patient-oriented research

Higher plasma docosahexaenoic acid is associated with reduced progression of coronary atherosclerosis in women with CAD

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Abstract **Fish intake, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and in some cases α-linolenic acid (ALA) have been associated with reduced risk of cardiovascular events and death. The association between n-3 fatty acids in plasma lipids and the progression of coronary artery atherosclerosis was assessed among women with established coronary artery disease (CAD). A prospective cohort study involved postmenopausal women (n = 228) participating in the Estrogen Replacement and Atherosclerosis Trial. Quantitative coronary angiography was performed at baseline and after 3.2 ± 0.6 (mean ± SD) years. Women with plasma phospholipid (PL) DHA levels above the median, compared with below, exhibited less atherosclerosis progression, as expressed by decline in minimum coronary artery diameter (−0.04 ± 0.02 and −0.10 ± 0.02 mm, respectively; *P* = 0.007) or increase in percentage stenosis (1.34 ± 0.76% and 3.75 ± 0.74%, respectively; *P* = 0.006), and had fewer new lesions [2.0% (0.5–3.5%) of measured segments (95% confidence interval) and 4.2% (2.8–5.6%), respectively; *P* = 0.009] after adjustments for cardiovascular risk factors. Similar results were observed for DHA in the triglycerides (TGs). EPA and ALA in plasma lipids were not significantly associated with atherosclerosis progression. Consistent with higher reported fish intake, higher levels of plasma TG and PL DHA are associated with less progression of coronary atherosclerosis in postmenopausal women with CAD.—** Erkkilä, A. T., N. R. Matthan, D. M. Herrington, and A. H. Lichtenstein. **Higher plasma docosahexaenoic acid is associated with reduced progression of coronary atherosclerosis in women with CAD. *J. Lipid Res.* 2006.** 47: **2814–2819.**

Supplementary key words polyunsaturated fatty acids • eicosapentaenoic acid • angiography • coronary artery disease

Fish intake and fish oil supplements have been associated with lower risk of cardiovascular events and mortality (1–4). Circulating levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are good independent biomarkers of fish intake because their occurrence in the diet is limited to marine products and the rate of elongation and desaturation of α-linolenic acid (ALA) to form EPA and DHA is very low, even when ALA is present at relatively high levels (5). Similarly, ALA, which cannot be synthesized de novo in humans, is a good independent marker of dietary plant-derived n-3 fatty acids (6, 7). Plasma levels of EPA and DHA have been associated with reduced risk of sudden death (8, 9), fatal ischemic heart disease (10), and myocardial infarction (11). The association between fish intake and reduced cardiovascular disease (CVD) risk has been attributed to a number of potential mechanisms, including effects on platelet function, plasma triglyceride (TG) concentrations, inflammatory factors, and arrhythmia (12–14). An increase in the stability of the atherosclerotic plaque itself has been reported after fish oil supplementation (15). However, longitudinal data in humans relating actual lesion progression and n-3 fatty acid intakes are limited, especially in women (16, 17). Furthermore, the impact of ALA on CVD risk remains unresolved (18–21).

We have previously demonstrated in women participating in the Estrogen Replacement and Atherosclerosis (ERA) Trial that self-reported fish intake, especially dark-fleshed and tuna fish, was inversely associated with the progression of coronary atherosclerosis (22). This report is an assessment of circulating levels of plasma n-3 fatty acids, ALA, EPA, and DHA, in women with habitual fish intake and no interventional supplementation. The relationship of these biomarkers to change in mean minimum coro-

Manuscript received 9 June 2006 and in revised form 14 August 2006 and in re-revised form 18 September 2006.

Published, JLR Papers in Press, September 18, 2006.
DOI 10.1194/jlr.P600005-JLR200

Abbreviations: ALA, α-linolenic acid; CAD, coronary artery disease; CE, cholesteryl ester; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PL, phospholipid; TG, triglyceride.

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nary artery diameter, percentage stenosis, and new lesion formation was studied. The aim of this study was to examine the association between plasma n-3 fatty acids and the progression of angiographically defined coronary atherosclerosis in a group of postmenopausal women with preexisting coronary artery disease (CAD) who underwent coronary angiography twice, 3.2 years apart (23).

