NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Intakes of long-chain n-3 polyunsaturated fatty acids and fish in relation to measurements of subclinical atherosclerosis

Ka He,
University of North Carolina at Chapel Hill, School of Public Health, Chapel Hill, NC

Kiang Liu,
Northwestern University, Feinberg School of Medicine, Chicago, IL

Martha L. Daviglus,
Northwestern University, Feinberg School of Medicine, Chicago, IL

Elisabeth Mayer-Davis,
University of North Carolina at Chapel Hill, School of Public Health, Chapel Hill, NC

Nancy Swords Jenny,
University of Vermont College of Medicine, Colchester, VT

Rui Jiang,
Columbia University Medical Center, New York, NY

Pamela Ouyang,
Johns Hopkins University, School of Medicine, Baltimore, MD

Lyn M. Steffen,
University of Minnesota, School of Public Health, Minneapolis, MN

David Siscovick,
University of Washington, School of Medicine, Seattle, WA

Colin Wu,
National Heart, Lung and Blood Institute, Bethesda, MD

R. Graham Barr,
Columbia University Medical Center, New York, NY

Michael Tsai, and
University of Minnesota, School of Public Health, Minneapolis, MN

Gregory L. Burke

Corresponding author: Ka He, MD, ScD, Departments of Nutrition and Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, 2202 McGavran-Greenberg Hall, CB# 7461, Chapel Hill, NC 27599, Phone: 919-843-2476, Fax: 919-966-7216, kahe@unc.edu.

Wake Forest University Health Sciences, Winston-Salem, NC

Abstract

Background—Data on the relations of different types of fish meals and long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs) with measures of atherosclerosis are sparse.

Objective—We examined intakes of long-chain n-3 PUFAs and fish in relation to clinical measures of subclinical atherosclerosis.

Design—A cross-sectional study was conducted in 5,488 multiethnic adults aged 45–84 years and free of clinical cardiovascular disease. Diet was assessed using self-administered food frequency questionnaires. Subclinical atherosclerosis was determined by common carotid intima-media thickness (cCIMT, >80th percentile), internal CIMT (iCIMT, >80th percentile), coronary artery calcium score (CAC, >0) or ankle-brachial index (ABI, <0.90), respectively.

Results—After adjustment for potential confounders, intakes of long-chain n-3 PUFAs and non-fried (broiled, steamed, baked or raw) fish were inversely related to subclinical atherosclerosis determined by cCIMT but not iCIMT, CAC or ABI. The multivariable odds ratio comparing the highest to the lowest quartile of dietary exposures in relation to subclinical atherosclerosis determined by cCIMT was 0.69 (95% CI: 0.55, 0.86; *p* for trend<0.01) for n-3 PUFA intake, 0.80 (95% CI: 0.64, 1.01; *p*=0.054) for non-fried fish and 0.90 (95% CI: 0.73, 1.10; *p*=0.33) for fried fish consumption.

Conclusions—This study indicates that dietary intake of long-chain n-3 PUFAs or non-fried fish is associated with lower prevalence of subclinical atherosclerosis classified by cCIMT although significant changes in iCIMT, CAC and ABI were not observed. Our findings also suggest that the association of fish and atherosclerosis may vary depending on the type of fish meal consumed and the measures of atherosclerosis.

Keywords

INTRODUCTION

Observational studies and randomized trials have indicated that higher intakes of long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs) including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and fish are associated with lower risk of cardiovascular disease.(1–4) Possible mechanisms underlying this cardioprotective effect include reduction of serum triglycerides,(5) decreased platelet aggregability,(6) and anti-arrhythmic effects.(6) While the hypotriglyceridemic effects of supplementation with high dose long-chain n-3 PUFAs are well established,(7) these fatty acids may also increase low density lipoprotein (LDL) cholesterol(8, 9) and enhance oxidation of LDL cholesterol.(10, 11) Indeed, previous studies do not reveal a clear picture of the effects of long-chain n-3 PUFAs or fish on subclinical atherosclerosis. Data from both animal models(12–14) and humans(15–17) are inconsistent.

Computed tomography scanning of the coronary arteries for calcium is widely used as a non-invasive method for assessing early coronary artery disease. The presence of coronary artery calcium (CAC) is a predictor of increased risk for future coronary events.(18, 19) The ultrasonographic evaluation of the carotid intimal-medial thickness (CIMT) is also utilized to assess carotid atherosclerosis.(20, 21) In addition, a low ankle-brachial index (ABI) has been reported to be associated with an increased risk of death, total cardiovascular disease, coronary heart disease, congestive heart failure, and symptomatic peripheral arterial disease. (22–25) Although these measurements are widely employed as markers of subclinical atherosclerosis in various epidemiologic studies, few studies have used these measurements simultaneously in a single cohort.

To determine whether the previous inconsistent findings on fish and subclinical atherosclerosis can be partially explained by the different clinical measures used and how different types of fish meals related to atherosclerosis, we examined fish consumption and long-chain n-3 PUFA intake in relation to clinical measures of atherosclerosis including common CIMT (cCIMT), internal CIMT (iCIMT), CAC and ABI in a cohort of multi-ethnic, middle-aged and older, men and women.

