SEX DIFFERENCES IN THE RELATIONSHIP OF POLYUNSATURATED FATTY ACIDS AND NONINVASIVE IMAGING MEASURES OF SUBCLINICAL CARDIOVASCULAR DISEASE

BY

JENNIFER S. ANDERSON

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Approved By:

David Herrington, MD, MHS, Advisor

Examinining Committee:

Mara Vitolins, DrPH, MPH, RD, Examining Committee Chair

Ronny Bell, PhD

W. Gregory Hundley, MD, MPH

Tim Morgan, PhD
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<td>AA</td>
<td>arachidonic acid</td>
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<tr>
<td>ALA</td>
<td>$\alpha$-linolenic acid</td>
</tr>
<tr>
<td>CMR</td>
<td>cardiac magnetic resonance imaging</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>DHA</td>
<td>docosahexaenoic acid</td>
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<tr>
<td>EF</td>
<td>ejection fraction</td>
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<tr>
<td>EPA</td>
<td>eicosapentaenoic acid</td>
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<tr>
<td>LA</td>
<td>linoleic acid</td>
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<td>LV</td>
<td>left ventricular</td>
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<tr>
<td>MESA</td>
<td>Multi-Ethnic Study of Atherosclerosis</td>
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ABSTRACT

Background: The association between plasma phospholipid omega-6 levels and cardiovascular disease (CVD) morbidity and mortality is unclear, and discrepancy remains concerning the cardiovascular benefit of the omega-3 fatty acid alpha-linolenic acid (ALA).

Objective: To determine the associations between plasma phospholipid omega-6 (arachidonic acid (AA), linoleic acid (LA)) and omega-3 levels (eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), ALA) with cardiac magnetic resonance imaging (CMR) measures of left ventricular and aortic dysfunction.

Design: Cross-sectional associations of plasma phospholipid levels with CMR measures of LV mass, LV volumes, ejection fraction, stroke volume, and aortic distensibility were investigated in 1,274 adults from four racial/ethnic groups, aged 45-84 years, free of clinical CVD who underwent CMR at baseline examination.

Results: Results of multivariate analysis adjusted for age, race/ethnicity, gender, BMI, smoking, education, field center, physical activity, systolic blood pressure, total:HDL cholesterol, and total energy intake, showed no statistically significant associations of plasma phospholipid omega-6 or omega-3 levels with CMR measures at the a priori-specified level of \( p < 0.01 \). However, in women, plasma phospholipid DHA was positively associated with LV mass (\( \beta = 1.89, p = 0.02; p \) interaction = 0.003). Conversely, a trend for a positive association between plasma phospholipid DHA and ejection fraction was noted in men (\( \beta = 0.009, p = 0.05; p \) interaction = 0.03).

Conclusions: Results suggest the association between plasma phospholipid DHA and CMR measures of LV mass and ejection fraction vary by gender. Additional research is warranted to clarify the effects of omega-3 fatty acids on cardiac structure and function in women versus men.
I. LITERATURE REVIEW

a. Overview

Cardiovascular disease (CVD) remains the major cause of morbidity and mortality in industrialized nations, accounting for approximately one death every 38 seconds in the United States every day,¹ and is known to be influenced by environmental factors including diet. Yet despite over a century of research, the relationship between diet and cardiovascular disease remains poorly understood. The classic diet-heart hypothesis, which postulated a relationship between saturated fat intake and atherosclerosis primarily through the mechanism of influencing serum cholesterol levels, has been recently challenged.²,³ Indeed, evidence from individual randomized controlled trials (RCTs) that have studied the relationship between saturated fat and CVD events have been mixed.²,⁴,⁵ Furthermore, dietary recommendations to reduce saturated fat have been relatively nonspecific, i.e., whether saturated fat should be replaced with carbohydrate, protein, or unsaturated fats. As most Americans grumble about “health foods [that tastes like] cardboard” and voice frustrations in the seemingly ever-changing consensus on what constitutes a heart healthy diet, many do not realize that simple and even pleasurable changes in the foods they eat can rival medication in terms of cardiovascular benefit.

Dietary fat in the form of polyunsaturated fatty acids (PUFAs) may confer several benefits, including improved serum cholesterol profiles,⁶ systemic inflammation,⁷ and insulin resistance⁸. Although the American Heart Association Scientific Advisory has recently published recommendations for ranges of “minimum” PUFA intakes,⁹,¹⁰ dietary recommendations are quite general (i.e., nonspecific for gender, age, etc.), and optimal intakes remain unclear. Thus, the proposed research is innovative because it (1) addresses the association between PUFAs and measures of subclinical CVD in both men and women of mixed race/ethnicity; (2) examines the association between plasma phospholipid PUFAs, an objective measure of dietary PUFA intake, and measures of subclinical CVD; and (3) examines the association between PUFAs and
measures of subclinical CVD as assessed by CMR, a novel and arguably more accurate technology. This contribution will be significant because it will be the next step in a continuum of research that may lead to the development of specific dietary recommendations, targets for nutraceutical therapies, and/or targets for optimal plasma fatty acid levels that may more effectively address CVD prevention in men and women. Important advances in effective dietary strategies for CVD prevention may not only be expected to decrease associated disease morbidity and mortality, but would also be expected to reduce the total direct and indirect cost of CVD, currently estimated at $503.2 billion.11

b. Polyunsaturated fatty acids (PUFAs): dietary sources, diet-plasma correlation

Dietary sources

Dietary fatty acids may be found in plant or animal fats, oils, or waxes. In biochemistry, a fatty acid is defined as a carboxylic acid with a long, unbranched chain of carbon atoms known as an aliphatic tail.12 This aliphatic tail may be “saturated” (meaning saturated with hydrogen atoms, i.e. no carbon-carbon double bonds), “monounsaturated” (defined by one carbon-carbon double bond), or “polyunsaturated” (defined by more than one carbon-carbon double bond). The standard nomenclature for fatty acids includes the number of carbon atoms, followed by the number of double bonds (after a colon), then the location of the first double bond (if present) as counted from the carboxyl group. For example, eicosapentaenoic acid (20:5ω-3) is a polyunsaturated fatty acid that is 20 carbons in length, contains five carbon-carbon double bonds, and contains its first double bond at the third carbon from the carboxyl end of the chain (Figure 1). Of additional note, the Greek letter ω (omega) is the last letter of the Greek alphabet; thus “ω-3” is simply a means for denoting “third carbon from the last” in this example (i.e. third carbon from the terminal carboxyl end of the fatty acid).
Figure 1. Biochemical structure and nomenclature of fatty acids. Individual carbons are represented by kinks in a straight line, and carbon-carbon double bonds are shown as double lines between these kinks.

Humans lack the ability to introduce double bonds beyond the 9th and 10th carbon position. Dietary consumption of two fatty acids, linoleic acid (18:2ω-6) and α-linolenic acid (18:3ω-3), are thus considered essential for human body systems. Linoleic acid (LA) is ubiquitous in the diet, found in vegetable oils such as corn, sunflower, and safflower, as well as animal fats. Alpha-linolenic acid (ALA) is found in vegetable oils such as flaxseed, walnut, canola, and soybeans, as well as some leafy green vegetables. Although humans are able to convert these essential fatty acids into longer-chain fatty acids (Figure 2), it is felt that the conversion of ALA to the long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is relatively inefficient. Thus, consumption of omega-3 fatty acids found in marine oils (particularly high in fatty fish) are considered by some to be the “other” essential fatty acids, as they have been shown to have multiple beneficial properties on cardiovascular health. Furthermore, LA and other omega-6 fatty acids compete with omega-3 fatty acids’ position in cell membranes, and are known to have differential physiological effects on inflammation, thrombosis, and vascular reactivity (Figure 2). Optimal dietary “omega-6/omega-3 ratio” as well as plasma “omega-3 index” measures have therefore been proposed, though clinical implications of these recommendations remain controversial.
Figure 2: Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and potential actions in cardiovascular disease.

α-Linolenic Acid (18:3ω3)  
*flaxseed, walnut, hemp oils; soybeans, leafy greens*

Stearidonic Acid (18:4ω3)

Eicosatetraenoic Acid (20:4 ω3)

Eicosapentaenoic Acid (20:5 ω3)  
*fatty fish, fish eggs*

Docosapentaenoic Acid (22:5ω3)  
*seal; organ meats*

Docosahexaenoic Acid (22:6 ω3)  
*fatty fish; organ meats; egg yolks; human milk*

Linoleic Acid (18:2ω6)  
*canola, sunflower, safflower oils; animal products*

γ-linolenic Acid (18:3ω6)

Dihomo-γ-linolenic Acid (20:3ω6)

Arachidonic Acid (20:4ω6)  
*animal fats, organ meats*

Docosatetraenoic Acid (22:4ω6)

Docosapentaenoic Acid (22:5ω6)

Inhibition of Inflammatory Cytokines

Favorable Effects on Cardiac Ion Channels

Fatty Acid Gene Expression (oxidation >> synthesis)

Favorable Eicosanoid Production:  
3-series prostaglandins, thromboxanes; 5-series leukotrienes

Unfavorable Eicosanoid Production:  
2-series prostaglandins, thromboxanes; 4-series leukotrienes

Atherosclerosis ↔ Myocardial Infarction ↔ Arrhythmia ↔ Sudden Cardiac Death
**Diet-plasma correlations**

The amount and type of dietary fats differ tremendously due to the variation in fatty acids contained in foods, the hidden nature of many fats, cultural differences in food preparation, and individual interpretation of questions about fat consumption. For these reasons, biomarkers of fat consumption are particularly appealing. Fatty acid levels may be measured in blood (including erythrocytes, platelets, or free fatty acids) and adipose tissue via gas-liquid chromatography or HPLC. Results are usually expressed as a percentage of total fatty acids, and though interaction of fatty acids with subsequent effects on metabolism is known to exist and may confound interpretation, it is generally believed that fatty acid profiles are reflective of relative patterns of dietary intake. However, considerable discrepancy exists regarding the relative contribution of individual fatty acids to each of these substrates, and to further complicate interpretation, measurements may be made in the cholesterol ester, phospholipid, and/or triglyceride fractions, with varying results. Though it is beyond the scope of this review to discuss the details of the variation in each fatty acid as determined by methodology, it is important to note three major points when considering the data available for our current analysis: (1) adipose tissue is considered the most reliable long-term marker of dietary intake (reflective of years of intake), though this is also the most invasive means of measurement and probably unrealistic in a large epidemiological study; (2) erythrocyte and plasma phospholipid measures are considered to reflect more short-term measures of dietary intake (1-2 weeks versus 1-2 months, respectively), but are more easily obtained than from adipose tissue and therefore the focus of this research project; and (3) while results are dependent on multiple variables (such as time lapse between dietary assessment and plasma measures), we may roughly expect estimated correlations between erythrocyte/plasma phospholipid measures vs dietary sources of EPA, DHA, and LA to range from 0.20-0.50. It is also important to note that while all dietary long-chain PUFAs (including arachidonic acid [AA], LA, EPA, or DHA) correlate well with plasma cholesterol
esters and plasma or erythrocyte phospholipids, the medium-chain omega-3 fatty acid ALA correlates well with plasma cholesterol esters only.\textsuperscript{26,24}

\begin{quote}
In summary, humans have limited capability of endogenously synthesizing PUFAs, and consumption of specific polyunsaturated fatty acids (PUFAs) has been implicated in cardiovascular health. While plasma PUFAs are considered to be generally reflective of dietary intake, measurement methodologies and strength of correlations vary considerably. This variation in methodology may therefore influence results, as in assessment of the relationship between plasma PUFAs and various cardiovascular outcomes.
\end{quote}

c. \textbf{Relationship of dietary vs plasma PUFAs and cardiovascular disease}

\textit{Omega-3 fatty acids and cardiovascular disease}

Multiple epidemiological, observational and interventional studies have established a relationship between increased long-chain omega-3 PUFA intake and reduced cardiovascular events and death.\textsuperscript{27-31} Intake of long-chain omega-3 PUFAs in the form of fish oil supplements have been shown to improve lipid profiles,\textsuperscript{32} exhibit antioxidant actions,\textsuperscript{33} reduce biomarkers of inflammation,\textsuperscript{34-36} and demonstrate anti-arrhythmogenic properties.\textsuperscript{37,38} Fish oil intake has also been shown to decrease platelet aggregation and demonstrate favorable effects on blood pressure and autonomic tone.\textsuperscript{18} Many of the latter effects may be the result of omega-3 fatty acids’ ability to selectively interfere with the production of atherogenic/thrombotic eicosanoids (Figure 2).\textsuperscript{28}

