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Marine-derived n-3 fatty acids and atherosclerosis in Japanese, Japanese Americans, and Whites: a cross-sectional study

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Abstract

Objectives—To examine whether marine-derived n-3 fatty acids (FAs) are associated with less atherosclerosis in Japanese than Whites in the United States.

Background—Marine-derived n-3 FAs at low levels are cardioprotective through their anti-arrhythmic effect.

Methods—A population-based cross-sectional study in 281 Japanese, 306 White, and 281 Japanese American men aged 40–49 was conducted to assess intima-media thickness of the carotid artery (IMT), coronary artery calcification (CAC), and serum FAs.

Results—Japanese in Japan had the lowest levels of atherosclerosis whereas Whites and Japanese Americans had similar levels. Japanese in Japan had twofold higher levels of marine-derived n-3 FAs than Whites and Japanese Americans. Japanese in Japan had significant and non-significant inverse associations of marine-derived n-3 FAs with IMT and CAC prevalence, respectively. The

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There is no conflict of interest.

significant inverse association with IMT remained after adjusting for traditional cardiovascular risk factors. Neither Whites nor Japanese Americans had such associations. Significant differences between Japanese in Japan and Whites in multivariable-adjusted IMT (mean difference 39 μm (95% confidence interval (CI), 21 to 57), p<0.001) and CAC prevalence (mean difference 10.7% (95% CI, 2.9 to 18.4), p=0.007) became non-significant after further adjusting for marine-derived n-3 FAs (22 μm (95% CI, −1 to 46), p=0.065 and 5.0 % (95% CI, −5.3 to 15.4), p=0.341, respectively).

Conclusions—Very high levels of marine-derived n-3 FAs have anti-atherogenic properties independent of traditional cardiovascular risk factors and may contribute to lower burden of atherosclerosis in Japanese in Japan, which is unlikely due to genetic factors.

Keywords

Atherosclerosis; epidemiology; n-3 fatty acids; coronary artery calcification; intima-media thickness; Japanese

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The Seven Countries Study showed that Japan had the lowest coronary heart disease (CHD) mortality among developed countries. This was largely attributed to very low population-levels of serum total cholesterol, 165 mg/dL, in Japan in the 1960s.(1) However, CHD mortality in Japan has been decreasing since the 1970s.(2) In other Asian countries it has been increasing with the rise in serum total cholesterol.(3,4) This is despite changes in lifestyle toward westernization in Japan after World War II that have brought continuous increase in dietary fat intake, serum total cholesterol, and mortality from colon, prostate, and breast cancer.(2,5) Large longitudinal studies in Japan showed that serum total cholesterol is significantly associated with the risk of CHD.(6,7) Moreover the relative but not absolute risks of CHD associated with serum total cholesterol and other risk factors in the Japanese in Japan are similar to those in white populations.(8) The low CHD mortality is not due to the misclassification of causes of death.(9) Even in men born after World War II, who adopted westernized lifestyle from childhood, CHD mortality in the Japanese in Japan is very low and less than a half of that in United States (U.S.) Whites.(10) We have shown that there have been similar lifetime levels of serum total cholesterol and blood pressure in the post-World-War-II birth cohort of Japanese and White men.(11) However, smoking rates are higher in Japan and prevalence of type 2 diabetes is similarly high.(11) Migrant studies of the Japanese to the U.S. demonstrated a rise in CHD mortality.(12) However, Japanese Americans have lower CHD mortality than U.S. Whites,(13) suggesting that the Japanese may have protective factors against CHD.

Evidence from epidemiological and autopsy studies indicates that in men in the post-World-War-II birth cohort, the Japanese in Japan have significantly lower levels of atherosclerosis than U.S. Whites. We reported that in men aged 40–49, the Japanese in Japan have significantly lower levels of atherosclerosis than Whites,(11) assessed by intima-media thickness (IMT) of the carotid artery and coronary artery calcification (CAC), independent predictors of cardiovascular event.(14,15) A recent large autopsy-based study of atherosclerosis showed that percentages of surface involvement with raised lesions in the coronary artery in men aged 30–34 are about 15% for the Japanese versus 50% for U.S. Whites.(16)

Recent studies in Japan, where fish intake is one of the highest in the world,(11,17) showed that additional supplementation or intake of marine-derived n-3 fatty acids (FAs) is significantly associated with reduced risk of nonfatal coronary events.(18,19) These findings suggest that very high intake of marine-derived n-3 FAs have anti-atherogenic effects.

