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Dietary α-Linolenic Acid and Total ω-3 Fatty Acids Are Inversely Associated with Abdominal Aortic Calcification in Older Women, but Not in Older Men[1,2]

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Abstract

Background: Associations of α-linolenic acid (ALA), eicosapentaenoic acid (EPA) plus decosahexaenoic acid (DHA), and total omega-3 (n–3) fatty acid (FA) intakes with abdominal aortic calcification (AAC) are not well understood.

Objective: This study explored the associations between baseline and long-term changes in ω-3 FA consumption and AAC severity among community-dwelling older men and women.

Methods: The present study used a subset of the Melbourne Collaborative Cohort Study in which participants were interviewed in 1990–1994 and again in 2010–2011. Dietary intake was evaluated at both baseline and follow-up with use of food-frequency questionnaires. AAC severity was assessed by both lateral thoraco-lumbar radiography and dual-energy X-ray absorptiometry (DXA) at follow-up.

Results: A total of 312 participants aged 45–64 y old at baseline were followed for a duration of (mean ± SD) 18 ± 1 y. Baseline energy-adjusted ALA intake tended to be inversely associated with AAC severity by radiography [OR (95% CI) for tertile 3 vs. tertile 1: 0.49 (0.23, 1.02), *P*-trend: 0.06] and was inversely associated with AAC severity by DXA [OR (95% CI) for tertile 3 vs. tertile 1: 0.37 (0.16, 0.83)] in women, after adjustment for confounders. Women in the third tertile of total ω-3 FA intake had significantly lower AAC severity by radiography [OR (95% CI): 0.33 (0.16, 0.71)] and DXA [OR (95% CI): 0.27 (0.12, 0.62)] than those in the first tertile. Changes in tertile of ω-3 FA intake over 18 y were not found to be associated with AAC severity in either men or women.

Conclusion: The results of our study suggest that dietary ALA and total ω-3 FA intakes are both important predictors of the development of AAC in older women, but not in older men. *J Nutr* 2015;145:1778–86.

Keywords: α-linolenic acid, eicosapentaenoic acid, decosahexaenoic acid, ω-3 fatty acids, abdominal aortic calcification.

Introduction

Dietary omega-3 (n–3) FAs primarily consist of α-linolenic acid (ALA)[10], EPA, and DHA (1). ALA is mainly derived from plant sources, such as canola, soybean, walnuts, and flaxseed, and fish is rich in EPA and DHA (2). To reduce the risk of cardiovascular disease (CVD), the National Heart Foundation and National Health and Medical Research Council of Australia recommend a combined EPA and DHA intake of at least 0.61 g/d for men and 0.42 g/d for women and an ALA intake of 1.3 g/d for men and 0.8 g/d for women (3, 4). There is increasing evidence from epidemiologic studies and clinical trials that finds higher dietary ALA or EPA plus DHA or total ω-3 FA consumption is associated with a lower risk of CVD and CVD risk factors (5–11).

Vascular calcification has been considered a consequence of aging (12) and an important predictor of arterial elasticity reduction or atherosclerosis (13). With the application of DXA, lateral lumbar radiography, echocardiography, and computed

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[10] Abbreviations used: AAC, abdominal aortic calcification; ALA, α-linolenic acid; CVD, cardiovascular disease; MCCS, Melbourne Collaborative Cohort Study; TC, total cholesterol.
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tomography technologies in the assessment of vascular calcification, an increasing number of studies have investigated its mechanisms and clinical consequences (14–16). Abdominal aortic calcification (AAC) was shown to be positively associated with traditional CVD risk factors (17). Furthermore, AAC is a strong predictor of CVD (18–20) independent of traditional CVD risk factors. Thus, AAC assessment may identify patients who are without traditional risk factors for CVD but may be at increased risk of a cardiovascular event (21).

Over the past 2 decades, growing evidence suggests that vascular calcification is a regulated cell-mediated process (14), which is potentially modifiable and reversible (22). Animal experiments have demonstrated that the long-chain ω-3 FAs EPA and DHA could help mitigate vascular calcification (23, 24). To our knowledge, only 2 population-based studies have examined the associations of EPA and DHA intakes with vascular calcification, and these studies investigated coronary calcification as the outcome. They did not report a protective effect of EPA and DHA intakes on calcification (25, 26). The potential relation between ALA or total ω-3 FA intake and AAC has not been investigated. Given strong evidence supportive of the preventive effects of ω-3 FA intake on CVD (5–11) and that AAC is a risk factor for CVD, and AAC and CVD share many risk factors (20), we hypothesized that a high intake of ω-3 FAs would be associated with lower AAC in community-dwelling older adults. Thus, our aim was to explore the association between baseline and change in ALA, EPA plus DHA, and total ω-3 FA intakes and AAC severity in an 18-y follow-up study.

Methods

Study population. The present study used a subset of the Melbourne Collaborative Cohort Study (MCCS), and the design has been previously described in detail elsewhere (27). Briefly, the MCCS is a prospective cohort study including 41,514 participants at baseline (1990–1994). For the present study, 956 subjects who resided close to Western Hospital, were aged between 45 and 64 y old (at baseline), were English speakers, and had calcium intake <500 mg/d or ≥1300 mg/d were approached to participate in this substudy (**Figure 1**). A total of 407 participants were eligible for the study after screening; 353 of them completed DXA scans and 346 of them completed radiographs at follow-up (2010–2011). Of the 346 participants who completed radiographs, 312 with readable radiographs were included in our data analysis for the association between ω-3 FA intake and AAC severity by radiography. Of the 312 participants with readable radiographs, 272 participants having readable DXA images were included in the analysis for the association between ω-3 FA intake and AAC severity by DXA. For the analysis of changes in ω-3 FA consumption over 18 y and AAC severity, 303 participants were available because 9 of the 312 participants did not complete the FFQ at follow-up. Informed consent was obtained from all participants. The study protocol was approved by the Cancer Council Victoria's Human Research and Melbourne Health Human Research Ethics Committees.

Assessment of dietary intake. Dietary intake for each participant was assessed at baseline with use of a FFQ with 121 food items and at follow-up by the Dietary Questionnaire for Epidemiological Studies version 3.1 with 98 food items. Both questionnaires were designed especially for the MCCS (28). Information on dietary habits including types and frequency of fats and oils used and use of milk, sugar, and diet supplements was collected. Intake of ω-3 FAs, including ALA, EPA, and DHA, from diet was calculated with use of Australian food composition data (29), and intake of other nutrients such as energy, fiber, and calcium was calculated based on Australian nutrient data (30).

