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# Associations of Dietary Long-Chain n-3 Polyunsaturated Fatty Acids and Fish with Biomarkers of Inflammation and Endothelial Activation (From the Multi-Ethnic Study of Atherosclerosis [MESA])

**Ka He, MD, ScD**[1], **Kiang Liu, PhD**[2], **Martha L Daviglus, MD, PhD**[2], **Nancy Swords Jenny, PhD**[3], **Elizabeth Mayer-Davis, PhD**[1], **Rui Jiang, MD, DrPH**[4], **Lyn Steffen, PhD, RD, MPH**[5], **David Siscovick, MD, MPH**[6], **Michael Tsai, PhD**[5], and **David Herrington, MD, MHS**[7]

1 *University of North Carolina at Chapel Hill, Chapel Hill, NC*

2 *Northwestern University, Chicago, IL*

3 *University of Vermont, Colchester, VT*

4 *Columbia University, New York, NY*

5 *University of Minnesota, St. Paul, MN*

6 *University of Washington, Seattle, WA*

7 *Wake Forest University, Winston-Salem, NC*

## Abstract

Cardioprotective effects of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) and fish consumption have been observed. However, data on the specific associations of these dietary factors with inflammation and endothelial activation are sparse. We conducted a cross-sectional study of 5,677 men and women from the MESA cohort including African Americans, Caucasians, Chinese and Hispanics, aged 45-84 years, and free of clinical cardiovascular disease. Dietary information was collected by self-administered food frequency questionnaire. Multivariable linear regression analyses were used to examine relations between intake of LC n-3 PUFAs, non-fried fish and fried fish and biomarkers of inflammation and endothelial activation. LC n-3 PUFA intakes were inversely associated with plasma concentrations of interleukin-6 (IL-6, P=0.01) and matrix metalloproteinase-3 (MMP-3, P=0.03) independent of age, body mass index, physical activity, smoking, alcohol consumption and dietary variables. Non-fried fish consumption was found inversely related to C-reactive protein (CRP, P=0.045) and IL-6 (P<0.01); and fried fish was observed being inversely related to soluble intercellular adhesion molecules-1 (sICAM-1) (P<0.01) but not associated with other biomarkers after adjustment for potential confounders. In conclusion, this study suggests that dietary intakes of LC n-3 PUFAs and fish are inversely associated with concentrations of some biomarkers reflecting lower levels of inflammation and endothelial activation. These results may partially explain the cardioprotective effects of fish consumption.

Corresponding author: Ka He, MD, ScD, Department of Nutrition, School of Public Health, University of North Carolina at Chapel Hill, 2202 McGavran-Greenberg, Campus Box: 7461, Chapel Hill, NC 27599, Phone: 919-8432476, Fax: 919-9667216, Email: E-mail: kahe@unc.edu.

**Keywords**

long-chain n-3 polyunsaturated fatty acids; fish oil; biomarker; inflammation; endothelial function

The findings from previous studies on intake of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) and fish and inflammation and endothelial activation are inconsistent. Also, the fatty acid content in fish may change as a result of deep frying.[1] However, little is known about how frying fish modifies associations of fish consumption with inflammatory markers. Therefore, we investigated associations of fish consumption and LC n-3 PUFA intake with markers of inflammation and endothelial activation in men and women aged 45-84 years in the Multi-Ethnic Study of Atherosclerosis (MESA). We hypothesized that intake of LC n-3 PUFAs and non-fried fish would be inversely associated with markers of inflammation and endothelial activation.