Studies examining surrogate endpoints for cardiovascular disease have also demonstrated favorable effects of long-chain omega-3 PUFAs. Randomized trials examining the effects of fish oil supplementation on brachial artery flow-mediated dilation (FMD), a noninvasive measure of early atherosclerosis that is predictive of incident cardiovascular events,\textsuperscript{39,40} has shown improvement after just 2 weeks’ duration.\textsuperscript{41,42} Others have observed inverse associations between long-chain omega-3 fatty acids and carotid intimal media thickness.\textsuperscript{43-45} Consumption of long-
chain omega-3 PUFAs has also been associated with measurable improvements in cardiac hemodynamics, including left ventricular diastolic function, stroke volume, and systemic vascular resistance as assessed by echocardiography. However, evidence of cardioprotective effects of plasma ALA and other PUFAs, including omega-6 fatty acids, remain mixed. Indeed, some studies have shown a positive relationship between plasma ALA and incident coronary heart disease as well as sudden cardiac death. These findings are inconsistent with multiple other studies that demonstrate a cardioprotective effect of dietary ALA intake, and coupled with the fact that many measures of plasma ALA correlate poorly with diet, suggest that the positive relationship between plasma ALA and CVD may be indicative of an enzymatic inefficiency predictive of CVD rather than an indication of a diet-CVD relationship.

Lastly, several lines of evidence suggest there may be important sex differences in metabolic, physiologic, and/or clinical consequences of dietary fat consumption. Unfortunately, there are few studies in women that have examined the relationship between omega-3 fatty acid or fish intake and clinical correlates of cardiovascular disease and/or incident events. While the Nurse’s Health Study clearly demonstrated a cardioprotective effect of non-fried fish consumption in a large population of Caucasian women, results from a recent 12-year prospective cohort of men and women of mixed racial/ethnic groups failed to demonstrate a significant protective effect of non-fried fish in women. Population surveys from New Zealand, Norfolk and Quebec have shown women to have greater tissue DHA content compared to men, regardless of dietary omega-3 intakes, and some have shown variable tissue EPA and docosapentaenoic acid (DPA) contents as well. Evidence from stable isotope feeding studies have also shown differences in omega-3 metabolism, with women demonstrating a higher capacity to metabolize ALA to DHA compared to men. Clinical implications of these findings suggest that greater amounts of dietary omega-3 PUFAs may have less impact in women compared to men, with less observed cardiovascular benefit.
Omega-6 fatty acids and cardiovascular disease

It has been postulated that excessive amounts of omega-6 PUFAs and a very high omega-6/omega-3 ratio, as found in today's Western diets, may be central in the pathogenesis of cardiovascular disease. High omega-6 PUFA intake in the form of arachidonic acid (AA) or linoleic acid (LA), a precursor for AA, may theoretically promote the production of atherogenic, pro-inflammatory eicosanoids (2- and 4-series prostaglandins, leukotrienes, thromboxane, and prostacyclin; Figure 2). In addition, because omega-6 and omega-3 PUFAs are known to compete for positions within cell membranes, higher intake of dietary omega-6 PUFA could displace the [more cardioprotective] omega-3 PUFAs. However, more recent analyses have challenged this viewpoint, and several large prospective trials have described an inverse association between LA (either dietary or plasma measures) and the risk of CVD. Similarly, intake of LA has been associated with reduction in the incidence of coronary heart disease in high LA intake intervention trials, and a recent meta-analysis of randomized controlled trials provides compelling evidence that replacing dietary saturated fats with PUFAs lowers CVD risk.

In contrast to the apparent benefit of LA, associations between arachidonic acid (AA) and CVD outcomes have been mixed: Block et al. described a u-shaped relationship between blood cell AA content and acute coronary syndrome case status; a meta-analysis of 25 case-control and prospective cohort studies found that increased AA content was significantly associated with coronary heart disease events only when measured in adipose tissue; and others have questioned the relationship of dietary versus plasma/tissue AA, have described a strong link between AA and BMI, and contend AA cannot be considered an independent CVD risk factor.

Finally, sex differences are again noted in the relationship of omega-6/other PUFAs and CVD. In the above meta-analysis that described an inverse association between PUFA intake and CVD, only two of the eight studies in this analysis included women, and neither of these studies
individually showed a significant relationship between increased PUFA intake and CVD risk. Other studies have suggested potential negative effects of increased PUFA intake in women, with decreased HDL cholesterol levels, evidence of atherosclerotic progression, and even nonsignificant trends toward more CVD events and higher total mortality in women compared to men. Taken with the sex differences in omega-3 PUFA metabolism as discussed previously, these findings further highlight the need for more research in understanding the differential effects of dietary versus plasma/tissue PUFAs in cardiovascular outcomes among women versus men.

In summary, though dietary and plasma long-chain omega-3 PUFAs have demonstrated cardiovascular benefit in the vast majority of studies, this relationship may vary by sex. Furthermore, there appears to be discrepancy in the association between the dietary versus plasma medium-chain omega-3 PUFA α-linolenic acid and cardiovascular disease, which may be reflective of the plasma lipid subfraction tested. Data examining the association between plasma omega-6 fatty acids, particularly arachidonic acid, and cardiovascular endpoints are mixed. More research examining relationships of individual PUFAs with cardiovascular endpoints, with emphasis on potential sex differences, is warranted.

d. Noninvasive measures of cardiovascular structure and function in prediction of clinical events

Several noninvasive measures of cardiac structure and function assessed via ultrasonography, electron beam computed tomography, and/or cardiac magnetic resonance imaging have been evaluated in their ability to predict incident coronary heart disease, heart failure, and/or cardiovascular death. These measures include (but are not limited to): left ventricular (LV) mass/hypertrophy; LV ejection fraction and stroke volume; and arterial/aortic stiffness. The relationships between each of these measures and cardiovascular events will be examined below. Particular emphasis will be placed on these measures as assessed via echocardiography (which is
simple, easy to use, and relatively inexpensive) and CMR (a newer technology, but generally considered more reliable and accurate), as these modalities do not require the use of ionizing radiation. Additional measures including left atrial size, diastolic function, carotid intimal media thickness, brachial artery flow-mediated dilation, coronary calcium scores and coronary flow reserve have also shown various levels of utility in predicting cardiovascular events, but are not the focus of our current analysis and therefore will not be discussed.

**Left ventricular mass**

Increased LV mass of the heart, also known as left ventricular hypertrophy (LVH), has been positively associated with increased incidence of chronotropic incompetence, acute coronary syndrome, and sudden cardiac death. The Framingham Heart Study was among the first to show a statistically significant, risk-factor adjusted positive relationship between increased LV mass (as assessed by echocardiography) and incident coronary heart disease: each 50 g per meter increase in LV mass increased risk of incident coronary heart disease by 67% in men, and 60% in women. Each incremental increase in LV mass was also associated with a markedly elevated risk of cardiovascular death in this cohort. Studies of patients with hypertension, renal failure, congestive heart failure, and patients with or without significant coronary artery obstruction at cardiac catheterization have additionally observed independent, positive relationships between echocardiographic measures of increased LV mass and cardiovascular morbidity and mortality.

The accuracy of LV mass measurement has been challenging in the past, partly because of the oblique angle at which the heart lies within the thorax, its continuous movement, and the lack of a technique for fully capturing the left ventricle. While the vast majority of studies have employed electrocardiographic and/or echocardiographic means of assessment for LVH, cardiac magnetic resonance imaging is considered a superior method of LV mass and volume assessment,
and is considered the gold standard of accuracy and reproducibility. Yet because CMR is a relatively new technology, there are few data that have directly examined the relationship of LV mass as assessed by CMR and incident cardiovascular events. To date, one recent population study has demonstrated an independent positive association between increased LV mass and incidence of heart failure.

**LV mass/volume ratio**

Geometric changes in the left ventricle, also known as remodeling, have been associated with cardiovascular events as assessed by echocardiography. Increased LV mass and wall thickness, known as “concentric” LVH remodeling, has been associated with higher risk of cardiovascular events when compared with other geometric patterns. Because CMR is able to assess 3-dimensional measures of size, shape, and function, patterns of geometric remodeling may be more easily identified than via 2-D or M-mode echocardiography, and as previously mentioned, carries the added benefit of imposing no ionizing radiation as compared to other 3-D modalities. Though data examining CMR and events are generally limited as also discussed previously, a recent prospective study found a positive relationship between LV mass/volume ratio (indicative of concentric LVH) and incident coronary heart disease.

**Ejection fraction and stroke volume**

LV ejection fraction (LVEF) and stroke volume are parameters of cardiac pump function (i.e., systolic function). Estimation of LVEF is a well-accepted expression of global LV function, and is a measure of the fraction of end-diastolic (i.e., end-relaxation) volume that is ejected from the LV with each contraction.

Assessment of LVEF using volumetric measurements, obtainable via all modalities, is calculated as \( \frac{\text{LV end-diastolic volume} [\text{LVEDV}] - \text{LV end-systolic volume} [\text{LVESV}]}{\text{LVEDV}} \). Stroke volume (SV) makes up the numerator in this calculation, and is the
volume of blood ejected with each contraction (SV = LVEDV – LVESV, thus LVEF = SV/LVEDV). The gold standard of measuring LVEF has historically utilized ventriculography obtained via angiography, which is invasive and requires use of contrast media. Complications of angiographic ventriculography include life-threatening cardiac arrhythmias, air or thrombus emboli, and/or contrast-related complications including allergic reactions or renal failure. In comparison, noninvasive LVEF assessment via echocardiography does not require contrast media, and has been found to be a strong predictor of clinical outcome in most cardiovascular conditions. However, this method is considered relatively crude, particularly when measured via “eyeball assessment,” and demonstrates markedly wide variances in comparison to other modalities. CMR assessment of LVEF is considered much more accurate than echocardiography and is also noninvasive. Studies comparing different LVEF imaging modalities have suggested that the superior reproducibility of CMR makes it more suitable than other modalities for individual or population follow-up of LVEF.

