% (HR = 0.72, 95% CI = 0.59–0.90), percutaneous coronary intervention was reduced 28% (HR = 0.78, 95% CI = 0.63–0.95), fatal myocardial infarction was reduced 50% (HR = 0.50, 95% CI = 0.26–0.97) and a composite of myocardial infarction, coronary revascularization (percutaneous coronary intervention or CABG) and death from coronary heart disease was reduced 17% (HR = 0.83, 95% CI = 0.71–0.97) [14]. In subgroup analysis, participants with low fish consumption (< 1.5 servings per week and presumably lower baseline omega-3 fatty acid plasma levels) had a 19% reduction in major cardiovascular events (HR = 0.81, 95% CI = 0.67–0.98), a 40% reduction in myocardial infarction (HR = 0.60, 95% CI = 0.45–0.81) and a trend toward a reduction in total mortality (HR = 0.87, 95% CI = 0.73–1.04) [14]. In contrast, those with higher baseline fish consumption had no benefit in cardiovascular risk reduction. African-Americans receiving omega-3 fatty acids had a 77% reduction (HR = 0.23, 95% CI = 0.09–0.62) in myocardial infarction regardless of baseline level of fish intake. Measurement of plasma levels of omega-3 fatty acids in VITAL may provide additional insight into benefit.

In the REDUCE-IT trial, 8179 patients with established CVD or diabetes with at least one other risk factor and who had elevated triglyceride levels despite statin treatment were randomized to 2 g of icosapent ethyl twice daily or placebo. Compared to placebo, those on icosapent ethyl had a 25% lower risk of developing the composite endpoint of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization or unstable angina after 4.9-year follow-up [16]. Both treatment groups had an EPA level of 26.1 μg/mL at baseline. However, at 1-year follow-up, patients randomized to icosapent ethyl had a significant 393.5% increase in their EPA levels to 144 μg/mL while the placebo group had a significant 2.9% decrease in EPA to a level of 23.3 μg/mL (*p* value for between group difference < 0.001) [16]. These findings from JELIS and REDUCE-IT, along with our findings, suggest the importance of taking into account the attained blood level of omega-3 fatty acids when interpreting trials. Furthermore, if omega-3 fatty acid plasma measurements are performed early in a trial, adjustment in omega-3 fatty acid dose might increase

Table 4
Baseline characteristics according to quartiles of eicosapentaenoic acid plus docosahexaenoic acid (μg/mL) for total group.