Potential confounders. At baseline, participants completed questionnaires documenting country of birth (Australia/New Zealand, United Kingdom/Malta, or other), physical activity (4 levels from low to high)

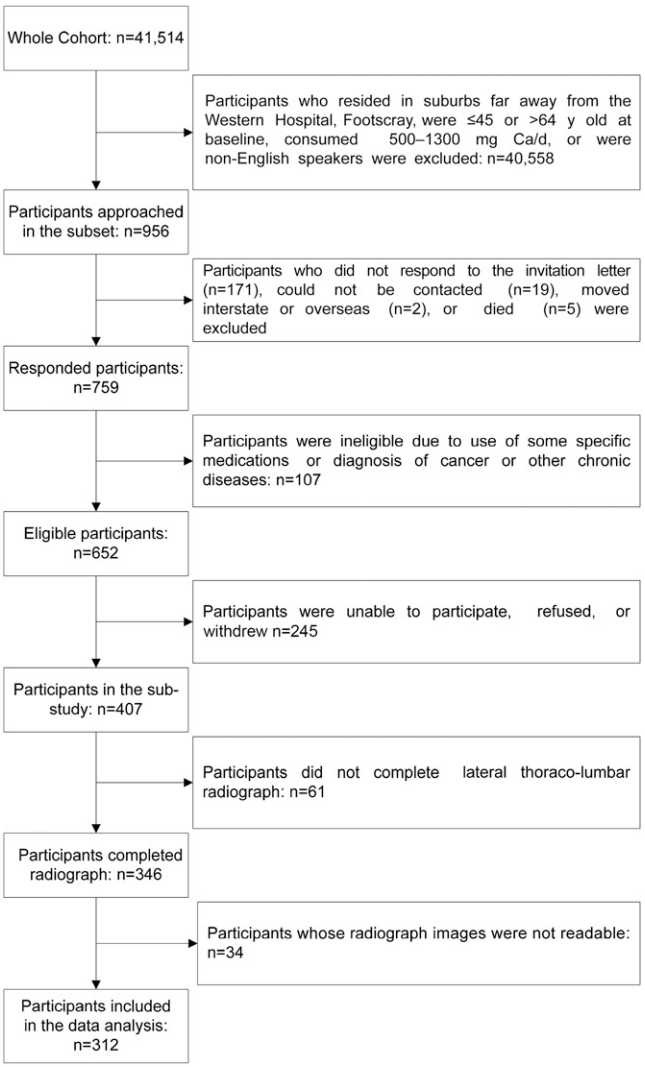

FIGURE 1 Flowchart of the study population selection.

(31), alcohol consumption (grams per day based on a beverage-specific quantity frequency questionnaire) (30), and smoking status (current, former, or never). Socioeconomic status was evaluated according to the Index of Relative Socioeconomic Advantage and Disadvantage with the lowest quintile representing the greatest socioeconomic disadvantage (32).

Height, weight, waist circumference, hip circumference, and blood pressure were measured by trained staff at baseline (27). BMI was calculated as weight in kilograms divided by the square of height in meters. Plasma total cholesterol (TC) concentration was measured immediately with use of Kodak Ektachem DT60 desktop analyzers.

Assessment of AAC. AAC deposits were assessed at follow-up with use of lateral thoraco-lumbar radiography, which has been as the standard tool to detect aortic calcification (19, 20). In addition, AAC was also assessed in lateral vertebral DXA scans with use of a Hologic bone densitometer (QDR 4500 W; Hologic Inc.), which uses a fan-beam. The AAC score was obtained from both the digitized lateral radiography and DXA of lumbar spine with use of a semiquantitative visually based technique (33). The score was assessed by B Khan and confirmed by another trained radiologist (N Khan). The severity score was used to estimate both the extent and the severity of calcific deposits anterior to the first 4 lumbar vertebrae (L1 to L4). The scores for anterior and posterior walls of the abdominal aorta in each vertebral segment were then summed as a composite AAC score in all the thoraco-lumbar radiographs or DXA images. AAC was scored as follows: no calcification—0, one-third or less of the aortic wall in that vertebral segment was calcified—1,

between one-third and two-thirds of the aortic wall was calcified—2, or more than two-thirds of the aortic wall was calcified—3 (as shown in **Figure 2**). The final composite score after combining the scores of 4 vertebral segments ranged from a minimum of "0" to maximum of "24," with each vertebral segment contributing a maximum of 6 points to the final. According to the AAC score, participants were categorized into "no calcification" (AAC score = 0), "moderate calcification" (AAC score between 1 and 5), and "high calcification" (AAC score ≥6) (20).

Statistical analyses. EPA and DHA were added together as long-chain marine ω-3 FAs in the analysis. Energy-adjusted ALA, EPA plus DHA, and total ω-3 FAs were calculated with use of regression models (34), and these energy-adjusted nutrients were used to analyze associations with AAC. Continuous values, skewed variables, and categorical variables in the text were expressed as means ± SDs, medians (IQRs), and frequency and percentages separately. Baseline characteristics of the study population are presented according to energy-adjusted tertiles of total ω-3 FA intake. To evaluate the differences in baseline characteristics by tertiles of total ω-3 FA intake, we used ANOVA for continuous variables, Kruskal-Wallis test for skewed variables, and chi-square test for categorical variables. Post hoc tests were then performed to identify the pairwise differences if the overall Kruskal-Wallis or chi-square tests were significant. Wilcoxon's rank-sum test was used to evaluate whether the consumption of ALA, EPA plus DHA, or total ω-3 FAs were different between men and women.

Men had significantly higher intake of ALA and total ω-3 FAs and more severe AAC than women (*P*-interaction between total ω-3 FA intake and sex for AAC severity: 0.04), therefore, the analyses of the association of ω-3 FA intake and AAC severity were performed separately for men and women. To evaluate if the baseline intakes of ALA, EPA plus DHA, and ω-3 FAs were associated with AAC severity after 18 y, ordinal logistic regression was performed. Univariate logistic regression models were performed and variables including country of birth, relative socioeconomic disadvantage, and alcohol intake were not included in the multivariate models because of the nonsignificance of their associations with AAC. Different models were adjusted for different confounders. *P*-trend was calculated for the AAC severity across energy-adjusted tertiles of ALA, EPA plus DHA, and ω-3 FA intake.