## Methods

The MESA cohort was established in 2000-02 with 6814 men and women aged 45 to 84 years, without clinical cardiovascular disease (CVD), recruited from 6 U.S. communities. This cohort is 38.5% Caucasian, 27.8% African American (AA), 21.9% Hispanic, and 11.8% Chinese. Details of sampling and recruitment procedures have been published elsewhere.[2] Participants were requested to complete a set of comprehensive measurements of subclinical CVD and assessment of standard CVD risk factors, sociodemographic factors, diet, lifestyle, and psychosocial factors. In the present study, we excluded those who had missing data on diet (n=577), missing data on all biomarkers (n=26), extreme total caloric intake (<600 or >6000 kcal/day, n=293), and had no information on covariates used in analyses (n=227). Some biomarkers were measured in sub-cohorts including CD40L, E-selectin, sTNF-R1, sICAM-1, MMP-3 and MMP-9. After these exclusions, the total numbers of participants remaining in the analyses by biomarkers are: CD40L, n=860; CRP, n=5677; IL-6, n=5569; E-selectin, n=860; fibrinogen, n=5676; sTNF-R1, n=860; sICAM-1, n=2266; MMP-3, n=860; and MMP-9, n=860.

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

Self-administered 127-item food frequency questionnaire (FFQ) and dietary supplement form in Block format[3] were used to collect dietary information. Participants were asked to identify their average consumption of each selected food during the previous year with 9 frequency options ranging from never/rare to frequently and also the serving size as either small, medium or large. Food consumption was adjusted for serving size, for example, the gram weight for small, medium and large portion, specific to age group (middle or old) and gender (i.e. small = 0.5 × medium; medium = standard age- and gender-specific serving size; large = 1.5 × medium).[4] For fish consumption, which we 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). Fish consumption was classified in 2 groups: fried fish and non-fried fish (broiled, steamed, baked or raw fish). In addition, the questionnaire contained three questions related to mixed fish dishes (for example, Chinese food with stir-fried shrimp or fish with vegetables; pasta with seafood; and seafood gumbo). Because of the limited information, we were not able to determine the exact portion of seafood in the mixed fish dishes. Thus, we did not include mixed fish dishes in the main analyses. Instead, we used this information in the secondary analyses by assigning a weight 40%, 50% or 60% respectively

for fish in the mixed fish dishes. Nutrients including LC n-3 PUFAs were derived from the Minnesota nutrition data system (NDS-R version 4.02/30).[5]

The FFQ was adopted from the FFQ used in the Insulin Resistance Atherosclerosis Study (IRAS), which has been validated in AA, Hispanics and Caucasians.[6] Compared to the average of eight 24-hour recalls, the mean correlation coefficients of nutrient intake were 0.62 for non-Hispanic Caucasians, 0.50 for AA, and 0.41 for Hispanics. The apparent lower degree of validity in Hispanics was accounted for by lower education attainment in that group. For reliability, the mean correlation coefficient for nutrients evaluated was 0.62 and did not differ by ethnic subgroup. To accommodate the MESA population, the FFQ was modified to include Chinese foods and culinary practices using the same approach as used previously to enhance ethnic sensitivity for the validated IRAS.

Blood was collected, processed and samples were stored using standardized protocols at the Laboratory for Clinical Biochemistry Research at University of Vermont, Burlington, VT, USA.[7] Participants were asked to fast for 12 hours, avoid smoking on the morning of the exam and avoid heavy exercise 12 hours before the exam. CRP and fibrinogen antigen were measured using the BNII nephelometer; average analytical coefficients of variation (CVs) are 3.6% and 2.7%, respectively. IL-6 and sTNF-R1 were measured by ultra-sensitive ELISA; average laboratory analytical CVs for these assays were 6.3% and 5.0%, respectively. sICAM-1, soluble E-selectin, and CD40L were measured by sandwich enzyme immunoassay; average analytical CVs were 5.0% and 5.9%, respectively. MMP-9 was measured by a high sensitivity quantitative sandwich enzyme immunoassay; average analytical CV was 4.9%. MMP-3 was measured by an ultra-sensitive, solid-phase sandwich ELISA using a polyclonal antibody specific for both the pro- and active forms of MMP-3; average analytical CV was 6.9%.

Demographic and major lifestyle variables were collected through questionnaire. Body weight and height were directly measured. 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.[2] Total physical activity was computed as the total of all light, moderate, and vigorous activities (minutes per week) multiplied by the activities' individual metabolic equivalent values.