Characteristics	1st Quartile < 92.2 (n = 54)	2nd Quartile 92.3 to 137.8 (n = 55)	3rd Quartile 137.9 to 205.9 (n = 55)	4th Quartile ≥206.0 (n = 54)	*p* for trend
Control, No. (%)	41 (75.9%)	39 (70.9%)	16 (29.1%)	3 (5.6%)	< 0.001
Lovaza, No. (%)	13 (24.1%)	16 (29.1%)	39 (70.9%)	51 (94.4%)	
Age, mean (SD), y	61.2 (8.1)	63.6 (8.0)	63.2 (7.9)	63.8 (7.2)	0.117
Male, No. (%)	46 (85.2%)	48 (87.3%)	51 (92.7%)	37 (68.5%)	0.050
Inclusion criteria (may have more than one), No. (%)					
History of MI	29 (53.7%)	23 (41.8%)	26 (47.3%)	21 (38.9%)	0.202
History of PCI	35 (64.8%)	33 (60.0%)	40 (72.7%)	26 (48.1%)	0.213
CABG	14 (25.9%)	22 (40.0%)	8 (14.5%)	10 (18.5%)	0.068
Cardiovascular risk factors, No. (%)					
Hypertension	47 (87.0%)	49 (89.1%)	38 (69.1%)	44 (81.5%)	0.117
Diabetes	20 (37.0%)	19 (34.5%)	11 (20.0%)	14 (25.9%)	0.083
Anthropometric and blood pressure, mean (SD)					
Weight, kg	90.5 (13.0)	92.2 (16.3)	92.2 (12.4)	88.4 (13.2)	0.467
Body mass index, kg/m^2	30.5 (3.6)	30.9 (4.0)	30.0 (3.3)	30.4 (3.3)	0.632
Waist circumference, cm	106.5 (10.1)	107.0 (10.8)	105.8 (9.0)	105.3 (11.0)	0.442
Systolic BP, mmHg	120.3 (14.1)	126.1 (13.1)	124.7 (14.4)	123.1 (15.7)	0.431
Diastolic BP, mmHg	70.7 (8.8)	72.7 (8.4)	75.7 (10.7)	72.1 (10.2)	0.208
Complete blood count, mean (SD)					
WBC, 10^9 cells/L	6.8 (2.1)	6.3 (1.3)	6.7 (1.8)	6.5 (1.4)	0.492
Monocytes, cells/μL	552.4 (179.9)	495.5 (161.3)	537.8 (166.2)	521.9 (172.5)	0.640
Neutrophils, cells/μL	4402.4 (1690.0)	4039.1 (1245.8)	4282.0 (1681.5)	3933.2 (1154.9)	0.194
Lymphocytes, cells/μL	1651.1 (536.6)	1565.2 (477.0)	1648.3 (739.1)	1804.0 (601.0)	0.137
Platelets, cells/μL	192.6 (48.3)	190.6 (59.3)	177.4 (40.4)	201.6 (51.7)	0.659
Lipids, mean (SD)					
Total cholesterol, mg/dL	146.2 (37.4)	152.3 (38.1)	143.5 (26.3)	170.6 (35.8)	0.003
Triglyceride, mg/dL	107.5 [72.0, 142.0]	114.0 [78.0, 166.0]	121.0 [79.0, 159.0]	139.5 [89.0, 204.0]	0.037
HDL-C, mg/dL	46.6 (14.8)	46.9 (15.7)	44.2 (10.9)	52.5 (16.9)	0.100
LDL-C, mg/dL	74.9 (26.9)	78.7 (29.9)	73.7 (19.9)	87.2 (30.1)	0.058
Biochemical profile, mean (SD)					
Glucose, mg/dL	108.4 (36.1)	107.8 (31.1)	100.1 (18.3)	107.0 (33.7)	0.514
HbA1c, %	6.3 (1.1)	6.2 (0.9)	6.0 (0.7)	6.2 (0.8)	0.451
Medication, No. (%)					
Statin	51 (94.4%)	51 (92.7%)	55 (100.0%)	51 (94.4%)	0.563
Aspirin	52 (96.3%)	52 (94.5%)	55 (100.0%)	50 (92.6%)	0.648
ACE-I	32 (59.3%)	34 (61.8%)	23 (41.8%)	29 (53.7%)	0.224
ARB	7 (13.0%)	12 (21.8%)	14 (25.5%)	9 (16.7%)	0.540
Hydrochlorothiazide	9 (16.7%)	8 (14.5%)	11 (20.0%)	11 (20.4%)	0.478
Furosemide	5 (9.3%)	7 (12.7%)	3 (5.5%)	4 (7.4%)	0.453
Calcium channel blocker	7 (13.0%)	14 (25.5%)	14 (25.5%)	13 (24.1%)	0.188
Beta blockers	38 (70.4%)	43 (78.2%)	40 (72.7%)	35 (64.8%)	0.421

ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; CABG, coronary artery bypass grafting; HbA1c, hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; MI, myocardial infarction; PCI, percutaneous coronary intervention; WBC, white blood cell count.
Values expressed as mean (SD) except triglyceride which is median [interquartile range].
To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129.

the omega-3 fatty acid index to the target level in subjects with initially low omega-3 fatty acid plasma levels.

