

**Citation:**

Erkkilä AT, Matthan NR, Herrington DM, Lichtenstein AH. Higher plasma docosahexaenoic acid is associated with reduced regression of coronary atherosclerosis in women with CAD. *J Lipid Res.* 2006; 47: 2,814-2,819.

**Study Design:**

Cohort

**Class:**

B - [Click here](#) for explanation of classification scheme.

**Research Design and Implementation Rating:**

POSITIVE: See Research Design and Implementation Criteria Checklist below.

**Research Purpose:**

To examine the association between plasma n-3 fatty acids and the progression of angiographically defined coronary atherosclerosis in a group of post-menopausal women with pre-existing coronary artery disease (CAD) followed for a mean of 3.2 years.

**Inclusion Criteria:**

- Enrolled in the Estrogen Replacement and Atherosclerosis (ERA) Trial
  - Post-menopausal women
  - Age less than 80 years
  - Not currently receiving estrogen-replacement therapy
  - One or more epicardial coronary stenoses of at least 30% of the luminal diameter
- Baseline and end of study coronary angiography available.

**Exclusion Criteria:**

Not described

**Description of Study Protocol:****Recruitment**

Participants were enrolled in the ERA trial; recruitment was not described for ERA Trial.

**Design**

- ERA Trial
  - Women were randomized to one of three groups:
    - 0.625mg conjugated equine estrogen
    - 0.625mg conjugated equine estrogen plus 2.5mg medroxyprogesterone acetate
    - Placebo
  - Followed for 3.2±0.6 (mean±SD) years
  - Neither of the treatments had a significant effect on the progression of CAD
- Current study assessed the relationship between circulating levels of plasma n-3 fatty acids to change in coronary artery diameter, percentage stenosis and new lesion formation.

**Blinding used**

Measurements of coronary artery diameter, percentage stenosis and new lesion formation were conducted by operators who were unaware of the woman's temporal sequence of the films.

**Intervention**

No intervention

**Statistical Analysis**

- Subjects were divided into categories according to median proportions of alpha linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in triglycerides (TG) and phospholipid (PL).
  - Differences between the groups were tested using the T-test, the Wilcoxon test or Chi-square.
- Associations of n-3 fatty acids in plasma lipids to changes in mean minimum coronary artery diameter and mean percentage stenosis were assessed with mixed model analysis of covariance
  - Adjusted for age, location of coronary segment, BMI, 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 above, except location of segment
- P<0.05 (two-tailed) was considered statistically significant.

## Data Collection Summary:

### Timing of Measurements

- Baseline
  - Questionnaires completed describing health status, medical history and cardiovascular risk factors
  - Clinical exam
  - Physical activity score
  - Serum lipids
  - Coronary angiography
- Follow-up at 3.2 years
  - Coronary angiography.

### Dependent Variables

- Mean minimum coronary artery diameters
  - Reference, minimum and average luminal diameters
  - Segments totally occluded or intervened with coronary artery bypass surgery were excluded from analysis
- Percentage stenosis
- New lesion formation
  - Presence of one or more segments with less than 15% stenosis at baseline and an increase of at least 15% at follow-up.

### Independent Variables

- Plasma lipids
  - Phospholipid
  - Triglyceride
  - Cholesterol ester
- n-3 fatty acids
  - ALA
  - EPA
  - DHA.

### Control Variables

- Age
- Location of coronary segment
- BMI
- Education
- Time of follow-up
- Study clinic
- Coronary artery bypass grafting
- Percutaneous transluminal coronary angioplasty
- Race
- Smoking
- Use of cholesterol-lowering medication
- Hormone replacement therapy
- Alcohol intake.

## Description of Actual Data Sample:

### Initial N

228 women

### Attrition (final N)

228 women

### Age

See table below

### Ethnicity

Not described

### Other relevant demographics

See table below

### Anthropometrics

See table below

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	
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Characteristic	Less than the Median (n=114)	≥ Median (n=114)	Less than the Median (n=114)	≥ Median (n=114)	Less than the Median (n=114)	≥ Median (n=114)	Less than the Median (n=114)	≥ Median (n=114)	Less than the Median (n=114)	≥ Median (n=114)	<Median (n=114)	≥ Median (n=114)
Age (year)	64.9 (7.3)	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)
BMI	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)§	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)§	211 (42)	211 (41)	219 (45)	213 (38)
HDL-cholesterol (mg/dl)	46 (13)	43 (11)§	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)§	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 (percentage)	43	42	43	42	40	45	38	46	45	40	37	48
Physical activity score	126 (90)	115 (71)	108 (71)	132 (89)§	116 (72)	124 (90)	122 (94)	118 (69)	104 (67)	134 (90)§	121 (73)	120 (89)
Smoking (percentage)	24	20	24	20	29	15§	22	21	25	19	30	14§
Education (percentage)												
Less than high school	43	38	46	35	42	39	41	40	49	33§	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.