Means and standard deviations or proportions were computed for selected variables by quartiles of non-fried fish consumption. All biomarkers were log-transferred to better approximate normal distributions. Multiple linear regressions were used to examine fish consumption and LC n-3 PUFA intake in relation to biomarkers of inflammation and endothelial activation. Based on the previous literature and univariate tests, we considered a number of potential confounders in the multivariate models. All $p$ values were 2-sided. A $p$ value $<0.05$ was considered statistically significant. We used SAS (version 9; SAS Institute, Inc., Cary, North Carolina) software for analyses.

## Results

The characteristics of the study population according to non-fried fish consumption are presented in Table 1. Participants in the highest quartile were more likely to be female, physically active, have higher education level, household income and lower BMI and were less likely to be current smokers than those in the lowest quartile. Also, consumption of non-fried fish was positively associated with intakes of LC n-3 PUFAs and total energy, and inversely related to intake of saturated fat, polyunsaturated fat, alpha-linolenic acid, linoleic acid and *trans* fatty acids.

In age-adjusted linear regression models, LC n-3 PUFA intake was significantly inversely related to CRP, IL-6, fibrinogen, TNF-R1, sICAM-1 and MMP3. Non-fried fish consumption

was significantly inversely associated with CRP, IL-6, fibrinogen and sICAM-1. Fried fish consumption was positively related to CRP and IL-6 but inversely associated with CD40L and sICAM-1 (Table 2).

After adjustment for multiple covariates, significant inverse associations remained between LC n-3 PUFA intake and IL-6 and MMP3 and between non-fried fish consumption and CRP and IL-6. Also, the inverse relation between fried fish consumption and sICAM-1 persisted after adjustment for multiple covariates (Table 2).

Since linoleic acid (n-6) may compete with n-3 fatty acids in the eicosanoid synthesis pathway,[8] we stratified analyses according to median intake of linoleic acid. We also stratified data according to median intake of α-linolenic acid because α-linolenic acid can be desaturated and elongated to LC n-3 PUFAs and high levels of α-linolenic acid intake may compensate for low intake of LC n-3 PUFAs. Although the observed associations of LC n-3 PUFA intake and biomarkers became statistically non-significant in low intake groups of linoleic acid and α-linolenic acid, the tests for interactions were not significant. (Table 3).

## Discussion

In this cross-sectional study, we found that intake of LC n-3 PUFAs derived from marine products was inversely related to some, but not all biomarkers of inflammation and endothelial activation. The observed inverse associations were independent of major lifestyle factors and potential dietary and non-dietary confounders.

Data from experimental studies have provided some evidence that LC n-3 PUFAs are anti-inflammatory.[9] Some epidemiological studies also indicate that these fatty acids may have beneficial effects on inflammation and endothelial function.[10-15] Of note, there was no information on type of fish meal in the previous studies relating fish to inflammation and endothelial function.

Few intervention studies have examined effects of fish oil supplementation on inflammation and endothelial activation and the findings were inconsistent. Hjerkinn et al. found significantly reduced concentrations of sICAM-1 in a fish oil supplementation group compared to placebo control group in elderly men with long-standing hyperlipidemia.[16] Also, in a double-blinded intervention study among 60 patients with coronary heart disease, Seierstad et al. found that IL-6 was significantly reduced by fish oil compared to rapeseed oil supplementation.[17] However, in a trial among 54 patients with coronary heart disease, Johansen et al. observed that patients who had taken 5.1g/d fish oil for 4 weeks had higher levels of sE-selectin compared to the patients who had taken corn oil as placebo.[18] In another randomized, double-blind trial among 300 acute myocardial infarction patients, Grundt et al. found no effects on sE-selectin, sICAM-1 and CRP by fish oil supplementation compared to corn oil.[19] Also, in an open-labeled randomized controlled trial among 77 post-myocardial infarction patients, Lee et al. reported no associations between fish oil supplementation and IL-6 and fibrinogen.[20] In addition, Berstad et al. found that a moderate dietary increase in LC n-3 PUFAs might have an effect on decreasing inflammatory markers, whereas fish oil supplementation might increase inflammation in vascular endothelium in a 18-month intervention in 171 men aged between 65 and 75 years.[21] Of note, these intervention studies were conducted in patients with clinical CVD and the sample sizes were generally quite small.