We next examined which fatty acid was the best predictor of change in coronary plaque for the nondiabetic subjects. The omega-3 fatty acid index predicted change of all plaque subtypes except fatty. Although the 3rd and 4th quartiles of EPA plus DHA had similar predictive ability for prevention of progression of fibrous, noncalcified and total plaque compared to the omega-3 fatty acid index, EPA plus DHA did not predict change in calcified plaque whereas the omega-3 fatty acid index did. Both the omega-3 index and EPA plus DHA were stronger predictors than EPA alone. Moreover, those in the 3rd quartile of EPA alone had prevention of only fibrous plaque at a much lower level of significance than EPA and DHA. This is an important finding because it suggests a better outcome with the combination of EPA and DHA than EPA alone. The lowest quartile of the omega-3 fatty acid index (< 3.43%) had significant within group progression of fibrous, calcified and total plaque compared to baseline whereas the lowest quartile in the other omega-3 fatty acid groups did not have significant within group progression. Taken together, the omega-3 fatty acid index appears to be the best predictor of prevention of coronary plaque change with an index ≥4% likely predicting prevention of progression. Various ratios of omega-3 fatty acids have been reported in prior studies. In the prospective Physicians Health Study, the highest quartile of the omega-3 fatty acid index (> 4.98%) and the third quartile (> 4.17%) were associated with 81% and 72% reductions in risk of sudden cardiac death, respectively, compared to the lowest quartile (< 3.45%) [30]. In the prospective University of California San Francisco Heart and Soul study, a plasma omega-3 fatty acid index > 3.6% was significantly associated with reduced all-cause mortality at 5.9 year follow-up [31]. In Korean acute myocardial infarction patients, an erythrocyte omega-3 fatty acid index > 4.74% was associated with reduced all-cause and CVD mortality [32]. Our study suggests benefit on prevention of coronary plaque progression at an omega-3 fatty acid index ≥4% and that blood levels of omega-3 fatty acids are better predictors of benefit than assignment to a fixed dose of omega-3 fatty acid supplementation. Therefore, blood levels may be able to help guide a personalized heart disease prevention strategy.

Potential beneficial mechanisms for omega-3 fatty acids include improvement in blood pressure and heart rate and anti-thrombotic and anti-inflammatory properties [33,34]. By quenching reactive oxygen species in lipoproteins and cellular membranes, EPA has antioxidant effects leading to less oxidation of LDL particles; non-oxidized LDL particles are more efficiently cleared by the LDL receptor and thus less atherogenic [35,36]. EPA treatment has also been associated with a

Table 5
Percent change in plaque volume stratified by quartiles of fatty acids at 30 months for nondiabetic subjects.