The change in tertile of ω-3 FA intakes was calculated as the tertile of energy-adjusted ω-3 FA intake at follow-up minus the tertile of energy-adjusted ω-3 FA intake at baseline. Participants were then divided into 3 groups: decrease in tertile, no change in tertile, and increase in tertile. Ordinal logistic regression models were used to examine the association between changes in tertiles of ω-3 FA intake and AAC severity. Multivariate models included baseline ω-3 FA intake, age, smoking, physical activity, BMI, systolic blood pressure, diastolic blood pressure, TC, and consumption of total energy and calcium. In the ordinal logistic regression models, the response variable AAC severity was valued 0, 1, and 2. To examine whether CVD could change the association between ω-3 FA intake and AAC severity, sensitivity analysis was performed. It was considered significant if the *P* value was <0.05 by a two-tailed test. All statistical analyses were performed with SAS 9.3 for Windows (SAS Institute Inc.).

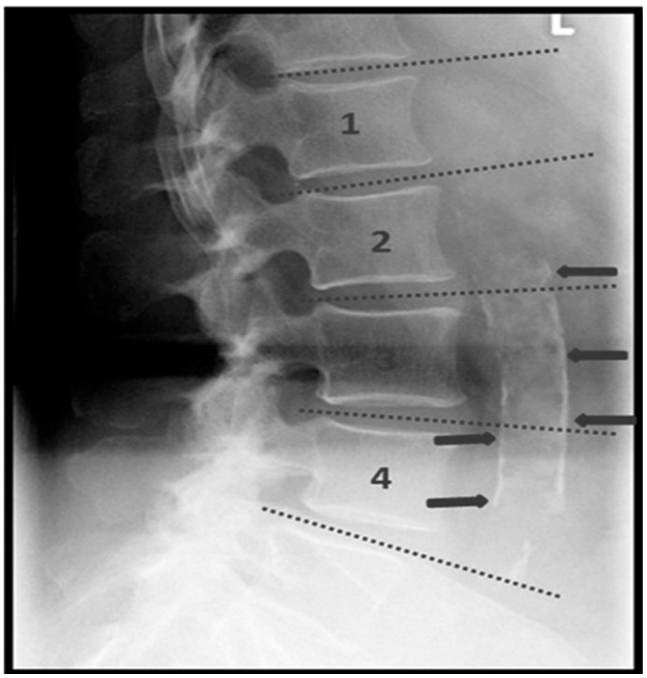

FIGURE 2 Assessment of abdominal aortic calcific deposits by radiography. The numbers 1, 2, 3, and 4 stand for the first 4 lumbar vertebral segments (L1 to L4). Each vertebral segment was defined using the midpoint of the intervertebral space above and below the vertebrae as boundaries. Score was graded as 0 for aortic calcifications in both posterior and anterior walls of the L1 segment, 1 each for the posterior and anterior walls of the L2 segment, 3 each for the anterior and posterior walls of the L3 segment, and 2 each for the anterior and posterior walls of the L4 segment.

Results

A total of 312 participants (124 men and 188 women) aged 45–64 y (mean ± SD: 52.9 ± 5.4 y) at baseline were followed up for a duration of (mean ± SD) 18 ± 1 y. A high agreement of AAC severity between radiography and DXA (weighted κ coefficient = 0.75, *P* = 0.019) was seen among the 272 participants with both readable radiographs and DXA images (data not shown), but radiographs are more sensitive than DXA in detecting aortic calcification. Men had a greater prevalence of high calcification than women (40% for men, 29% for women, *P* = 0.01).

The proportion of male participants whose lumbar spine radiographs were not readable (16 of 41 participants, 39%) was similar to those who had useable radiographs (124 men out of 312 participants, 40%, *P* = 0.93). There was no significant difference in age (included: 52.9 ± 5.4; excluded: 54.4 ± 5.3, *P* = 0.11) between included and excluded participants. ALA, EPA plus DHA, and total ω-3 FA intakes at baseline were not significantly different between included and excluded participants when Wilcoxon's rank-sum test was performed [ALA: median (IQR) = 0.76 (0.57–1.20) for included, median (IQR) = 0.91 (0.59–1.27) for excluded, *P* = 0.17; EPA plus DHA: median (IQR) = 0.21 (0.14–0.31) for included, median (IQR) = 0.21 (0.13–0.29) for excluded, *P* = 0.88; total ω-3 FAs: median (IQR) = 1.03 (0.69–1.53) for included, median (IQR) = 1.16 (0.81–1.68) for excluded, *P* = 0.18].

There was no difference in country of birth, relative socioeconomic disadvantage, smoking, physical activity, age, alcohol intake, height, weight, waist circumference, hip circumference, BMI, blood pressure, or TC across total ω-3 FA intake tertiles for men or women. Participants in the third tertile of energy-adjusted ω-3 FAs had higher intake of energy, fiber, calcium, fruit, vegetable, fish, and meat than those in the first tertile.

A higher intake of energy-adjusted ω-3 FAs was associated with a lower proportion of participants with high AAC severity by both radiography and DXA in women (**Table 1**).

As **Figure 3** demonstrates, men consumed significantly higher amounts of ALA (*P* < 0.001) and total ω-3 FAs (*P* = 0.01) than women. There was no significant difference in EPA plus DHA intake between men and women (*P* = 0.94). ALA accounted for a mean of 80% in men and 76% in women of total ω-3 FAs.

Neither assessment of AAC severity was associated with ALA, EPA plus DHA, or total ω-3 FA consumption in men (**Table 2**).