One possible biological mechanism underlying the beneficial effects of LC n-3 PUFAs on inflammation and endothelial function is that these fatty acids compete with n-6 fatty acids for prostaglandin and leukotriene synthesis at the cyclooxygenase and lipoxygenase level. LC n-3 PUFAs from fish or fish oil modulate prostaglandin metabolism by increasing prostaglandin $E_3$ (an active vasodilator and inhibitor of platelet aggregation), thromboxane $A_3$ (a weak

platelet aggregator and a weak vasoconstrictor) and leukotriene $B_5$ (a weak inducer of inflammation) and by decreasing production of thromboxane $A_2$ (a potent platelet aggregator and vasoconstrictor) and leukotriene $B_4$ formation (an inducer of inflammation and a powerful inducer of leukocyte chemotaxis and adherence).[22] Another suggested mechanism is that LC n-3 PUFAs may react with active oxygen species because of their multiple double bonds and lead to a decreased production of hydrogen peroxide. Hydrogen peroxide is a critical activator of the nuclear factor-κB system of transcription factors that controls the coordinated expression of adhesion molecules and of leukocyte-specific chemoattractants upon cytokine stimulation.[23]

In the present study, we found independent inverse associations of LC n-3 PUFAs and non-fried fish with IL-6, CRP and MMP3. The relevance of these inflammatory and endothelial activation biomarkers in the atherogenic process has been recognized. Studies suggest that CRP and IL-6, 2 systemic inflammatory markers, are independent predictors of CVD and may play important role in atherogenesis.[24] In addition, MMP3 is suggested an independent prognostic factor in stable coronary artery disease.[25] Several lines of evidence supports the important role of MMPs in plaque stability.[26]

sICAM-1 is thought to be a key factor in the adherence of monocytes to the endothelium and subsequently transmigration into the intima. The role of sICAM-1 in the pathogenesis of inflammation and atherosclerosis has been confirmed by experimental studies.[27] Studies also indicate that sICAM-1 is a predictor of CVD independent of other traditional risk factors.[28] In the present study, we observed that fried fish but not non-fried fish consumption was significantly inversely related to sICAM-1. This finding is not expected since the frying process may reduce the content of LC n-3 PUFAs and produce *trans*-fatty acids. One possible explanation is that fried fish consumption may be a marker of relatively unhealthy lifestyle; those who had high fried fish consumption were more likely to suffer from dyslipidemia and were likely to be under treatment or medication (e.g., taking statin), which may lower the level of sICAM-1.[29] However, in this cohort, the inverse association between fried fish consumption and sICAM-1 level remained significant with adjustment for or exclusion of participants using cholesterol-lowering medication. Alternatively, the observed inverse association between fried fish and sICAM-1 in this study may be simply due to chance. Future studies are warranted.

In addition, some other limitations merit consideration. Because of the cross-sectional design, the possibility of reverse causation can not be completely excluded. Also, we could not account for any effects of recent change in diet or medication on inflammatory markers. For instance, participants at high risk of CVD may have changed their diets (i.e. increased fish intake) because of obtaining advice from health care workers or receiving information via media. This diet change would be expected to attenuate associations between LC n-3 PUFAs or fish and biomarkers of inflammation and endothelial activation. Inevitable measurement errors in estimating fish consumption and LC n-3 PUFA intake as well as in the biomarker measures could bias our results and would also be expected to attenuate associations. Although we adjusted for a number of potential confounders in the analysis, the possibility of confounding from unmeasured dietary and non-dietary factors can 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.[30] Thus, mercury in fish might confound our findings.