We hypothesized that in men aged 40–49 higher levels of serum marine-derived n-3 FAs in the Japanese in Japan than Whites are associated with lower levels of atherosclerosis in the Japanese in Japan. We also hypothesized that in men aged 40–49 Japanese Americans have levels of atherosclerosis lower than Whites but higher than the Japanese in Japan. We tested these hypotheses in the electron-beam tomography, risk factor assessment among Japanese and U.S. men in the post-World-War-II birth cohort (ERA JUMP Study), a population-based-cross-sectional study of 868 men aged 40–49 in the Japanese in Japan, U.S. Whites, and Japanese Americans.(11,20)

Methods

Subjects and basic measurements

During 2002 to 2006, 926 men aged 40–49 were randomly selected; 313 Japanese from Kusatsu, Shiga, Japan, 310 Whites from Allegheny County, Pennsylvania, U.S.,(11,20) and 303 Japanese Americans from a representative sample of offspring of fathers who participated in the Honolulu Heart Program,(21) Honolulu, Hawaii, U.S. These offspring were the third or fourth generation of Japanese Americans without ethnic admixture.(20) All participants were without clinical cardiovascular disease, type 1 diabetes, or other severe diseases.(11) The current study excluded 50 subjects who drank > 69 g per day of alcohol because very heavy drinking was associated with both serum marine-derived n-3 FAs and CAC.(22) We also excluded eight subjects with missing data. Our final sample was 281 Japanese in Japan, 306 Whites, and 281 Japanese Americans. Informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of Shiga University of Medical Science, Otsu, Japan, University of Pittsburgh, Pittsburgh, U.S., and Kuakini Medical Center, Honolulu, U.S.

All participants underwent a physical examination, lifestyle questionnaire, and laboratory assessment as described previously.(11,20) Serum samples were stored at −80°C, shipped on dry ice to University of Pittsburgh, and determined for levels of low-density-lipoprotein (LDL) cholesterol, high-density-lipoprotein (HDL) cholesterol, triglycerides, glucose, insulin, and C-reactive protein.(11)

Determination of serum fatty acids

To determine percentages of serum fatty acids and total fatty acid amounts, lipids were extracted from the stored serum samples.(23) The 100-μl samples plus 1, 2-dinonadecanoyl-sn-glycero-3-phosphocholine (50-μg C19:0), as an internal standard, were homogenized in 4-ml methanol, 2-ml chloroform, and 1.5-ml water. After 15 minutes, 2-ml chloroform and 2-ml water were added and the samples were vortexed. Then the tubes were centrifuged at 1200 g for 30 minutes at 16°C and the upper phase was discarded. The lower phase was dried under nitrogen and was re-suspended in 1.5- ml-14%-boron-trifluoride methanol. The samples were heated at 90°C for 40 minutes and were extracted after cooling with 4.0-ml pentane and 1.5-ml water. The mixtures were vortexed and the organic phase was recovered. The extracts were dried under nitrogen, re-suspended in 50-μl heptane and 2 μl was injected into a capillary column (SP-2380 105m × 0.53mm × 0.20μm, Suple1co Inc., Bellefonte, PA). The gas chromatograph was a PerkinElmer Clarus 500 equipped with a flame ionization detector and an autosampler. The oven temperature was 140°C for 35 minutes. The temperature was raised at 8°C per minutes to 220°C and then was held for 12 minutes. Injector and detector temperatures were both at 260°C and helium, the carrier gas, was at 15 psi. Components were identified by comparison of retention time with those of authentic standards (Sigma, St. Louis, MO). Measurement of fatty acids in the serum eliminates the need to isolate specific fractions. Similar conclusions are reached with the serum as with fractions.(24)

The coefficients of variation between runs for major marine-derived n-3 FAs, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) were 4.5% and 7.2%, respectively. The coefficients of variation for other major FAs: palmitic (16:0), stearic (18:0), oleic (18:1n-9), linoleic (18:2n-6), alpha-linolenic, arachidonic (20:4n-6), docosapentaenoic (22:5n-3) acids, and total fatty acid amount were 1.2%, 4.0%, 2.3%, 1.6%, 7.9%, 2.8%, 4.5%, and 5.7%, respectively. Marine-derived n-3 FAs were defined as a sum of eicosapentaenoic, docosahexaenoic and docosapentaenoic acids.