TABLE 1 Baseline characteristics by gender-specific energy-adjusted total ω-3 FA intake in older men and women[1]

	Men				Women			
	Tertile 1 (n = 41)	Tertile 2 (n = 41)	Tertile 3 (n = 42)	P	Tertile 1 (n = 62)	Tertile 2 (n = 63)	Tertile 3 (n = 63)	P
Energy-adjusted total ω-3 FAs, g/d	1.16 [0.16–1.27]	1.39 [1.28–1.48]	1.64 [1.49–3.04]		0.98 [0–1.07]	1.16 [1.07–1.27]	1.52 [1.27–2.63]	
Country of birth				0.30[2]				0.12[2]
Australia/New Zealand	33 (80)	39 (95)	37 (88)		59 (97)	62 (98)	57 (90)	
United Kingdom/Malta	8 (20)	2 (5)	5 (12)		2 (3)	1 (2)	6 (10)	
Relative socioeconomic disadvantage				0.22[2]				0.37[2]
1st quintile	11 (27)	13 (32)	4 (10)		11 (18)	15 (24)	12 (19)	
2nd quintile	6 (15)	2 (5)	5 (12)		16 (26)	12 (19)	15 (24)	
3rd quintile	12 (29)	11 (27)	12 (29)		19 (31)	15 (24)	13 (21)	
4th quintile	6 (15)	8 (20)	14 (33)		10 (16)	12 (19)	10 (16)	
5th quintile	6 (15)	7 (17)	7 (17)		5 (8)	9 (14)	13 (21)	
Smoking status				0.35[2]				0.20[2]
Current	6 (15)	4 (10)	2 (5)		3 (5)	7 (11)	4 (6)	
Former	14 (34)	21 (51)	16 (38)		13 (21)	19 (30)	20 (32)	
Never	21 (51)	16 (39)	24 (57)		45 (74)	37 (59)	39 (62)	
Physical activity score				0.16[2]				0.43[2]
1st quartile	11 (27)	13 (32)	6 (14)		9 (15)	9 (14)	9 (14)	
2nd quartile	8 (20)	5 (12)	8 (19)		10 (16)	16 (25)	10 (16)	
3rd quartile	13 (32)	13 (32)	13 (31)		26 (43)	26 (41)	21 (33)	
4th quartile	9 (22)	10 (24)	15 (36)		16 (26)	12 (19)	23 (37)	
Age, y	53.5 ± 5.7	52.3 ± 5.0	53.3 ± 5.2	0.85[3]	53.8 ± 5.7	52.3 ± 5.0	52.5 ± 5.7	0.17[3]
Height, cm	174.4 ± 7.0	173.9 ± 6.0	174.7 ± 7.9	0.87[3]	161.5 ± 6.3	161.2 ± 6.4	161.8 ± 5.4	0.82[3]
Weight, kg	80.4 ± 14.2	81.3 ± 10.5	83.5 ± 14.5	0.27[3]	66.1 ± 10.2	65.8 ± 10.0	70.0 ± 15.6	0.08[3]
Waist circumference, cm	90.9 ± 10.2	91.6 ± 10.2	93.7 ± 12.6	0.25[3]	76.0 ± 8.9	75.5 ± 8.8	79.0 ± 12.8	0.11[3]
Hip circumference, cm	99.8 ± 8.7	99.8 ± 7.6	101.9 ± 8.8	0.25[3]	99.4 ± 8.8	99.1 ± 7.4	102.0 ± 11.3	0.11[3]
BMI, kg/m^2	26.3 ± 3.5	27.0 ± 3.8	27.4 ± 4.6	0.22[3]	25.3 ± 3.6	25.3 ± 3.4	26.8 ± 6.1	0.08[3]
SBP, mm Hg	138.6 ± 14.7	136.1 ± 15.3	135.6 ± 16.7	0.40[3]	133.9 ± 19.1	130.7 ± 14.4	133.2 ± 18.3	0.85[3]
DBP, mm Hg	82.2 ± 8.4	80.6 ± 9.6	82.5 ± 9.5	0.88[3]	73.1 ± 11.0	72.2 ± 11.5	73.4 ± 9.7	0.88[3]
Plasma TC, mmol/L	5.5 ± 0.9	5.6 ± 1.4	5.3 ± 0.7	0.34[3]	5.4 ± 1.0	5.6 ± 1.0	5.4 ± 1.2	0.81[3]
Alcohol, g/d	19.4 ± 20.5	21.4 ± 20.5	14.0 ± 15.9	0.19[3]	6.0 ± 11.4	8.4 ± 14.5	7.6 ± 11.7	0.49[3]
Energy, kcal/d	2700 (1400–3610)[a]	1890 (1590–2880)[a]	3520 (2430–4110)[b]	<0.001[4]	2440 (1230–3360)[a]	1410 (1220–2790)[a]	2720 (1500–3340)[b]	0.002[4]
Fiber, g/d	26.6 (17.4–42.0)[a]	20.4 (16.9–31.7)[a]	43.6 (33.3–53.8)[b]	<0.001[4]	29.1 (17.0–37.9)[a]	20.4 (14.7–34.1)[a]	35.3 (26.3–48.7)[b]	<0.001[4]
Calcium,[5] mg/d	1350 (430–1580)[a]	460 (390–1330)[b]	1400 (1320–1470)[a]	0.002[4]	1340 (450–1620)[a]	450 (390–1320)[b]	1380 (460–1510)[a]	0.001[4]
ALA,[5] g/d	0.91 (0.52–1.18)[a]	0.84 (0.63–1.13)[a]	1.50 (1.14–1.90)[b]	<0.001[4]	0.69 (0.49–1.00)[a]	0.65 (0.55–0.92)[a]	1.11 (0.85–1.46)[b]	<0.001[4]
EPA plus DHA, g/d	0.13 (0.09–0.18)[a]	0.21 (0.15–0.24)[a]	0.36 (0.24–0.53)[b]	<0.001[4]	0.12 (0.08–0.19)[a]	0.20 (0.15–0.24)[a]	0.34 (0.25–0.64)[b]	<0.001[4]
Fruit, times/wk	12.0 (4.5–23.0)[a]	10.0 (6.0–21.5)[a]	23.8 (10.5–33.5)[b]	0.005[4]	23.5 (9.5–35.0)	19.0 (9.0–29.5)	23.5 (14.0–42.5)	0.06[4]
Vegetables, times/wk	22.5 (16.5–38.0)[a]	26.5 (18.5–41.0)[a]	38.8 (30.5–53.0)[b]	<0.001[4]	34.0 (25.5–46.5)[a]	34.5 (23.0–46.0)[a]	49.5 (35.5–65.5)[b]	<0.001[4]
Fish, times/wk	1.0 (0.5–1.5)[a]	1.5 (1.0–2.0)[b]	3.0 (2.0–4.0)[b]	<0.001[4]	1.0 (0.5–1.5)[a]	1.5 (1.0–2.0)[b]	3.0 (1.5–5.0)[b]	<0.001[4]
Meat, times/wk	8.0 (5.0–11.5)[a]	9.5 (6.5–12.5)[a]	12.3 (9.0–19.0)[b]	0.001[4]	6.5 (4.0–9.0)[a]	8.0 (6.5–11.5)[b]	10.5 (6.5–15.5)[b]	<0.001[4]
Nuts, times/wk	0.5 (0.0–1.5)[a]	1.0 (0.5–3.5)[a]	1.3 (1.0–6.0)[b]	0.002[4]	1.0 (0.5–3.0)	1.0 (0.5–3.0)	1.0 (0.0–5.5)	0.92[4]
AAC by radiography				0.72[2]				0.02[2]
None	11 (27)	7 (17)	11 (26)		18 (29)[a]	18 (29)[a]	30 (48)[b]	
Moderate	12 (29)	18 (44)	15 (36)		21 (34)[a]	29 (46)[a]	18 (29)[b]	
High	18 (44)	16 (39)	16 (38)		23 (37)[a]	16 (25)[a]	15 (24)[b]	
AAC by DXA				0.19[2]				0.01[2]
None	13 (32)	11 (27)	14 (33)		14 (23)[a]	23 (37)[b]	28 (44)[b]	
Moderate	11 (27)	14 (34)	11 (26)		19 (31)[a]	20 (32)[b]	15 (24)[b]	
High	17 (41)	11 (27)	9 (21)		21 (34)[a]	8 (13)[b]	13 (21)[b]	

