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CLINICAL RESEARCH | Atherosclerosis

Marine-Derived n-3 Fatty Acids and Atherosclerosis in Japanese, Japanese-American, and White Men

A Cross-Sectional Study

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Objectives We sought to examine whether marine-derived n-3 fatty acids are associated with less atherosclerosis in Japanese versus white populations in the U.S.

Background Marine-derived n-3 fatty acids at low levels are cardioprotective through their antiarrhythmic effect.

Methods A population-based cross-sectional study in 281 Japanese (defined as born and living in Japan), 306 white (defined as white men born and living in the U.S.), and 281 Japanese-American men (defined as Japanese men born and living in the U.S.) ages 40 to 49 years was conducted to assess intima-media thickness (IMT) of the carotid artery, coronary artery calcification (CAC), and serum fatty acids.

Results Japanese men had the lowest levels of atherosclerosis, whereas whites and Japanese Americans had similar levels. Japanese had 2-fold higher levels of marine-derived n-3 fatty acids than whites and Japanese Americans in the U.S. Japanese had significant and nonsignificant inverse associations of marine-derived n-3 fatty acids with IMT and CAC prevalence, respectively. The 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 and whites in multivariable-adjusted IMT (mean difference 39 μm, 95% confidence interval [CI]: 21 to 57μm, $p < 0.001$) and CAC prevalence (mean difference 10.7%, 95% CI: 2.9% to 18.4%, $p = 0.007$) became nonsignificant after we adjusted further for marine-derived n-3 fatty acids (22 μm, 95% CI: –1 to 46 μm, $p = 0.065$ and 5.0%, 95% CI: –5.3% to 15.4%, $p = 0.341$, respectively).

Conclusions Very high levels of marine-derived n-3 fatty acids have antiatherogenic properties that are independent of traditional cardiovascular risk factors and may contribute to lower the burden of atherosclerosis in Japanese, a lower burden that is unlikely the result of genetic factors. (J Am Coll Cardiol 2008;52:417–24) © 2008 by the American College of Cardiology Foundation

The Seven Countries Study showed that Japan had the lowest rate of coronary heart disease (CHD) mortality among developed countries, which 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 increase

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Abbreviations and Acronyms

CAC = coronary artery calcification

CCA = common carotid artery

CCS = coronary calcium score

CHD = coronary heart disease

CI = confidence interval

HDL = high-density lipoprotein

IMT = Intima-media thickness

in serum total cholesterol (3,4). This is despite changes in life-style toward Westernization in Japan after World War II that have brought a 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 Japanese men born in Japan after World War II, who adopted Westernized life-styles from childhood, CHD mortality is very low and less than one-half of that in white men in the U.S. (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 greater in Japan, and the prevalence of type 2 diabetes is similarly high (11). Migrant studies of Japanese to the U.S. demonstrated an increase in CHD mortality (12). However, Japanese Americans have lower rates of CHD mortality than white populations in the U.S. (13), suggesting that the Japanese populations may have protective factors against CHD.

Evidence from epidemiological and autopsy studies indicate that of the men in the post-World War II birth cohort, Japanese men have significantly lower levels of atherosclerosis than white men residing in the U.S. We reported that in men ages 40 to 49 years, Japanese men have significantly lower levels of atherosclerosis than white men in the U.S. (11), assessed by intima-media thickness (IMT) of the carotid artery and coronary artery calcification (CAC), which are independent predictors of cardiovascular events (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 ages 30 to 34 are approximately 15% for the Japanese men versus 50% for white men in the U.S. (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 is significantly associated with a reduced risk of nonfatal coronary events (18,19). These findings suggest that very high intake of marine-derived n-3 fatty acids has anti-atherogenic effects.

We hypothesized that, in men ages 40 to 49 years, greater levels of serum marine-derived n-3 fatty acids in the Japanese men (defined as men born and living in Japan) versus white men (defined as white men living in the U.S.) are associated with lower levels of atherosclerosis. We also hypothesized that, in men ages 40 to 49 years, Japanese-American men (defined as Japanese men born and living in the U.S.) have levels of atherosclerosis that are lower than white men in the U.S. but greater than Japanese men. We tested these hypotheses in the ERA JUMP (Electron-Beam Tomography, Risk Factor Assessment Among Japanese and U.S. Men in the Post-World War II Birth Cohort) study, a population-based, cross-sectional study of 868 men ages 40 to 49 years in Japanese, white-American, and Japanese-American men (11,20).