METHODS

Subjects

The Estrogen Replacement and Atherosclerosis Trial was a randomized, double-blind, placebo-controlled trial of hormone replacement therapy in postmenopausal women. The study design and primary results have been reported previously (23). Briefly, postmenopausal women younger than 80 years who were not currently receiving estrogen-replacement treatment and had one or more epicardial coronary stenoses of at least 30% of the luminal diameter were eligible for the study. The subjects were randomized into three groups and received *1*) 0.625 mg of conjugated equine estrogen (n = 100), *2*) 0.625 mg of conjugated equine estrogen plus 2.5 mg of medroxyprogesterone acetate (n = 104), or *3*) placebo (n = 105). The subjects were followed for 3.2 ± 0.6 (mean ± SD) years. Neither of the treatments had a significant effect on the progression of coronary atherosclerosis.

The study protocol was approved by the Institutional Review Board at the participating sites (23) and at New England Medical Center and Tufts University. All subjects gave their informed written consent before participation in the study.

Covariate measurements

At baseline, subjects completed questionnaires on their health status, medical history, and cardiovascular risk factors and underwent clinical examination. Subjects were classified as having diabetes if fasting glucose was ⩾126 mg/dl or 2 h glucose was ⩾200 mg/dl during an oral glucose tolerance test, glycated hemoglobin was >7% (24), or they self-reported having diabetes or reported use of diet, oral hypoglycemic agents, or insulin as treatment for diabetes. Physical activity score was calculated as described previously (25). Serum lipids were analyzed using standardized enzymatic methods (23).

Analysis of fatty acids in plasma lipids

Lipids were extracted from plasma (26) after the addition of an internal standard (25 μg each of cholesteryl heptadecanoate, triheptadecanoin glyceride, and 1,2-diheptadecanoyl-glycero-3-phosphocholine). Phospholipid (PL), TG, and cholesteryl ester (CE) subfractions were separated by solid-phase extraction using aminopropyl columns (27), saponified, and then methylated (28). The fatty acid methyl esters were analyzed using an Autosystem XL gas chromatograph (Perkin-Elmer, Boston MA) equipped with a 30 m × 0.25 mm inner diameter (film thickness, 0.25 μm) capillary column (HP-INNOWAX; Agilent Technologies). Helium was used as the carrier gas (2 ml/min), and the split ratio was 2:1. Injector and flame ionization detector temperatures were 250°C and 260°C, respectively. The oven temperature was programmed at 80°C, held for 2 min, and then increased to 160°C at a rate of 10°C/min. After 5 min, the temperature was increased to 222°C at a rate of 2°C/min, then held for 5 min. The final temperature was 252°C, held for 5 min. Peaks of interest were identified by comparison with authentic fatty acid standards (Nu-Chek Prep, Inc.) and expressed as molar percentage proportions of fatty acids relative to the internal standard. Plasma for fatty acid analysis was available for 228 of the original 248 women who had data from both the baseline and follow-up angiography.

Outcome measurements

Quantitative coronary angiography was performed using standardized techniques at baseline and at the end of follow-up (mean, 9.3 segments per woman) (23). Review and analysis of the paired films were performed using a previously validated system of cine projectors (SME-3500; Sony, Park Ridge, NJ) and software (QCAPlus; Sanders Data Systems, Palo Alto, CA). With this system, the mean intraoperator difference between blinded duplicate measurements of minimum diameter for vessels with lesions is 0.02 mm. The reference, minimum, and average luminal diameters, as well as the degree of stenosis as a percentage of the reference diameter, were assessed in proximal epicardial segments (23). All measurements were performed by operators unaware of the woman's temporal sequence of the films. Segments totally occluded or intervened with coronary artery bypass surgery of angioplasty were excluded from the analyses. Changes in minimum luminal diameter and percentage stenosis during follow-up were calculated. Development of a new lesion was defined as the presence of one or more segments with <15% stenosis at baseline and an increase of at least 15% at follow-up.