Electron-beam computed tomography (EBT)

The scanning procedures were described elsewhere.(11) Scanning was performed using the standardized protocol with a GE-Imatron C150 EBT scanner (GE Medical Systems, South San Francisco, U.S.). A total of 30 to 40 contiguous 3-mm-thick transverse images were obtained from the level of the aortic root to the apex of the heart. Images were recorded during a maximal breath hold using ECG-guided triggering of 100-m-second exposures during the same phase of the cardiac cycle. CAC was considered to be present with three contiguous pixels (area=1 mm^2) ≥130 Hounsfield Units. One trained reader at the Cardiovascular Institute, University of Pittsburgh, read the images, using a Digital-Imaging-and-Communications-in-Medicine workstation and software by the AccuImage Diagnostic Corporation, San Francisco, which calculates coronary calcium score (CCS) with the Agatston scoring method.(25) To define the presence of CAC, CCS ≥10 was used.(11,26) The reader was blinded to participant's characteristics and the study centers. The reproducibility of the EBT scans had an intra-class correlation of 0.98.

IMT of the carotid artery

The scanning procedures were described elsewhere.(11,27) Before the study began, sonographers at all centers received training for carotid scanning provided by the Ultrasound Research Laboratory, University of Pittsburgh.(28) A Toshiba 140A scanner (Japan and Pittsburgh) and a Siemens Acuson Cypress scanner (Honolulu), equipped with a 7.5 MHz-linear-array imaging probe were used. The sonographers scanned the right and left common carotid arteries (CCA), the carotid bulbs, and the internal carotid arteries. For the CCA segment, both near and far walls were examined 1-cm proximal to the bulb. For the bulb and internal carotid artery areas, only far walls were examined. The scans were recorded on videotape and sent to the laboratory for scoring. Trained readers digitized the best image for scoring and then measured the average IMT across 1-cm segments of near and far walls of the CCA and the far wall of the carotid bulb and internal carotid arteries on both sides. Plaque was defined as a distinct area identified with either a focal area of hyperechogenecity or a focal protrusion into the lumen of the vessel.(27) Our current study used CCA because prevalence of the carotid plaque in CCA was similarly low both in the Japanese in Japan and Whites. The readers were blinded to participant's characteristics and the study centers. Correlation coefficients of IMT between sonographers and between readers were 0.96 and 0.99, respectively.(28)

Statistical analyses

To compare risk factors or levels of atherosclerosis among populations, analysis of variance for continuous variables or the Mantel-Haenszel test for categorical variables was used. To examine associations of marine-derived n-3 FAs with atherosclerosis, we made tertile groups of marine-derived n-3 FAs for each population and compared age- and multivariable-adjusted tertile-specific levels of atherosclerosis. To examine the linear trend of tertile-specific levels of atherosclerosis and whether marine n-3 FAs were associated with less atherosclerosis in the Japanese in Japan than Whites, general-linear-model analyses were used. All P-values were two-tailed.

Results

The Japanese in Japan had a less favorable or similar profile of many risk factors compared to Whites, including blood pressure, hypertension, LDL-cholesterol, triglycerides, glucose, diabetes, and cigarette smoking (Table 1). However, the Japanese in Japan were significantly less obese than Whites. Japanese Americans and Whites were similarly obese. Japanese Americans had significantly higher rates of hypertension and diabetes and levels of triglycerides and glucose than Whites.

The Japanese in Japan had the lowest levels of atherosclerosis (Table 2). Japanese Americans had significantly higher (IMT) and similar levels (CAC) compared to Whites (Table 2). Even among those without diabetes, hypertension, and lipid lowering medications, Japanese Americans (n=185) had significantly higher (IMT) and similar levels (CAC) compared to Whites (n=247) (IMT (μm, mean (standard error) 711 (70) vs. 665 (60), $p<0.001$ and CAC prevalence 26.6% vs. 21.1%, $p=0.184$, for Japanese Americans and Whites, respectively).

The Japanese in Japan had twofold higher levels of marine-derived n-3 FAs than both U.S. populations (Table 3). The lower 5th percentile of marine-derived n-3 FAs (4.97%) in the Japanese in Japan was still higher than the mean levels in Whites and Japanese Americans. Total fat amount was comparable across populations.