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## References

1. Candela M, Astiasaran I, Bello J. Deep-fat frying modifies high-fat fish lipid fraction. J Agric Food Chem 1998;46:2793–2796.
2. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR Jr, Kronmal R, Liu K, Nelson JC, O'Leary D, Saad MF, Shea S, Szklo M, Tracy RP. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol 2002;156:871–881. [PubMed: 12397006]
3. Block G, Woods M, Potosky A, Clifford C. Validation of a self-administered diet history questionnaire using multiple diet records. J Clin Epidemiol 1990;43:1327–1335. [PubMed: 2254769]
4. 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]
5. Minnesota Nutrition Coordinating Center. Minnesota Nutrition Data System Program 4.02 [computer program]. Version Nutrient Database Version 30. 1999.
6. Mayer-Davis EJ, Vitolins MZ, Carmichael SL, Hemphill S, Tsaroucha G, Rushing J, Levin S. Validity and reproducibility of a food frequency interview in a Multi-Cultural Epidemiology Study. Ann Epidemiol 1999;9:314–324. [PubMed: 10976858]
7. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the Cardiovascular Health Study. Clin Chem 1995;41:264–270. [PubMed: 7874780]
8. Nair SS, Leitch JW, Falconer J, Garg ML. Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action. J Nutr 1997;127:383–393. [PubMed: 9082021]
9. Calder PC. Polyunsaturated fatty acids, inflammation, and immunity. Lipids 2001;36:1007–1024. [PubMed: 11724453]
10. Pischon T, Hankinson SE, Hotamisligil GS, Rifai N, Willett WC, Rimm EB. Habitual dietary intake of n-3 and n-6 fatty acids in relation to inflammatory markers among US men and women. Circulation 2003;108:155–160. [PubMed: 12821543]
11. Lopez-Garcia E, Schulze MB, Manson JE, Meigs JB, Albert CM, Rifai N, Willett WC, Hu FB. Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. J Nutr 2004;134:1806–1811. [PubMed: 15226473]
12. Niu K, Hozawa A, Kuriyama S, Ohmori-Matsuda K, Shimazu T, Nakaya N, Fujita K, Tsuji I, Nagatomi R. 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;84:223–229. [PubMed: 16825699]
13. Fernandez-Real JM, Broch M, Vendrell J, Ricart W. Insulin resistance, inflammation, and serum fatty acid composition. Diabetes Care 2003;26:1362–1368. [PubMed: 12716789]
14. Madsen T, Skou HA, Hansen VE, Fog L, Christensen JH, Toft E, Schmidt EB. C-reactive protein, dietary n-3 fatty acids, and the extent of coronary artery disease. Am J Cardiol 2001;88:1139–1142. [PubMed: 11703959]
15. Zampelas A, Panagiotakos DB, Pitsavos C, Das UN, Chrysohoou C, Skoumas Y, Stefanadis C. Fish consumption among healthy adults is associated with decreased levels of inflammatory markers related to cardiovascular disease: the ATTICA study. J Am Coll Cardiol 2005;46:120–124. [PubMed: 15992645]
16. Hjerkinn EM, Seljeflot I, Ellingsen I, Berstad P, Hjermann I, Sandvik L, Arnesen H. Influence of long-term intervention with dietary counseling, long-chain n-3 fatty acid supplements, or both on circulating markers of endothelial activation in men with longstanding hyperlipidemia. Am J Clin Nutr 2005;81:583–589. [PubMed: 15755826]
17. Seierstad SL, Seljeflot I, Johansen O, Hansen R, Haugen M, Rosenlund G, Froyland L, Arnesen H. Dietary intake of differently fed salmon; the influence on markers of human atherosclerosis. Eur J Clin Invest 2005;35:52–59. [PubMed: 15638820]

18. Johansen O, Seljeflot I, Hostmark AT, Arnesen H. The effect of supplementation with omega-3 fatty acids on soluble markers of endothelial function in patients with coronary heart disease. Arterioscler Thromb Vasc Biol 1999;19:1681–1686. [PubMed: 10397685]

19. Grundt H, Nilsen DW, Mansoor MA, Hetland O, Nordoy A. Reduction in homocysteine by n-3 polyunsaturated fatty acids after 1 year in a randomised double-blind study following an acute myocardial infarction: no effect on endothelial adhesion properties. Pathophysiol Haemost Thromb 2003;33:88–95. [PubMed: 14624050]

20. Lee KW, Blann AD, Lip GY. Effects of omega-3 polyunsaturated fatty acids on plasma indices of thrombogenesis and inflammation in patients post-myocardial infarction. Thromb Res 2006;118:305–312. [PubMed: 16154181]