PARTICIPANTS AND METHODS

Study Population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a population based study of 6814 men and women aged 45 to 84 years, without clinical cardiovascular disease, recruited from 6 U.S. communities. Participants were enrolled between August 1, 2000, and July 30, 2002. The self-identified ethnic background of the cohort is 38.5% Caucasians, 27.8% African Americans, 21.9% Hispanics, and 11.8% Chinese. The primary goal of the MESA Study is to determine the characteristics associated with subclinical cardiovascular disease and its progression. Details of sampling and recruitment procedures have been published else where.(26) Participants completed a set of measurements of subclinical cardiovascular disease and assessment of standard cardiovascular disease risk factors, sociodemographic factors, diet, lifestyle, and psychosocial factors. In the present analysis, we excluded those who had missing data on diet (n=577), ABI (n=70), cCIMT (n=74), iCIMT (n=98), and who had extreme total energy intake (<600 or >6000 kcal/day, n=285). We also excluded participants without information on covariates used in the analysis (n=222). After these exclusions, a total of 5488 participants remained in the analysis.

The MESA protocol was approved by institutional review boards at all participating sites, and all participants provided informed consent.

Dietary Assessment

Dietary information was assessed using a self-administered food frequency questionnaire (FFQ) and dietary supplement form. The FFQ was based on a FFQ used in the tri-ethnic (non-Hispanic Caucasians, African Americans, and Hispanics) Insulin Resistance Atherosclerosis Study (IRAS) and was modified for the Diabetes Prevention Program to include foods typically consumed by a Chinese population and to collect supplemental information.(26, 27) Participants were asked to identify usual frequency of consumption of

the food items during the past year and the average serving size consumed. Nine frequency responses were available in the questionnaire, ranging from "never/rare" to "2+ per day", and three serving sizes were specified: small, medium, and large. In this study, fish consumption was defined as fish and other seafood intake. Participants were asked to indicate how often they consumed 1) fried fish or fish sandwich, fried shrimp, calamari; 2) shrimp, lobster, crab, oysters, mussels (not fried); 3) tuna, salmon, sardines (including sashimi or sushi); and 4) other broiled, steamed, baked or raw fish (such as trout, sole, halibut, poke, grouper). Studies suggested that the preparation method particularly frying might substantially alter the fatty acid content of a fish meal(28) and the associations with cardiovascular disease risk might be different.(29) Also, long-chain n-3 PUFA contents in shell fish are relatively low. Therefore, we divided fish consumption into three groups: fried fish (including fried shell fish), non-fried fish (broiled, steamed, baked or raw fish) and non-fried fish + shell fish. Because near 50% participants reported no shell fish consumption, we were not able to analyze shell fish separately. In the questionnaire, there are also three questions related to mixed fish dish such as Chinese food with stir-fried shrimp or fish with vegetables; pasta with seafood; and seafood gumbo. Because we were not able to determine the exact portion of seafood in the mixed fish dish, we did not include mixed fish dishes in the main analyses. However, we included mixed fish dishes in the secondary analyses by assigning a weight 0.4, 0.5 or 0.6 respectively to the mixed fish dish. We converted fish consumption into servings per day and adjusted for serving size (i.e. small = $0.5 \times$ medium; medium = standard age- and gender-specific serving size; large = $1.5 \times$ medium).(30) Nutrients including long-chain n-3 PUFAs were derived from the Minnesota nutrition data system (NDS-R version 4.02/30).(31) Information on the dose of supplements including fish oil was acquired. Also, dose of supplements was included in estimation of nutrient intake.

Although the FFQ has not been validated in MESA, the IRAS questionnaire has demonstrated reasonable validity and reliability in a diverse cohort.(32) Compared to the average of eight 24-hour recalls, the mean correlation coefficients of nutrient intake were 0.62 for non-Hispanic Caucasians, 0.5 for African Americans, and 0.41 for Hispanics. The relatively lower observed correlations among Hispanics were largely accounted for by differences in education across the ethnicities. For reliability, the mean correlation coefficient for nutrients evaluated was 0.62 and did not differ by ethnic subgroup.

Measures of Subclinical Atherosclerosis

In this study, subclinical atherosclerosis was defined by CAC, cCIMT, iCIMT and ABI, respectively. CAC was measured using 2 scans obtained on the same occasion in all participants during the clinical examination using electron beam computed tomography or multi-detector computed tomography.(33) The presence of CAC (Agatston score > 0) was considered as a marker of subclinical atherosclerosis.

IMT was measured between lumen-intima and media-adventitia interfaces of near and far walls of the common carotid artery (the 1 cm segment proximal to the bifurcation) and the internal carotid artery (including the bifurcation and 1cm distal to the bifurcation) with B-mode ultrasound (Logiq 700 ultrasound machine; General Electric Medical Systems).(34)

Subclinical atherosclerosis was defined by the values of cCIMT or iCIMT index of greater than 80th percentile values.

All participants were evaluated in the supine position after at least 5 minutes of rest. Systolic blood pressure was measured in arms and in the right and left posterior tibial and dorsalis pedis artery. The resting ABI was determined for each leg by dividing the systolic blood pressure at the ankle by the brachial pressure; the lower of the ABI values obtained in each leg was used in the data analysis.(35) An ABI value less than 0.9 was classified as subclinical atherosclerosis in this analysis.

Other Covariates Assessment

Demographic and major lifestyle variables were collected through questionnaire; including age, gender, race/ethnicity, education level, household income, smoking status, medical conditions, and current medication use. Body weight and height were directly measured according to standard procedures. Body mass index (BMI) was calculated as the ratio of weight (kg) to squared height (m). Physical activity was measured using a detailed, semi-quantitative questionnaire adopted from the Cross-Culture Activity Participation Study.(26) Total physical activity was computed as the total intentional exercise (all light, moderate, and vigorous activities [minutes per week]) multiplied by the activities' individual metabolic equivalent values.

