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Association of blood levels of marine omega-3 fatty acids with coronary calcification and calcium density in Japanese men

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Abstract

Background/Objective: Clinical trials of eicosapentaenoic acid (EPA) among high-risk groups in Japan in which consumption of mairne-omega-3 fatty acids (OM3) is much higher than other countries showed slower progression of coronary atherosclerosis. We aimed to determine the cross-sectional associations of coronary artery calcification (CAC) and calcium density with OM3, EPA and docosahexaenoic acid (DHA), two principal OM3, in the general population in Japan.

Subjects/Methods: The Shiga Epidemiological Study of Subclinical Atherosclerosis examined a population-based sample of 1,074 men aged 40–79 in 2006–08 for computed-tomography-measured CAC score (CCS), a well-established biomarker of coronary atherosclerosis, CAC density score (CDS), a potential marker of plaque stabilization, serum levels of OM3, and risk factors.

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Conflict of interest

None to declare

Results: Prevalence of CCS >0, ≥100, and ≥300 was 65.8%, 25.9% and 12.9%, respectively; the mean (SD) OM3, EPA and DHA were 10.1% (3.2), 3.2% (1.7), and 5.9% (1.6), respectively. Odds ratios (95% CI, p-value) of CCS 0, 100 and 300 in ordinal logistic regression associated with 1 SD increase of OM3, EPA, and DHA were 0.91 (0.81–1.03, p=0.12), 0.99 (0.88–1.11, p=0.87) and 0.84 (0.74–0.94, p=<0.01), respectively. The inverse association of DHA with CCS remained significant in multivariate-adjusted model: odds ratio of 0.87 (0.77–0.99, p=0.03). Blood levels of OM3, EPA or DHA did not have any significant associations with CDS.

Conclusions—DHA but not EPA had a significant inverse association with coronary atherosclerosis in the general population with high levels of OM3. Future trials are warranted comparing the effect of high-dose DHA and EPA on atherosclerosis and cardiovascular outcomes.

Keywords

Atherosclerosis; coronary artery calcification; coronary calcium density; docosahexaenoic acid; eicosapentaenoic acid; epidemiology; Japanese

Introduction

Recent long-term large randomized clinical trials (RCTs) of marine omega-3 fatty acids (OM3) on coronary heart disease (CHD) among high-risk groups all failed to show clinical benefit of OM3, *i.e.*, Alpha-Omega Trial, Outcome Reduction With Initial Glargine Intervention Trial (ORIGIN), Supplementation en Folates et Aomega-3 (SU.FOM.OM3) and the Risk and Prevention Study (1–4) of which doses of administered OM3 ranged from 0.3 to 0.9 g/day. In contrast, JELIS (Japan Eicosapentaenoic acid (EPA) Lipid Intervention Study), which randomized 18,465 patients with hypercholesterolemia with and without cardiovascular disease to receive a statin plus 1.8 g/day of EPA, one of the two principal OM3, or statin alone, showed a significant 19% relative reduction in major coronary events with no significant changes between treatment groups in low-density lipoprotein cholesterol (LDL-C).(5) Another RCT of 241 Japanese patients with acute coronary syndrome showed a significant 11% relative reduction in cardiovascular events in a statin plus 1.8 g/day of EPA compared to statin alone.(6) Of note, we and others pointed out that 1.8 g/day of EPA in Japanese trials (on top of the high consumption of OM3 in Japan) will generate blood levels of OM3 that would require about 3.6 g/day of OM3 on a US background diet (7, 8) (the median background dietary intake of OM3 (g/day) in Japan is 1.1 compared to <0.1 in the US.(9, 10)) Additionally, three recent RCTs of 1.8 g/day of EPA conducted in Japan showed significantly slower progressions of coronary plaque among patients with CHD(11, 12) and carotid intima-media thickness (CIMT) among patients with diabetes.(13) These results indicate that high-dose OM3 is anti-atherogenic in high-risk groups. A cross-sectional study of 261 inhabitants in the fishing village and 209 in the farming village in Japan showed a significant inverse association of blood levels of OM3 with the number of carotid plaque(14) implying that OM3 may be anti-atherogenic even in the general population with high consumption of OM3.