Methods

Subjects and basic measurements. During 2002 to 2006, 926 men ages 40 to 49 years were randomly selected: 313 Japanese men from Kusatsu, Shiga, Japan; 310 white men from Allegheny County, Pennsylvania (11,20); and 303 Japanese-American men from a representative sample of offspring of fathers who participated in the Honolulu Heart Program (21), Honolulu, Hawaii. 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/day of alcohol because very heavy drinking was associated with both serum marine-derived n-3 fatty acids and CAC (22). We also excluded 8 subjects with missing data. Our final sample was 281 Japanese men, 306 white men, and 281 Japanese-American men. 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, Pennsylvania; and Kuakini Medical Center, Honolulu, Hawaii.

All participants underwent a physical examination, life-style 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 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 of methanol, 2 ml of chloroform, and 1.5 ml of water. After 15 min of homogenization, 2 ml of chloroform and 2 ml of water were added and the samples were vortexed. Then, the tubes were centrifuged at 1,200 *g* for 30 min at 16°C and the upper phase was discarded. The lower phase was dried under nitrogen and was resuspended in 1.5 ml of 14% borontrifluoride methanol. The samples were heated at 90°C for 40 min and were extracted after cooling with 4.0 ml of pentane and 1.5 ml of water. The mixtures were vortexed, and the organic phase was recovered. The extracts were dried under nitrogen, resuspended in 50 μl of heptane, and 2 μl was injected into a capillary column (SP-2380 105 m × 0.53 mm × 0.20 μm, Suple1co Inc., Bellefonte, Pennsylvania). The gas chromatograph was a PerkinElmer Clarus 500 (Waltham, Massachusetts) equipped with a flame ionization detector and an autosampler. The oven temperature was 140°C for 35 min. The temperature was increased at 8°C per min to 220°C and then was held for 12 min. 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, Missouri). 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 fatty acids, 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 fatty acids were as follows: palmitic (16:0), stearic (18:0), oleic (18:1n-9), linoleic (18:2n-6), alpha-linolenic, arachidonic (20:4n-6), and 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 fatty acids were defined as a sum of eicosapentaenoic, docosahexaenoic, and docosapentaenoic acids.

Electron beam computed tomography. The scanning procedures were described elsewhere (11). Scanning was performed with the standardized protocol with a GE-Imatron C150 EBT scanner (GE Medical Systems, South San Francisco, California). 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 with the use of electrocardiogram (ECG)-guided triggering of 100-ms exposures during the same phase of the cardiac cycle. We considered CAC to be present with 3 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, California), 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 electron beam computed tomography scans had an intraclass correlation of 0.98.

Intima-media thickness 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 (Tokyo, Japan, and Pittsburgh, Pennsylvania) and a Siemens Acuson Cypress scanner (Honolulu, Hawaii) equipped with a 7.5-MHz linear-array imaging probe were used. The sonographers scanned the right and left common carotid arteries (CCAs), 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). We used CCA in our current study because the prevalence of the carotid plaque in CCA was similarly low both in Japanese and white men. 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 fatty acids with atherosclerosis, we made tertile groups of marine-derived n-3 fatty acids 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 fatty acids were associated with less atherosclerosis in the Japanese men than white men, general linear model analyses were used. All p values were 2-tailed.

Results

Japanese men had a less favorable or similar profile of many risk factors compared with white men, including blood pressure, hypertension, low-density lipoprotein cholesterol, triglycerides, glucose, diabetes, and cigarette smoking (Table 1). However, Japanese men were significantly less obese than white men. Japanese-American men and white men were similarly obese. Japanese-American men had significantly greater rates of hypertension and diabetes and levels of triglycerides and glucose than white men.

Japanese men had the lowest levels of atherosclerosis (Table 2). Japanese-American men had significantly greater IMT and similar levels of CAC compared with white men (Table 2). Even among those without diabetes, without hypertension, or taking lipid-lowering medications, Japanese-American men (n = 185) had significantly greater IMT and similar levels of CAC compared with white men (n = 247) (IMT, mean [standard error]: 711 μm [70 μm] vs. 665 μm [60 μm], $p < 0.001$ and CAC prevalence: 26.6% vs. 21.1%,

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Table 1 Basic Characteristics of the Study Participants in 2002 to 2006