**Aortic stiffness**

Alterations in aortic wall structure resulting in decreased compliance with subsequent increased pulse pressure have been associated with CVD. Quantitative assessment of aortic elasticity has been investigated via several echocardiographic and CMR measures, and include aortic root distensibility, aortic stiffness index, and pulse wave velocity, among others. In general, an increase in aortic stiffness and/or a decrease in aortic distensibility are felt to be reflective of the widespread nature of atherosclerosis. CMR measures of compliance in the ascending aorta (= change in volume normalized to pulse pressure) and pulse wave velocity around the aortic arch (= rate of propagation of the flow wave in early systole) are abnormal in early atherosclerosis, and have been shown to be predictive of cardiac events. However, there remains disagreement in the literature regarding the ability of aortic stiffening to predict the presence of subclinical CVD.
In summary, several imaging modalities have been evaluated in prediction of cardiovascular events. Of these modalities, cardiac magnetic imaging resonance is a noninvasive measure that generally provides the greatest accuracy and reproducibility without requiring the use of ionizing radiation. However, because it is a relatively new technology, studies assessing the relationship of various CMR measures with cardiovascular outcomes or events are currently lacking.

e. The relationship of PUFAs and noninvasive imaging modalities of subclinical CVD

This section will review data that emphasize the relationship of PUFAs and imaging modalities that encompass the specific aims of our present analysis and ancillary studies, including echocardiographic and CMR modalities. Observations exploring the relationship of PUFAs and other imaging modalities, including carotid intimal media thickening (CIMT) and angiography, have observed inverse relationships with long-chain omega-3 PUFAs. The few studies that have examined associations between other types of PUFAs and CIMT generally show inverse relationships between medium- and long-chain omega-3 as well as some omega-6 fatty acids. In contrast, studies that have examined the association between omega-3 PUFAs and coronary calcium scores have generally shown no significant relationship. There are also data that suggest possible sex differences in the relationship of PUFAs and atherosclerosis: a positive association between dietary PUFAs (in comparison to other fats) and angiographic evidence of atherosclerotic progression in postmenopausal women has been observed.

PUFAs and echocardiographic measures

Mozaffarian et al. found that consumption of fish or fish oil is associated with several improvements in cardiovascular hemodynamics, including echocardiographic measures of diastolic filling and stroke volume. These results were corroborated in a randomized trial of healthy men, where men that consumed high doses of fish oil (4 g/day) showed significant
improvements in LV diastolic filling compared to men that consumed similar amounts of corn oil. In addition, a recent study of men and women diagnosed with acute coronary syndrome found that moderate fish intake (1-2 servings/week) was associated with 53% lower likelihood of developing LV systolic dysfunction compared to no/rare fish intake. Rodent studies further support the protective effect of fish oils on cardiovascular function: fish oil has shown protective effects on cardiac hypertrophy in genetically predisposed mice, as well as pressure overload-induced LV dysfunction in rats. Lastly, though one study by Rupp et al. found that extent of LV dilatation (assessed by echocardiographic measures of LV end-diastolic diameter) was associated with reductions in both plasma DHA and AA, data examining the relationship of cardiovascular structure/function and other types of PUFAs are lacking.

**PUFAs and cardiac magnetic imaging measures**

As mentioned previously, there are few data that comprehensively examine the relationship of dietary or plasma PUFAs and noninvasive measures of subclinical CVD, and there are even fewer data that have examined associations between diet and CMR measures. A recent MESA analysis examined associations of dietary patterns, metabolic dysfunction, and CMR measures of LV mass and function, and found that dietary patterns that included high intake of foods with a high glycemic index, high-fat meats, cheeses, and processed foods were associated with unfavorable effects on LV structure and function (including LV mass, stroke volume, and ejection fraction). Conversely, low intakes of vegetables, soy, fruit, green and black tea, low-fat dairy desserts, seeds and nuts, and fish were favorably associated with measures of LV structure and function, though further adjustment for components associated with the Metabolic Syndrome attenuated these effects. To date, similar associations between CMR measures and specific macronutrients have not been defined.
In summary, PUFAs in the form of supplemental fish oil show strong evidence for a beneficial effect on all noninvasive measures of subclinical cardiovascular disease. Dietary omega-3 fatty acids, particularly in the form of non-fried fish, generally show similar patterns. Few data exist examining the relationship of other types of PUFAs and noninvasive measures of subclinical CVD, potential sex differences need to be further explored, and no studies to date have examined the association between individual PUFAs and CMR measures of CVD.

GLOBAL SUMMARY:

Consumption of polyunsaturated fatty acids has been implicated in cardiovascular health. Plasma phospholipid PUFAs are an objective means of quantifying dietary PUFA intake, and though some plasma phospholipid PUFAs have also shown CVD benefit (particularly long-chain omega-3 PUFAs), others PUFA types have not been thoroughly studied and/or have shown discrepant results. In addition, some studies suggest sex differences in the relationship between dietary PUFAs and cardiovascular endpoints, highlighting the need for more studies that include female participants. Finally, cardiac MRI is a novel means of evaluating associations between plasma phospholipid PUFAs and subclinical measures of cardiovascular disease among women and men.
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CHAPTER II:
ASSOCIATIONS OF PLASMA OMEGA-6 AND OMEGA-3 FATTY ACID LEVELS AND CARDIAC MAGNETIC RESONANCE IMAGING MEASURES OF CARDIOVASCULAR STRUCTURE AND FUNCTION: THE MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS

Jennifer S. Anderson, Jennifer A. Nettleton, Gregory Hundley, Michael Y. Tsai, Lyn M. Steffen, Rozenn N. Lemaitre, David Siscovick, João Lima, Martin R. Prince, David M. Herrington

INTRODUCTION

Contemporary work suggests that consumption of polyunsaturated fatty acids (PUFAs), in place of saturated fats, decreases the risk of cardiovascular disease (CVD).\(^1\) Dietary fats in the form of PUFAs have several potential cardiovascular benefits, including improvements in serum cholesterol profiles,\(^2\) systemic inflammation,\(^3\) and insulin resistance.\(^4\) In addition, consumption of long-chain omega-3 PUFAs (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA], both found in fatty fish) has been associated with measurable improvements in cardiac hemodynamics, including left ventricular (LV) diastolic function, stroke volume, and systemic vascular resistance as assessed by echocardiography.\(^5\)

It is generally believed that plasma phospholipid fatty acid profiles are reflective of dietary intake,\(^6\) and indeed, an inverse association between plasma phospholipid DHA and arterial stiffness as measured by pulse wave velocity has also been observed.\(^7\) Arterial stiffness has been shown to be an independent predictor of cardiovascular mortality\(^8\) and is thought to be in the causal pathway leading to increased LV mass.\(^9\) Consumption of fatty fish has been associated with a trend toward decreased LV mass as determined by electrocardiography.\(^5\) However, more accurate objective measures assessing the relationship of omega-3 PUFAs and LV mass as well as LV mass/volume ratio (the latter associated with both non-heart failure cardiovascular events as well as diastolic dysfunction\(^10\)) in human subjects are lacking. Furthermore, similar associations among other types of PUFAs (including alpha-linolenic acid [ALA], a medium-chain omega-3 fatty acids obtained from plant sources such as flaxseed and canola oil, as well as omega-6 fatty acids, ubiquitous in the diet and found in meat and vegetable sources) have not been defined.
The purpose of this study is to determine the associations between plasma phospholipid omega-6 fatty acids (arachidonic acid [AA] and linoleic acid [LA]) and plasma phospholipid omega-3 fatty acids (EPA, DHA, and ALA) with cardiac magnetic resonance (CMR) measures of cardiovascular structure and function, including aortic distensibility, LV mass, LV mass/volume ratio, ejection fraction, and stroke volume. The Multi-Ethnic Study of Atherosclerosis (MESA) comprises four unique ethnic groups (Caucasian, African-American, Chinese, and Hispanic) allowing for examination of the associations of plasma fatty acid levels with imaging measures of cardiovascular structure and function in men and women of different racial/ethnic groups.
MATERIALS AND METHODS

Study Population and Data Collection

MESA is a prospective cohort study that began in July 2000, to investigate the prevalence, correlates and progression of subclinical CVD in individuals without known CVD at baseline.\textsuperscript{11} The main cohort included 6,814 women and men aged 45-84 years old at baseline recruited from 6 US communities (Baltimore, Md.; Chicago, Ill.; Forsyth County, N.C.; Los Angeles County, Calif.; northern Manhattan, N.Y.; and St. Paul, Minn.). MESA cohort participants were 38% Caucasian (n=2624), 28% African American (n=1895), 22% Hispanic (n=1492), and 12% Chinese (n=803). Details of the MESA study design have been documented previously.\textsuperscript{11} A variety of non-invasive measures of subclinical disease, including magnetic resonance imaging of cardiac structure and function, were obtained from volunteers during the first examination of the MESA cohort (July 2000-August 2002). In the present study, we excluded those who had missing data on both plasma phospholipid fatty acids (n = 5,172) and CMR measures (n = 4,404 for measures of LV mass, LV mass/volume ratio, ejection fraction and stroke volume; n = 5,136 for aortic distensibility), as well as those with missing covariates used in the study (n = 2). Because plasma phospholipid fatty acids and CMR measures were secondary measures, these assessments were not performed on the full cohort at baseline. Total sample size of subjects who had data on plasma phospholipid measures, selected covariates, and CMR measures of LV mass, LV mass/volume ratio, ejection fraction and stroke volume was 1,276. Two outliers were excluded from the plasma phospholipid ALA sample due to suspected error, thus the sample size for this subset was 1,274. Total sample size of subjects who had data on plasma phospholipid measures, selected covariates and CMR measures of aortic stiffness was 914. This study was approved by the Institutional Review Boards of each study site and written informed consent was obtained from all participants.
**Plasma phospholipids extraction**

Fasting blood samples were collected and fatty acid analyses were performed at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN) as previously described. In brief, plasma phospholipids were extracted with chloroform/methanol, and thin layer chromatography was used to separate the lipid fractions. The fatty acids in the phospholipid fraction were transmethylated and separated by gas chromatography equipped with a flame ionization detector. The concentration of each fatty acid was expressed as a percentage of total fatty acids.