Statistical analysis

All statistical analyses were performed using SAS (version 8; SAS Institute, Cary, NC). The normality of continuous variables was checked, and log transformations were applied as needed. Subjects were divided into categories according to median of proportions of ALA, EPA, and DHA in TG and PL. Differences in baseline characteristics were tested between the groups defined by the median proportions of n-3 fatty acids using the *t*-test, the Wilcoxon test, or the Chi-square test, as appropriate. The associations of n-3 fatty acids in plasma lipids were assessed with the test parameters of changes in mean minimum coronary artery diameter and mean percentage stenosis using mixed-model analysis of covariance. These measurements were adjusted, as indicated, for age, location of coronary segment, body mass index, education, time of follow-up, study clinic, coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, race, smoking, use of cholesterol-lowering medication and hormone replacement therapy, and alcohol intake. Differences in the development of new lesions among the fatty acid categories were tested with General linear model, adjusting for the factors listed above, with the exception of location of segment. A value of $P < 0.05$ (two-tailed) was considered statistically significant.

RESULTS

The distribution of ALA, EPA, and DHA differed markedly among the plasma lipid subfractions (TG, CE, and PL) (**Fig. 1**). DHA and EPA were the most abundant in PL (0.52 ± 0.22 and 2.55 ± 0.82 mol%, respectively), and ALA and DHA were most abundant in TG (1.22 ± 0.52 and 0.63 ± 0.38 mol%, respectively). The abundance of these fatty acids tended to be lowest or intermediate in the CE subfraction. On the basis of these data, the TG and PL subfractions were the focus of subsequent statistical analysis. The subjects were subclassified according to the median plasma level of fatty acids in these two subfractions (**Table 1**).

Fig. 1. The proportions of α-linolenic, eicosapentaenoic, and docosahexaenoic acids in plasma triglycerides (TG), cholesteryl esters (CE), and phospholipids (PL). Values shown are means ± SD.

The baseline characteristics of the subjects were assessed in groups defined by the median of plasma n-3 fatty acids in TG and PL (**Table 2**). There were few consistent differences on the basis of this data stratification.

Women with levels of DHA above the median had significantly less progression of coronary atherosclerosis over a 3.2 year period, as measured by changes in minimum coronary artery diameter and change in percentage stenosis regardless of whether DHA was assessed in the TG or PL subfraction of plasma (**Table 3**). Additionally, women with higher levels of PL DHA had fewer new lesions (P = 0.009). There were no significant relationships between EPA or ALA and atherosclerosis progression, regardless of measure. No significant relationships were identified when the association between measures of atherosclerotic lesion progression and any of the three n-3 fatty acids in the CE subfraction of plasma was assessed (data not shown).

DISCUSSION

This is the first report of a direct association between a biomarker of fish intake and disease progression and confirms the prior observation that self-reported fish intake was associated with atherosclerosis progression in this

TABLE 1. Proportions of ALA, EPA, and DHA above and below the median value

Fatty Acid	Median	Subjects < Median (n = 114)	Subjects ⩾ Median (n = 114)
TGs			
ALA	1.12	0.81 ± 0.21[a]	1.62 ± 0.48
EPA	0.18	0.12 ± 0.04	0.28 ± 0.11
DHA	0.53	0.36 ± 0.11	0.90 ± 0.38
PLs			
ALA	0.17	0.13 ± 0.02	0.23 ± 0.06
EPA	0.49	0.34 ± 0.09	0.68 ± 0.19
DHA	2.50	1.90 ± 0.37	3.20 ± 0.60

ALA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PL, phospholipid; TG, triglyceride.
[a] Mean ± SD.

TABLE 2. Baseline characteristics according to categories below and above median of n-3 fatty acids in plasma TGs and PLs