21. Berstad P, Seljeflot I, Veierod MB, Hjerkinn EM, Arnesen H, Pedersen JI. Supplementation with fish oil affects the association between very long-chain n-3 polyunsaturated fatty acids in serum non-esterified fatty acids and soluble vascular cell adhesion molecule-1. Clin Sci (Lond) 2003;105:13–20. [PubMed: 12589702]

22. Leaf A, Weber PC. Cardiovascular effects of n-3 fatty acids. N Engl J Med 1988;318:549–557. [PubMed: 3277056]

23. De Caterina R, Zampolli A. n-3 fatty acids: antiatherosclerotic effects. Lipids 2001;36(Suppl):S69–78. [PubMed: 11837996]

24. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836–843. [PubMed: 10733371]

25. Wu TC, Leu HB, Lin WT, Lin CP, Lin SJ, Chen JW. Plasma matrix metalloproteinase-3 level is an independent prognostic factor in stable coronary artery disease. Eur J Clin Invest 2005;35:537–545. [PubMed: 16128859]

26. Nanni S, Melandri G, Hanemaaijer R, Cervi V, Tomasi L, Altimari A, Van Lent N, Tricoci P, Bacchi L, Branzi A. Matrix metalloproteinases in premature coronary atherosclerosis: influence of inhibitors, inflammation, and genetic polymorphisms. Transl Res 2007;149:137–144. [PubMed: 17320799]

27. Bourdillon MC, Poston RN, Covacho C, Chignier E, Bricca G, McGregor JL. ICAM-1 deficiency reduces atherosclerotic lesions in double-knockout mice (ApoE(-/-)/ICAM-1(-/-)) fed a fat or a chow diet. Arterioscler Thromb Vasc Biol 2000;20:2630–2635. [PubMed: 11116064]

28. Ridker PM, Hennekens CH, Roitman-Johnson B, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet 1998;351:88–92. [PubMed: 9439492]

29. Ray KK, Morrow DA, Shui A, Rifai N, Cannon CP. Relation between soluble intercellular adhesion molecule-1, statin therapy, and long-term risk of clinical cardiovascular events in patients with previous acute coronary syndrome (from PROVE IT-TIMI 22). Am J Cardiol 2006;98:861–865. [PubMed: 16996863]

30. Guallar E, Sanz-Gallardo MI, van't Veer P, Bode P, Aro A, Gomez-Aracena J, Kark JD, Riemersma RA, Martin-Moreno JM, Kok FJ. Mercury, fish oils, and the risk of myocardial infarction. N Engl J Med 2002;347:1747–1754. [PubMed: 12456850]

**Table 1**

Characteristics of the study population according to quartiles of non-fried fish consumption, MESA, 2000

| Characteristics | Non-fried fish consumption | | | | P value |
|---|---|---|---|---|---|
| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | |
| Non-fried (broiled, steamed, baked or raw) fish, median (servings/week) | 0.0 | 0.3 | 0.8 | 2.0 | -- |
| Fried fish, median (servings/week) | 0.1 | 0.2 | 0.2 | 0.2 | -- |
| Number of participants | 1482 | 1293 | 1532 | 1384 | -- |
| Age, (years) | 62.3 (10.5) | 61.9 (10.2) | 62.1 (10.2) | 62.4 (10.0) | 0.52 |
| Men | 49.2% | 46.6% | 45.0% | 46.3% | 0.13 |
| Race/ethnicity | | | | | |
| African American | 19.5% | 25.3% | 26.9% | 24.6% | <0.01 |
| Caucasian | 32.9% | 40.0% | 43.5% | 47.0% | |
| Chinese | 9.2% | 12.4% | 13.2% | 18.0% | |
| Hispanic | 38.5% | 22.4% | 16.4% | 10.4% | |
| Education | | | | | |
| Less than high school | 29.4% | 14.8% | 14.0% | 11.6% | <0.01 |
| High school | 22.9% | 19.3% | 16.4% | 12.4% | |
| Some college | 26.7% | 30.5% | 29.5% | 24.3% | |
| College or higher | 20.9% | 35.5% | 40.1% | 51.7% | |
| Household annual income | | | | | |
| ≤ $16,000 | 25.6% | 16.6% | 15.5% | 16.1% | <0.01 |
| $16,001-$39,999 | 37.0% | 33.4% | 29.4% | 25.4% | |
| ≥$40,000 | 37.4% | 50.0% | 55.1% | 58.5% | |
| BMI, ($kg/m^2$) | 28.7 (5.4) | 28.3 (5.4) | 28.1 (5.5) | 27.6 (5.2) | <0.01 |
| Intentional exercise, (MET-min/week) | 1247 (1913) | 1491 (2303) | 1666 (2431) | 1890 (2624) | <0.01 |
| Smoking status | | | | | |
| Never smoker | 51.2% | 50.1% | 50.8% | 53.0% | <0.01 |
| Former smoker | 33.3% | 38.6% | 37.2% | 37.6% | |
| Current smoker | 15.5% | 11.3% | 12.0% | 9.4% | |
| Pack-years of cigarette smoking | 12.8 (28.7) | 10.8 (19.6) | 10.4 (18.3) | 10.4 (21.0) | <0.01 |
| Alcohol consumption, (drinks/week) | 4.1 (7.4) | 3.6 (6.0) | 3.6 (5.8) | 4.1 (5.8) | 0.23 |