For statistical analyses, plasma phospholipid fatty acids were divided into quartiles. When appropriate, analyses were performed with and without inclusion of outliers, defined as greater than three standard deviations from the mean. Log transformation was performed when deviation from normality was detected (noted for plasma EPA only). Additional analyses including comparisons of CMR measures with (a) each fatty acid as a continuous variable as well as (b) each fatty acid classified into quartile ranks based on gender-specific sample ranges were also examined within this cohort, and similar patterns were observed (data not shown).

**Cardiac magnetic resonance imaging measures**

CMR imaging was performed with 1.5-T whole-body MRI systems, Signa CV/I or Signa LX (General Electric Medical Systems), at the first examination. Determination of LV mass and volumes have been previously described, and were adjusted for body size. In brief, a stack of short axis images covering the entire left ventricle was acquired with TR/TE 8-10 msec/ 3-5 msec, flip angle 20 degrees, 6 mm slice thickness, 4 mm gap, flow compensation, in plane resolution 1.4-1.6 mm (frequency) x 2.2-2.5 mm. LV mass was determined by the summation of myocardial area (the difference between endocardial and epicardial contour) times slice thickness plus image gap in the end-diastolic phase multiplied by the specific gravity of myocardium (1.05
LV end-diastolic volume and LV end-systolic volume were calculated by the summation of areas on each separate slice multiplied by the summation of slice thickness and image gap. LV stroke volume was calculated as the difference between LV end-diastolic volume and LV end-systolic volume. LV ejection fraction was calculated as LV stroke volume divided by LV end-diastolic volume multiplied by 100.

Evaluation of aortic distensibility has also been previously described. In brief, MRI scans of the aorta were obtained using gradient echo phase-contrast cine MRI with ECG gating. Images of the ascending and descending aorta were acquired in the transverse plane at the level of the right pulmonary artery perpendicular to the vessel lumen. Imaging parameters were as follows: repetition time: 10 ms; echo time, 1.9 ms; field of view, 34 cm; slice thickness, 8 mm; matrix: 256x224; 2 signal averages; temporal resolution, 20 ms; velocity encoding gradient: 150 cm/s; and receiver bandwidth: ±32 kHz. The following formula was used for calculation of aortic distensibility: aortic distensibility = (maximum area–minimum area)/[(Minimum area)x*pulse pressure]x1000. The minimum and maximum cross-sectional areas of the ascending aorta were determined using an automated contour routine using the software FLOW (Medis; Raleigh, N.C.). Pulse pressure was the difference between systolic and diastolic measurements of blood pressure, obtained immediately before and after the MRI aortic measurements while the patient was in the supine position in the MRI scanner.

Statistical Analysis

Means and standard deviations or proportions were calculated for selected variables according to quartiles of plasma fatty acids (Table 1). For the primary analysis, linear regression models were used to examine the association between plasma phospholipid fatty acid levels and CMR measures of cardiovascular structure and function. Minimally-adjusted (age, gender, race/ethnicity, field center) and multivariable-adjusted models were used to examine these
relationships. A number of potential confounders for inclusion in the fully-adjusted models were evaluated based on clinical relevance, previously published associations, or associations with exposures or outcomes in the current data set. The final multivariable-adjusted model included age (continuous), gender, race/ethnicity (Caucasian/white, African American/black, Hispanic, Chinese American), BMI (continuous), current smoking status (current, past, never), education level (less than high school, high school with or without some college [no degree] or technical school, associate’s or bachelor’s degree, graduate or professional school), field center (Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University, University of California at Los Angeles), total:HDL cholesterol (continuous), systolic blood pressure (continuous), and total energy intake (continuous). For parsimony in model construction, the following covariates that did not appreciably alter the relationship between plasma fatty acids and CMR measures of cardiovascular structure and function were excluded: diastolic blood pressure (continuous), diabetes diagnosis (2003 American Diabetes Association fasting criteria algorithm, including both self-reported diabetes as well as diabetes medication; categorized as treated diabetes, untreated diabetes, and impaired fasting glucose), hypertension medication (yes/no), current hormone replacement therapy (in women; yes/no), serum triglyceride level (continuous), lipid-lowering medication (yes/no), tobacco pack-years (continuous), income (<$50k, 50-99,999, $100k), alcohol intake (number of drinks/week, continuous), fruit intake (servings/week, continuous), whole grains intake (servings/week, continuous), cruciferous vegetable intake (servings/week, continuous), and nut/seed consumption (servings/week, continuous).

As our previous work has suggested a gender difference in the association between omega-3 fatty acids and endothelial function (Anderson JS et al., Am J Clin Nutr 2010), and because gender differences in fatty acid metabolism and tissue levels have been described, we also chose to stratify analyses by gender. When there appeared to be a significant difference in plasma
phospholipid fatty acid levels and CMR measures of LV structure and function, formal tests for interaction were performed using the product of [plasma phospholipid fatty acid x gender] in the model. Because fatty acids have also been shown to have differential effects on vascular reactivity by age, we also explored whether there were significant differences in the relationship between plasma phospholipid fatty acid levels and CMR measures of cardiovascular structure and function after stratification by age (<65 years vs. ≥65 years). Because many of our outcomes are correlated, we felt that a Bonferroni’s adjustment would be overly conservative in this analysis; thus a more stringent p value of < 0.01 was considered statistically significant, and all p values were 2-sided. We used JMP version 8.0 (SAS Institute Inc., Cary, N.C.) for analyses.

RESULTS

Participant Characteristics
Characteristics of participants according to plasma phospholipid omega-6 fatty acid LA and omega-3 fatty acid DHA quartiles are presented in Table 1. The highest plasma phospholipid LA quartile was associated with male gender, Chinese race/ethnicity, lower BMI, lower blood lipid levels, lower prevalence of diabetes diagnosis, lower alcohol intake, and less fruit intake compared to the lower plasma phospholipid LA quartiles. In comparison, the highest plasma phospholipid DHA quartile was associated with older age, female gender, Chinese race/ethnicity, lower BMI, higher education, less tobacco use, lower systolic blood pressure, lower blood lipid levels, higher cholesterol medication use, lower total energy intake, lower alcohol intake, less saturated fat intake and greater cruciferous vegetable intake compared to the lower plasma phospholipid DHA quartiles.

Plasma Omega-6 fatty acids and CMR Measures of Cardiovascular Structure and Function
After adjustment for age, gender, race/ethnicity, and field center, plasma phospholipid AA showed no association with LV mass, LV mass/volume ratio, EF, stroke volume, or aortic
distensibility as assessed by CMR. These results were unchanged after adjustment for additional covariates (Table 3). Plasma phospholipid LA showed a statistically significant inverse association with ejection fraction in the minimally-adjusted model ($p = 0.001$) with a trend for significance in the fully-adjusted model ($p = 0.02$; Table 3), but this association was not robust after additional adjustment for plasma phospholipid saturated fatty acid levels ($p = 0.07$, data not shown). No other significant associations between plasma phospholipid LA and CMR measures of cardiovascular structure and function were found in either minimally- or fully-adjusted models (Table 3). Stratification by age and gender revealed no heterogeneity in the relationship between either plasma phospholipid AA or LA and CMR measures of cardiovascular structure and function (data not shown). Stratification by race/ethnicity revealed some statistically significant associations that were difficult to interpret due to small sample sizes (supplement, Tables 1s & 2s).

**Plasma Omega-3 fatty acids and CMR Measures of Cardiovascular Structure and Function**

After adjustment for age, gender, race/ethnicity, and field center, plasma phospholipid DHA showed no significant association with LV mass, LV mass/volume ratio, EF, stroke volume, or aortic distensibility in either minimally- or fully-adjusted models (Table 4a). Though trends for positive associations between plasma phospholipid EPA and LV mass as well as stroke volume were noted in minimally-adjusted models ($p = 0.02$ and $p = 0.03$, respectively; data not shown), these associations became insignificant after adjustment for additional covariates (Table 4a). Similarly, plasma phospholipid ALA showed no statistically significant associations with LV mass, LV mass/volume ratio, EF, stroke volume, or aortic distensibility as assessed by CMR. These associations were unchanged after adjustment for additional covariates (Table 4b), including adjustment for other plasma fatty acids.
When analyses were stratified by gender, a positive trend was noted between plasma phospholipid DHA and LV mass in women ($p = 0.02$; Figure 3a) but not in men, and a statistically significant [gender x DHA] interaction was observed ($p = 0.003$). A similar pattern between plasma phospholipid EPA and LV mass in women was observed, but again, results were not statistically significant ($p = 0.06$, fully-adjusted model); tests for [gender x EPA] interactions were also nonsignificant. In contrast to the findings noted in women, higher plasma phospholipid DHA was associated with a trend for increased ejection fraction in men ($p = 0.05$; Figure 3b); formal [gender x DHA] interaction tests also showed a trend toward significance ($p = 0.03$).

Finally, stratification by age revealed no heterogeneity in the relationship between plasma phospholipid omega-3 fatty acid levels and CMR measures of cardiovascular structure and function (data not shown). Stratification by race/ethnicity revealed some statistically significant associations that were difficult to interpret due to small sample sizes (data not shown).

**DISCUSSION**

Plasma phospholipids are a marker of dietary patterns, with higher levels reflecting greater consumption of polyunsaturated fats and less cardiovascular disease. These data from 1,274 healthy, multi-ethnic volunteers corroborate this concept by showing that men within higher plasma phospholipid DHA demonstrated higher ejection fraction compared to men within lower plasma phospholipid DHA.

The trend for a positive association between plasma phospholipid DHA and ejection fraction in men is supported by the literature.$^{5,20}$ The beneficial effects of dietary, as well as plasma phospholipid EPA and DHA, in prevention of sudden cardiac death, acute coronary syndrome, and heart failure are well documented.$^{21-25}$ Others have demonstrated an association between long-chain omega-3 fatty acids and improvements in stroke volume, systemic vascular resistance,
and improved E/A ratio (a marker for diastolic dysfunction) as assessed by echocardiography,\textsuperscript{5} and have shown an inverse association between plasma DHA and measures of arterial stiffness including pulse wave velocity.\textsuperscript{7} These findings are biologically plausible, as experimental evidence suggest that long-chain fatty acids may impact membrane fluidity, nitric oxide production, and/or shift of eicosanoid pathways involved in inflammation and vasoconstriction.\textsuperscript{26-29}

In contrast to the results in men, the observed positive association between plasma phospholipid DHA and increased LV mass among women within in this cohort initially appears surprising. However, there remains considerable discrepancy in the literature regarding gender differences in the association between PUFAs and cardiovascular outcomes. Among population-based cohorts, the Nurses’ Health Study demonstrated a significantly negative association between fish consumption and incidence of CHD, the ARIC study showed an inverse relationship in plasma phospholipid omega-3 fatty acids (particularly DHA) and incident heart failure in women,\textsuperscript{30} and the Infarction Prognosis Study found that lower plasma level of EPA (though not DHA) was an independent predictor for all-cause-mortality in women with acute myocardial infarction.\textsuperscript{31} In addition, a recent study examining the relationship of plasma fatty acids and echocardiographic assessment of cardiac function in heart failure patients showed that decreased DHA was associated with increased LV dilatation, and stratification by gender revealed no heterogeneity.\textsuperscript{20} However, the Rotterdam study observed no relationship between fish consumption and incident heart failure in a population of predominantly women.\textsuperscript{32} The Women’s Health Initiative also did not observe an association between fish consumption and atrial fibrillation.\textsuperscript{33} Results from other studies suggest potential negative effects of PUFA intake, where women with greater PUFA intake had lower HDL cholesterol concentrations,\textsuperscript{34} evidence of atherosclerotic progression,\textsuperscript{35} and nonsignificant trends toward more CHD events and higher total mortality in women compared to men.\textsuperscript{36} In a previous report, we found that women within the highest quartile of non-fried fish
consumption had decreased brachial artery flow-mediated dilation (FMD), a noninvasive measure of endothelial function, compared to women within the lowest quartile of non-fried fish consumption (Anderson JS et al, Appendix A). Endothelial dysfunction is an independent predictor of cardiovascular events,\textsuperscript{37,38} and may induce an increased in LV mass via increased systemic vascular resistance.\textsuperscript{39} These data suggest there may be gender differences in the effects of omega-3 fatty acids on vascular reactivity and/or arterial stiffness, and highlight the need for more research that better elucidates differential effects of PUFAs in cardiovascular outcomes among women versus men.