	ALA in TG		EPA in TG		DHA in TG		ALA in PL		EPA in PL		DHA in PL	
Characteristic	<Median (n = 114)	⩾Median (n = 114)	<Median (n = 114)	⩾Median (n = 114)	<Median (n = 114)	⩾Median (n = 114)	<Median (n = 114)	⩾Median (n = 114)	<Median (n = 114)	⩾Median (n = 114)	<Median (n = 114)	⩾Median (n = 114)
Age (years)	64.9 ± 7.3[a]	64.2 ± 6.9	63.6 ± 7.3	65.4 ± 6.8	64.0 ± 7.6	65.1 ± 6.5	64.3 ± 7.5	64.8 ± 6.7	63.7 ± 7.3	65.3 ± 6.9	63.6 ± 7.6	65.5 ± 6.5
Waist (cm)	94 ± 15	93 ± 16	92 ± 16	95 ± 15	92 ± 16	95 ± 14	94 ± 16	93 ± 14	92 ± 15	95 ± 15	92 ± 16	95 ± 14
Body mass index (kg/m^2)	29.8 ± 6.0	29.7 ± 8.2	28.9 ± 6.0	30.5 ± 8.1	29.1 ± 6.3	30.4 ± 8.0	30.0 ± 8.7	29.5 ± 5.6	28.6 ± 5.9	30.7 ± 8.1[b]	29.6 ± 8.2	29.9 ± 6.1
Total cholesterol (mg/dl)	216 ± 41	216 ± 42	215 ± 44	217 ± 40	216 ± 44	216 ± 39	221 ± 45	211 ± 38[b]	211 ± 42	211 ± 41	219 ± 45	213 ± 38
HDL-cholesterol (mg/dl)	46 ± 13	43 ± 11[b]	43 ± 11	46 ± 13	44 ± 12	44 ± 12	45 ± 11	44 ± 13	45 ± 13	44 ± 11	44 ± 12	44 ± 12
LDL-cholesterol (mg/dl)	133 ± 37	136 ± 37	135 ± 40	134 ± 34	136 ± 39	133 ± 35	141 ± 42	129 ± 32[b]	133 ± 38	137 ± 37	138 ± 38	131 ± 36
TG (mg/dl)	184 ± 107	201 ± 108	197 ± 109	188 ± 106	195 ± 110	190 ± 105	181 ± 101	203 ± 113	182 ± 101	202 ± 113	191 ± 99	194 ± 116
Total to HDL-cholesterol ratio	4.99 ± 1.59	5.41 ± 1.84	5.29 ± 1.58	5.11 ± 1.86	5.21 ± 1.62	5.19 ± 1.83	5.16 ± 1.62	5.23 ± 1.82	5.06 ± 1.54	5.33 ± 1.88	5.29 ± 1.87	5.11 ± 1.57
Systolic blood pressure (mm Hg)	134 ± 18	134 ± 17	132 ± 19	136 ± 16	132 ± 19	136 ± 16	131 ± 19	136 ± 16	133 ± 20	135 ± 16	132 ± 19	136 ± 17
Diastolic blood pressure (mm Hg)	74 ± 9	74 ± 8	74 ± 9	74 ± 8	73 ± 9	75 ± 8	74 ± 9	74 ± 8	74 ± 9	75 ± 8	74 ± 9	75 ± 8
Diabetes (%)	43	42	43	42	40	45	38	46	45	40	37	48
Physical activity score	126 ± 90	115 ± 71	108 ± 71	132 ± 89[b]	116 ± 72	124 ± 90	122 ± 94	118 ± 69	104 ± 67	134 ± 90[b]	121 ± 73	120 ± 89
Smoking (%)	24	20	24	20	29	15[b]	22	21	25	19	30	14[b]
Education (%)												
Less than high school	43	38	46	35	42	39	41	40	49	33[b]	44	37
High/vocational school	34	44	38	40	44	35	36	42	39	40	40	38
At least college	23	18	16	25	14	27	22	18	12	28	16	25

Median values are as follows in TG: ALA, 1.12; EPA, 0.18; DHA, 0.53; median values are as follows in PL: ALA, 0.17; EPA, 0.49; DHA, 2.50.
[a] Mean ± SD.
[b] $P < 0.05$ compared with the <median category (*t*-test, Wilcoxon test, or Chi-square test).