[1] Values are means ± SDs, medians (IQRs), medians [ranges], or n (%). Within sex, labeled values in a row without a common letter differ, $P < 0.05$. AAC, abdominal aortic calcification; ALA, α-linolenic acid; DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol.
[2] Chi-square test was used to test the differences of categorical variables among tertiles of energy-adjusted total ω-3 FA intake.
[3] ANOVA was performed to test the differences of continuous variables among tertiles of energy-adjusted total ω-3 FA intake.
[4] Kruskal-Wallis test was used to evaluate the differences of skewed variables among tertiles of energy-adjusted total ω-3 FA intake.
[5] The low median calcium intake in tertile 2 occurred because there was a higher proportion of participants with calcium intake <500 g/d in this tertile in both men and women. The IQRs for ALA between tertiles overlapped because total ω-3 FA intake was adjusted for energy but ALA was not.

In women, a weak inverse relation between energy-adjusted ALA intake and AAC severity by radiography (P-trend: 0.13) was seen. The relation was strengthened (P-trend: 0.06) after adjustment for age, smoking, physical activity, BMI, blood pressure, TC, and intakes of energy and calcium in Model 4. Women in the third tertile of energy-adjusted total ω-3 FA intake had a significantly lower AAC severity by radiography than those in the first tertile. The association

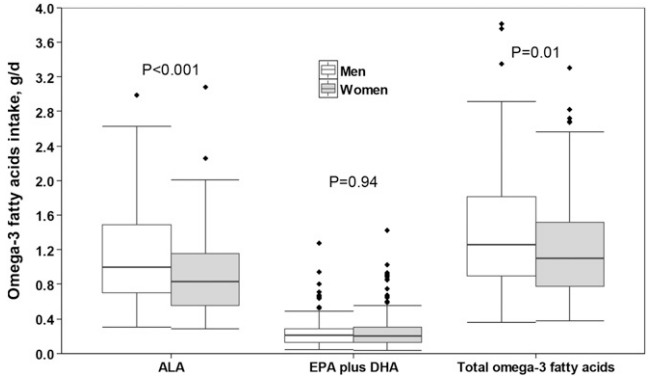

FIGURE 3 Dietary ALA, EPA plus DHA, and total ω-3 FA intakes in older men and women. *P* values for gender difference were calculated by Wilcoxon's rank-sum test. The horizontal lines that form the top and bottom of the box refer to the 75th percentile (Q3) and 25th percentile (Q1). The horizontal line in the middle of the box is the median. Horizontal lines above and below the box represent maximum [Q3 + 3.5 × (Q3 − Q1)] and minimum [Q3 − 3.5 × (Q3 − Q1)] values. The dots represent outliers, which are values greater than the maximum value. ALA, α-linolenic acid; Q, quartile.

remained significant after controlling for the confounders in Model 4.

An inverse association between energy-adjusted ALA consumption and AAC severity by DXA was also observed (*P*-trend: 0.016) after adjustment for all confounders in women. Higher consumption of total ω-3 FAs was significantly associated with lower risk of AAC severity by DXA (*P*-trend: 0.029) in women, and this association was strengthened when more confounders were taken into account (*P*-trend: 0.001).

EPA plus DHA intake was not found to be significantly associated with AAC severity by radiography ($P = 0.70$) or DXA ($P = 0.69$) among women (**Table 3**).

Changes in tertile of energy-adjusted ALA ($P = 0.16$ for men; $P = 0.62$ for women), EPA plus DHA ($P = 0.61$ for men; $P = 0.58$ for women), and total ω-3 FA intakes ($P = 0.91$ for men; $P = 0.38$ for women) were not associated with AAC severity among either men or women, after adjustment of baseline ω-3 FA intake, age, smoking, physical activity, BMI, systolic blood pressure, diastolic blood pressure, TC, and consumption of total energy and calcium (**Figure 4**).

Sensitivity analysis. To determine whether CVD could change the association between ω-3 FA intake and AAC severity, we repeated the analyses among participants (106 men, 171 women) who did not report diabetes, heart disease, or stroke at baseline. The significant inverse relation between baseline dietary ω-3 FA intake and AAC severity by radiography was observed among women but not among men [OR (95% CI) for tertile 3 vs. tertile 1 among women: 0.39 (0.19, 0.82); OR (95% CI) for tertile 3 vs. tertile 1 among men: 0.70 (0.28, 1.76)] after adjustment for confounders.