The Japanese in Japan had a significant inverse association of marine-derived n-3 FAs with IMT (Table 4). The significant association remained after adjusting for blood pressure, HDL-cholesterol, and triglycerides (model II) and further adjusting for other potential confounders (models III and IV). The Japanese in Japan appeared to have an inverse association of marine-derived n-3 FAs with CAC prevalence. The association appeared to remain after adjusting for potential confounders (model III and IV). In contrast, neither Whites nor Japanese Americans had a significant inverse association with marine-derived n-3 FAs with IMT or CAC prevalence (Table 4). Although Whites had significant and non-significant age-adjusted inverse association of IMT and CAC prevalence, respectively with marine-derived n-3 FAs, the associations were attenuated after adjusting for blood pressure, HDL-cholesterol, and triglycerides (model II). It is noted that the median of marine-derived n-3 FAs in the lowest tertile in the Japanese in Japan was still higher than that of the highest tertile in Whites and Japanese Americans.

Significant differences in both IMT and CAC prevalence between the Japanese in Japan and Whites were attenuated and became non-significant after adjusting for serum marine-derived n-3 FAs (Table 5). The mean difference in aged-adjusted IMT was 56 μm (95% confidence interval (CI), 42 to 70 μm $p<0.001$) (model I), which became 39 μm (95% CI, 22 to 57 μm, $p<0.001$) after further adjusting for traditional risk factors (model II) and 39 μm (95% CI, 21 to 57 μm, $p<0.001$) after further adjusting for novel risk and other factors (model III). After further adjusting for marine-derived n-3 FAs, the mean difference became non-significant (22 μm, 95% CI, −1 to 46 μm, $p=0.065$) (model IV). Similarly the significant mean difference in CAC prevalence after adjusting for traditional risk and other factors (9.4%, 95% CI, 1.4 to 17.3%, $p=0.021$) (model III) became non-significant after further adjusting for marine-derived n-3 FAs (5.0%, 95% CI, −5.3 to 15.4%, $p=0.341$). Adjusting for FAs other than marine-derived n-3, *i.e*., alpha linolenic, total n-6, saturated, monounsaturated, or trans FAs, did not attenuate the significant difference (data now shown). The differences in multivariable-adjusted levels of atherosclerosis between the Japanese in Japan and Japanese Americans were attenuated but remained significant after further adjusting for serum marine-derived n-3 or other FAs (data not shown).

Discussions

Our study in men aged 40–49 shows that the Japanese in Japan had twofold higher levels of marine-derived n-3 FAs than Whites and that the marine-derived n-3 FAs attenuated the significant differences in multivariable-adjusted carotid IMT and CAC prevalence between the Japanese in Japan and Whites. Our study also shows significant and non-significant inverse

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associations of marine-derived n-3 FAs with IMT and CAC prevalence, respectively, in the Japanese in Japan, which remained after adjusting for cardiovascular risk and other factors. Our study also shows that in men aged 40–49 Japanese Americans as compared to Whites had higher or similar levels of atherosclerosis. Our results suggest that marine-derived n-3 FAs have anti-atherogenic effects, especially at high levels observed in the Japanese in Japan.

The results of two recent studies in Japan(18,19) support the hypothesis that high marine-derived n-3 FAs have anti-atherogenic effects. The Japan Eicosapentaenoic acid Lipid Interventions Study,(18) a randomized trial of 18,645 Japanese in Japan examining the effectiveness of 1.8 g eicosapentaenoic acid per day plus a statin in reducing CHD rates reported that after a follow-up of 4.6 years, the hazard ratio in eicosapentaenoic acid versus control groups was 0.81 (95% CI, 0.68 to 0.96) for nonfatal coronary events. The Japan Public Health center-Based Study,(19) a 10-year prospective cohort study of 41,578 middle-aged Japanese, reported that dietary intake of marine-derived n-3 FAs has significant inverse associations with nonfatal coronary events. The multivariate-adjusted hazard ratio in the highest versus lowest quintiles of marine-derived n-3 FAs intake (median intake = 2.1 versus 0.3 g/day, respectively) was 0.33 (95% CI, 0.16 to 0.63) for nonfatal coronary events.