Coronary artery calcification (CAC) is a well-established biomarker of coronary atherosclerosis. CAC predicts future cardiovascular events much better than carotid plaque. (15) In studies of Western population, CAC shows no significant association with dietary

intake of OM3(16, 17) potentially due to much lower consumption of OM3 in Western than Japanese populations. A recent observational study documents that calcium density of the coronary plaque is inversely associated with cardiovascular events at a given level of CAC, (18) suggesting that higher calcium density represents more stable plaque. RCTs of OM3 among patients with endarterectomy reported that OM3 increases plaque stability.(19) (20)

In this study, we hypothesized that blood levels of OM3 in Japanese would have a significant inverse association with CAC and that the inverse association of OM3 with CAC is primarily attributed to docosahexaenoic acid (DHA), another principal OM3, based on our previous study in Japanese showing that blood levels of DHA but not EPA had a significant inverse association with CIMT.(21) We also hypothesized that blood levels of OM3 would have a significant positive association with CAC density. We tested these hypotheses in a population-based sample of 1,094 Japanese from the Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA).

Subjects and methods

SESSA is a population-based study of a random sample from a general Japanese population as previously described in detail.(22–26) Briefly, a total of 1,094 men aged 40 to 79 years were recruited in 2006–2008. After excluding eight participants with missing data (n=7 for LDL-C and n=1 for volume score), a total of 1,086 participants were analyzed for the current study. There were 102 participants with a history of myocardial infarction (MI), stroke or chronic kidney disease (CKD). The study was approved by the Institutional Review Boards of Shiga University of Medical Science and University of Pittsburgh. All participants provided written informed consent.

CAC measurements

CAC was assessed using either electron-beam computed tomography (EBCT, n=756) using a C-150 scanner (Imatron, South San Francisco, CA, US) or 16-channel multi-detector-row computed tomography (MDCT, n=330) using an Aquilion scanner (Toshiba, Tokyo, Japan). (24–26) Images were obtained from the level of the root of the aorta through the heart at a 3-mm slice thickness with a scan time of 100 milliseconds (EBCT) or 320 milliseconds (MDCT). CAC was quantified using AccuImage software (AccuImage Diagnostics, South San Francisco, CA, US). The presence of CAC was defined as a minimum of three contiguous pixels (area=1 mm^2) with density ≥ 130 Hounsfield Units. CAC score (CCS) was calculated according to the Agatston method.(27) A volume score was also calculated.(28) CAC density score (CDS) was calculated as Agatston score divided by area score where the area score was calculated as the volume score divided by the slice thickness: 3 mm.(18) All images were read by a single physician trained at the Cardiovascular Institute, University of Pittsburgh, who was blinded to participant's characteristics. The reproducibility of the scans showed an intraclass correlation of 0.98 for both CCS and CDS.

Blood levels of OM3 and other fatty acids

Venipuncture was performed early in the clinic visit after a 12-hour fast. Serum samples were stored at −80°C and shipped on dry ice to University of Pittsburgh. Fatty acids were

analyzed by capillary-gas-liquid chromatography (PerkinElmer Clarus 500).(29) OM3 was defined as the sum of EPA, docosapentaenoic acid (DPA) and DHA. The coefficients of variation for EPA, DPA and DHA were 4.5 %, 4.5% and 7.2%, respectively. Those for plant-based omega-3 fatty acid: alpha-linolenic acid (ALA), and two major omega-6 fatty acids: linoleic (LA) and arachidonic fatty acids (ARA), were 7.9%, 1.6% and 4.5%, respectively.

Other measurements

A self-administered questionnaire was used to obtain information from participants on demography, smoking, alcohol drinking, medication (hypertension, dyslipidemia, and diabetes mellitus), and medical history as previously described.(22–26) Pack-years of smoking were calculated as years of smoking multiplied by the number of cigarettes per day divided by 20. Alcohol drinking was assessed based on the quantity and frequency of beer, wine, liquor, or sake (Japanese rice wine) consumption by the participant. An alcohol drinker was defined as an individual who consumed alcohol ≥ two times a week. Ethanol consumption per day was estimated, assuming that concentrations of alcohol were 5%, 12%, 40%, and 16% for beer, wine, liquor, and sake, respectively.