Statistical analysis

Quartiles of fish consumption and long-chain n-3 PUFA intake were created and used in the analysis. Age-, sex- and race-adjusted means and standard errors or proportions were computed for selected variables by quartiles of long-chain n-3 PUFA intake. Logistic regression analyses were used to examine the associations of fish consumption and long-chain n-3 PUFA intake with subclinical atherosclerosis, which are classified by cCIMT (>80th percentile), iCIMT (>80th percentile), CAC (Agatston score >0), or ABI (<0.90). We considered a number of potential confounders in the multivariable models including age, race/ethnicity, sex, BMI, physical activity, household income, smoking status, systolic blood pressure, antihypertensive medication use, and several dietary variables. We first ran a basic model by including a few demographic variables. Other major lifestyle factors and dietary variables were included in the analysis as potential confounders based on previous studies and statistical tests. Covariates were added to the basic regression model in a hierarchical fashion so that we can assess the impact of adjusting for specific confounders on the association of each combination of dietary exposure and clinical measures of atherosclerosis. Also, we adjusted for fried fish when examining non-fried fish and *verse visa*. Test for trends across quartiles was evaluated using the median values of each quartile of fish and long-chain n-3 PUFA intake. In a sensitivity analysis, different cut-off points (10, 50, 100, and 200) were used for CAC to see if the results on fish or long-chain n-3 PUFAs and CAC were robust. All p values were two-sided with the p value less than 0.05 considered statistically significant. SAS (version 9; SAS Institute, Inc., Cary, North Carolina) software was used for all analyses.

RESULTS

Table 1 shows age-, sex- and race- adjusted characteristics of the study population according to dietary intake of long-chain n-3 PUFA. The median daily intakes of long-chain n-3 PUFA across quartiles were 40 mg, 80 mg, 120 mg and 220 mg respectively. Compared to participants in the lowest quartile of long-chain n-3 PUFA intake, those who in the highest quartile were more likely to have higher education and household income; they exercised more, and were less likely to be current smokers. Also, participants in the highest quartile of long-chain n-3 PUFA intake had lower intakes of alcohol, total energy, saturated fat and *trans* fat, and higher intake of alpha-linolenic acid.

In multivariable analysis, a significant inverse association was observed between long-chain n-3 PUFA intake and subclinical atherosclerosis determined by cCIMT. The odds of having cCIMT greater than 80th percentile was 31% lower (odds ratio [OR], 0.69; 95% confidence interval [CI], 0.55, 0.86; *p* for trend = 0.005) for participants in the highest quartile of long-chain n-3 PUFA intake as compared to those who in the lowest quartile (Table 2). Increasing non-fried fish consumption was related to decreased odds of having cCIMT greater than 80th percentile (OR, 0.80; 95% CI, 0.64, 1.01; *p* for trend = 0.054) but this inverse association was attenuated by including shell fish (OR, 0.84; 95% CI, 0.67, 1.04; *p* for trend = 0.15). Fried fish was not related to cCIMT. In addition, no inverse associations were observed, however, between long-chain n-3 PUFA intake, non-fried fish, non-fried fish + shell fish, and fried fish consumption with subclinical atherosclerosis classified by iCIMT, CAC or ABI (Table 2).

After adjustment for potential confounders, long-chain n-3 PUFA intake and fish consumption were significantly inversely related to blood triglyceride levels. Also, intakes of long-chain n-3 PUFAs and non-fried fish were positively associated with blood high-density lipoprotein (HDL) cholesterol levels. The ratio of total and HDL cholesterol has been suggested as a powerful predictor of coronary heart disease.(36) In this study, dietary long-chain n-3 PUFA intake but not fish consumption was significantly inversely related to the ratio of total and HDL cholesterol (Table 3).

By stratifying data, we found that the inverse associations between non-fried fish consumption or long-chain n-3 PUFA intake and cCIMT were not appreciably modified by gender. The p values for interaction tests of gender with long-chain n-3 PUFAs and non-fried fish were 0.95 and 0.57 respectively. In addition, the observed inverse associations remained in Caucasians but not other ethnicities (data not shown).

In sensitivity analyses (data not shown), we used different cut-off points (10, 50, 100, and 200) for CAC, the results were not materially altered. In addition, approximately 4% participants used fish oil supplements. The results remained when we excluded the supplement users in the analyses. In this study, we were not able to analyze shell fish separately because near half of the participants reported no shell fish consumption. Nevertheless, no association with any of the clinical markers was observed by dichotomizing shell fish consumption. Moreover, we included mixed fish dishes in the

analysis by assigning a weight 0.4, 0.5 or 0.6 respectively to mixed fish dish. The observed inverse associations were slightly attenuated.

DISCUSSION

In this cross-sectional study, we found that dietary intake of long-chain n-3 PUFAs and non-fried fish consumption were associated with a lower prevalence odds ratio of subclinical atherosclerosis classified by cCIMT but not iCIMT, CAC or ABI. The inverse associations were consistent in men and in women.