Our data do not contradict the anti-arrhythmic effect of marine-derived n-3 FAs, associated with reduced risk of cardiac death. Once- or twice-weekly consumption of fish, *i.e.*, 30 g of fish per day, is associated with reduced risk of cardiac death in Western countries.(29) Increasing fish intake above this level has little benefit.(30) In contrast, the Japanese men consume more than 100 g of fish everyday on average from early in life.(31) Thus the Japanese in Japan consume fish far above the threshold for preventing cardiac death attributable to the anti-arrhythmic effect. In fact, neither the Japan Public Health center-Based Study nor the Japan Eicosapentaenoic acid Lipid Interventions Study observed a significant association of marine-derived n-3 FAs with cardiac death.(18,19)

Our finding that Japanese Americans had similar or higher levels of atherosclerosis as compared to U.S. Whites is unexpected. The significantly higher prevalence of diabetes in Japanese Americans than U.S. Whites was expected based on the well-known observation that Asian including Japanese Americans are more susceptible to developing diabetes than U.S. Whites.(32) However, even after excluding those with diabetes, hypertension, and taking lipid-lowering medications, Japanese Americans still had the similar or higher levels of atherosclerosis. Our results may suggest that the third or fourth generation Japanese Americans have similar or higher CHD rates in the future compared to U.S. Whites.(13) Further study is needed to carefully monitor CHD rates in third or fourth generation Japanese Americans. Our findings indicate that anti-atherogenic effect of marine-derived n-3 FAs is likely to be present only in populations with fish intake well above those of most western populations. It thus appears unlikely that short term supplementation in a low fish intake population would show such a relationship.

It is now widely recognized that inflammation plays a critical role in initiation and progression of atherosclerosis as well as plaque rupture.(33) Marine-derived n-3 FAs have a variety of anti-inflammatory effects.(34) Lifelong high intake of marine-derived n-3 FAs in the Japanese in Japan may be related to lower levels of atherosclerosis through their anti-inflammatory effects. It is also possible that lifelong high levels of marine-derived n-3 FAs in the Japanese in Japan may be associated with improved endothelial or platelet function, lower levels of thrombosis or oxidative stress, plaque stability,(35–39) or combinations of these factors.

Although the Japanese in Japan were much less obese, the significant differences in atherosclerosis between the Japanese in Japan and Whites remained after adjusting for body-mass index and risk factors associated with obesity, *i.e.*, blood pressure, lipids, glucose, and insulin. Another possibility is adipocytokines. However, adiponectin, an adipocytokine which has a potential anti-atherogenic properties and is inversely associated with obesity, is paradoxically lower in the Japanese in Japan than in Whites.(40)

This unique study is the first population-based international study to compare non-invasively evaluated atherosclerosis, *i.e*., carotid IMT and CAC. The inclusion of the Japanese in Japan, whose intake of fish is one of the highest in the world, enables us to analyze the associations of wide range of marine-derived n-3 FAs with atherosclerosis. The fact that Japanese Americans as compared to U.S. Whites had higher or similar levels of atherosclerosis indicates that the low atherosclerosis in the Japanese in Japan is unlikely due primarily to genetic factors. The finding in the current study will be difficult to replicate in other ongoing U.S. studies. For example, the Multi-Ethnic Study on Atherosclerosis (41) does not have a Japanese cohort. It has Chinese, Hispanic, and African Americans with various stages of acculturation(42) whereas Japanese Americans in the current study are third or fourth generation considered to be acculturated.

Some limitations of the study warrant consideration. The sample size is relatively small. Our study included men and only those aged 40–49. However, we focused on this specific sex- and age-group because, unlike older age groups or women, in this birth cohort levels of serum total cholesterol and blood pressure have been similar between Japanese men in Japan and U.S. White men throughout their lifetime.(11) Our cross-sectional analyses are likely to underestimate the lifelong effects of marine-derived n-3 FAs on atherosclerosis because levels of marine-derived n-3 FAs are markedly different between the Japanese in Japan and U.S. populations throughout their lifetime. Serum marine-derived n-3 FAs reflect short-term dietary fat intake and may not reflect long-term dietary intake. However, because the variation in serum marine-derived n-3 FAs occurs randomly, the actual association of marine-derived n-3 FAs with atherosclerosis is likely to be stronger than was observed in the current study. The study is observational and we cannot exclude the possibility of residual or unmeasured confounding, *i.e*., total energy intake.(43)

If the high intake of marine-derived n-3 FAs in the Japanese in Japan is the primary reason for their extremely low CHD mortality in the face of high traditional cardiovascular risk factors, dietary interventions to increase marine-derived n-3 FAs in the U.S. and other countries where fish intake is not as high as in Japan could have a very substantial impact on CHD. Foods are being modified to increase marine-derived n-3 FAs in the diet(44) so that sources other than fish can be used to increase marine-derived n-3 FAs to prevent CHD. Long-term primary prevention trials of high-dose marine-derived n-3 FAs comparable to levels in Japan on atherosclerosis and CHD would be needed to test whether intake of marine-derived n-3 FAs markedly lowers CHD rates.