TABLE 3. Progression of coronary atherosclerosis and new lesions according to categories below and above median of n-3 fatty acids in plasma TGs and PLs

n-3 Fatty Acids	Change in Mean Minimum Coronary Artery Diameter			Change in Mean Percentage Stenosis			New Lesions		
	<Median	⩾Median	*P*	<Median	⩾Median	*P*	<Median	⩾Median	*P*
	mm			%			*% of measured segments*		
TG*s*									
ALA									
Baseline	1.95 ± 0.03[a]	1.90 ± 0.03	0.22	29.4 ± 0.8	30.6 ± 0.8	0.33			
Change	−0.09 ± 0.02	−0.09 ± 0.02	0.82	3.33 ± 0.65	3.28 ± 0.65	0.95			
Adjusted change[b]	−0.08 ± 0.02	−0.6 ± 0.02	0.27	3.13 ± 0.74	2.00 ± 0.78	0.19	3.2 (1.8–4.6)[c]	3.6 (2.1–5.1)	0.67
EPA									
Baseline	1.93 ± 0.03	1.92 ± 0.03	0.80	29.4 ± 0.8	30.6 ± 0.8	0.34			
Change	−0.10 ± 0.02	−0.09 ± 0.02	0.71	3.53 ± 0.65	3.08 ± 0.65	0.62			
Adjusted change	−0.08 ± 0.02	−0.06 ± 0.02	0.49	3.17 ± 0.77	2.12 ± 0.75	0.22	3.3 (1.8–4.7)	3.5 (2.0–4.9)	0.82
DHA									
Baseline	1.93 ± 0.03	1.91 ± 0.03	0.51	29.1 ± 0.8	30.9 ± 0.8	0.12			
Change	−0.11 ± 0.02	−0.07 ± 0.02	0.02	3.94 ± 0.65	2.68 ± 0.65	0.16			
Adjusted change	−0.10 ± 0.02	−0.04 ± 0.02	0.01	3.53 ± 0.74	1.66 ± 0.76	0.03	3.3 (1.9–4.7)	3.5 (2.0–5.0)	0.85
PLs									
ALA									
Baseline	1.91 ± 0.03	1.94 ± 0.03	0.40	31.1 ± 0.8	29.0 ± 0.8	0.06			
Change	−0.10 ± 0.02	−0.09 ± 0.02	0.71	3.72 ± 0.67	2.94 ± 0.63	0.38			
Adjusted change	−0.07 ± 0.02	−0.07 ± 0.02	0.99	3.05 ± 0.78	2.24 ± 0.74	0.35	3.8 (2.3–5.2)	2.8 (1.4–4.2)	0.24
EPA									
Baseline	1.93 ± 0.03	1.92 ± 0.03	0.77	29.4 ± 0.8	30.5 ± 0.8	0.38			
Change	−0.11 ± 0.02	−0.08 ± 0.02	0.19	3.52 ± 0.66	3.11 ± 0.70	0.65			
Adjusted change	−0.09 ± 0.02	−0.06 ± 0.02	0.10	3.09 ± 0.81	2.25 ± 0.73	0.35	4.0 (2.5–5.4)	2.6 (1.1–4.0)	0.10
DHA									
Baseline	1.94 ± 0.03	1.90 ± 0.03	0.28	28.9 ± 0.8	31.1 ± 0.8	0.06			
Change	−0.11 ± 0.02	−0.07 ± 0.02	0.09	4.05 ± 0.65	2.56 ± 0.65	0.10			
Adjusted change	−0.10 ± 0.02	−0.04 ± 0.02	0.007	3.75 ± 0.74	1.35 ± 0.76	0.006	4.2 (2.8–5.6)	2.0 (0.5–3.5)	0.009

[a] Mean ± SEM.

[b] Baseline and change values adjusted for age and location of coronary segment and adjusted change values also for body mass index, smoking, cholesterol-lowering medication and hormone replacement therapy use, diabetes, education, clinic, time of follow-up, revascularization procedures, and alcohol intake. Location of coronary segment was not included in the models used to analyze differences in the appearance of new lesions.

[c] Mean (95% confidence interval).

group of women (22). The results demonstrate that higher levels of plasma DHA in the TG and PL subfractions was significantly associated with less progression of coronary atherosclerosis in postmenopausal women with established CAD after a 3.2 year follow-up period. On a physiological level, these results are plausible. Although the underlying biochemical mechanism(s) for the association of n-3 fatty acids and CAD risk have yet to be fully elucidated, potential mechanisms include changes in membrane fluidity, precursors of bioactive compounds involved in cellular signaling systems (prostaglandins, leukotrienes, prostacyclins, thromboxanes, and lipoxins), and the regulation of gene expression related to e.g. endothelial activation and inflammation (29, 30).