In spite of potential benefits of dietary long-chain n-3 PUFAs in the reduction of coronary heart disease mortality,(3) it is not clear whether long-chain n-3 PUFAs have a direct effect on the pathogenesis of atherosclerosis. A randomized controlled trial among 59 patients with angiographically documented coronary heart disease reported that fish oil treatment (6 grams EPA and DHA daily) for 2 years had no major favorable effect on the diameter of atherosclerotic coronary arteries.(15) Another randomized, double-blind, placebo-controlled trial in 223 patients with angiographically proven coronary artery disease found that fish oil intake, approximately 1.65 grams EPA and DHA daily for 2 years, modestly mitigated the course of human coronary atherosclerosis,(16) but had no effect on progression of carotid atherosclerosis.(37) Change in atherosclerosis extent in carotid arteries and in coronary arteries in these patients during the same period was not correlated.(37) The association of fish or long-chain n-3 PUFAs with atherosclerosis was also examined in observational studies. A cross-sectional study conducted in 470 Japanese found that serum long-chain n-3 PUFA level was inversely related to probability of common carotid plaques.(38) Also, a prospective cohort study of postmenopausal women with coronary artery disease (n=229) found that fish consumption was associated with significantly less progression of coronary atherosclerosis.(17)

The effects of long-chain n-3 PUFAs on atherosclerosis may be mediated through their roles in lipoprotein metabolism. In this study, we found that fish and long-chain n-3 PUFA intakes were associated with lower triglyceride levels. Long-chain n-3 PUFAs and non-fried fish were positively related to HDL. Long-chain n-3 PUFAs were also inversely associated with the ratio of total and HDL cholesterol. No statistically significant associations were found for LDL and total cholesterol. A recent meta-analysis of 21 randomized controlled trials summarized the effects of fish oil supplementation on lipid values.(39) The meta-analysis suggests that fish oil consumption significantly decreases serum triglycerides and modestly improves HDL. However, fish oil intake increases the level of LDL cholesterol and has no effect on total cholesterol. It is uncertain how these combined effects of long-chain n-3 PUFAs on lipid values affect the development or progression of atherosclerosis. In addition, long-chain n-3 PUFAs may have an anti-platelet aggregation effect,(40, 41) and have a modest hypotensive effect.(42) Moreover, studies indicate that long-chain n-3 PUFAs have anti-inflammatory effects and may improve endothelial function.(43, 44) The associations of long-chain n-3 PUFAs with atherosclerosis might reflect the integrative effects of long-chain n-3 PUFAs with lipids, thrombosis, inflammation, and endothelial function.

A major strength of our study is that we have multiple clinical measures of subclinical atherosclerosis. However, in this study, we only observed an inverse association between long-chain n-3 PUFAs and cCIMT. Although all of these measurements are recognized as markers of subclinical atherosclerosis, they may be different surrogates of the atherosclerotic process. For example, ABI is generally thought of as the best clinical marker of diffuse atherosclerosis. CAC may reflect atherosclerosis in coronary arteries. cCIMT and iCIMT are considered as markers of carotid atherosclerosis. We found that dietary long-chain n-3 PUFAs were associated with cCIMT but not iCIMT. One possible explanation is that iCIMT mainly reflects hypertension status but not carotid atherosclerosis *per se*. Of note, intakes of long-chain n-3 PUFAs and fish were relatively low in this cohort. The observed null associations in this study might be partially explained by the low long-chain n-3 PUFA intake or fish consumption. Nevertheless, significant risk reduction of ischemic stroke with similar amount of long-chain n-3 PUFA intake was reported.(45) In addition, we examined the associations among multiple ethnic groups. Although qualitatively similar associations were observed across ethnicities, the statistically significant inverse associations between long-chain n-3 PUFAs and cCIMT only remained in Caucasians. Notably, Caucasian is the largest ethnic group in MESA. The relatively small numbers of participant identified having cCIMT greater than 80th percentile in other ethnicities especially in Chinese and Hispanics might limit our ability to observe any possible significant association. Another strength of this study is that we examined separately fried fish, non-fried fish and non-fried fish + shell fish consumption in relation to measures of atherosclerosis. Shell fish contains relatively low levels of long-chain n-3 PUFAs. In this study, the observed inverse association between non-fried fish and cCIMT was attenuated by including shell fish. Although it was modestly inversely related to blood triglyceride levels, we found no beneficial effects of fried fish consumption on atherosclerosis. Our findings are biologically plausible since frying may affect a fish meal's fatty acid composition(28) including reducing long-chain n-3 PUFA content and producing *trans*-unsaturated fatty acids. These changes in fatty acid profile of cooked fish meals may somewhat attenuate or cancel the potential benefit of fish consumption.

Our study has some limitations. First, given the cross-sectional analysis, the possibility of reverse causation can not be completely excluded. Also, we could not account for the effects of changes in diet on atherosclerosis. For example, some participants who were at high risk of cardiovascular disease might have changed their diet (e.g., increased fish consumption or took fish oil supplement) based on advice from physicians or information via media sources. This diet change could dilute any inverse association of long-chain n-3 PUFAs or fish and subclinical atherosclerosis. Second, dietary intake was self-reported in this study, the inevitable measurement errors in estimating fish consumption as well as long-chain n-3 PUFA intake could bias our results. Third, although we adjusted for a number of potential confounders in the analysis, residual confounding and possible confounding from unmeasured dietary and non-dietary factors could not be ruled out. For example, information on mercury and other contaminants in fish was not available. Study suggests that mercury may increase risk of myocardial infarction.(46) Thus, mercury in fish might confound our findings. Fourth, near 20% participants were excluded in the analyses mainly because of the missing data. Although the characteristics of these two groups were not significantly

different, the results might be affected by the exclusions. Finally, the relatively small numbers of participant with ABI < 0.90 might limit our capability to examine the associations using the ABI as the measure of subclinical atherosclerosis.