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Abbreviations list

CAC
: Coronary artery calcification

CCS
: Coronary calcium score

CCA
: Common carotid artery

CHD
: Coronary heart disease

CI
: Confidence interval

FA
: fatty acid

HDL
: High density lipoprotein

IMT
: Intima-media thickness

LDL
: low density lipoprotein

U.S
: United States

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Table 1

Basic characteristics of the study participants in 2002 – 2006.

	Japanese in Japan (n=281)	Whites (n=306)	Japanese Americans (n=281)	P
Age (years)	45.0 (2.8)	45.0 (2.8)	46.2 (2.8)	†§
Body-mass index (kg/m^2)	23.6 (3.1)	27.9 (4.3)	28.0 (4.5)	¶§
Systolic blood pressure (mmHg)	124.2 (15.8)	122.5 (11.2)	127.3 (12.6)	†§
Diastolic blood pressure (mmHg)	75.7 (11.8)	73.1 (8.6)	77.6 (9.4)	¶†
Hypertension (%)	24.2	14.7	32.4	¶†
LDL-cholesterol (mg/dL)	133.6 (35.5)	134.7 (33.6)	121.6 (32.4)	†§
Triglycerides (mg/dL)	152.2 (77.0)	151.3 (100.0)	184.1 (140.7)	†§
HDL-cholesterol (mg/dL)	53.3 (13.1)	47.5 (12.7)	49.8 (10.8)	¶§
Glucose (mg/dL)	106.1 (17.7)	101.3 (13.5)	111.7 (20.7)	¶†§
Insulin (μIU/mL)	10.2 (4.2)	15.3 (8.4)	15.2 (9.3)	¶§
C-reactive protein (mg/L)	0.65 (1.04)	1.64 (2.32)	1.34 (2.32)	¶§
Fibrinogen (mg/dL)	253.7 (63.3)	291.2 (70.4)	317.7 (72.8)	¶†§
Diabetes mellitus (%)	5.0	3.2	13.6	†§
Current cigarette smoker (%)	47.3	7.2	12.8	¶§
Alcohol drinker (%)	63.3	43.8	33.1	¶†§
Ethanol consumption (g/day)	19.9 (19.9)	9.6 (11.7)	11.6 (16.8)	¶§
Exercise (%)	25.6	73.1	n.a.	
Hypertension medications (%)	4.3	8.2	19.9	†§
Lipid-lowering medications (%)	2.8	12.4	22.8	¶†§
Diabetes medications (%)	1.1	1.0	6.0	§

Values are means (standard deviations) unless stated otherwise.

LDL: low density lipoprotein. HDL: high density lipoprotein. n.a.: not assessed.

Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or hypertensive medication. Diabetes was defined as fasting glucose ≥ 124 mg/dL or diabetes medication.

Alcohol drinker was defined as those who drank alcohol two days per week or more. Those who exercised were defined as those who regularly exercised ≥ one hour per week.

¶P<0.01 between the Japanese in Japan versus Whites

†P<0.01 between Whites and Japanese Americans

§P<0.01 between the Japanese in Japan versus Japanese Americans

Table 2

Extent of atherosclerosis in Japanese in Japan, Whites, and Japanese Americans in 2002 – 2006

	Japanese in Japan (n=281)	Whites (n=306)	Japanese Americans (n=281)	P
Coronary artery				
CCS (median (IQR))	0 (0, 1.0)	1.0 (0, 12.3)	0 (0, 30.9)	¶§
Prevalence of CAC (%)	9.3	26.1	31.4	¶§
Carotid artery				
CCA IMT (μm) (mean (SD))	614 (80)	670 (94)	720 (115)	¶†§
Prevalence of CCA plaque (%)	0	0.7	16.7	†§

CCS: Coronary calcium score, IQR: inter quartile range, CAC: coronary artery calcification, CCA: common carotid artery, IMT: intima-media thickness, SD: standard deviation

¶P<0.01 between the Japanese in Japan versus Whites

†P<0.01 between Whites and Japanese Americans

§P<0.01 between the Japanese in Japan versus Japanese Americans

Table 3

Serum levels of fatty acids in Japanese in Japan, Whites, and Japanese Americans in 2002 – 2006