Body-mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Blood pressure (BP) was measured in the right arm of the seated participant after the participant sat quietly for five minutes, using an appropriate-sized cuff, with an automated sphygmomanometer (BP-8800, Omron Health Care Co. Ltd, Tokyo, Japan). The average of two measurements was used to determine BP. Hypertension was defined as systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or use of anti-hypertensive medication. Diabetes mellitus was defined as a hemoglobin A1c ≥ 6.5%,(30) a fasting blood glucose ≥7.0 mmol/L or use of anti-diabetic medication. CKD was defined as an estimated glomerular filtration rate <30 mL/min.(31)

Total cholesterol and triglycerides were measured using enzymatic assays, and high-density lipoprotein cholesterol (HDL-C) using a direct method. LDL-C was estimated using Friedewald's formula.(32) When triglycerides exceeded 4.52 mmol/L, we did not estimate LDL-C and excluded these participants from the study (n=7). Lipid measurements were standardized according to the protocol for the US Centers for Disease Control and Prevention/Cholesterol Reference Method Laboratory Network. C-reactive protein (CRP) was measured by nephelometry using a BN II Analyzer.

Statistics

Participant characteristics were shown as means and standard deviations (SDs) for continuous variables with approximately normal distributions, medians and interquartile ranges for continuous variables with skewed distributions, and frequencies and percentages for categorical variables. To examine the univariate associations of the CCS with participant's characteristics, we used a general linear model to compare characteristics across four groups of CCS using cutoff points of CCS 0, 100 and 300 (CCS=0, CCS <100, CCS >300 and CCS ≥300), adjusting for age because CCS was age dependent. These cutoff points are widely used and clinically relevant.(33, 34) To examine multivariable associations of CCS with each of OM3, EPA and DHA, we used both ordinal logistic and robust linear

regressions because the distribution of CCS was highly skewed. For ordinal logistic regression, we chose cutoff points of CCS 0, 100 and 300. For multivariable adjustments in both ordinal logistic and robust linear regressions, we first adjusted for age and CT type (Model I), then further adjusted for hypertension, diabetes, LDL-C, HDL-C, pack-years of smoking and BMI (Model II) based on previous studies.(35, 36) In Model III, we additionally adjusted for CRP, triglycerides, ethanol intake and medications for lipids and hypertension as well as cardio-renal disease (a past history of MI, stroke or CKD). To examine the univariate association of CDS with the participant's characteristics, we used a general linear model to compare characteristics across three even groups of CDS adjusting for age. To examine multivariable associations of CDS with each of OM3, EPA and DHA at a given CAC score, we used robust linear regression using the same modeling strategy as described above except that we adjusted for log-transformed CCS in Model I. As sensitivity analyses, the association was examined excluding those with cardio-renal disease. Additionally, the associations of CDS with each of OM3, EPA, and DHA were analyzed excluding participants on lipid-lowering medication because statin therapy might increase calcium density.(18) As our supplemental analyses, the associations of CCS and CDS with each of ALA, LA, and ARA were examined. Two-tailed P values of $< .05$ were considered to indicate statistical significance. Though we had two outcomes (CAC and CDS) no adjustment was made for the multiple testing. All these statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC, US).

Results

Among 1,086 participants the mean (SD) age was 64.1 (9.9) years (Table 1); 56.1% were hypertensive, 22.2% had diabetes, and 31.9% were current smokers. The mean (SD) LDL-C was 3.22 (0.82) mmol/L. CAC was present in 714 subjects (65.8%), and prevalence of CCS ≥100 and ≥300 was 25.9% and 12.9%, respectively. The mean (SD) OM3, EPA, and DHA were 10.1 (3.2), 3.2 (1.7), and 5.9 (1.6), respectively.

Generally, cardiovascular risk factors had a positive significant association with the CCS categories including age, BMI, systolic BP, total cholesterol, CRP, pack-years of smoking, and rates of hypertension and diabetes (Table 2). In contrast, neither OM3, EPA, DHA nor other fatty acids had significant associations with the CCS categories.

In contrast to CCS, the category of CDS had no significant associations with cardiovascular risk factors (Table 3). Likewise, levels of fatty acids including OM3, EPA, and DHA were very similar across the three groups. Though rates of participants on lipid-lowering medication tended to be higher in the higher categories of CDS, the association did not reach statistical significance.