| Characteristics | Non-fried fish consumption | | | | P value |
|---|---|---|---|---|---|
| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | |
| Use of fish oil supplements | 3.4% | 4.0% | 3.8% | 5.9% | <0.01 |
| Daily dietary intake | | | | | |
| EPA + DHA, (% energy) | 0.035 (0.027) | 0.053 (0.028) | 0.076 (0.036) | 0.149 (0.080) | <0.01 |
| Saturated fat, (% energy) | 11.3 (3.1) | 11.0 (3.0) | 10.4 (2.9) | 9.8 (3.0) | <0.01 |
| Polyunsaturated fat, (% energy) | 8.2 (3.1) | 7.8 (2.8) | 7.6 (2.7) | 7.8 (2.7) | <0.01 |
| Alpha-linolenic acid, (% energy) | 0.666 (0.267) | 0.654 (0.231) | 0.648 (0.216) | 0.663 (0.217) | 0.13 |
| Linoleic acid, (% energy) | 6.7 (2.9) | 6.4 (2.7) | 6.2 (2.6) | 6.2 (2.6) | <0.01 |
| *Trans* fatty acid, (% energy) | 1.8 (0.8) | 1.8 (0.7) | 1.7 (0.7) | 1.5 (0.7) | <0.01 |
| Total energy intake, (kcal) | 1589 (681) | 1563 (659) | 1604 (644) | 1747 (687) | <0.01 |

Data are mean (standard deviation), unless otherwise specified.

BMI: body mass index; MET: metabolic equivalent-minutes; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

**Table 2**

Relations of long-chain n-3 fatty acid and fish intake and biomarkers of inflammation and endothelial activation