	Japanese Men (n = 281)	White Men (n = 306)	Japanese-American Men (n = 281)	p Value
Age (yrs)	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 (mm Hg)	124.2 (15.8)	122.5 (11.2)	127.3 (12.6)	*†
Diastolic blood pressure (mm Hg)	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	NA	
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 mean (SD) unless stated otherwise. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or hypertensive medication. Diabetes was defined as fasting glucose ≥124 mg/dl or diabetes medication. Alcohol drinker was defined as those who drank alcohol 2 days/week or more. Those who exercised were defined as those who regularly exercised ≥1 h/week. *p <0.01 between white and Japanese-American men. †p <0.01 between Japanese versus Japanese-American men. ‡p <0.01 between Japanese versus white men.
HDL = high-density lipoprotein; LDL = low-density lipoprotein; NA = not assessed.

p = 0.184, for Japanese-American men and white men, respectively).

Japanese men had 2-fold higher levels of marine-derived n-3 fatty acids than both U.S. populations (Table 3). The lower 5th percentile of marine-derived n-3 fatty acids (4.97%) in Japanese men was still greater than the mean levels in white and Japanese-American men. Total fat amount was comparable across populations.

Japanese men had a significant inverse association of marine-derived n-3 fatty acids 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). Japanese men appeared to have an inverse association of marine-derived n-3 fatty acids with CAC prevalence. The association appeared to remain after adjusting for potential confounders (model III and IV). In contrast, neither white nor Japanese-American men had a significant inverse association with marine-derived n-3 fatty acids with IMT or CAC prevalence (Table 4). Although white men had a significant and nonsignificant age-adjusted inverse association of IMT and CAC prevalence, respectively, with marine-derived n-3 fatty acids, 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 fatty acids in the lowest tertile in Japanese men was still greater than that of the highest tertile in white and Japanese-American men.

Significant differences in both IMT and CAC prevalence between Japanese men and white men were attenuated and became nonsignificant after adjusting for serum marine-derived n-3 fatty acids (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

Table 2 Extent of Atherosclerosis in Japanese, White, and Japanese-American Men in 2002 to 2006

	Japanese Men (n = 281)	White Men (n = 306)	Japanese-American Men (n = 281)	p Value
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	†‡

*p < 0.01 between Japanese versus white men. †p < 0.01 between Japanese versus Japanese-American men. ‡p < 0.01 between white and Japanese-American men.
CAC = coronary artery calcification; CCA = common carotid artery; CCS = coronary calcium score; IMT = intima-media thickness; IQR = interquartile range.

Table 3 Serum Levels of Fatty Acids in Japanese Men, White Men, and Japanese-American Men in 2002 to 2006

	Japanese Men (n = 281)	White Men (n = 306)	Japanese-American Men (n = 281)	p Value
Total fatty acids (mg/dl)	245 (53)	237 (51)	243 (85)	NS
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 (SD). *p < 0.01 between the Japanese and white men. †p < 0.01 between white and Japanese-American men. ‡p < 0.01 between the Japanese and Japanese-American men. 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).
NS = not significant.

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 fatty acids, the mean difference became nonsignificant (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 nonsignificant after further adjusting for marine-derived n-3 fatty acids (5.0%, 95% CI: −5.3% to 15.4%, p = 0.341). Adjusting for fatty acids other

Table 4 Multivariable-Adjusted Associations of Marine n-3 Fatty Acids With Intima-Media Thickness and Prevalence of Coronary Artery Calcification in Japanese Men, White Men, and Japanese-American Men