In summary, our study indicates that dietary intakes of long-chain n-3 PUFAs and non-fried fish are inversely associated with subclinical atherosclerosis determined by cCIMT but not iCIMT, CAC or ABI. Our findings also suggest that results of clinical and epidemiologic research may differ according to the measures employed to assess atherosclerosis and the type of fish meal consumed.

Acknowledgments

References

1. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet. 1999; 354:447–55. [PubMed: 10465168]

2. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation. 2002; 106:2747–57. [PubMed: 12438303]

3. He K, Song Y, Daviglus ML, et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation. 2004; 109:2705–11. [PubMed: 15184295]

4. Bucher HC, Hengstler P, Schindler C, Meier G. N-3 polyunsaturated fatty acids in coronary heart disease: a meta-analysis of randomized controlled trials. Am J Med. 2002; 112:298–304. [PubMed: 11893369]

5. Roche HM, Gibney MJ. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. Am J Clin Nutr. 2000; 71:232S–7S. [PubMed: 10617977]

6. Kang JX, Leaf A. Prevention of fatal cardiac arrhythmias by polyunsaturated fatty acids. Am J Clin Nutr. 2000; 71:202S–7S. [PubMed: 10617972]

7. Harris WS. n-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr. 1997; 65:1645S–1654S. [PubMed: 9129504]

8. Reis GJ, Silverman DI, Boucher TM, et al. Effects of two types of fish oil supplements on serum lipids and plasma phospholipid fatty acids in coronary artery disease. Am J Cardiol. 1990; 66:1171–5. [PubMed: 2239719]

9. Fumeron F, Brigant L, Ollivier V, et al. n-3 polyunsaturated fatty acids raise low-density lipoproteins, high-density lipoprotein 2, and plasminogen-activator inhibitor in healthy young men. Am J Clin Nutr. 1991; 54:118–22. [PubMed: 2058572]

10. Harats D, Dabach Y, Hollander G, et al. Fish oil ingestion in smokers and nonsmokers enhances peroxidation of plasma lipoproteins. Atherosclerosis. 1991; 90:127–39. [PubMed: 1759984]

11. Whitman SC, Fish JR, Rand ML, Rogers KA. n-3 fatty acid incorporation into LDL particles renders them more susceptible to oxidation in vitro but not necessarily more atherogenic in vivo. Arterioscler Thromb. 1994; 14:1170–6. [PubMed: 8018674]

12. Harker LA, Kelly AB, Hanson SR, et al. Interruption of vascular thrombus formation and vascular lesion formation by dietary n-3 fatty acids in fish oil in nonhuman primates. Circulation. 1993; 87:1017–29. [PubMed: 8443878]

13. Rich S, Miller JF Jr, Charous S, et al. Development of atherosclerosis in genetically hyperlipidemic rabbits during chronic fish-oil ingestion. Arteriosclerosis. 1989; 9:189–94. [PubMed: 2923575]

14. Fincham JE, Gouws E, Woodroof CW, et al. Atherosclerosis. Chronic effects of fish oil and a therapeutic diet in nonhuman primates. Arterioscler Thromb. 1991; 11:719–32. [PubMed: 1851432]

15. Sacks FM, Stone PH, Gibson CM, Silverman DI, Rosner B, Pasternak RC. HARP Research Group. Controlled trial of fish oil for regression of human coronary atherosclerosis. J Am Coll Cardiol. 1995; 25:1492–8. [PubMed: 7759696]

16. von Schacky C, Angerer P, Kothny W, Theisen K, Mudra H. The effect of dietary omega-3 fatty acids on coronary atherosclerosis. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 1999; 130:554–62. [PubMed: 10189324]

17. Erkkila AT, Lichtenstein AH, Mozaffarian D, Herrington DM. Fish intake is associated with a reduced progression of coronary artery atherosclerosis in postmenopausal women with coronary artery disease. Am J Clin Nutr. 2004; 80:626–32. [PubMed: 15321802]

18. Shaw LJ, Raggi P, Schisterman E, Berman DS, Callister TQ. Prognostic value of cardiac risk factors and coronary artery calcium screening for all-cause mortality. Radiology. 2003; 228:826–33. [PubMed: 12869688]

19. Greenland P, LaBree L, Azen SP, Doherty TM, Detrano RC. Coronary artery calcium score combined with Framingham score for risk prediction in asymptomatic individuals. Jama. 2004; 291:210–5. [PubMed: 14722147]

20. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident myocardial infarction: the Rotterdam Study. Circulation. 2004; 109:1089–94. [PubMed: 14993130]

21. Salonen JT, Salonen R. Ultrasound B-mode imaging in observational studies of atherosclerotic progression. Circulation. 1993; 87:II56–65. [PubMed: 8443925]

22. McKenna M, Wolfson S, Kuller L. The ratio of ankle and arm arterial pressure as an independent predictor of mortality. Atherosclerosis. 1991; 87:119–28. [PubMed: 1854359]

23. Newman AB, Shemanski L, Manolio TA, et al. The Cardiovascular Health Study Group. Ankle-arm index as a predictor of cardiovascular disease and mortality in the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol. 1999; 19:538–45. [PubMed: 10073955]

24. Jager A, Kostense PJ, Ruhe HG, et al. Microalbuminuria and peripheral arterial disease are independent predictors of cardiovascular and all-cause mortality, especially among hypertensive subjects: five-year follow-up of the Hoorn Study. Arterioscler Thromb Vasc Biol. 1999; 19:617–24. [PubMed: 10073965]

25. McDermott MM. Ankle brachial index as a predictor of outcomes in peripheral arterial disease. J Lab Clin Med. 1999; 133:33–40. [PubMed: 10385479]

26. Bild DE, Bluemke DA, Burke GL, et al. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol. 2002; 156:871–81. [PubMed: 12397006]

27. Mayer-Davis EJ, Sparks KC, Hirst K, et al. Dietary intake in the diabetes prevention program cohort: baseline and 1-year post randomization. Ann Epidemiol. 2004; 14:763–72. [PubMed: 15573453]

28. Candela M, Astiasaran I, Bello J. Deep-fat frying modifies high-fat fish lipid fraction. J Agric Food Chem. 1998; 46:2793–2796.