Finally, while there is discrepancy in the literature concerning the cardiovascular benefit of the medium-chain omega-3 fatty acid ALA,\textsuperscript{40-45} the present analysis does not provide evidence of either benefit or harm in the association between plasma phospholipid ALA and CMR measures of cardiovascular structure and function. Additionally, potential harm of a diet rich in omega-6 fatty acids has been long debated.\textsuperscript{46,47} AA has been associated with mediators of inflammation, thrombosis, vascular constriction, and a greater risk of myocardial infarction.\textsuperscript{46,48,49} Dietary LA, a precursor for AA, has also been postulated to be associated with increased inflammation and displacement of cardioprotective omega-3 fatty acids, with subsequent increased risk of CVD.\textsuperscript{47} However, LA has also shown beneficial effects on plasma lipid profiles, and a more recent meta-analysis has suggested an inverse association between dietary LA and CVD.\textsuperscript{46} Results from the present analysis do not provide strong evidence of either cardiovascular benefit or harm in the association between plasma phospholipid omega-6 fatty acid levels and CMR measures of cardiovascular structure and function.
The strengths of this study include the ethnically diverse population, the inclusion of both men and women, and the availability of objective plasma phospholipid fatty acid measures as well as CMR measures of cardiovascular structure and function. The limitations include the observational cross-sectional study design with its known inability to infer causation and potential for temporal bias, and like any observational study, there remains the possibility of residual confounding for factors (such as health status) that could have meaningful impact on the observed results. In addition, the CMR results we report in this study were obtained using a fast gradient-echo MR pulse sequence, which may contain slight differences in cardiac volume, mass, and EF obtained in comparison to more updated technology.\textsuperscript{13,50} Lastly, though we chose a more conservative \textit{a priori} \( p \) value in an attempt to correct for any findings that may be reflective of spurious associations resulting from multiple exposure-outcome testing, the vast majority of our observations exceeded this threshold, and must be interpreted with caution.

In conclusion, this study is the first to examine the relationship between plasma phospholipid fatty acid levels and cardiac magnetic resonance imaging measures of cardiovascular structure and function. Within this cohort of mixed ethnic/racial groups without known coronary artery disease, a positive association between plasma phospholipid docosahexaenoic acid (DHA) and LV mass was found among women but not men, and a statistically significant [gender x DHA] interaction was observed. A gender difference was also suggested in the association between plasma phospholipid DHA and ejection fraction: a positive association between DHA and ejection fraction was observed in men but not women, and a trend for a [gender x DHA] interaction was noted. Additional research is warranted to clarify the effects of omega-3 fatty acids on cardiac structure and function in women versus men.
References:


Table 1. Relation between baseline characteristics and plasma phospholipid DHA, LA.¹

<table>
<thead>
<tr>
<th></th>
<th>LA quartile (n)</th>
<th>DHA quartile (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1</td>
<td>Q2</td>
</tr>
<tr>
<td></td>
<td>n = 324</td>
<td>n = 316</td>
</tr>
<tr>
<td>Age in years, mean±SE</td>
<td>65.4±0.7</td>
<td>63.7±0.7</td>
</tr>
<tr>
<td>Gender, %female</td>
<td>65.5</td>
<td>61.1</td>
</tr>
<tr>
<td>Race/ethnicity, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>20.2</td>
<td>12.9</td>
</tr>
<tr>
<td>Black</td>
<td>22.9</td>
<td>16.5</td>
</tr>
<tr>
<td>Chinese</td>
<td>12.7</td>
<td>19.4</td>
</tr>
<tr>
<td>Hispanic</td>
<td>44.2</td>
<td>51.1</td>
</tr>
<tr>
<td>BMI, mean±SE</td>
<td>28.1±0.3</td>
<td>28.4±0.3</td>
</tr>
<tr>
<td>Education ≥ high school, %</td>
<td>82</td>
<td>79.5</td>
</tr>
<tr>
<td>Cigarette Smoking, % current</td>
<td>15.1</td>
<td>16.5</td>
</tr>
<tr>
<td>Phys activity, mod-heavy mean±SE MET-h/wk</td>
<td>5143±374</td>
<td>5343±362</td>
</tr>
<tr>
<td>SBP, mean±SE mmHg</td>
<td>128±1.4</td>
<td>126±1.3</td>
</tr>
<tr>
<td>Total:HDL cholesterol</td>
<td>4.3±0.08</td>
<td>4.3±0.08</td>
</tr>
<tr>
<td>Cholesterol medication, %</td>
<td>25.7</td>
<td>15</td>
</tr>
<tr>
<td>Diabetic, %</td>
<td>15.5</td>
<td>17</td>
</tr>
<tr>
<td>Total energy, mean kcal/day</td>
<td>1591±53</td>
<td>1616±52</td>
</tr>
<tr>
<td>Alcohol, avg drinks/wk</td>
<td>4.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Saturated fat, %kcal</td>
<td>18.9</td>
<td>20.3</td>
</tr>
<tr>
<td>Fruits, avg srv/day</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Cruciferous vegetables, avg srv/day</td>
<td>0.35</td>
<td>0.37</td>
</tr>
</tbody>
</table>

¹adjusted for age, gender, race/ethnicity, education, and site.
Table 2. Mean plasma phospholipid fatty acid levels by gender.\textsuperscript{1}

<table>
<thead>
<tr>
<th>Plasma phospholipid, %total fatty acids</th>
<th>Men n = 597</th>
<th>Women n = 679</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eicosapentaenoic acid</td>
<td>0.94±0.08</td>
<td>0.98±0.08</td>
<td>0.50</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>4.05±0.11</td>
<td>4.33±0.10</td>
<td>0.0005</td>
</tr>
<tr>
<td>Alpha-linolenic acid</td>
<td>0.16±0.006</td>
<td>0.18±0.005</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>12.16±0.18</td>
<td>12.37±0.17</td>
<td>0.12</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>21.69±0.25</td>
<td>20.96±0.23</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

\textsuperscript{1}mean±SE adjusted for age, race/ethnicity, education, smoking, site, BMI, energy intake
Table 3. Relation between plasma phospholipid omega-6 fatty acid levels and measures of cardiac structure and function.\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Measure</th>
<th>Plasma AA (n)\textsuperscript{3}</th>
<th>Plasma LA (n)\textsuperscript{3}</th>
<th>P</th>
<th>Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1  n = 319</td>
<td>Q2  n = 319</td>
<td>Q3  n = 320</td>
<td>Q4  n = 318</td>
</tr>
<tr>
<td>Aortic distensibility, 10(-3) mmHg(-1)</td>
<td>1.25</td>
<td>1.25</td>
<td>1.22</td>
<td>1.23</td>
</tr>
<tr>
<td>LV mass, grams</td>
<td>143.3</td>
<td>144.4</td>
<td>145.8</td>
<td>143.8</td>
</tr>
<tr>
<td>LV mass:volume</td>
<td>0.12</td>
<td>0.11</td>
<td>0.11</td>
<td>0.14</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>68.32</td>
<td>68.05</td>
<td>68.01</td>
<td>68.61</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>84.92</td>
<td>86.10</td>
<td>86.82</td>
<td>83.86</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Multivariate linear regression analysis with adjustment for age, gender, race/ethnicity, BMI, current smoking status, education level, site, total:HDL cholesterol, systolic blood pressure, and total energy intake.

\textsuperscript{2}AA, arachidonic acid; LA, linoleic acid; LV, left ventricular.

\textsuperscript{3}Sample sizes are for each plasma phospholipid quartile associated with LV mass, LV mass/volume ratio, ejection fraction, and stroke volume. Sample sizes for each plasma phospholipid quartile associated with aortic distensibility varied slightly due to fewer available aortic distensibility measures, and were as follows for AA: Q1 = 204, Q2 = 232, Q3 = 140, Q4 = 238; for LA: Q1 = 255, Q2 = 227, Q3 = 223, Q4 = 209.
Figures 3a and 3b. Relations between plasma phospholipid DHA, left ventricular (LV) mass, and ejection fraction, by gender.¹

Results shown are least squares means ± SE after adjustment for age, race/ethnicity, BMI, current smoking status, education level, site, total:HDL cholesterol, systolic blood pressure, and total energy intake. Concentrations of plasma phospholipid fatty acid levels (expressed as percentages of total fatty acids) including DHA were summed and ranked into quartiles from lowest to highest based on sample range. Significant [gender x DHA] interactions were noted for both LV mass ($p = 0.003$) and ejection fraction ($p = 0.03$).

¹
Table 4a. Relation between plasma phospholipid omega-3 fatty acid levels and measures of cardiac structure and function.1,2

<table>
<thead>
<tr>
<th>Measure</th>
<th>Plasma EPA (n)3</th>
<th>Plasma DHA (n)3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q1 n= 305</td>
<td>Q2 n= 298</td>
</tr>
<tr>
<td>Aortic distensibility, 10(-3) mmHg(-1)</td>
<td>1.22 1.26 1.23 1.26</td>
<td>0.26</td>
</tr>
<tr>
<td>LV mass, grams</td>
<td>143.14 143.61 146.48 145.38</td>
<td>0.14</td>
</tr>
<tr>
<td>LV mass:volume</td>
<td>0.12 0.12 0.14 0.12</td>
<td>0.31</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>67.94 68.50 68.22 68.68</td>
<td>0.26</td>
</tr>
<tr>
<td>Stroke volume, mL</td>
<td>84.52 85.93 85.15 87.04</td>
<td>0.12</td>
</tr>
</tbody>
</table>

1 Multivariate linear regression analysis with adjustment for age, gender, race/ethnicity, BMI, current smoking status, education level, site, total:HDL cholesterol, systolic blood pressure, and total energy intake.
2 EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ALA, alpha-linolenic acid; LV, left ventricular.
3 Sample sizes are for each plasma phospholipid quartile associated with LV mass, LV mass/volume ratio, ejection fraction, and stroke volume. Sample sizes for each plasma phospholipid quartile associated with aortic distensibility varied slightly due to fewer available aortic distensibility measures, and were as follows for EPA, Q1 = 225, Q2 = 212, Q3 = 245, Q4 = 232; and for DHA, QQ = 213, Q2 = 239, Q3 = 220, Q4 = 242.
Table 4b. Relation between plasma phospholipid omega-3 fatty acid levels and measures of cardiac structure and function.\(^1,2\)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Plasma ALA (n)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Q1</td>
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<tr>
<td>Aortic distensibility, 10(-3) mmHg(-1)</td>
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<tr>
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<tr>
<td>Stroke volume, mL</td>
<td>84.20</td>
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</table>

\(^1\)Multivariate linear regression analysis with adjustment for age, gender, race/ethnicity, BMI, current smoking status, education level, site, total:HDL cholesterol, systolic blood pressure, and total energy intake.