Ordinal logistic regressions showed that DHA had a significant inverse association with CCS after adjusting for age and CT type (odds ratio (OR): 0.84, 95% confidence interval (CI): 0.74–0.94, $p < 0.01$) (Table 4). The inverse association remained significant after further adjusting for traditional cardiovascular risk factors (OR: 0.88, 95% CI: 0.0.78–0.99, $p=0.045$) and other factors (OR: 0.87, 95% CI 0.77–0.99, $p=0.03$). Robust linear regression also showed a significant inverse association of DHA with CCS, which remained after

adjusting for various risk factors. In contrast, EPA had almost null associations with CCS in all models and in both ordinal logistic and robust regression analyses. OM3 had an inverse association with CCS in all models without statistical significance. The above results did not materially change when we excluded individuals with cardio-renal disease (Supplemental Table 1). The association of ALA, LA, or ARA with CCS was null and non-significant (Supplemental Table 2).

The associations of CDS with each of OM3, EPA and DHA were inverse (Table 5) without statistical significance except for Model II for OM3 and EPA. The above results did not change when we excluded individuals with cardio-renal disease (Supplemental Table 3). Even after excluding participants on lipid-lowering medications, the association was non-significant (Supplemental Table 4). The association of ALA, LA, or ARA with CDS was virtually null (Supplemental Table 5).

Discussion

This population-based cross-sectional study of 1,086 men showed that serum levels of OM3 had a non-significant inverse association with CCS. When the association of EPA and DHA was examined separately, DHA but not EPA had a significant inverse association with CCS. The significant inverse association of DHA with CCS remained after adjusting for various factors or after excluding participants with cardio-renal disease. In contrary to our hypothesis, serum levels of OM3, EPA or DHA had no significant associations with CDS. The associations of serum levels of ALA, LA, and ARA with CCS and CDS were virtually null. This study is the first to document the significant inverse association of DHA with CCS in the general population.

Numerous preclinical studies have documented the anti-atherogenic properties of OM3,(37) yet epidemiological studies in Western countries do not show any significant association of OM3 with CAC. The Multi-Ethnic Study of Atherosclerosis found no significant cross-sectional association of dietary intake of OM3 with CAC in 5,488 subjects.(17) The Rotterdam Study found no significant longitudinal association of dietary intake of OM3 with CAC in 1,570 subjects.(16) Median dietary intake of OM3 in these two populations was about 100 mg/day which is less than one tenth of the Japanese general population.(9, 10)

It is perceived that increasing dietary intake of OM3 more than 1.0 g/day has no cardiovascular benefit in the general population.(38) Three recent large prospective cohort studies in Japan, however, showed that higher dietary intake of OM3 (median dietary intake ranges from 1.7 to 2.3 g/day in the highest quartile or quintile) was inversely associated with cardiovascular outcomes. A 10-year prospective cohort study of 41,578 men and women documented a significant inverse association of dietary intake of OM3 with non-fatal MI (multivariable-adjusted hazard ratio (m-HR) of 0.33, 95% CI: 0.17–0.63, comparing the highest to the lowest quintile).(39) A 12.7-year prospective cohort study of 57,972 men and women showed a significant inverse association of dietary intake of OM3 with cardiovascular events (m-HR of 0.81, 95% CI: 0.67–0.98, comparing the highest to the lowest quartile).(40) A 24-year prospective cohort study of 9,190 men and women demonstrated a significant inverse association of dietary intake of OM3 with cardiovascular

death (m-HR of 0.80, 95% CI: 0.66–0.96, comparing the highest to the lowest quintile).(41) The latter two studies reported an inverse but non-significant association of dietary intake of OM3 with CHD, yet the non-significant association was likely due to the relatively small numbers of CHD cases.(42) These results were consistent with our finding that serum levels of OM3 had a significant inverse association with CAC the general population in Japan.

We observed a significant inverse association of CAC with DHA but not EPA. This observation was consistent with some previous observational studies reporting significant inverse associations of DHA but not EPA with coronary atherosclerosis in women with CHD (longitudinal)(43) and CIMT in the general population in Japan (cross sectional).(21) Additionally, a review of case-control studies reported that DHA had stronger inverse association with CHD than EPA.(44) However, a more recent systematic review on observational studies and RCTs showed that the relative risks of EPA and DHA associated with CHD were very similar.(45) Short-term (6 weeks) RCTs of high-dose OM3 (4g/day) documented somewhat differential effects of purified EPA and DHA on cardiovascular risk factors in patients without CHD, *e.g.*, DHA but not EPA significantly increased HDL-C and reduced BP and heart rate.(46–48) No RCTs have compared the differential effect of EPA and DHA on atherosclerosis or cardiovascular outcomes.