| | EPA + DHA | | Non-fried fish | | Fried fish | |
|---|---|---|---|---|---|---|
| **Markers** | **Age-adjusted** | **Multivariate-adjusted** | **Age-adjusted** | **Multivariate-adjusted** | **Age-adjusted** | **Multivariate-adjusted** |
| Log CD40L (ng/mL) * | -0.23 (0.54) | 0.42 (0.27) | -0.08 (0.57) | 0.17 (0.24) | -0.49 (0.04) | -0.11 (0.68) |
| Log hsCRP (mg/L)** | -1.24 (<0.01) | -0.24 (0.31) | -0.38 (<0.01) | -0.17 (0.03) | 0.74 (<0.01) | 0.16 (0.35) |
| Log IL-6 (pg/mL)*** | -0.88 (<0.01) | -0.37 (<0.01) | -0.28 (<0.01) | -0.16 (<0.01) | 0.29 (<0.01) | -0.07 (0.46) |
| Log E-selectin (ng/mL)* | -0.31 (0.24) | 0.07 (0.79) | -0.13 (0.19) | 0.01 (0.88) | 0.16 (0.36) | 0.08 (0.66) |
| Log fibrinogen (mg/dL) **** | -0.09 (0.03) | -0.05 (0.24) | -0.04 (<0.01) | -0.03 (0.84) | 0.06 (0.06) | -0.01 (0.80) |
| Log sTNF-R1 (pg/mL) * | -0.53 (<0.01) | -0.26 (0.06) | -0.09 (0.07) | -0.05 (0.31) | -0.11 (0.22) | -0.09 (0.35) |
| Log sICAM-1 (ng/mL) ***** | -0.55 (<0.01) | -0.16 (0.06) | -0.08 (<0.01) | 0.01 (0.96) | -0.24 (<0.01) | -0.17 (0.01) |
| Log MMP3 (ng/mL) * | -0.79 (0.02) | -0.69 (0.03) | -0.14 (0.25) | -0.18 (0.12) | 0.02 (0.92) | -0.05 (0.81) |
| Log MMP9 (ng/mL) * | -0.31 (0.29) | 0.50 (0.11) | 0.09 (0.41) | 0.16 (0.14) | -0.23 (0.22) | -0.07 (0.74) |

Values are β coefficients (P-values);

Non-fried fish includes broiled, steamed, baked or raw fish.

Adjusted for age, race, gender, household income, education, BMI, physical activity, smoking status, aspirin use, NSAID use, alcohol consumption, and intakes of saturated fat, polyunsaturated fat, α-linolenic acid, *trans-fatty* acids and total energy. For fish consumption, additionally adjusted for either fried fish or non-fried fish.

* Biomarker available in: n=860,

** Biomarker available in: n=5677,

*** Biomarker available in: n=5569,

**** Biomarker available in: n=5676,

***** Biomarker available in: n=226

**Table 3**

Relations of long-chain n-3 PUFA intake and biomarkers stratified by linoleic fatty acid (n-6) or α-linolenic fatty acid (n-3) intake, MESA, 2000

| Markers | Linoleic acid intake below median (< 5.77 % energy) | Linoleic acid intake above median (≥ 5.77 % energy) | P for interaction | α-linolenic acid intake below median (< 0.61 % energy) | α-linolenic acid intake above median (≥ 0.61 % energy) | P for interaction |
|---|---|---|---|---|---|---|
| Log CD40L (ng/mL) | 0.14 (0.83) | 0.30 (0.54) | 0.55 | 0.73 (0.26) | 0.18 (0.71) | 0.78 |
| Log hsCRP (mg/L) | 0.02 (0.96) | -0.40 (0.21) | 0.85 | 0.05 (0.88) | -0.43 (0.15) | 0.45 |
| Log IL-6 (pg/mL) | -0.10 (0.62) | -0.50 (0.007) | 0.44 | -0.26 (0.20) | -0.39 (0.02) | 0.67 |
| Log sE-selectin (ng/mL) | 0.03 (0.94) | 0.23 (0.53) | 0.46 | 0.50 (0.25) | 0.04 (0.92) | 0.57 |
| Log fibrinogen (mg/dL) | -0.05 (0.40) | -0.05 (0.37) | 0.46 | -0.10 (0.14) | -0.03 (0.55) | 0.22 |
| Log sTNF-R1 (pg/mL) | -0.25 (0.25) | -0.19 (0.31) | 0.39 | -0.39 (0.09) | -0.16 (0.35) | 0.12 |
| Log sICAM-1 (ng/mL) | -0.11 (0.37) | -0.15 (0.21) | 0.78 | -0.21 (0.12) | -0.11 (0.32) | 0.57 |
| Log MMP3 (ng/mL) | -0.51 (0.31) | -0.75 (0.06) | 0.98 | -0.13 (0.79) | -1.00 (0.02) | 0.32 |
| Log MMP9 (ng/mL) | 0.07 (0.89) | 0.89 (0.03) | 0.33 | 0.51 (0.32) | 0.62 (0.12) | 0.61 |

Values are β coefficients (P values);

Adjusted covariates and number of participants for each biomarker are listed in Table 2 except α-linolenic acid.