29. Mozaffarian D, Lemaitre RN, Kuller LH, Burke GL, Tracy RP, Siscovick DS. Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the Cardiovascular Health Study. Circulation. 2003; 107:1372–7. [PubMed: 12642356]

30. Cummings SR, Block G, McHenry K, Baron RB. Evaluation of two food frequency methods of measuring dietary calcium intake. Am J Epidemiol. 1987; 126:796–802. [PubMed: 3661527]

31. Minnesota Nutrition Coordinating Center. Minnesota Nutrition Data System, Program 4.02 [computer program]. Version Nutrient Database Version 3.0. 1999

32. Mayer-Davis EJ, Vitolins MZ, Carmichael SL, et al. Validity and reproducibility of a food frequency interview in a Multi-Cultural Epidemiology Study. Ann Epidemiol. 1999; 9:314–24. [PubMed: 10976858]

33. Carr JJ, Nelson JC, Wong ND, et al. Calcified coronary artery plaque measurement with cardiac CT in population-based studies: standardized protocol of Multi-Ethnic Study of Atherosclerosis (MESA) and Coronary Artery Risk Development in Young Adults (CARDIA) study. Radiology. 2005; 234:35–43. [PubMed: 15618373]

34. O'Leary DH, Polak JF, Wolfson SK Jr, et al. Use of sonography to evaluate carotid atherosclerosis in the elderly. The Cardiovascular Health Study. CHS Collaborative Research Group. Stroke. 1991; 22:1155–63. [PubMed: 1926258]

35. Vogt MT, McKenna M, Wolfson SK, Kuller LH. The relationship between ankle brachial index, other atherosclerotic disease, diabetes, smoking and mortality in older men and women. Atherosclerosis. 1993; 101:191–202. [PubMed: 8379964]

36. Shai I, Rimm EB, Hankinson SE, et al. Multivariate assessment of lipid parameters as predictors of coronary heart disease among postmenopausal women: potential implications for clinical guidelines. Circulation. 2004; 110:2824–30. [PubMed: 15492318]

37. Angerer P, Kothny W, Stork S, von Schacky C. Effect of dietary supplementation with omega-3 fatty acids on progression of atherosclerosis in carotid arteries. Cardiovasc Res. 2002; 54:183–90. [PubMed: 12062374]

38. Yamada T, Strong JP, Ishii T, et al. Atherosclerosis and omega-3 fatty acids in the populations of a fishing village and a farming village in Japan. Atherosclerosis. 2000; 153:469–81. [PubMed: 11164437]

39. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: A systematic review. Atherosclerosis. 2006

40. Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? Lancet. 1978; 2:117–9. [PubMed: 78322]

41. Agren JJ, Vaisanen S, Hanninen O, Muller AD, Hornstra G. Hemostatic factors and platelet aggregation after a fish-enriched diet or fish oil or docosahexaenoic acid supplementation. Prostaglandins Leukot Essent Fatty Acids. 1997; 57:419–21. [PubMed: 9430389]

42. Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation. 1993; 88:523–33. [PubMed: 8339414]

43. Lopez-Garcia E, Schulze MB, Manson JE, et al. Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. J Nutr. 2004; 134:1806–11. [PubMed: 15226473]

44. Niu K, Hozawa A, Kuriyama S, et al. Dietary long-chain n-3 fatty acids of marine origin and serum C-reactive protein concentrations are associated in a population with a diet rich in marine products. Am J Clin Nutr. 2006; 84:223–9. [PubMed: 16825699]

45. He K, Rimm EB, Merchant A, et al. Fish consumption and risk of stroke in men. Jama. 2002; 288:3130–6. [PubMed: 12495393]

46. Guallar E, Sanz-Gallardo MI, van't Veer P, et al. Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med. 2002; 347:1747–54. [PubMed: 12456850]

Table 1

Age-, sex- and race-adjusted characteristics of the study population according to quartiles of long-chain n-3 PUFA intake, MESA baseline examination, 2000–2002[1]