Plaque type	A. Quartiles of Omega-3 Fatty Acid Index					
	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	*p* value[a]	*p* value[b]
	< 3.43%	3.43 to < 5.41%	5.41 to < 7.67%	≥7.67%		
	(n = 38)	(n = 39)	(n = 38)	(n = 39)		
	Median [IQR]	Median [IQR]	Median [IQR]	Median [IQR]		
Fatty	4.3 [-9.9, 28.5]	5.0 [-7.4, 14.8]	2.0 [-8.4, 25.0]	−0.2 [-14.9, 18.3]	0.876	0.303
Fibrous	11.3 [0.3, 28.3][c]	0.1 [-11.5, 15.2][d]	−0.8 [-16.9, 14.9]	0.4 [-10.3, 15.3]	0.007	0.011
Noncalcified	13.1 [-3.8, 28.1]	2.0 [-3.9, 12.7][e]	0.2 [-15.4, 18.1]	−4.1 [-9.4, 14.4]	0.050	0.020
Calcified	104.5 [22.0, 268.7][f]	31.0 [-0.8, 133.4]	47.5 [-1.5, 102.3]	59.6 [-18.5, 244.2]	0.016	0.210
Total	20.6 [7.1, 34.7][g]	8.9 [-0.6, 27.5][h]	5.9 [-6.8, 21.8]	8.4 [-6.9, 19.0]	0.012	0.005
	B. Quartiles of eicosapentaenoic acid (ug/mL)					
	1st Quartile < 29.4 (n = 38) Median [IQR]	2nd Quartile 29.4 to 55.7 (n = 39) Median [IQR]	3rd Quartile 55.8 to < 89.0 (n = 39) Median [IQR]	4th Quartile ≥89.0 (n = 38) Median [IQR]	*p* value[a]	*p* value[b]
Fatty	7.3 [-8.9, 23.1]	2.8 [-10.5, 16.4]	5.2 [-8.5, 22.8]	−1.4 [-13.3, 16.2]	0.791	0.314
Fibrous	11.3 [-7.7, 27.7]	4.4 [-4.4, 15.8]	2.9 [-11.5, 16.2]	−4.3 [-11.3, 11.0]	0.050	0.031
Noncalcified	8.4 [-6.4, 27.1]	2.5 [-3.9, 16.3]	3.9 [-9.6, 18.9]	−4.1 [-12.3, 14.2]	0.154	0.037
Calcified	95.5 [13.0, 235.6]	82.6 [1.0, 171.5]	40.0 [-0.6, 125.9]	66.9 [-8.0, 138.5]	0.150	0.304
Total	17.2 [3.1, 34.7]	8.9 [2.9, 27.5]	13.0 [-6.8, 21.4]	7.5 [-7.1, 17.8]	0.073	0.032
	C. Quartiles of docosahexaenoic acid (ug/mL)					
	1st Quartile < 61.0 (n = 38) Median [IQR]	2nd Quartile 61.0 to 87.1 (n = 39) Median [IQR]	3rd Quartile 87.2 to 112.1 (n = 39) Median [IQR]	4th Quartile ≥112.2 (n = 38) Median [IQR]	*p* value[a]	*p* value[b]
Fatty	1.2 [-13.0, 17.4]	6.8 [-7.4, 16.0]	4.6 [-12.7, 22.9]	0.1 [-8.1, 19.1]	0.669	0.693
Fibrous	7.6 [-2.8, 27.8]	2.9 [-11.5, 18.0]	0.7 [-15.2, 17.6]	0.6 [-9.2, 14.9]	0.114	0.140
Noncalcified	5.6 [-8.2, 26.1]	2.5 [-6.0, 17.1]	0.5 [-14.6, 18.2]	−1.0 [-9.5, 15.1]	0.250	0.329
Calcified	99.3 [8.0, 249.7]	43.3 [4.3, 215.0]	41.8 [-0.6, 127.6]	53.7 [-18.8, 133.8]	0.119	0.074
Total	15.8 [1.3, 30.5]	9.0 [-2.4, 37.2]	13.9 [2.0, 20.9]	7.0 [-6.9, 19.0]	0.262	0.119
	D. Quartiles of eicosapentaenoic acid plus docosahexaenoic acid (ug/mL)					
	1st Quartile < 93.4 (n = 38) Median [IQR]	2nd Quartile 93.4 to 146.6 (n = 39) Median [IQR]	3rd Quartile 146.7 to 208.4 (n = 39) Median [IQR]	4th Quartile ≥208.4 (n = 38) Median [IQR]	*p* value[a]	*p* value[b]
Fatty	4.3[-9.9,23.0]	6.8[-10.3,23.5]	0.8[-8.5,14.7]	−0.9[-13.3,18.5]	0.661	0.582
Fibrous	9.7[-0.5,27.8]	6.6[-7.1,18.3]	−2.4[-16.1,15.5]	0.1[-9.6,12.8]	0.004	0.016
Noncalcified	11.3[-3.4,26.1]	5.0[-4.1,18.2]	−3.7[-10.7,13.4]	−2.0[-9.6,14.4]	0.019	0.038
Calcified	101.6[17.6251.5]	49.8[0.1161.5]	41.6[3.4125.9]	57.8[-14.7138.5]	0.075	0.062
Total	19.1[5.9,34.7][e]	8.9[2.3,37.2]	10.0[-7.0,21.4]	6.8[-6.9,16.5]	0.017	0.005
	E. Quartiles of eicosapentaenoic acid to arachidonic acid ratio					
	1st Quartile < 0.102 (n = 38) Median [IQR]	2nd Quartile 0.102 to 0.237 (n = 39) Median [IQR]	3rd Quartile 0.238 to 0.444 (n = 39) Median [IQR]	4th Quartile ≥0.445 (n = 38) Median [IQR]	*p* value[a]	*p* value[b]
Fatty	4.1 [-8.9, 22.8]	8.7 [-7.3, 23.5]	2.8 [-8.1, 16.7]	−5.3 [-16.9, 16.6]	0.879	0.253
Fibrous	9.1 [-7.7, 27.7]	7.3 [-3.2, 18.0]	2.9 [-12.2, 20.3]	−2.2 [-10.5, 7.6]	0.081	0.022
Noncalcified	5.6 [-6.4, 27.1]	6.5 [-3.2, 17.2]	3.9 [-14.6, 20.7]	−4.5 [-10.4, 12.8]	0.206	0.020
Calcified	95.5 [17.7, 235.6]	82.6 [0.1, 147.0]	18.5 [-7.6, 97.2]	68.8 [-4.7, 149.0]	0.016	0.391
Total	17.2 [3.1, 37.9][e]	14.3 [4.2, 29.5]	8.3 [-7.0, 20.3]	6.8 [-7.3, 17.8]	0.024	0.021