\* Mean±SD § P<0.05 compared with the "less than"median category

#### Location

Multi-center study but sites not described

#### Summary of Results:

Distribution of ALA, EPA and DHA

1. Differed among the plasma lipid subfractions (TG, CE and PL)
2. DHA and EPA were most abundant in PL (0.52±0.22 and 2.55±0.82mol percentage, respectively)
3. ALA and DHA were most abundant in TG (1.22±0.52 and 0.63±0.38mol percentage, respectively)
4. Tended to be lowest or intermediate in the CE subfraction so TG and PL were focus of subsequent statistical analysis.

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

Change									

	<b>in Mean Minimum Coronary Artery Diameter</b>			<b>Change in Mean Percentage Stenosis</b>			<b>New Lesions</b>		
<b>n-3 Fatty Acids</b>	Less than the Median	≥ Median	P	Less than the Median	≥Median	P	Less than the Median	≥Median	P
	mm			Percentage			Percentage measured segments		
<b>TGs</b>									
<b>ALA</b>									
<b>Baseline</b>	1.95 (0.03)	1.90 (0.03)	0.22	29.4 (0.08)	30.6 (0.8)	0.33			
<b>Change</b>	-0.09 (0.02)	-0.09 (0.02)	0.82	3.33 (0.65)	3.28 (0.65)	0.95			
<b>Adjusted change</b>	-0.08 (0.02)	-0.06 (0.02)	0.27	3.13 (0.74)	2.00 (0.78)	0.19	3.2 (1.8-4.6)§	3.6 (2.1-5.1)	0.67
<b>EPA</b>									
<b>Baseline</b>	1.93 (0.03)	1.92 (0.03)	0.80	29.4 (0.8)	30.6 (0.8)	0.34			
<b>Change</b>	00.10 (0.02)	-0.09 (0.02)	0.71	3.53 (0.65)	3.08 (0.65)	0.62			
<b>Adjusted change</b>	-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
<b>DHA</b>									
<b>Baseline</b>	1.93 (0.03)	1.91 (0.03)	0.51	29.1 (0.8)	30.9 (0.8)	0.12			
<b>Change</b>	-0.11 (0.02)	-0.07 (0.02)	0.02	3.94 (0.65)	2.68 (0.65)	0.16			
<b>Adjusted change</b>	-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
<b>PLs</b>									
<b>ALA</b>									
<b>Baseline</b>	1.91 (0.03)	1.94 (0.03)	0.40	31.1 (0.8)	29.0 (0.8)	0.06			
<b>Change</b>	-0.10 (0.02)	-0.09 (0.02)	0.71	3.72 (0.67)	2.94 (0.65)	0.38			
<b>Adjusted change</b>	-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
<b>EPA</b>									
<b>Baseline</b>	1.93 (0.03)	1.92 (0.03)	0.77	29.4 (0.8)	30.5 (0.8)	0.38			
<b>Change</b>	-0.11 (0.02)	-0.08 (0.02)	0.19	3.52 (0.66)	3.11 (0.70)	0.65			
<b>Adjusted change</b>	-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
<b>DHA</b>									
<b>Baseline</b>	1.94 (0.03)	1.90 (0.03)	0.28	28.9 (0.8)	31.1 (0.8)	0.06			
<b>Change</b>	-0.11 (0.02)	-0.07 (0.02)	0.09	4.05 (0.65)	2.56 (0.65)	0.10			

<b>Adjusted change</b>	-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 (.5-3.5)	0.009
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\* Mean (SEM) § Mean (95% CI)

1. Women with levels of DHA above the median had significantly less progression of coronary atherosclerosis 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.
2. Women with higher levels of PL DHA had fewer new lesions.

#### Other Findings

#### Author Conclusion:

- Higher levels of DHA in plasma phospholipid and triglyceride were significantly associated with the reduced progression of coronary atherosclerosis over the three-year follow up in post-menopausal women with established CAD
- These results support the dietary recommendations to increase the intake of fatty fish to reduce CAD risk.

#### Reviewer Comments:

- EPA and DHA serve as biomarkers of fish intake
- ALA serves as a biomarker of plant-derived n-3 fatty acids.

#### Research Design and Implementation Criteria Checklist: Primary Research

##### Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

##### Validity Questions

<b>1.</b>	<b>Was the research question clearly stated?</b>	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
<b>2.</b>	<b>Was the selection of study subjects/patients free from bias?</b>	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	???
<b>3.</b>	<b>Were study groups comparable?</b>	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	N/A
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	Yes

3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
<b>4.</b>	<b>Was method of handling withdrawals described?</b>	<b>Yes</b>
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
<b>5.</b>	<b>Was blinding used to prevent introduction of bias?</b>	<b>Yes</b>
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	Yes
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
<b>6.</b>	<b>Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were intervening factors described?</b>	<b>Yes</b>
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	N/A
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	Yes
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	N/A
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
<b>7.</b>	<b>Were outcomes clearly defined and the measurements valid and reliable?</b>	<b>Yes</b>
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	???
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
<b>8.</b>	<b>Was the statistical analysis appropriate for the study design and type of outcome indicators?</b>	<b>Yes</b>
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes

8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	Yes
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
<b>9.</b>	<b>Are conclusions supported by results with biases and limitations taken into consideration?</b>	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
<b>10.</b>	<b>Is bias due to study's funding or sponsorship unlikely?</b>	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes

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