\(^2\)ALA, alpha-linolenic acid; LV, left ventricular.

\(^3\)Sample sizes are for each plasma phospholipid quartile associated with LV mass, LV mass:volume ratio, ejection fraction, and stroke volume. Sample sizes for each ALA quartile associated with aortic distensibility varied slightly due to fewer available aortic distensibility measures, and were as follows: Q1 = 258, Q2 = 226, Q3 = 199, Q4 = 231.
CHAPTER III:

ADDITIONAL DISCUSSION AND FUTURE DIRECTIONS
ADDITIONAL DISCUSSION AND FUTURE DIRECTIONS

Our observations within the MESA cohort suggest sex differences in the relationship of polyunsaturated fatty acids with imaging measures of subclinical cardiovascular disease. Specifically, the highest quartiles of plasma phospholipid DHA were associated with higher LV mass in women. A threshold benefit of this long-chain omega-3 PUFA within women was also suggested, as the second lowest quartile of plasma DHA was associated with decreased LV mass. In contrast, favorable associations between high amounts of plasma phospholipid DHA and measures of subclinical CVD (specifically, ejection fraction) were noted in men. These results show similar characteristics to a previous analysis where we examined associations of dietary long-chain omega-3 PUFAs and noninvasive measures of endothelial function: women who consumed more long-chain omega-3 PUFAs in the form of non-fried fish were noted to have decreased brachial artery flow-mediated dilation compared to women who consumed less long-chain omega-3 PUFAs (Appendix A). Furthermore, there was no significant relationship between dietary long-chain omega-3 PUFAs and flow-mediated dilation in men in this analysis, though there was a suggestion of a beneficial relationship between long-chain omega-3 PUFAs and baseline artery diameter. Thus these findings suggest the possibility of sex differences in the metabolism of dietary substrates and/or potential sex steroid-PUFA interactions with subsequent effects on cardiovascular physiology. The goal of this discussion is to further examine experimental evidence for and explore mechanisms related to these observed sex differences.

Evidence for effects of gender/sex steroids in fatty acid metabolism

Observational studies have indicated that, irrespective of diet, women have significantly higher DHA levels (measured in plasma phosphatidylecholine, triacylglycerol, and nonesterified fatty acids) compared to men.1-3 The difference in DHA has been shown to be substantial, and equivalent to what can be achieved by an extra intake of approximately one regular fish-oil
capsule every other day, or 1–2 fatty fish meals per month. Experimental studies utilizing stable isotope tracing techniques have further demonstrated that women have a higher capacity for converting ALA to DHA compared to men.\(^5,7\) Similarly, Palowsky et al.\(^8\) found that women provided a beef-based diet had a three-fold greater conversion of DPA to DHA compared to men (with a similar trend noted for \textit{ad libitum} diets), but, interestingly, there was no difference in DPA to DHA conversion when both genders consumed a fish-based diet. Such differences may be relevant with respect to pregnancy, when the developing fetus depends on DHA supplied by the mother, whose diet may vary considerably over the course of gestation.

The influence of exogenous sex steroids in regulation of DHA production has also been described. Several studies conducted in women using oral contraceptives have indicated that OCP users had higher plasma DHA compared to non-users, and studies in post-menopausal women have demonstrated increased plasma EPA and DHA with hormone replacement users versus non-users.\(^5,4,6,9\) In addition, estradiol has been specifically indicated in conversion of ALA to DPA and EPA,\(^10-12\) and studies in trans-sexuals have shown higher DHA levels in male-to-female trans-sexuals consuming oral estradiol.\(^4\) This increase in DHA has been observed with oral but not transdermal estradiol, therefore it has been speculated that hepatic synthesis of DHA represents the major source of the increase in DHA in women compared with men.\(^4\) Indeed, in vitro studies have demonstrated up-regulation of hepatic desaturase enzymes in the presence of estradiol.\(^10\)

Whether enhanced hepatic biosynthesis of DHA also translates to an increase in phospholipid content of PUFAs within human cardiomyocytes has yet to be determined. One study examining the effect of DHA on rat cardiomyocytes showed that DHA inhibited conversion of ALA to higher metabolites.\(^13\) Others have also observed DHA-driven inhibition of fatty acid synthesis via down-regulation of the transcription factor sterol regulatory element-binding protein-1 (SREBP-1).\(^14\) Of additional note, SREBP-1 is also involved in interconversion of
The effects of estrogen (specifically, 17beta-estradiol) on left ventricular remodeling have been well-established, and potentially include several mediators that may function via multiple receptors. Thus perhaps the interplay between SREBP-1 or other transcription factors involved in both fatty acid and hormone metabolism is a potential site of interaction that may affect vascular reactivity and/or left ventricular remodeling, and could be potential explanation for our own observations (Figure 4).

* SREBP-1 = steroid-receptor binding protein 1; 17beta-HSD12 = 17beta-hydroxysteroid dehydrogenase type 12; p38 MAPK = p38 mitogen-activated protein kinase.
In summary, women have higher plasma DHA levels, and sex steroids likely play a role in facilitating increased DHA production, possibly via upregulation of hepatic enzymes that promote conversion from ALA and/or other precursors. Whether this enhanced DHA biosynthesis also translates to an increase versus a decrease in cardiomyocyte phospholipid content with subsequent effects on physiology remains unclear.

**Sex steroids, DHA, and physiological/biochemical parameters associated with CVD**

Experimental studies that have included both genders and/or have examined sex steroids in the relationship of fish oil/EPA/DHA supplementation and various surrogate endpoints of cardiovascular disease have shown mixed results. While some randomized clinical trials including men and women have shown beneficial effects of EPA/DHA supplementation on endothelial function and hemodynamic parameters,\textsuperscript{22-24} others have shown no effect.\textsuperscript{25,26} Though estrogen’s positive effects on nitric oxide synthesis/production in vitro have been well-documented,\textsuperscript{27} similar in vitro action for DHA’s influence on nitric oxide has been mixed,\textsuperscript{28,29} and data that have examined interactive effects of these substrates are lacking. Others have shown that DHA has differential effects on lipoprotein profiles in male versus female apoE knockout mice, noting that more favorable effects were seen in female mice, though no significant differences in atherosclerotic-like lesions were demonstrated between sexes.\textsuperscript{30} Lastly, Phang et al.\textsuperscript{31} observed differential effects of EPA and DHA on platelet aggregation by gender: DHA was shown to significantly reduce platelet aggregation in women but not men, while EPA showed a significant effect in men but not women. Thus, while experimental data examining the influence of sex steroids on the relationship between DHA and various surrogate endpoints for cardiovascular disease are relatively scarce, none of these studies suggest that high DHA levels may be associated with harmful effects in women compared to men.
Future directions: Taken together, these findings highlight the need for more research in understanding the differential effects of PUFAs in cardiovascular outcomes among women versus men. Next steps for analyses may include longitudinal associations between long-chain omega-3 PUFAs and incident cardiovascular events among MESA women and men, as well as replication of our findings within other epidemiological cohorts, if available. Translational studies might further examine the relationship between diet, PUFA content within cardiac myocytes, and variation of this relationship in the presence of hormones. Such exploration of sex differences in the relationship between dietary PUFAs and CVD endpoints may provide further insight for future studies directed toward investigation of PUFAs’ mechanisms of action for CVD development in women versus men (e.g., nitric oxide production, influence on renin-angiotensin action, collagen formation/fibrosis), and may ultimately lead to gender-specific dietary recommendations and/or nutraceutical therapies that could more effectively target disease prevention.
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APPENDIX A

RELATIONSHIP OF OMEGA-3 FATTY ACIDS AND DIETARY FISH INTAKE WITH BRACHIAL ARTERY FLOW-MEDIATED VASODILATION IN THE MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS (MESA)

Jennifer S. Anderson\textsuperscript{a}; Jennifer A. Nettleton\textsuperscript{b}; David M. Herrington\textsuperscript{a}; W. Craig Johnson\textsuperscript{c}; Michael Y. Tsai\textsuperscript{d}; and David Siscovick\textsuperscript{e}

\textsuperscript{a} Wake Forest University, Winston-Salem, NC
\textsuperscript{b} University of Texas Health Sciences Center at Houston, Houston, TX
\textsuperscript{c} University of Washington, Seattle, WA
\textsuperscript{d} University of Minnesota, Minneapolis, MN

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ABSTRACT

Background: The relationship of dietary fish intake and brachial artery measures, including brachial artery flow-mediated dilation (FMD), has not been well-established across gender and racial/ethnic groups.

Objective: We hypothesized that consumption of non-fried fish as well as plasma phospholipid measures of long-chain omega-3 fatty acids would be positively associated with larger FMD in men and women across racial/ethnic groups.

Design: Cross-sectional associations of brachial artery measures with fish intake (ascertained by food frequency questionnaire), and plasma phospholipid omega-3 levels were investigated in 3,045 adults aged 45-84 years, free of clinical cardiovascular disease (CVD).

Results: In overall multivariate-adjusted analyses, we found no significant associations between fish intake or any brachial artery measures. However, when stratified by sex, the highest quartile of non-fried fish consumption was associated with 0.10 mm lower [1 SD] brachial artery diameter in men ($p = 0.01$) and 0.27% smaller flow-mediated dilation (FMD) in women ($p = 0.02$) compared to the lowest quartile of non-fried fish intake in each respective sex strata. When stratified by race/ethnicity as well as race/ethnicity by sex, additional heterogeneity was noted, but results were difficult to interpret due to small sample sizes. Plasma phospholipid omega-3 levels showed similar directionality of association with brachial artery measures as observed for non-fried fish, though statistical significance was not achieved in fully-adjusted models.

Conclusions: This study indicates that the association between non-fried fish intake and baseline brachial artery size varies by sex, with suggestive evidence of sex differences in the association between non-fried fish intake and FMD.