[a] *p* value comparing the first to third quartile.
[b] *p* value comparing the first to fourth quartile.
[c] Within group *p* value at 30 months compared to baseline = 0.012.
[d] *p* = 0.016 compared to 1st quartile.
[e] *p* = 0.067 compared to 1st quartile.
[f] Within group *p* value at 30 months compared to baseline = 0.042.
[g] Within group *p* value at 30 months compared to baseline < 0.001.
[h] *p* = 0.055 compared to 1st quartile.

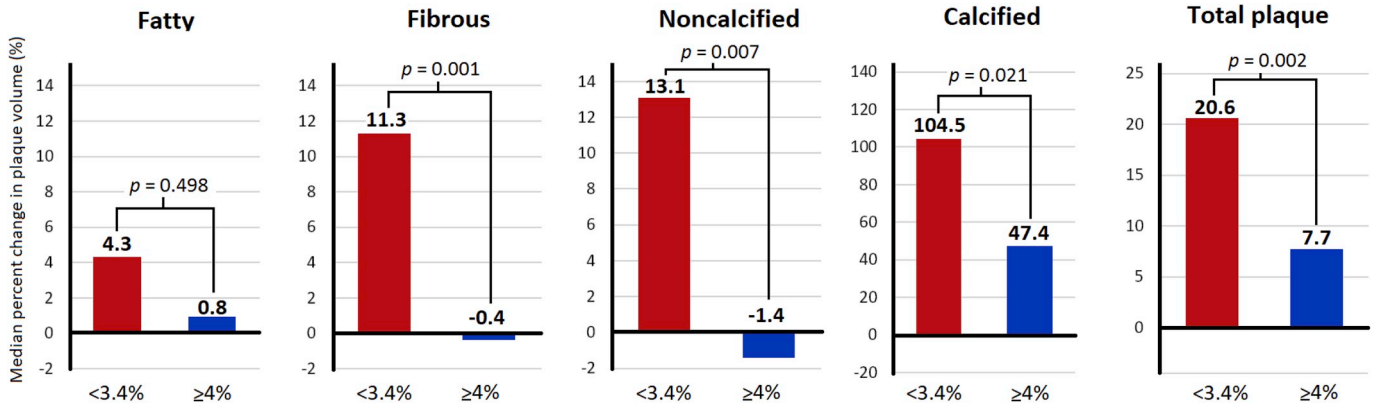

Fig. 1. Percent change in coronary plaque volume at 30 months in nondiabetic subjects with an omega-3 fatty acid index < 3.4% compared to ≥4%.

reduction in inflammation and improved stabilization of plaque [37]. Oxidized lipoproteins and hyperglycemia lead to endothelial dysfunction which is associated with atherosclerosis [38,39] and which EPA has been shown to reverse [40]. EPA inhibits the formation of cholesterol crystals [36] which can destabilize the fibrous cap of plaque and lead to local inflammation and thrombus formation [41]. EPA has favorable effects on unstable plaque by increasing fibrous cap thickness, lumen diameter and plaque stability as well as reducing thrombosis and platelet activation [37,42]. Although the mechanism responsible for benefit in the REDUCE-IT trial is unknown, our results suggest that prevention of progression of coronary artery plaque may be one potential mechanism.