Evidence from RCTs in Japan demonstrated that high-dose pure EPA had anti-atherogenic properties in patients with CHD(11, 12) and diabetes.(13) In these Japanese RCTs,(12, 13) blood levels of EPA increased whereas those of DHA decreased in the intervention group indicating that the anti-atherogenic properties of OM3 in these RCTs among high-risk groups were attributed to EPA. Whether high-dose (4.0 g/day) purified EPA or primarily EPA is anti-atherogenic (EVAPORATE: NCT02926027) or reduces cardiovascular outcome (REDUCE IT: NCT 01492361, STRENGTH: NCT02104817) is currently under investigation in high-risk groups. Our results suggest that DHA may have more potent anti-atherogenic properties than EPA in the general population. Future trials are warranted comparing the effect of DHA and EPA on atherosclerosis and cardiovascular outcomes.

Contrary to our hypothesis that OM3 would have a positive association with CDS based on an RCT of OM3 among patients with endarterectomy which reported that OM3 increases plaque stability,(19) we observed inverse associations of OM3 with CDS. In this RCT, plaque stabilization was partly associated with well-formed fibrous caps in the invention group and increasing calcium density may not necessarily be a mechanism for plaque stabilization of OM3.

Our study showed no significant association of CAC or CDS with serum levels of ALA, LA or ARA. This finding is consistent with the results from a recent meta-analysis of blood levels of these fatty acids with coronary outcomes in 10 large prospective cohort studies and RCTs(49) except for ARA. This meta-analysis documented a significant inverse association of coronary outcomes with ARA. Our finding of the null association of ARA with CCS or CDS might suggest that ARA may be cardio-protective through mechanisms other than anti-atherosclerosis.

Limitations of this study include first: blood levels of OM3 reflect short-term dietary intake and may not reflect long-term dietary intake. However, in populations where intake of OM3 is high like Japanese, a single measurement of blood levels of EPA and DHA reflects habitual intake of EPA and DHA, respectively.(50) Second, the study was observational thus we cannot rule out the possibility of residual or unmeasured confounding. Third, the participants were men only and the results cannot be extended to women. Third, we did not make any adjustment to alpha level to conserve the power, though we had two outcomes (CAC and CDS). However, even after the Bonferroni correction to alpha for two different outcomes (i.e. 0.05/2= 0.025), the result did not change materially: the inverse association of DHA with CAC remained significant. Fourth, although pure EPA and pure DHA are available to conduct intervention studies in human (46), pure DPA is not. Thus we did not show the results of DPA. However, DPA did not have any significant association with CAC or CAC density (data not shown).Finally, about 25% of individuals had CDS slightly greater than 4.0 though the score theoretically ranges from 1.0 to 4.0. The reason for this is unknown. The results did not materially change when we excluded participants with CDS >4.0 (data not shown).

Three RCTs of high-dose pure EPA conducted in Japan demonstrated the anti-atherogenic properties of EPA in high-risk subjects.(11–13) This study documents the significant inverse association of DHA but not EPA with coronary atherosclerosis in the general population with high consumption of OM3 suggesting that DHA may be more anti-atherogenic than EPA. Studies of comparing high-dose DHA and EPA to prevent atherosclerotic cardiovascular disease are justified.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Basic Characteristics of Participants in the Shiga Epidemiological Study of Subclinical Atherosclerosis in 2006–08.