Characteristics	Long-chain n-3 PUFA intake				P value[2]
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Median n-3 PUFA intake,[3] mg/day	40	80	120	220	--
Number of participants	1372	1372	1372	1372	--
Education, %					<0.01
Less than high school	22.1	17.6	13.9	15.8	
High school	21.9	18.5	16.8	14.0	
Some college	28.9	27.3	28.9	26.2	
College or higher	27.1	36.6	40.4	44.1	
Household annual income, %					<0.01
$16,000	21.0	15.1	17.8	19.5	
$16,001–$39,999	34.9	32.0	29.3	28.9	
$40,000	44.1	52.9	52.9	51.6	
BMI,[3] kg/m^2, mean	28.4 (0.14)	28.3 (0.14)	28.0 (0.14)	27.4 (0.14)	<0.01
Total intentional exercise, MET[3]-min/week	1442 (63)	1452 (63)	1565 (63)	1873 (63)	<0.01
Smoking status, %					<0.01
Never smoker	49.2	50.7	50.5	55.3	
Former smoker	35.9	38.0	37.4	34.0	
Current smoker	14.9	11.3	12.1	10.6	
Pack-years of cigarette smoking	13.6 (0.6)	10.9 (0.6)	10.4 (0.6)	9.5 (0.6)	<0.01
Alcohol consumption, drinks/week	4.4 (0.2)	3.9 (0.2)	3.6 (0.2)	3.5 (0.2)	<0.01
Use of fish oil supplements, %	3.1	4.1	4.5	5.4	0.43
Seated systolic blood pressure, mmHg	126.1 (0.53)	126.0 (0.53)	125.4 (0.53)	126.2 (0.53)	0.70
Seated diastolic blood pressure, mmHg	71.4 (0.26)	71.5 (0.26)	71.8 (0.26)	72.4 (0.26)	0.02
Anti-hypertensive medication use, %	28.3	30.8	34.0	32.8	0.28
Diabetes history, %	12.9	12.5	13.7	15.6	0.41

Characteristics	Long-chain n-3 PUFA intake				P value[2]
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	
LDL cholesterol,[3] mg/dl	117.9 (0.8)	118.8 (0.8)	116.2 (0.8)	116.0 (0.8)	0.05
HDL cholesterol,[3] mg/dl	50.1 (0.4)	50.9 (0.4)	51.3 (0.4)	52.2 (0.4)	<0.01
Triglycerides, mg/dl	145.2 (2.3)	131.2 (2.2)	128.8 (2.2)	121.5 (2.2)	<0.01
Daily dietary intake					
Non-fried (broiled, steamed, baked or raw) fish, servings/week	0.1 (0.03)	0.5 (0.03)	0.9 (0.03)	2.3 (0.03)	<0.01
Fried fish, servings/week	0.1 (0.02)	0.3 (0.02)	0.4 (0.02)	0.6 (0.02)	<0.01
Total energy intake, kcal	1768 (17)	1691 (17)	1592 (17)	1470 (17)	<0.01
Saturated fat, % energy	11.6 (0.08)	10.9 (0.08)	10.4 (0.08)	9.6 (0.08)	<0.01
Trans fatty acid, % energy	1.8 (0.02)	1.8 (0.02)	1.7 (0.02)	1.5 (0.02)	<0.01
Alpha-linolenic acid, % energy	0.65 (0.01)	0.64 (0.01)	0.65 (0.01)	0.68 (0.01)	<0.01

[1] Data are means (standard error), unless otherwise specified; and data were obtained by general linear model (GLM).

[2] P-values for differences across quartiles were obtained using general linear models for continuous variables, logistic regression for binary variables, and polytomous logistic regression for categorical variables.

[3] PUFA: polyunsaturated fatty acid; BMI: body mass index; MET: metabolic equivalent-minutes; LDL: low density lipoprotein; HDL: high density lipoprotein.

Table 2

Multivariable-adjusted odds ratios (95% confidence intervals) of subclinical atherosclerosis determined by various biomarkers according to quartiles of dietary intake of fish and long-chain n-3 PUFA, MESA 2000–2002.[1]

Quartiles (N of participants)[2]	Median Intake	cCIMT		iCIMT		CAC		ABI	
		N with cCIMT > 80th percentiles	OR (95% CI)	N with iCIMT > 80th percentiles	OR (95% CI)	N with CAC > 0	OR (95% CI)	N with ABI < 0.90	OR (95% CI)
N-3 PUFA[3]	mg/day								
Q1 (1372)	40	317	1.00	299	1.00	690	1.00	49	1.00
Q2 (1372)	80	275	0.77 (0.63, 0.95)	268	0.89 (0.72, 1.09)	674	0.96 (0.80, 1.14)	44	0.91 (0.59, 1.42)
Q3 (1372)	120	263	0.78 (0.63, 0.96)	285	1.07 (0.87, 1.31)	665	0.95 (0.80, 1.14)	40	0.90 (0.56, 1.43)
Q4 (1372)	220	234	0.69 (0.55, 0.86)	245	0.97 (0.78, 1.22)	698	1.14 (0.94, 1.38)	50	1.28 (0.81, 2.02)
P for trend			0.005		0.87		0.09		0.18
Non-fried fish[3],[4]	Servings/week								
Q1 (1422)	0	316	1.00	310	1.00	709	1.00	62	1.00
Q2 (1257)	0.3	256	0.95 (0.77, 1.18)	252	1.00 (0.82, 1.24)	621	1.03 (0.86, 1.23)	35	0.72 (0.46, 1.12)
Q3 (1468)	0.8	281	0.88 (0.72, 1.09)	281	0.95 (0.78, 1.17)	719	1.00 (0.84, 1.20)	46	0.84 (0.55, 1.29)
Q4 (1341)	2.0	236	0.80 (0.64, 1.01)	254	0.98 (0.78, 1.22)	678	1.02 (0.84, 1.24)	40	0.92 (0.58, 1.47)
P for trend			0.054		0.80		0.89		0.94
Non-fried fish and shell fish[3]	Servings/week								
Q1 (1491)	0	344	1.00	334	1.00	751	1.00	60	1.00
Q2 (1243)	0.5	237	0.90 (0.73, 1.11)	231	0.91 (0.74, 1.12)	606	1.04 (0.87, 1.25)	40	0.97 (0.63, 1.49)
Q3 (1383)	1.0	261	0.83 (0.67, 1.02)	273	0.97 (0.79, 1.19)	681	0.97 (0.81, 1.16)	41	1.00 (0.65, 1.55)
Q4 (1371)	2.5	247	0.84 (0.67, 1.04)	259	0.97 (0.79, 1.21)	689	1.11 (0.92, 1.34)	42	1.15 (0.73, 1.82)
P for trend			0.15		0.98		0.26		0.49
Fried fish[3],[4]	Servings/week								
Q1 (2240)	0	435	1.00	442	1.00	1145	1.00	69	1.00