(250 words)
**Key words:** fish, n-3 polyunsaturated fatty acids, omega-3, phospholipids, eicosapentaenoic acid, docosahexaenoic acid, brachial artery flow-mediated dilation, flow-mediated dilation, endothelial function, Multi-Ethnic Study of Atherosclerosis
INTRODUCTION

Multiple epidemiological, observational and interventional studies have shown an inverse relationship between fish consumption and cardiovascular events and death.\textsuperscript{1-6} Several mechanisms have been reported that may be responsible for these beneficial associations.\textsuperscript{7-10} One purported mechanism includes omega-3 (n-3) fatty acids’ impact on lipid bilayer composition and subsequent improvement in membrane fluidity, which may play an important role in endothelial function.\textsuperscript{11-13}

Flow-mediated dilation (FMD) is a noninvasive surrogate for endothelial function, which is, in part, dependent on endothelial production and release of nitric oxide.\textsuperscript{14-17} Large, prospective cohort studies suggest that FMD may be a useful tool in evaluating early atherosclerotic disease, particularly in healthy individuals with low Framingham risk scores, and larger FMD has been shown to be predictive of decreased incident cardiovascular events (fully-adjusted hazard ratios ranging 0.84-0.91).\textsuperscript{18-22} FMD has also been shown to be influenced by intake of fish oils in randomized trials as short as 2 weeks’ duration.\textsuperscript{23,24} However, there are few studies that examine the effects of dietary fish consumption on FMD, and the relationship between fish intake and FMD amongst different racial/ethnic groups are presently lacking.

The main objective of the present study was to examine the relationships of endothelial dysfunction, as measured by FMD, with fish intake and also plasma phospholipid omega-3 fatty acid levels (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) using data from the Multi-Ethnic Study of Atherosclerosis (MESA). We hypothesized that consumption of non-fried fish foods as well as plasma phospholipid EPA and DHA would be positively associated with larger FMD in men and women across racial/ethnic groups. We additionally examined the relationship of other brachial artery measures (baseline brachial diameter, maximal diameter) with fish intake/plasma phospholipid omega-3 fatty acid levels for exploratory purposes, as others
have also observed an association of increased cardiovascular events with larger baseline brachial artery diameter.18,19

MATERIALS AND METHODS

Study Population and Data Collection

MESA is a prospective cohort study that began in July 2000 to investigate the prevalence, correlates and progression of subclinical cardiovascular disease (CVD) in individuals without known CVD at baseline. The main cohort included 6,814 women and men aged 45-84 years old at baseline recruited from 6 US communities (Baltimore, Md; Chicago, Ill.; Forsyth County, N.C.; Los Angeles County, Calif.; northern Manhattan, N.Y.; and St. Paul, Minn.). MESA cohort participants were 38% white (n = 2,624), 28% black (n = 1,895), 22% Hispanic (n = 1,492), and 12% Chinese (n = 803). Details of MESA study design have been documented previously.25 A variety of non-invasive measures of subclinical disease, including brachial FMD, were obtained during the first examination of the MESA cohort (July 2000-August 2002). In the present study, we excluded those who had missing data on both fish intake (n = 364) and brachial flow-mediated dilation (n = 3,589, see below), as well as those with missing covariates used in the study (n = 92). Total sample size after these exclusions was 3,045. This study was approved by the Institutional Review Boards of each study site and written informed consent was obtained from all participants.

Dietary Assessment

A detailed description of the MESA FFQ has been described previously.26 At baseline participants recorded the serving size (small, medium, or large) and frequency (times per day, week, or month) they consumed each FFQ item. Responses were converted to servings/day by multiplying consumption frequency by reported serving size, with weights of 0.5, 1.0, and 1.5 applied to small, medium, and large serving sizes, respectively.26
The MESA FFQ was modified from the FFQ used in the Insulin Resistance Atherosclerosis Study (IRAS) to include unique Chinese foods and to collect supplemental information.\textsuperscript{25,27} Validity of the FFQ was assessed by comparison with 8, 24-hour dietary recalls in non-Hispanic white, black, and Hispanic persons in IRAS.\textsuperscript{27} Ten FFQ items included information about fish and shellfish consumption, which were then categorized by type as follows: non-fried fish (2 items)—tuna, salmon, sardines, sashimi or sushi; other broiled, steamed, baked, or raw fish such as trout, sole, halibut, poke, and grouper; shellfish (1 item)—non-fried shrimp, lobster, crab, oysters, and mussels; fish-containing mixed dishes (6 items)—fish in enchiladas, burritos, quesadilla, pasta, stew, gumbo, paella, and salad; and (4) fried fish (1 item)—any fried fish or fish sandwich, fried shrimp, and calamari. A recent validation study on dietary macronutrient intake and plasma lipids demonstrated criterion performance of the MESA FFQ,\textsuperscript{28} and associations between fish intake and plasma fatty acids were previously reported.\textsuperscript{29} We additionally confirmed a positive, statistically significant association between non-fried fish intake and plasma phospholipid EPA and DHA levels within our sample population (overall $r = 0.29, p < .0001$; data not shown). The strength of this relationship was similar to that observed by others,\textsuperscript{30} and other types of fish did not show statistically significant relationships with fatty acids after adjustment for non-fried fish.

For statistical analysis, frequency of fish consumption was further classified into four main categories for each fish type: (1) rare/never, (2) 1-3x/month, (2) 1-2x/week, and (4) >2x/week. Quartile ranks were created based on the sample range. This strategy was adopted based on previous data that have demonstrated a threshold effect of non-fried fish intake, with consumption of non-fried fish once weekly resulting in remarkably lower incidence of sudden cardiac death compared to little/no non-fried fish, as well as data suggesting a plateau effect at a non-fried fish intake of twice weekly.\textsuperscript{29,31} Additional analyses including comparisons of $<1$ serving vs $\geq 1$
serving of non-fried fish per week on brachial measures were also examined within this cohort, and similar patterns were observed (data not shown).

*Plasma phospholipids extraction*

Fasting blood samples were collected and plasma phospholipid fatty acid analyses were performed at the Collaborative Studies Clinical Laboratory at Fairview-University Medical Center (Minneapolis, MN) as previously described (n = 1,642). In brief, plasma phospholipids were extracted with chloroform/methanol, and thin layer chromatography was used to separate the lipid fractions. The fatty acids were assessed by gas chromatography with a flame ionization detector, and the concentration of each fatty acid was expressed as a percentage of total fatty acids. Because EPA and DHA are found together in fatty fish and plasma phospholipid values are therefore highly correlated, plasma phospholipid concentrations of EPA and DHA were summed for each subject. For statistical analyses, quartile ranks were created based on the sample range of these sums.

*Brachial flow-mediated dilation measurement*

Brachial FMD was measured in the MESA cohort during the first examination. Participants were excluded if they had uncontrolled hypertension (158 MESA participants were excluded), had blood pressures in the left and right arms that differed by more than 15 mmHg, or if they had a history of Raynaud’s phenomenon (55 MESA participants were excluded), a congenital abnormality of the arm or hand (12 MESA participants were excluded), or a radical mastectomy on either side (100 MESA participants were excluded). Participants were examined in the supine position after 15 minutes rest and after at least a six-hour fast. A standard blood pressure cuff was positioned around the right arm, 2 inches below the antecubital fossa, and the artery was imaged 5 to 9 cm above the antecubital fossa. A linear-array multifrequency transducer operating at 9 MHz (GE Logiq 700 Device) was used to acquire images of the right brachial artery. After obtaining
baseline images, the cuff was inflated to 50 mmHg above the participant’s systolic blood pressure for 5 minutes. Digitized images of the right brachial artery were captured continuously for 30 seconds before cuff inflation, and for two minutes beginning immediately before cuff deflation to document the vasodilator response. A detailed description of the scanning and reading protocol can be found at the MESA website (www.mesa-nhlbi.org).

Analysis of the brachial artery videotapes was performed in a nested case-cohort of MESA participants involving a random sample of MESA participants (subcohort, n = 2,844) and all those who had an adjudicated cardiovascular event by October 10, 2005 (cases, n = 182). The videotapes were analyzed at the Wake Forest University Cardiology Image Processing Laboratory using a previously validated semi-automated system. The semi-automated readings (media-adventitial interfaces to media-adventitial interfaces) of these digitized images generated the baseline and maximum diameters of the brachial artery from which % FMD was computed (%FMD = [(Maximum diameter – Baseline diameter)/ Baseline diameter] x 100%). Correlations for repeated measures of baseline diameter, maximum diameter and % FMD obtained with an original and a quality control read scanned on two separate days in 40 MESA participants (32 males, 18 Caucasians, 2 Chinese, 10 African American and 10 Hispanics) were 0.99, 0.99 and 0.81 respectively.

**Statistical Analysis**

Means and standard deviations or proportions were calculated for selected variables according to the four categories of non-fried fish consumption (Table 1). For the primary analysis, regression models were used to examine the association between fish consumption (separately by fish type) and brachial artery measures: baseline diameter, maximum diameter following the flow stimulus, and absolute and percent change in brachial diameter (% FMD). Minimally-adjusted (age) and fully-adjusted models were used to examine these relationships. On the basis of previously
published research and univariate tests, we considered a number of potential confounders for inclusion in the fully adjusted models, including BMI, systolic and diastolic blood pressure, diabetes diagnosis (2003 American Diabetes Association fasting criteria algorithm), hypertension medication, serum triglyceride level, ratio of serum total: HDL cholesterol, lipid-lowering medication, exercise, tobacco pack-years, current and former smoking status, alcohol intake, income, education, menopausal status, current hormone replacement therapy, total caloric intake, percent saturated fat intake, \(\alpha\)-linolenic acid intake, linoleic acid intake, vitamin/mineral supplement use (including vitamins C, E, and folate), and polyphenolic-rich food intake (highest quintile vs others). However, fish intake may influence brachial artery measures via modification of plasma lipid levels and blood pressure (i.e., inclusion of these variables might represent over-adjustment). Including or excluding these variables as covariates did not significantly alter the relationship between fish intake and brachial FMD. Thus, these covariates were not retained in the final multi-variable adjusted model, which included age, sex, race/ethnicity, BMI, current smoking status, education level, income level, and (in women only) current hormone replacement therapy. In exploratory analysis, similar methods were used stratifying by each sex and race/ethnicity. When there appeared to be significant differences in fish and brachial artery measures by sex as well as race/ethnicity, formal tests for interaction were performed using the product of [fish x sex] and [fish x race/ethnicity] in separate models. In additional analyses the association between plasma phospholipid EPA + DHA and brachial dimensions were examined using similar age-adjusted and fully-adjusted linear models. All \(p\) values were 2-sided, and a \(p\) value \(\leq 0.05\) was considered statistically significant. We used JMP version 8.0 (SAS Institute Inc., Cary, North Carolina) for analyses.

RESULTS

Participant Characteristics
Characteristics of participants according to intake of non-fried fish are presented in Table 1. Greater non-fried fish consumption was associated with older age, White and Chinese race/ethnicity, lower BMI, higher education, higher income, less tobacco use, lower systolic blood pressure, lower blood lipid levels, and lower prevalence of diabetes. Greater non-fried fish consumption was also associated with lower saturated fat intake and higher consumption of fruits and vegetables, although greater intake was also associated with lower physical activity levels.