Although our trial was not designed to assess cardiovascular events, it is worth noting that several studies assessing coronary plaque subtypes with coronary computed tomographic angiography have shown that higher volume of noncalcified plaque and total plaque are associated with higher rates of cardiac death, myocardial infarction and coronary revascularization [43] and higher rates of acute coronary syndrome [44,45]. Furthermore, evidence from intravascular ultrasound studies shows that progression of plaque atheroma volume is independently associated with higher rates of a composite of cardiac death, myocardial infarction and coronary revascularization ($p < 0.002$) and regression is associated with fewer events [3]. Taken together, these findings suggest that plaque composition and volume predict cardiovascular events and support the potential clinical importance of lack of progression of plaque volume observed in the omega-3 ethyl-ester arm in the current trial.

No difference in plaque change was observed in diabetic subjects in the current study due to the fact that those with an omega-3 fatty acid index < 4% had similar low rates of plaque progression compared to diabetics with an omega-3 fatty acid index ≥4%. A potential reason for lack of difference could be postulated due to use of antidiabetic medications and the higher utilization of cardioprotective medications including angiotensin-receptor blockers in diabetic subjects compared to nondiabetic subjects. A nontargeted metabolomics analysis in combination with targeted quantification of eicosanoids and endocannabinoids in a case-control study of the Carotid Intima Media Thickness (IMT) and IMT Progression as Predictors of Vascular Events in a High Risk European Population (IMPROVE) pan-European cohort reported that high concentrations of fatty acids were associated with a 35% reduction in CVD events in nondiabetic subjects (odds ratio [OR], 0.65; 95% CI, 0.27–0.80; $p = 0.030$) but not diabetic subjects [46]. DHA approached significance, but the investigators did not examine EPA alone or the omega-3 fatty acid index. Their results, along with ours of differential response by diabetes status, highlight the importance of stratifying populations on potentially important clinical features as diabetes status in designing future clinical trials and interpreting results.

More women achieved a higher omega-3 fatty acid level than men. A potential reason could be increased dietary intake of α-linolenic acid (ALA), EPA or DHA. ALA can be converted to EPA and less so, to DHA in the human. Women have an increased ability to convert ALA to omega-3 fatty acids compared to men [47]. Studies with stable isotope-labelled ALA have shown conversion rates of ALA to EPA of 21% versus 8% and of ALA to DHA of 9% versus 0% in women and men, respectively [48,49]. This finding could be one reason accounting for the higher prevalence of women in the highest omega-3 fatty acid quartiles in our study. Of note, HDL-C level trended toward significantly higher at baseline in the highest omega-3 index quartile, a finding which could be due to the higher prevalence of women in the highest omega-3 fatty acid quartiles. The role of HDL in cholesterol efflux capacity has been demonstrated to be more predictive than HDL-C concentration [50]. A limitation of our study is that we did not measure the potential effect of omega-3 fatty acid on HDL function in reverse cholesterol transport.

In conclusion, omega-3 fatty acids added to statins over 30 months prevented coronary plaque progression in nondiabetic subjects with well controlled LDL-C and triglyceride levels when an omega-3 fatty acid index ≥4% was achieved. Omega-3 plasma levels achieved were a better predictor of outcome than treatment allocation [17]. Low omega-3 index < 3.43% identified nondiabetic subjects at risk of significant coronary plaque progression despite statin therapy. These findings highlight the potential importance of measuring plasma levels of omega-3 fatty acids to guide personalized therapy. Future trials should target an omega-3 fatty acid index ≥4% in an attempt to maximize cardiovascular benefit and reduce residual risk of cardiovascular events. Finally, since not all subjects receiving EPA and DHA increase plasma level sufficiently, clinical trials of EPA and DHA should measure early response in plasma levels which may require an increase in dose to best assess change in clinical outcomes with EPA and DHA supplementation.

Clinical trial registration

URL: http//www.clinicaltrials.gov. Unique identifier: NCT01624727.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

Dr. Welty designed the study and obtained funding. Drs. Welty and Elajami recruited study subjects, carried out visits and did database entry. Dr. Alfaddagh did the analysis. Dr. Welty and Dr. Alfaddagh drafted the manuscript and all contributed to data interpretation and manuscript revision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.04.213.

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