Quartiles (N of participants)[2]	Median Intake	cCIMT		iCIMT		CAC		ABI	
		N with cCIMT > 80th percentiles	OR (95% CI)	N with iCIMT > 80th percentiles	OR (95% CI)	N with CAC > 0	OR (95% CI)	N with ABI < 0.90	OR (95% CI)
Q2 & Q3 (1789)[5]	0.2	341	0.92 (0.77, 1.11)	331	0.98 (0.82, 1.18)	890	1.03 (0.89, 1.20)	57	1.06 (0.72, 1.58)
Q4 (1459)	0.8	313	0.90 (0.73, 1.11)	324	1.17 (0.96, 1.43)	692	0.93 (0.78, 1.11)	57	1.07 (0.70, 1.65)
P for trend			0.38		0.09		0.34		0.78

[1] Data were obtained by logistic regression models. cCIMT: common carotid intima-media thickness; iCIMT: internal carotid intima-media thickness; CAC: coronary artery calcium score; ABI: ankle-brachial index; PUFA: polyunsaturated fatty acid.

[2] The numbers of participant in fish quartiles are different due to the tied values of the amount of fish consumption.

[3] Adjusted for age, race, gender, body mass index, physical activity, household income, smoking status (current, former and never smoker), systolic blood pressure, antihypertensive medication use, alcohol consumption, and intakes of saturated fat, α-linolenic acid, *trans*-fatty acids, and total energy intake; and further adjusted for fried fish and shell fish (for non-fried fish) or fried fish (for non-fried fish + shell fish) or non-fried fish and shell fish (for fried fish).

[4] Non-fried fish includes broiled, steamed, baked or raw fish; fried fish includes fried fish and fried shell fish.

[5] We combined quartile 2 and quartile 3 because of a small number of participants ranked into quartile 2 due to tied values of fried fish consumption.

Table 3

Blood lipid profile according to the quartiles of dietary intakes of fish and long-chain n-3 PUFA, MESA 2000–2002[1]

	Quartiles				
	Q1	Q2	Q3	Q4	P for trend
N-3 PUFAs					
Median (range), % energy	0.02 (0.00, 0.04)	0.05 (0.04, 0.06)	0.08 (0.06, 0.10)	0.14 (0.10, 0.91)	--
LDL, mg/dL	117.2	118.5	116.6	116.5	0.31
HDL, mg/dL	50.8	50.9	51.0	51.8	0.04
Triglycerides, mg/dL	138.6	131.0	131.1	126.0	< 0.01
TC, mg/dL	195.1	195.7	193.7	193.1	0.08
TC: HDL	4.08	4.11	4.06	4.00	0.03
Non-fried fish					
Median (range), servings/week	0.0 (0.0, 0.1)	0.3 (0.2, 0.5)	0.8 (0.6, 1.1)	2.0 (1.2, 19.5)	--
LDL, mg/dL	116.0	118.0	117.8	117.1	0.86
HDL, mg/dL	50.6	51.0	51.2	51.7	0.04
Triglycerides, mg/dL	134.9	135.0	130.1	126.9	< 0.01
TC, mg/dL	193.5	195.5	195.0	193.8	0.72
TC: HDL	4.07	4.10	4.07	4.02	0.15
Non-fried fish + shell fish					
Median (range), servings/week	0.0 (0, 0.2)	0.5 (0.3, 0.7)	1.0 (0.8, 1.6)	2.5 (1.6, 19.5)	--
LDL, mg/dL	117.0	118.5	116.3	117.1	0.77
HDL, mg/dL	50.7	50.8	51.5	51.4	0.13
Triglycerides, mg/dL	134.3	135.4	129.5	127.7	0.02
TC, mg/dL	194.5	196.1	193.7	193.6	0.29
TC: HDL	4.08	4.13	4.01	4.04	0.16
Fried fish					
Median (range), servings/week	0.0 (0.0, 0.0)	0.1 (0.1, 0.1)	0.2 (0.2, 0.5)	0.8 (0.6, 8.3)	--
LDL, mg/dL	117.2	115.8	116.8	118.1	0.32
HDL, mg/dL	51.3	51.4	50.8	51.0	0.69

	Quartiles				
	Q1	Q2	Q3	Q4	P for trend
Triglycerides, mg/dL	133.0	130.9	134.1	127.7	0.06
TC, mg/dL	194.7	192.9	194.1	194.8	0.82
TC: HDL	4.06	4.03	4.08	4.07	0.95

[1] Mean lipid levels across quartiles of dietary exposures were obtained by general linear models.

P values for trend were obtained by modeling the median values of the quartiles as continuous variables. PUFA: polyunsaturated fatty acid; LDL: low density lipoprotein cholesterol; HDL: high density lipoprotein cholesterol; TC: total cholesterol. Adjusted for variables listed in Table 2.