Long-chain n-3 fatty acids in serum or tissues have been associated with lower risk of fatal ischemic heart disease (10) and sudden death (8). Data from intervention studies have also identified a lower risk of cardiovascular end points after increased intakes of fatty fish or fish oil supplements (3, 31). Nonetheless, the data relating biomarkers of fish consumption to angiographic measures are limited. The results of secondary prevention trials using fish oil supplementation in CAD patients have been mixed, and the data are available mainly for men (16, 17). von Schacky et al. (17) reported a modestly reduced progression rate of coronary atherosclerosis over a 2 year observational period, whereas Sacks et al. (16), in a smaller study, reported no significant effect over a 28 month observational period. Autopsy studies have suggested that higher levels of long-chain n-3 fatty acids in adipose tissue or coronary artery PLs are associated with less atherosclerosis in coronary arteries (32, 33). Animal studies have demonstrated antiatherosclerotic effects of EPA and DHA (34, 35). Incorporation of n-3 fatty acids into atherosclerotic plaques has been reported to enhance their stability (15).

The significant inverse associations of atherosclerosis progression were identified for DHA but not EPA or ALA, similar to observations by other investigators (8, 36). There are a number of potential explanations for this observation. If the beneficial effects of n-3 fatty acids are related to the physical conformation of the acyl chain in plasma, cellular membranes, lipid droplets, or atherosclerotic plaque, the difference in length and number of double bonds would favor DHA. The conversion rate of EPA to DHA is slow (5), as is the conversion of ALA to EPA (37). Therefore, the circulating levels of ALA and EPA may be good indicators of plant and marine dietary intakes, but not CAD outcomes.

The role of ALA in cardioprotection has been controversial. A lower prevalence of calcified atherosclerotic

plaques (19) as well as lower carotid intima-media thickness (38) have been associated with higher dietary intakes of ALA in observational studies. No association was observed between ALA and CAD progression, despite the association between ALA in PL and serum total and LDL cholesterol concentrations in the current study. Likewise, other investigators have reported that levels of ALA in serum are not associated with the risk of CAD (8, 36, 39). With respect to intervention studies, Bemelmans et al. (40) reported that use of ALA-enriched margarine yielding 2.3% of energy from ALA for 2 years did not affect the progression rate of carotid or femoral intima-media thickness. A recent systemic review of n-3 fatty acids and CVD outcomes concluded that for both primary and secondary prevention, there was a significant effect of EPA and DHA but no significant effect of ALA (4).

A potential limitation of this study is that the 3 year follow-up may have been too short an observational period to fully address the association between CAD progression and all of the n-3 fatty acids. The study cohort was a group of older, predominantly white volunteers with established coronary disease. The extent to which these findings may apply to younger, healthier women or women of other ethnic backgrounds remains to be established. Plasma fatty acid measures at the end of the 3 year follow-up period would be of interest. However, plasma was not available for these measures, and long-term sustained changes in dietary habits were unlikely to have occurred. We also cannot exclude the possibility that changes in the number of PLs carrying DHA influenced the results, as we did not measure the plasma concentration of total PL. A strength of this study is that the data provide an independent assessment of n-3 fatty acids in plasma, free of selective dietary recall.

In conclusion, higher levels of DHA in plasma PL and TG were significantly associated with the reduced progression of coronary atherosclerosis over the 3 year follow-up in postmenopausal women with established CAD. These results support the dietary recommendations to increase the intake of fatty fish to reduce CAD risk.

The authors gratefully acknowledge Susan M. Jalbert and Nancy A. Resteghini for serum lipid fatty acid analysis. This study was supported by grants from the National Heart, Lung, and Blood Institute (Grants U01 HL-45488 and NIH HL-54727), the National Center for Research Resources General Clinical Research Center (Grant Mo1 rr07122), and the United States Department of Agriculture (Grant 58 1950 9 001).

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