Discussion

Our results demonstrated that baseline ALA and total ω-3 FA intakes were inversely associated with AAC severity over 18 y among community-dwelling older women, but not men. The decreased risk of AAC among women with higher intake of total

TABLE 2 Associations of ALA, EPA plus DHA, and total ω-3 FA intakes with AAC assessed by radiography and DXA in older men[1]

	Results from radiography				Results from DXA			
	Tertile 1	Tertile 2	Tertile 3	*P*-trend	Tertile 1	Tertile 2	Tertile 3	*P*-trend
ALA								
Participants, *n*	41	41	42		35	37	38	
Energy-adjusted intake,[2] g/d	0.97 [0.16–1.04]	1.12 [1.04–1.20]	1.30 [1.21–2.12]		0.95 [0.16–1.02]	1.09 [1.03–1.20]	1.28 [1.20–2.12]	
OR (95% CI),[3] Model 1	Ref	0.85 (0.37, 1.92)	0.89 (0.40, 2.02)	0.79	Ref	0.75 (0.32, 1.75)	0.81 (0.34, 1.91)	0.64
OR (95% CI),[3] Model 2	Ref	0.89 (0.39, 2.05)	0.87 (0.38, 2.00)	0.74	Ref	0.75 (0.32, 1.76)	0.78 (0.33, 1.86)	0.59
OR (95% CI),[3] Model 3	Ref	1.12 (0.46, 2.75)	1.32 (0.54, 3.27)	0.54	Ref	0.87 (0.35, 2.14)	1.02 (0.41, 2.55)	0.96
OR (95% CI),[3] Model 4	Ref	1.07 (0.44, 2.64)	1.47 (0.58, 3.71)	0.41	Ref	0.77 (0.30, 1.98)	1.16 (0.45, 2.97)	0.74
EPA plus DHA								
Participants, *n*	41	41	42		36	37	38	
Energy-adjusted intake,[2] g/d	0.14 [0.01–0.18]	0.22 [0.18–0.25]	0.35 [0.25–1.18]		0.13 [0.01–0.18]	0.21 [0.18–0.25]	0.34 [0.25–1.18]	
OR (95% CI),[3] Model 1	Ref	0.63 (0.28, 1.45)	1.20 (0.52, 2.73)	0.66	Ref	0.94 (0.40, 2.21)	1.00 (0.43, 2.35)	0.99
OR (95% CI),[3] Model 2	Ref	0.51 (0.22, 1.20)	1.05 (0.45, 2.45)	0.88	Ref	0.86 (0.36, 2.04)	0.97 (0.41, 2.31)	0.95
OR (95% CI),[3] Model 3	Ref	0.51 (0.20, 1.28)	1.00 (0.39, 2.58)	0.99	Ref	0.91 (0.37, 2.29)	1.05 (0.40, 2.74)	0.92
OR (95% CI),[3] Model 4	Ref	0.54 (0.22, 1.35)	1.28 (0.51, 3.20)	0.60	Ref	0.91 (0.36, 2.29)	1.20 (0.46, 3.10)	0.70
Total ω-3 FAs								
Participants, *n*	41	41	42		36	37	38	
Energy-adjusted intake,[2] g/d	1.16 [0.16–1.27]	1.39 [1.28–1.48]	1.64 [1.49–3.04]		1.11 [0.16–1.25]	1.35 [1.25–1.46]	1.62 [1.47–3.04]	
OR (95% CI),[3] Model 1	Ref	1.15 (0.50, 2.61)	0.84 (0.37, 1.89)	0.66	Ref	0.65 (0.28, 1.53)	0.62 (0.27, 1.47)	0.29
OR (95% CI),[3] Model 2	Ref	0.96 (0.42, 2.23)	0.80 (0.35, 1.85)	0.60	Ref	0.57 (0.24, 1.38)	0.62 (0.26, 1.49)	0.30
OR (95% CI),[3] Model 3	Ref	0.87 (0.35, 2.19)	1.04 (0.42, 2.55)	0.93	Ref	0.56 (0.21, 1.47)	0.83 (0.32, 2.15)	0.74
OR (95% CI),[3] Model 4	Ref	0.98 (0.39, 2.47)	1.32 (0.53, 3.31)	0.55	Ref	0.53 (0.20, 1.44)	1.05 (0.40, 2.74)	0.95

[1] AAC, abdominal aortic calcification; ALA, α-linolenic acid; Ref, reference.
[2] Values are medians [ranges].
[3] Ordinal logistic regression models were used to calculate ORs (95% CIs) and *P*-trend. Model 1 adjusted for age (continuous); Model 2 adjusted for Model 1 plus smoking (current, former, or never) and physical activity (quartiles); Model 3 adjusted for Model 2 plus BMI (continuous), systolic blood pressure (continuous), diastolic blood pressure (continuous), and plasma total cholesterol (continuous); and Model 4 adjusted for Model 3 plus total energy (in tertiles) and calcium (in tertiles) intakes.

TABLE 3 Associations of ALA, EPA plus DHA, and total ω-3 FA intakes with AAC assessed by radiography and DXA in older women[1]

	Results from radiography				Results from DXA			
	Tertile 1	Tertile 2	Tertile 3	*P*-trend	Tertile 1	Tertile 2	Tertile 3	*P*-trend
ALA								
Participants, *n*	62	63	63		50	54	54	
Energy-adjusted intake,[2] g/d	0.73 [0.17–0.82]	0.88 [0.82–0.94]	1.10 [0.94–2.55]		0.73 [0.32–0.81]	0.87 [0.82–0.94]	1.1 [0.94–2.55]	
OR (95% CI),[3] Model 1	Ref	0.85 (0.44, 1.66)	0.59 (0.30, 1.17)	0.13	Ref	0.62 (0.30, 1.27)	0.53 (0.26, 1.10)	0.088
OR (95% CI),[3] Model 2	Ref	0.83 (0.42, 1.61)	0.58 (0.29, 1.13)	0.11	Ref	0.60 (0.29, 1.24)	0.51 (0.25, 1.06)	0.072
OR (95% CI),[3] Model 3	Ref	0.82 (0.41, 1.64)	0.49 (0.24, 1.00)	0.05	Ref	0.51 (0.24, 1.09)	0.39 (0.18, 0.85)	0.018
OR (95% CI),[3] Model 4	Ref	0.56 (0.26, 1.20)	0.49 (0.23, 1.02)	0.06	Ref	0.42 (0.19, 0.96)	0.37 (0.16, 0.83)	0.016
EPA plus DHA								
Participants, *n*	62	63	63		52	54	54	
Energy-adjusted intake,[2] g/d	0.13 [0.01–0.18]	0.22 [0.18–0.27]	0.42 [0.27–1.38]		0.12 [0.01–0.17]	0.22 [0.18–0.27]	0.43 [0.27–1.38]	
OR (95% CI),[3] Model 1	Ref	1.47 (0.75, 2.89)	0.88 (0.45, 1.73)	0.70	Ref	1.31 (0.64, 2.70)	0.86 (0.41, 1.78)	0.69
OR (95% CI),[3] Model 2	Ref	1.40 (0.70, 2.77)	0.81 (0.40, 1.60)	0.53	Ref	1.25 (0.60, 2.60)	0.78 (0.37, 1.63)	0.52
OR (95% CI),[3] Model 3	Ref	1.35 (0.67, 2.75)	0.63 (0.30, 1.31)	0.23	Ref	0.99 (0.46, 2.12)	0.65 (0.29, 1.42)	0.29
OR (95% CI),[3] Model 4	Ref	1.01 (0.48, 2.12)	0.54 (0.25, 1.13)	0.10	Ref	0.85 (0.38, 1.89)	0.59 (0.27, 1.31)	0.20
Total ω-3 FAs								
Participants, *n*	62	63	63		52	54	54	
Energy-adjusted intake,[2] g/d	0.98 [0.30–1.07]	1.16 [1.07–1.27]	1.52 [1.27–2.63]		0.98 [0.33–1.07]	1.16 [1.07–1.28]	1.53 [1.28–2.63]	
OR (95% CI),[3] Model 1	Ref	0.86 (0.44, 1.68)	0.49 (0.25, 0.96)	0.04	Ref	0.43 (0.21, 0.89)	0.45 (0.22, 0.93)	0.029
OR (95% CI),[3] Model 2	Ref	0.85 (0.43, 1.66)	0.44 (0.22, 0.89)	0.02	Ref	0.41 (0.20, 0.85)	0.40 (0.19, 0.83)	0.013
OR (95% CI),[3] Model 3	Ref	0.74 (0.36, 1.50)	0.31 (0.15, 0.64)	0.002	Ref	0.25 (0.11, 0.55)	0.27 (0.12, 0.61)	0.001
OR (95% CI),[3] Model 4	Ref	0.60 (0.29, 1.25)	0.33 (0.16, 0.71)	0.004	Ref	0.22 (0.09, 0.50)	0.27 (0.12, 0.62)	0.001