Marine n-3 Fatty Acids

Intima-Media Thickness (μm)					Prevalence of Coronary Artery Calcification (%)				
Japanese Men					**Japanese Men**				
Median (%) (IQR)	Low 6.51 (4.16, 7.78)	Middle 8.71 (8.00, 9.72)	High 12.30 (10.05, 16.19)	p for Trend	Median (%) (IQR)	Low 6.51 (4.16, 7.78)	Middle 8.71 (8.00, 9.72)	High 12.30 (10.05, 16.19)	p for Trend
Model I	627 (8)	616 (8)	600 (8)	0.016	Model I	12.1 (3.0)	8.0 (3.0)	7.9 (3.0)	0.318
Model II	626 (8)	616 (8)	601 (8)	0.022	Model II	11.7 (3.0)	8.2 (3.0)	8.1 (3.0)	0.399
Model III	629 (8)	617 (8)	597 (8)	0.004	Model III	13.1 (3.0)	8.6 (3.0)	6.2 (3.0)	0.107
Model IV	630 (8)	618 (8)	596 (8)	0.004	Model IV	13.2 (2.9)	7.9 (2.9)	6.9 (2.9)	0.144
White Men					**White Men**				
Median (%) (IQR)	Low 2.42 (1.56, 2.82)	Middle 3.47 (2.90, 4.04)	High 5.23 (4.17, 9.42)	p for Trend	Median (%) (IQR)	Low 2.42 (1.56, 2.82)	Middle 3.47 (2.90, 4.04)	High 5.23 (4.17, 9.42)	p for Trend
Model I	685 (9)	672 (9)	653 (9)	0.014	Model I	32.0 (4.2)	22.1 (4.2)	22.8 (4.2)	0.127
Model II	676 (9)	673 (9)	661 (9)	0.271	Model II	29.3 (4.4)	22.1 (4.1)	25.4 (4.3)	0.545
Model III	676 (9)	674 (8)	661 (9)	0.258	Model III	28.8 (4.3)	22.0 (4.1)	26.0 (4.3)	0.656
Model IV	675 (9)	674 (9)	665 (9)	0.460	Model IV	30.5 (4.4)	22.4 (4.2)	26.0 (4.4)	0.491
Japanese-American Men					**Japanese-American Men**				
Median (%) (IQR)	Low 2.93 (1.79, 3.64)	Middle 4.40 (3.82, 5.12)	High 6.49 (5.12, 11.42)	p for Trend	Median (%) (IQR)	Low 2.93 (1.79, 3.64)	Middle 4.40 (3.82, 5.12)	High 6.49 (5.12, 11.42)	p for Trend
Model I	721 (12)	719 (12)	719 (12)	0.886	Model I	30.4 (4.8)	34.5 (4.8)	26.7 (4.8)	0.587
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, high-density lipoprotein cholesterol, and triglycerides. Model III: further adjusted for low-density lipoprotein cholesterol, smoking, glucose, insulin, and body mass index. Model IV: further adjusted for C-reactive protein, fibrinogen, alcohol, and medications for diabetes, hypertension, and hyperlipidemia.
IQR = interquartile range.

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Table 5 Multivariable-Adjusted Mean Differences in Intima-Media Thickness and Prevalence of Coronary Artery Calcification Between Japanese Men and White Men

	Japanese Men	White Men	Mean Difference (95% CI)	p Value
Intima-Media Thickness (μm)				
Model I	614 (5)	670 (5)	56 (42 to 70)	<0.001
Model II	623 (6)	662 (5)	39 (22 to 57)	<0.001
Model III	625 (6)	662 (6)	37 (18 to 56)	<0.001
Model IV	632 (7)	655 (7)	22 (−1 to 46)	0.065
Prevalence of Coronary Artery Calcification (%)				
Model I	9.3 (2.2)	26.1 (2.1)	16.8 (10.7 to 22.8)	<0.001
Model II	12.5 (2.5)	23.2 (2.4)	10.7 (2.9 to 18.4)	0.007
Model III	12.5 (2.7)	23.8 (2.6)	11.3 (2.8 to 19.8)	0.009
Model IV	15.5 (3.0)	20.5 (3.1)	5.0 (−5.3 to 15.4)	0.341

Values are expressed as mean and standard error. Model I: adjusted for age. Model II: further adjusted for blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, 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 hyperlipidemia. Model IV: further adjusted for marine n-3 fatty acids. Those who exercised were defined as those who regularly exercised ≥1 h/week.
CI = confidence interval.

than marine-derived n-3 (i.e., alpha linolenic, total n-6, saturated, monounsaturated, or trans fatty acids) did not attenuate the significant difference (data not shown). The differences in multivariable-adjusted levels of atherosclerosis between Japanese men and Japanese-American men were attenuated but remained significant after further adjusting for serum marine-derived n-3 or other fatty acids (data not shown).

Discussion

Our study in men ages 40 to 49 years shows that Japanese men had 2-fold greater levels of marine-derived n-3 fatty acids than white men and that the marine-derived n-3 fatty acids attenuated the significant differences in multivariable-adjusted carotid IMT and CAC prevalence between Japanese men and white men. Our study also shows significant and nonsignificant inverse associations of marine-derived n-3 fatty acids with IMT and CAC prevalence, respectively, in Japanese men, which remained after adjusting for cardiovascular risk and other factors. Our study also shows that in men ages 40 to 49 years, Japanese-American men compared with white men had greater or similar levels of atherosclerosis. Our results suggest that marine-derived n-3 fatty acids have antiatherogenic effects, especially at the high levels observed in Japanese men.