	Japanese in Japan (n=281)	Whites (n=306)	Japanese Americans (n=281)	P
Total fatty acids (mg/dL)	245 (53)	237 (51)	243 (85)	n.s.
Fatty acids proportion				
Marine-derived n-3 fatty acids (%)	9.2 (2.9)	3.9 (1.7)	4.8 (2.2)	¶†§
Eicosapentaenoic acid (%)	2.5 (1.3)	0.8 (0.6)	1.0 (1.0)	¶§
Docosahexaenoic acid (%)	5.9 (1.6)	2.4 (1.2)	3.2 (1.4)	¶†§
Alpha linolenic fatty acids (%)	0.2 (0.2)	0.3 (0.3)	0.4 (0.4)	¶†§
Total n-6 fatty acids (%)	35.1 (4.1)	41.4 (4.2)	41.4 (4.3)	¶§
Linoleic acid (%)	26.8 (4.0)	29.9 (4.1)	30.2 (4.2)	¶§
Arachidonic acid (%)	6.6 (1.3)	9.0 (1.9)	8.9 (2.3)	¶§
Saturated fatty acids (%)	31.6 (2.1)	30.9 (2.4)	30.8 (2.2)	¶§
Monounsaturated fatty acids (%)	19.4 (2.8)	18.9 (3.1)	18.0 (3.4)	†§
Trans fatty acids (%)	0.6 (0.2)	1.0 (0.5)	0.9 (0.4)	¶†§

Values are expressed as mean (standard deviation).

Marine-derived n-3 fatty acids were calculated as a sum of eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3), and docosahexaenoic acid (22:6n-3); total n-6 fatty acids as a sum of linoleic acid (18:2n-6), gamma-linoleic acid (18:3n-6), dihomo-gamma-linolenic acid (20:3n-6), and arachidonic acid (20:4n-6); saturated fatty acids as a sum of myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0); monounsaturated fatty acids as a sum of palmitoleic acid (16:1n-7), oleic acid (18:1n-9), and cis-vaccenic acid (18:1n-7), and trans fatty acids as a sum of palmitelaidic acid (16:1t), elaidic acid (18:1t) and linolelaidic acid (18:2tt). Plant-derived n-3 fatty acids were alpha linolenic fatty acid (18:3n-3).

n.s.: Not significant

[¶] P<0.01 between the Japanese in Japan versus Whites

[†] P<0.01 between Whites and Japanese Americans

[§] P<0.01 between the Japanese in Japan versus Japanese Americans

Table 4

Multivariable-adjusted associations of marine n-3 fatty acids with intima-media thickness (A) and prevalence of coronary artery calcification (B) in Japanese in Japan, Whites, and Japanese Americans

(A) Intima-media thickness (μm)

Japanese in Japan				
	Marine n-3 fatty acids			
	Low	**Middle**	**High**	
Median (%) (IQR)	**6.51 (4.16,7.78)**	**8.71 (8.00,9.72)**	**12.30 (10.05,16.19)**	**P for trend**
Model I	627 (8)	616 (8)	600 (8)	0.016
Model II	626 (8)	616 (8)	601 (8)	0.022
Model III	629 (8)	617 (8)	597 (8)	0.004
Model IV	630 (8)	618 (8)	596 (8)	0.004
Whites				
	Marine n-3 fatty acids			
	Low	Middle	High	
Median (%)(IQR)	2.42 (1.56,2,82)	3.47 (2.90,4.04)	5.23 (4.17.9.42)	P for trend
Model I	685 (9)	672 (9)	653 (9)	0.014
Model II	676 (9)	673 (9)	661 (9)	0.271
Model III	676 (9)	674 (8)	661 (9)	0.258
Model IV	675 (9)	674 (9)	665 (9)	0.460
Japanese Americans				
	Marine n-3 fatty acids			
	Low	Middle	High	
Median (%)(IQR)	2.93 (1.79, 3.64)	4.40 (3.82,5.12)	6.49 (5.12,11.42)	P for trend
Model I	721 (12)	719 (12)	719 (12)	0.886

(B) Prevalence of coronary artery calcification (%)