[1] AAC, abdominal aortic calcification; ALA, α-linolenic acid; Ref, reference.
[2] Values are medians [ranges].
[3] Ordinal logistic regression models were used to calculate ORs (95% CIs) and *P*-trend. Model 1 adjusted for age (continuous); Model 2 adjusted for Model 1 plus smoking (current, former, or never) and physical activity (quartiles); Model 3 adjusted for Model 2 plus BMI (continuous), systolic blood pressure (continuous), diastolic blood pressure (continuous), and total cholesterol (continuous); and Model 4 adjusted for Model 3 plus total energy (in tertiles) and calcium (in tertiles) intakes.

ω-3 FAs was significant after adjusting for traditional cardiovascular risk factors and some nutrient intakes. Baseline EPA plus DHA intake was not associated with AAC severity among either men or women. Associations between changes in tertile of total ω-3 FA intake over 18 y and AAC severity were not observed among either men or women. Comparison of the results from 2 methods of AAC assessment by radiography and DXA showed similar associations between ω-3 FA intake and AAC severity.

A higher concentration of serum long-chain n–3 FAs was previously found to be associated with a lower incidence of vascular calcification (35). Nevertheless, long-chain n–3 FA (EPA plus DHA) intake was not found to be a predictor of coronary artery calcium in 2 population-based studies (25, 26). Because of the absence of more data on the relation between ω-3 FA intake and vascular calcification, the remainder of the discussion relates to the associations of ω-3 FA intake with CVD and CVD risk factors.

The benefits of EPA and DHA supplements for protection against CVD events in participants with CVD were inconsistent among different clinical trials (10, 36, 37). Large-scale longitudinal cohort studies have reported dietary EPA and DHA to be inversely associated with cardiovascular risk factors including blood pressure, cardiac function, vascular reactivity, and lipids, and positively associated with antiplatelet, anti-inflammatory, and antioxidative actions (11). Dietary EPA and DHA may have primarily preventive effects in the general population rather than secondary preventive effects among patients with CVD. Nevertheless, the preventive effects of EPA plus DHA on vascular calcification have not been supported by general population studies (25, 26). Likewise, our study showed that higher consumption of EPA plus DHA was not associated with lower AAC severity, which might be attributable to the low intake of EPA plus DHA in our cohort. The preventive effects on CVD were only apparent [OR (95% CI) for CVD events: 0.78 (0.65, 0.93)] at a higher dose of EPA and DHA (>1 g/d) (37), so the benefits for AAC might be found only for high intake of dietary EPA and DHA.

We found that higher baseline ALA intake was associated with lower AAC severity over 18 y among women. Previous studies provided strong evidence to support the findings in the present study. A higher ALA consumption was demonstrated to be protective of CVD, and the protective effect was most evident among subjects with low fish intake (6). We did not examine whether the anticalcification effect of ALA intake was more evident among participants with low fish intake because of the small sample size of our study. In the Nurses' Health Study, 76,283 women with 10 y of follow-up were included in the data analysis. A higher dietary intake of ALA was associated with lower fatal, but not nonfatal, ischemic heart disease, after adjustment for age and standard coronary risk factors (7). Furthermore, a clinical trial with a mean follow-up of 27 mo showed that the Mediterranean diet abundant in ALA appeared to be more efficient than currently used postinfarct prudent diets in the secondary prevention of coronary heart disease and death (8). It is possible that other factors, including phosphorous and vegetable intake known to be associated with ALA intake, could confound the association with AAC (38).

In our cohort, the majority of ω-3 FA is ALA, which is mainly obtained from plant sources. Participants with more ALA intake might also have more nut or soybean intake, which may have other benefits with regard to AAC severity (39, 40). EPA plus DHA accounted for a small proportion of total ω-3 FAs. Fish is rich in EPA and DHA but is not the predominant source of EPA and DHA in Australians; red meat is the main source because of