Variable	Participants (n=1,086)
Age, mean (SD), years	64.1 (9.9)
Body mass index, mean (SD), kg/m^2	23.6 (3.0)
Systolic blood pressure, mean (SD), mm Hg	136.6 (19.1)
Hypertension, no. (%)	609 (56.1)
Total cholesterol, mean (SD), mmol/l	5.38 (0.87)
LDL-C, mean (SD), mmol/l	3.22 (0.82)
HDL-C, mean (SD), mmol/l	1.52 (0.44)
Triglycerides, median (IQR), mmol/l	1.20 (0.87, 1.69)
Lipid-lowering medication, no. (%)	165 (15.2)
Glucose, mean (SD), mmol/l	5.71 (1.18)
Diabetes, no. (%)	241 (22.2)
C-reactive protein, median (IQR), nmol/l	43.81 (20.95, 86.67)
Current smoker, no. (%)	346 (31.9)
Pack-years of smoking, median (IQR)	25.0 (5.5, 44.0)
Alcohol drinker, no. (%)	673 (61.9)
Ethanol amount, median (IQR), g/day	14.0 (0.5, 37.0)
CHD, Stroke or CKD, no. (%)	102 (9.4)
Prevalence of CAC score >0, no. (%)	714 (65.8)
≥ 100, no. (%)	281 (25.9)
≥ 300, no. (%)	140 (12.9)
CAC volume, median (IQR)	8.0 (0.0, 88.2)
CAC density, median (IQR)	3.8 (3.4, 4.1)
Total marine omega-3 fatty acids, mean (SD), %	10.1 (3.2)
Eicosapentaenoic acid, mean (SD), %	3.2 (1.7)
Docosahexaenoic acid, mean (SD), %	5.9 (1.6)
Alpha-linolenic acid, mean (SD), %	0.7 (0.2)
Linoleic acid, mean (SD), %	25.3 (4.2)
Arachidonic acid, mean (SD), %	6.2 (1.2)

CKD, chronic kidney disease; CAC, coronary artery calcium; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; IQR, inter-quartile range; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation

Table 2

Age-Adjusted Characteristics of Participants by the Distribution of Coronary Calcium Score.

Variable	CCS = 0 (n = 372)	CCS 1–99 (n = 433)	CCS 100–299 (n = 141)	CCS ≥300 (n = 140)	P for trend
Age, mean (SD), years	58.9 (11.3)	65.3 (7.9)	68.8 (7.3)	69.9 (6.6)	<.01
Body mass index, mean (SE), kg/m^2	22.7 (0.2)	24.0 (0.1)	23.9 (0.3)	24.3 (0.3)	<.01
Systolic blood pressure, mean (SE), mm Hg	132.5 (1.0)	137.5 (0.9)	138.8 (1.6)	142.1(1.6)	<.01
Hypertension, no. (%)	159 (42.6)	257 (59.5)	87 (61.8)	110 (78.2)	<.01
Total cholesterol, mean (SE), mmol/l	5.30 (0.05)	5.44 (0.04)	5.47 (0.07)	5.34 (0.07)	.02
LDL-C, mean (SE), mmol/l	3.16 (0.04)	3.27 (0.04)	3.30 (0.07)	3.19 (0.07)	.05
HDL-C, mean (SE), mmol/l	1.58 (0.02)	1.48 (0.02)	1.49 (0.04)	1.47 (0.04)	.20
Triglycerides, median (IQR), mmol/l	1.03 (0.78, 1.48)	1.34 (0.93, 1.83)	1.35 (0.91, 1.69)	1.25 (0.90, 1.79)	<.01
Lipid-lowering medication, no. (%)	39 (10.4)	47 (10.7)	30 (21.0)	44 (31.5)	<.01
Glucose, mean (SE), mmol/l	5.50 (0.07)	5.78 (0.06)	5.84 (0.10)	5.94 (0.10)	<.01
Diabetes, no. (%)	53 (14.1)	100 (23.1)	32 (22.6)	51 (36.6)	<.01
C-reactive protein, median (IQR), nmol/l	38.10 (20.00, 64.76)	45.71 (23.81, 90.48)	43.81 (21.90, 106.67)	58.10 (24.76, 117.14)	<.01
Current smoker, no. (%)	96 (25.8)	139 (32.1)	50 (35.2)	54 (38.6)	.01
Pack-years of smoking, median (IQR)	20.8 (2.4, 38.2)	26.5 (7.2, 44.4)	27.5 (5.9, 42.6)	36.0 (18.8, 48.6)	<.01
Alcohol drinker, no. (%)	213 (57.1)	276 (63.6)	94 (66.1)	91 (64.8)	.12
Ethanol amount, median (IQR), g/day	8.7 (0.8, 31.4)	16.3 (1.0, 40.6)	15.3 (1.0, 39.0)	17.4 (1.0, 45.0)	<.01
CHD, Stroke or CKD, no. (%)	16 (4.2)	26 (6.0)	13 (8.7)	31 (21.7)	<.01
CAC volume, mean (SE)	-	20.2(13.1)	143.0 (23.2)	827.4 (23.5)	<.01
CAC density, mean (SE)	-	3.9 (0.1)	3.9 (0.1)	3.9 (0.1)	.61
Total marine omega-3 fatty acids, mean (SE), %	10.0 (0.2)	10.3 (0.2)	9.8 (0.3)	9.8 (0.3)	.37
Eicosapentaenoic acid, mean (SE), %	3.1 (0.1)	3.3 (0.1)	3.1 (0.2)	3.3 (0.2)	.18
Docosahexaenoic acid, mean (SE), %	5.9 (0.1)	6.1 (0.1)	5.8 (0.1)	5.6 (0.1)	.10
Alpha-linolenic acid, mean (SE), %	0.7 (0.0)	0.8 (0.0)	0.8 (0.0)	0.7 (0.0)	.13
Linoleic acid, mean (SE), %	25.8 (0.2)	25.9 (0.2)	25.3 (0.4)	25.1 (0.4)	.19
Arachidonic acid, mean (SE), %	6.3 (0.1)	6.2 (0.1)	6.2 (0.1)	6.3 (0.1)	.14