The results of 2 recent studies in Japan (18,19) support the hypothesis that high marine-derived n-3 fatty acids have antiatherogenic effects. The JELIS (Japan Eicosapentaenoic acid Lipid Interventions Study) (18), a randomized trial of 18,645 Japanese subjects that examined the effectiveness of 1.8 g of 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 the eicosapentaenoic acid versus control groups was 0.81 (95% CI: 0.68 to 0.96) for nonfatal coronary events. The JPHC (Japan Public Health Center-Based Study) (19), a 10-year prospective cohort study of 41,578 middle-aged Japanese subjects, reported that dietary intake of marine-derived n-3 fatty acids has significant inverse associations with nonfatal coronary events. The multivariate-adjusted hazard ratio in the highest versus lowest quintiles of marine-derived n-3 fatty acids intake (median intake = 2.1 vs. 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 antiarrhythmic effect of marine-derived n-3 fatty acids, 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 every day on average from early in life (31). Thus, the Japanese men consume fish far above the threshold for preventing cardiac death attributable to the antiarrhythmic effect. In fact, neither the researchers from the JPHC study nor the JELIS study observed a significant association of marine-derived n-3 fatty acids with cardiac death (18,19).

Our finding that Japanese-American men had similar or greater levels of atherosclerosis compared with U.S. white men is unexpected. The significantly greater prevalence of diabetes in Japanese-American men than U.S. white men was expected based on the well-known observation that Asians, including Japanese Americans, are more susceptible to developing diabetes than U.S. white populations (32). However, even after excluding those with diabetes, with hypertension, and taking lipid-lowering medications, Japanese Americans still had similar or greater levels of atherosclerosis. Our results may suggest that the third- or fourth-generation Japanese Americans will have similar or greater CHD rates in the future compared with U.S. white subjects (13). Further study is needed to carefully monitor CHD rates in third- or fourth-generation Japanese Americans. Our findings indicate that the antiatherogenic effect of marine-derived n-3 fatty acids 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 fatty acids have a variety of anti-inflammatory effects (34). Lifelong high intake of marine-derived n-3 fatty acids in the Japanese population 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 fatty acids in the Japanese subjects 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 men in this study were much less obese, the significant differences in atherosclerosis between

Japanese and white men 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 that has potential antiatherogenic properties and is inversely associated with obesity, is paradoxically lower in the Japanese than in white men (40).

This unique study is the first population-based international study to compare noninvasively evaluated atherosclerosis (i.e., carotid IMT and CAC). The inclusion of Japanese men, whose intake of fish is one of the highest in the world, enables us to analyze the associations of a wide range of marine-derived n-3 fatty acids with atherosclerosis. The fact that Japanese-American compared with U.S. white men had greater or similar levels of atherosclerosis indicates that the low atherosclerosis in Japanese men is unlikely to be primarily a result of 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-American populations with various stages of acculturation (42), whereas the Japanese Americans in the current study are third or fourth generation and considered to be acculturated.

Study limitations. Some limitations of the study warrant consideration. The sample size is relatively small. Our study included men and only those ages 40 to 49 years. However, we focused on this specific gender- 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 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 fatty acids on atherosclerosis, because levels of marine-derived n-3 fatty acids are markedly different between Japanese and U.S. populations throughout their lifetimes. Serum marine-derived n-3 fatty acids reflect short-term dietary fat intake and may not reflect long-term dietary intake. However, because the variation in serum marine-derived n-3 fatty acids occurs randomly, the actual association of marine-derived n-3 fatty acids 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).

Conclusions

If the high intake of marine-derived n-3 fatty acids in Japanese men 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 fatty acids 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 fatty acids in the diet (44) so that sources other than fish can be used to increase marine-derived n-3 fatty acids to prevent CHD. Long-term primary prevention trials of high-dose marine-derived n-3 fatty acids comparable with levels in Japan on atherosclerosis and CHD would be needed to test whether intake of marine-derived n-3 fatty acids markedly lowers CHD rates.

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Key Words: atherosclerosis ■ epidemiology ■ n-3 fatty acids ■ coronary artery calcification ■ intima-media thickness ■ Japanese.