Japanese in Japan				
	Marine n-3 fatty acids			
	Low	**Middle**	**High**	
Median (%) (IQR)	**6.51 (4.16,7.78)**	**8.71 (8.00,9.72)**	**12.30 (10.05,16.19)**	**P for trend**
Model I	12.1 (3.0)	8.0 (3.0)	7.9 (3.0)	0.318
Model II	11.7 (3.0)	8.2 (3.0)	8.1 (3.0)	0.399
Model III	13.1 (3.0)	8.6 (3.0)	6.2 (3.0)	0.107
Model IV	13.2 (2.9)	7.9 (2.9)	6.9 (2.9)	0.144
Whites				
	Marine n-3 fatty acids			
	Low	Middle	High	
Median (%)(IQR)	2.42 (1.56, 2,82)	3.47 (2.90,4.04)	5.23 (4.17,9.42)	P for trend
Model I	32.0 (4.2)	22.1 (4.2)	22.8 (4.2)	0.127
Model II	29.3 (4.4)	22.1 (4.1)	25.4 (4.3)	0.545
Model III	28.8 (4.3)	22.0 (4.1)	26.0 (4.3)	0.656
Model IV	30.5 (4.4)	22.4 (4.2)	26.0 (4.4)	0.491
Japanese Americans				
	Marine n-3 fatty acids			
	Low	Middle	High	
Median (%)(IQR)	2.93 (1.79, 3.64)	4.40 (3.82,5.12)	6.49 (5.12,11.42)	P for trend
Model I	30.4 (4.8)	34.5 (4.8)	26.7 (4.8)	0.587

(A) Intima-media thickness (µm)					(B) Prevalence of coronary artery calcification (%)				
Japanese in Japan					**Japanese in Japan**				
	Marine n-3 fatty acids					**Marine n-3 fatty acids**			
	Low	**Middle**	**High**			**Low**	**Middle**	**High**	
Median (%) (IQR)	**6.51 (4.16,7.78)**	**8.71 (8.00,9.72)**	**12.30 (10.05,16.19)**	**P for trend**	**Median (%) (IQR)**	**6.51 (4.16,7.78)**	**8.71 (8.00,9.72)**	**12.30 (10.05,16.19)**	**P for trend**
Model II	720 (12)	719 (11)	719 (12)	0.959	Model II	30.1 (4.9)	34.7 (4.7)	26.2 (4.8)	0.579
Model III	717 (12)	715 (11)	727 (11)	0.552	Model III	28.7 (4.8)	34.0 (4.7)	28.2 (4.8)	0.951
Model IV	717 (12)	715 (11)	726 (11)	0.584	Model IV	29.0 (4.8)	34.0 (4.7)	27.9 (4.9)	0.877

Model I Adjusted for age

Model II Further adjusted for blood pressure, HDL-C, and triglycerides

Model III Further adjusted for LDL-C, smoking, glucose, insulin, and body-mass index

Model IV Further adjusted for C-reactive protein, fibrinogen, alcohol, and medications for diabetes, hypertension, and hyperlipidemia

Table 5

Multivariable-adjusted mean differences in intima-media thickness and prevalence of coronary artery calcification between the Japanese in Japan and whites

Intima media thickness (μm)

	Japanese in Japan	**Whites**	**Mean difference (95% CI)**	**p**
Model I	614 (5)	670 (5)	56 (42, 70)	<0.001
Model II	623 (6)	662 (5)	39 (22, 57)	<0.001
Model III	625 (6)	662 (6)	37 (18, 56)	<0.001
Model IV	632 (7)	655 (7)	22 (−1, 46)	0.065

Prevalence of coronary artery calcification (%)

	Japanese in Japan	Whites	Mean difference (95% CI)	p
Model I	9.3 (2.2)	26.1 (2.1)	16.8 (10.7, 22.8)	<0.001
Model II	12.5 (2.5)	23.2 (2.4)	10.7 (2.9, 18.4)	0.007
Model III	12.5 (2.7)	23.8 (2.6)	11.3 (2.8, 19.8)	0.009
Model IV	15.5 (3.0)	20.5 (3.1)	5.0 (−5.3, 15.4)	0.341

Values are expressed as mean and standard error.

CI: Confidence interval

Model I Adjusted for age

Model II Further adjusted for blood pressure, LDL-C, HDL-C, triglycerides, glucose, insulin, smoking and body-mass index

Model III Further adjusted for C-reactive protein, fibrinogen, alcohol, exercise, and medications for diabetes, hypertension, and hyerlipidemia

Model IV Further adjusted for marine n-3 fatty acids

Those who exercised were defined as those who regularly exercised ≥ one hour per week.