CKD, chronic kidney disease; CAC, coronary artery calcification; CCS, coronary calcium score; HD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; IQR, inter-quartile range; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation; SE, standard error

Table 3

Age-Adjusted Characteristics of Participants by the Distribution of CAC Density Score.

Variable	Tertile 1 (n= 237)	Tertile 2 (n=240)	Tertile 3 (n=237)	*p*-trend
Age, mean (SD), years	65.5 (7.4)	68.2 (7.5)	66.9 (8.4)	<.01
Body mass index, mean (SE), kg/m^2	24.1 (0.2)	23.7 (0.2)	24.0 (0.2)	.15
Systolic blood pressure, mean (SE), mm Hg	140.1 (1.2)	141.4 (1.2)	137.7 (1.2)	.13
Hypertension, no. (%)	152 (63.8)	174 (72.4)	154 (64.9)	.08
Total cholesterol, means (SE), mmol/l	5.41 (0.06)	5.39 (0.06)	5.36 (0.06)	.50
LDL-C, mean (SE), mmol/l	3.27 (0.05)	3.24 (0.05)	3.20 (0.05)	.37
HDL-C, mean (SE), mmol/l	1.47 (0.03)	1.48 (0.03)	1.49 (0.03)	.76
Triglycerides, median (IQR), mmol/l	1.26 (0.90, 1.70)	1.29 (0.89, 1.75)	1.30 (0.93, 1.89)	.42
Lipid medication, no. (%)	34 (14.1)	47 (19.3)	43 (18.2)	.27
Glucose, mean (SE), mmol/l	5.76 (0.08)	5.89 (0.08)	5.79 (0.08)	.24
Diabetes, no. (%)	59 (24.8)	84 (34.7)	51 (21.3)	<.01
C-reactive protein, median (IQR), nmol/l	47.62 (23.81, 92.38)	50.48 (23.81, 108.57)	51.43 (22.86, 100.00)	.53
Current smoker, no. (%)	68 (28.6)	86 (35.7)	67 (28.1)	.08
Pack-years of smoking, median (IQR)	28.6 (5.7, 45.6)	31.4 (15.2, 47.5)	27.4 (5.6, 46.7)	.36
Alcohol drinker, no. (%)	158 (65.8)	163 (67.6)	136 (57.2)	.048
Ethanol amount, median (IQR), g/day	15.5 (1.6, 36.0)	18.5 (1.9, 42.3)	12.3 (0.8, 40.4)	.23
CHD, Stroke or CKD, no. (%)	21 (8.7)	35 (14.3)	23 (9.7)	.11
CAC score	80.0(35.5)	519.4 (35.3)	184.1 (35.4)	<.01
CAC volume	70.3 (27.8)	410.3 (27.7)	132.8 (27.7)	<.01
Total marine omega-3 fatty acids, mean (SE), %	10.5 (0.2)	10.3 (0.2)	10.0 (0.2)	.10
Eicosapentaenoic acid, mean (SE), %	3.4 (0.1)	3.4 (0.1)	3.2 (0.1)	.13
Docosahexaenoic acid, mean (SE), %	6.1 (0.1)	6.0 (0.1)	5.9 (0.1)	.14
Alpha-linolenic acid, mean (SE), %	0.8 (0.0)	0.7 (0.0)	0.8 (0.0)	.03
Linoleic acid, mean (SE), %	24.9 (0.3)	24.8 (0.3)	24.9 (0.3)	.70
Arachidonic acid, mean (SE), %	6.1 (0.1)	6.1 (0.1)	6.1 (0.1)	.89