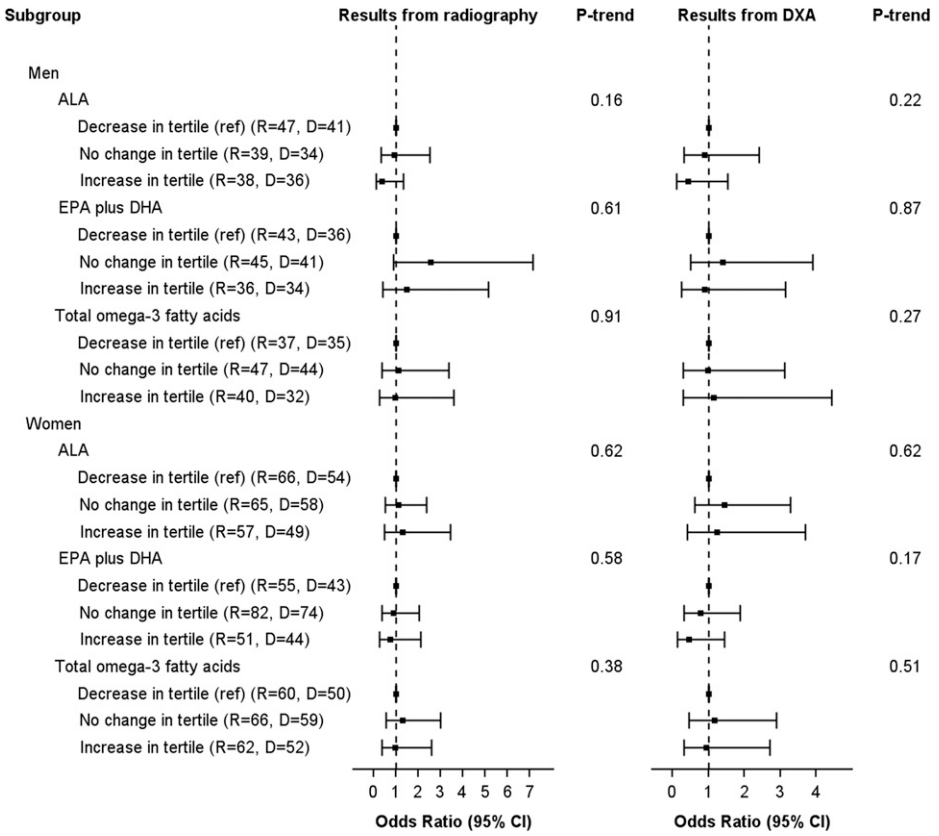

FIGURE 4 Associations of changes in energy-adjusted ALA, EPA plus DHA, and total ω-3 FA intakes with abdominal aortic calcification assessed by radiography and DXA in older men and women. With the decrease in tertile as reference, multivariate adjusted ORs (95% CIs) for no change and increase in tertile of ω-3 FA intake were derived from ordinal logistic regression models. Covariates included baseline ω-3 FA intake (in tertiles), age (continuous), smoking (current, former, or never), physical activity (quartiles), BMI (continuous), SBP (continuous), DBP (continuous), TC (continuous), and total energy (in tertiles) and calcium (in tertiles) intakes. ALA, α-linolenic acid; D, number of participants for DXA; DBP, diastolic blood pressure; R, number of participants for radiography; ref, reference; SBP, systolic blood pressure; TC, total cholesterol.

its more frequent consumption (41). This may have confounded the association of animal-derived EPA plus DHA intake with AAC in our study because fresh and processed meat may have other components, such as saturated fats, that may be less healthy (42). Further adjustment for meat and fish intake did not change this association.

Recently, a study based on the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (43) found no significant association between total ω-3 FA intake and risk of coronary heart disease. In contrast, the Japan Public Health Center–Based Study cohort (44) found that dietary intake of ω-3 FAs was significantly inversely associated with total coronary heart disease and nonfatal coronary heart events among men and women after adjustment for confounders. Our data also suggested an inverse relation between total ω-3 FA intake and AAC severity over 18 y in women, but not men. Consistent with our study, data from the Health Professionals Follow-up Study showed increasing dietary ω-3 FAs was not associated with the risk of coronary heart disease among men (45), whereas the Nurses' Health Study indicated potential benefits from higher ω-3 FA intake on the prevention of coronary heart disease among women (46). It is possible that the preventive effects of higher ω-3 FA intake on CVD are stronger among women than men. The benefit of higher ω-3 FA intake for prevention of AAC may have been more apparent in women than men because women appear to have a greater ability to metabolize ALA, the main ω-3 FA in the diet of participants, to DHA than men (47, 48). In addition, women exceeded, but men failed to meet, dietary guidelines for absolute ALA intake (data not shown) (3), which may partly explain why the association was observed in women but not men.

In our study, change in tertile of total ω-3 FA intake over 18 y was not shown to be associated with AAC severity. One explanation is that 2 different FFQs were used to assess ω-3 FA intake at baseline and follow-up, which meant we could only assess changes in intake tertiles relative to other study participants. For the analyses of the association between ω-3 FA intake at baseline and AAC, supplement use was not a confounder because only 7 of the 312 participants were using fish oil supplements at baseline. Although we collected data on supplement use, we were not able to ascertain the ω-3 FA dose at either time point. At follow-up, 40% of the participants were using fish oil supplements, and it is possible that this influenced our analysis of the changes over time.

The strengths of our study included a long-term follow-up of the cohort, which allowed us to determine the effects of baseline and changes in ω-3 FA intake on AAC severity over 18 y. Furthermore, AAC scores were assessed with use of both radiography and DXA. One limitation of our study was that AAC was not assessed at baseline. Therefore, it is uncertain whether the severe AAC developed before baseline or during the 18-y follow-up, although one study demonstrated that a high proportion of subjects developed vascular calcification between 50 and 70 y of age (12), suggesting that participants in our study possibly had a low prevalence of AAC severity at baseline. In addition, our analyses are entirely based on FA intake derived from FFQs, which are known to measure intake with considerable error. We have evidence to indicate that the baseline FFQ measured EPA, DHA, and total ω-3 FA intake moderately well (corrected correlation coefficients for EPA, DHA, and total ω-3 FAs were 0.40, 0.78, and 0.57, respectively), whereas the correlation between plasma ALA and dietary ALA intake was weak (corrected correlation coefficient: 0.24) (49). The data for the correlation between plasma FA composition and dietary FA intake derived from a follow-up FFQ is not available. Besides, we were unable to analyze AAC according to the food source of EPA and DHA, thus, we could not determine if EPA and DHA intake from different sources had different effects on AAC.

The primary aim of this study was to evaluate the impact of low and high dietary calcium intake on bone density, fractures, vascular events, and aortic calcifications. The exclusion of participants with moderate intake of calcium might have biased our observations on the association of ω-3 FAs with AAC, although further adjustment of calcium intake in the multivariate models did not change this association.

In conclusion, baseline dietary ALA and total ω-3 FA intakes are important predictors of the development of AAC in older women, but not in older men. However, EPA plus DHA intake is not associated with AAC severity in either men or women.

Acknowledgments

DRE, GGG, and PRE designed the research; BK and NK conducted the research; XS, KMS, DS, and AH analyzed the data; and XS, KMS, DS, AH, and PRE wrote the paper. All authors read and approved the final manuscript.

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