CKD, chronic kidney disease; CAC, coronary artery calcium; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; IQR, inter-quartile range; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation

Table 4

Association of Blood Levels of (A) Total Marine Omega-3 Fatty Acids, (B) Eicosapentaenoic Acid, and (C) Docosahexaenoic Acid With Coronary Calcium Score Among 1,086 Japanese Participants by Ordinal Logistic and Robust Linear Regression Analyses.

	Ordinal Logistic Regression			Robust Linear Regression		
	Odds ratio	**95% CI**	**P**	**Beta-coefficient**	**SE**	**P**
(A) Total omega-3 fatty acids						
Model I	0.91	0.81–1.03	0.12	-0.106	0.075	0.16
Model II	0.94	0.83–1.07	0.34	-0.096	0.075	0.20
Model III	0.91	0.74–1.04	0.17	-0.129	0.074	0.08
(B) Eicosapentaenoic acid						
Model I	0.99	0.88–1.11	0.87	0.006	0.073	0.94
Model II	1.00	0.89–1.13	0.97	-0.010	0.074	0.90
Model III	0.97	0.85–1.10	0.64	-0.057	0.074	0.44
(C) Docosahexaenoic acid						
Model I	0.84	0.74–0.94	<0.01	-0.220	0.075	<0.01
Model II	0.88	0.78–0.99	0.045	-0.185	0.075	0.01
Model III	0.87	0.77–0.99	0.03	-0.193	0.074	<0.01

Model I: Adjusted for age and CT type

Model II: Model I and further adjustment for hypertension, diabetes, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, pack-years of smoking and body mass index

Model III: Models I and II and further adjustment for C reactive protein, triglycerides, lipid medications, ethanol intake, and history of cardiovascular disease and chronic kidney disease.

CI, confidence interval; SE, standard error

Table 5

Association of Blood Levels of (A) Total Marine Omega-3 Fatty Acids, (B) Eicosapentaenoic Acid, and (C) Docosahexaenoic Acid With CAC Density Score Among 714 Japanese Participants by Robust Linear Regression Analysis.

	Beta-Coefficient	SE	P
(A) Total omega-3 fatty acids			
Model I	-0.0072	0.0047	0.12
Model II	-0.0099	0.0049	0.04
Model III	-0.0085	0.0051	0.09
(B) Eicosapentaenoic acid			
Model I	-0.0079	0.0046	0.09
Model II	-0.0100	0.0048	0.04
Model III	-0.0089	0.0050	0.08
(C) Docosahexaenoic acid			
Model I	-0.0054	0.0048	0.26
Model II	-0.0084	0.0050	0.10
Model III	-0.0068	0.0052	0.19

Model I: Adjusted for age, CT type, and CAC score

Model II: Model I and further adjustment for hypertension, diabetes, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, pack-years of smoking and body mass index

Model III: Models I and II and further adjustment for C reactive protein, triglycerides, lipid-lowering medications, ethanol intake, and history of cardiovascular disease and chronic kidney disease.

CI, confidence interval; CAC, coronary artery calcification; SE, standard error

For model III, with outcome = CAC density score with the main predictor of total Omega-3 Fatty Acids (OM3)

- β coefficient for a 1-SD of total OM3 was −0.0085
- β coefficient for a 1-SD of age was 0.0066
- β coefficient for a 1-SD of body mass index was −0.0031
- β coefficient for a 1-SD of high-density lipoprotein cholesterol was 0.0091
- β coefficient for a 1-SD of low-density lipoprotein cholesterol was −0.0032