The numbers of subjects in each of the non-fried fish categories were roughly similar overall and after stratification for sex. The median intake of the sex- and race/ethnicity-combined sample was approximately 3 servings per month, which corresponded to a median plasma phospholipid EPA+DHA level of 4.87%. Hispanics consumed non-fried fish the least frequently, with a median intake of 1x/month, followed by blacks (median intake 2-3x/month). Whites and Chinese consumed non-fried fish most frequently (median intakes 1x/week and 2x/week, respectively). When further stratified by sex as well as race/ethnicity, values were similar. Intakes of other fish groups also showed similar patterns (data not shown).

Fish consumption and Brachial Artery Measures

After adjustment for age, the highest quartiles of non-fried fish intake were significantly associated with smaller baseline brachial artery diameter compared to the lowest quartile of intake ($p < 0.0001$); however, after adjustment for other covariates, this association was no longer statistically significant (Table 2). There were no other statistically significant associations between non-fried fish intake and brachial artery measures in either the age-adjusted or fully-adjusted overall models.

When analyses were stratified by sex, a statistically significant inverse relationship between non-fried fish intake and baseline diameter was observed in men (Table 2). In contrast, in women, greater non-fried fish intake was associated with larger baseline diameters and smaller % FMD.
Simple inspection of the data suggest that frequency of non-fried fish intake presents as opposing U-shaped patterns for baseline artery diameter and FMD in men versus women (Figure 1). Tests of interaction between sex and non-fried fish intake for baseline diameter and %FMD were performed, and a statistically significant interaction was noted between sex and non-fried fish intake for baseline diameter ($p = 0.0001$ for baseline diameter; $p = 0.09$ for %FMD).

No other statistically significant associations of any brachial artery measure with consumption of other fish types were observed.

Data were additionally stratified by race/ethnicity, and a statistically significant inverse association was observed between non-fried fish intake and baseline artery diameter among Hispanics (Table 3). Tests of interaction by race/ethnicity were examined, and were nonsignificant. Data were further stratified by race/ethnicity and sex, and among these stratified analyses, an inverse association between non-fried fish intake and baseline artery diameter was statistically significant among Hispanic men ($p = 0.01$; data not shown), and an inverse relationship between non-fried fish intake and raw FMD (mm change) was statistically significant in Chinese women only ($p = 0.03$; data not shown). However, the numbers of participants within each of these subgroups were small.

**Plasma phospholipid EPA+DHA and FMD**

Similar to observations by other MESA investigators (using a different sample size and selection criteria), plasma phospholipid EPA+DHA was significantly positively correlated with non-fried fish intake across all races and both men and women within our subsample of participants ($r = 0.28$ for both men and women, $p<0.0001$; data not shown). Consistent with dietary non-fried fish consumption, there was an inverse relationship noted between baseline artery diameter and plasma phospholipid EPA+DHA in men, though results were only significant in the age-adjusted model ($p = 0.003$ in age-adjusted model, data not shown; $p = 0.16$ in fully-adjusted model, Figure
1). In women, a pattern that paralleled the relationship with dietary non-fried fish intake and FMD was also observed, but this was not statistically significant (Figure 1). When data were stratified by race/ethnicity, statistically significant inverse associations were observed between plasma phospholipid EPA+DHA and baseline diameter among Chinese and Hispanics in age-adjusted models but not fully-adjusted models. In addition, a borderline statistically significant association between plasma phospholipid EPA+DHA and FMD in blacks was observed in fully-adjusted models but not age-adjusted models (Table 4). Stratification by sex in addition to race/ethnicity revealed no statistically significant associations between plasma phospholipid EPA+DHA and brachial measures (data not shown).

DISCUSSION

In the present study examining the relationship between fish consumption and noninvasive measures of endothelial function across racial/ethnic groups, the main finding was that increasing quartiles of non-fried fish intake resulted in disparate responses in baseline artery diameter and FMD in men versus women. Visual inspection of the data suggested nearly a mirror-image relationship amongst these brachial measures by sex, though statistical significance was only observed for baseline artery diameter in men and FMD in women (Table 2; Figure 1). The second finding of this study was that stratification by race/ethnicity revealed a statistically significant inverse association between non-fried fish intake and baseline artery diameter among Hispanics (Table 3), and although some statistically significant associations were detected between non-fried fish and brachial artery measures after further stratification by sex, sample sizes were quite small and results difficult to interpret with confidence. Lastly, similar patterns were also observed between plasma phospholipid EPA+DHA and brachial artery measures, though results were not statistically significant in each case (Table 4; Figure 1).
Recently, greater FMD and smaller baseline artery diameter have been associated with decreased incident cardiovascular events within the MESA cohort, and an inverse relationship between FMD and left ventricular mass has also been noted. Other MESA investigators have demonstrated associations between non-fried fish consumption and lower concentrations of inflammatory biomarkers and markers of endothelial activation, and a positive correlation between non-fried fish consumption and concentrations of DHA and EPA in plasma phospholipids has been observed. Our subsample demonstrated a median plasma phospholipid EPA+DHA level that was comparable to that observed by others with similar intakes of non-fried fish, and would be expected to reduce incidence of sudden cardiac death substantially compared to those in the lowest quartile of non-fried fish intake. Thus the inverse association between non-fried fish intake and FMD observed in women in the current study is unexpected. Additional analyses are warranted to determine whether the relationship between non-fried fish intake (and/or plasma phospholipid levels) and other cardiovascular outcomes also vary by sex.

Several lines of evidence suggest there may be important sex differences in metabolic, physiologic, and/or clinical consequences of dietary fat consumption. Unfortunately, there are few studies in women that have examined the relationship between omega-3 fatty acid or fish intake and clinical correlates of cardiovascular disease and/or incident events, and even fewer that have included women of different racial/ethnic groups. While the Nurse’s Health Study clearly demonstrated a cardioprotective effect of non-fried fish consumption in a large population of Caucasian women, results from a recent 12-year prospective cohort of men and women of mixed racial/ethnic groups failed to demonstrate a significant protective effect of non-fried fish in women. Of interest, an increased risk in incident cardiovascular events in those who consumed more salted and dried fish was observed within this cohort.
Some previous data suggest that omega-3 fatty acid metabolism differs between men and women. Population surveys from New Zealand, Norfolk and Quebec have shown women to have greater tissue DHA content compared to men, regardless of dietary omega-3 intakes, and some have shown variable tissue EPA and docosapentaenoic acid (DPA) contents as well.\textsuperscript{42-45} Evidence from stable isotope feeding studies have also shown differences in omega-3 metabolism, with women demonstrating a higher capacity to metabolize $\alpha$-linolenic acid to DHA compared to men.\textsuperscript{46,47} However, it has also been postulated that women may have less ability to retro-convert DHA to EPA and DPA, and experimental studies have demonstrated that hormone replacement therapy inhibits such retroconversion.\textsuperscript{48} Clinical implications of these findings are unclear, as both EPA and DHA have shown beneficial effects on cardiovascular risk factors. It is possible that EPA versus DHA exhibit varying mechanisms of action on inflammation, lipid oxidation, nitric oxide or other modulators of endothelial function.\textsuperscript{49}

Our study is unique in that it examined fish consumption separately in men and women of four different racial/ethnic groups, and also examined plasma phospholipid EPA+DHA. While our results are not consistent with other research showing significant improvement in FMD after feeding fish oil supplements,\textsuperscript{23,24} our study differed in that dietary whole fish was examined. Furthermore, this was not a feeding trial that examined a clinical outcome but rather this was an ethnically diverse cohort consuming their own native diet. Although the observed differences between men and women was unexpected, the inconsistency between men and women of similar racial/ethnic groups suggests that this disparity is unlikely to be fully explained by food preparation methods alone (i.e. Chinese men and women, whom we expect would have similar methods of fish preparation, differed in FMD results; likewise for Hispanic men versus women). The similar results noted between brachial artery measures and plasma phospholipid EPA+DHA levels, which were correlated with non-fried fish intake, further support these findings.
The strengths of this study include the ethnically diverse population, the inclusion of both men and women, the large overall sample size, and the availability of detailed dietary information as well as objective measures of endothelial function. The limitations include the observational cross-sectional study design with its known inability to infer causation and potential for temporal bias, as well as several limitations in the MESA diet questionnaire. Because the diet questionnaire was not designed to specifically assess fish/long-chain fatty acid intake, some potentially key details are lacking, such as separate data on amount of oily fish consumed, freshness of fish, and fish preparation and cooking methods. Furthermore, like any observational study, there remains the possibility of residual confounding for factors (such as health status) or differential recall related to food intake that could have meaningful impact on the observed results. It is also possible that our findings are reflective of spurious associations resulting from multiple testing. Despite these potential shortcomings, however, previous data from MESA speak to the construct validity of the assessment of fish intake in this population. Finally, we must also consider the possibility of measurement error within the multiple brachial artery diameter measures which could diminish our ability to observe statistically significant associations. Though intra-individual variability of FMD is notable, the cross-sectional design of this large population study reduces the potential significance of such inconsistency.

In conclusion, this study adds to a small but growing body of evidence that diet may have differential effects on cardiovascular outcomes in women compared to men. Within this cohort of mixed racial/ethnic groups without known coronary artery disease, the associations of non-fried fish consumption with brachial artery measures varied by sex. Similar associations were observed between plasma phospholipid EPA+DHA levels and brachial artery measures. Additional analyses provided suggestive evidence of differences by race/ethnicity as well as race/ethnicity by sex. Future studies are warranted to clarify the extent to which sex may modify the relationship between polyunsaturated fatty acids and cardiovascular outcomes.
References:


15. Vogel RA, Corretti MC, Plotnick GD. A comparison of brachial artery flow-mediated vasodilation using upper and lower arm arterial occlusion in subjects with and without coronary


### Table 1. Sample characteristics, classified by frequency of non-fried fish intake.1

<table>
<thead>
<tr>
<th></th>
<th>Rare/Never (n = 618)</th>
<th>1-3x/mo (n = 918)</th>
<th>1-2x/wk (n = 803)</th>
<th>&gt;2x/wk (n = 706)</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, mean±SD</td>
<td>61±0.39</td>
<td>61±0.32</td>
<td>62±0.34</td>
<td>62±0.38</td>
<td>0.03</td>
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<tr>
<td>Gender, % male</td>
<td>52.7</td>
<td>48.3</td>
<td>49.1</td>
<td>48.2</td>
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<tr>
<td>Race, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;.0001</td>
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<tr>
<td>White</td>
<td>27.3</td>
<td>36.1</td>
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<td>38.2</td>
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<tr>
<td>Black</td>
<td>18.9</td>
<td>23.1</td>
<td>22.2</td>
<td>19.6</td>
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<tr>
<td>Chinese</td>
<td>11.4</td>
<td>12.7</td>
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<td>Hispanic</td>
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<td>28.1</td>
<td>18.1</td>
<td>12.1</td>
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<td>BMI, mean±SD</td>
<td>28.7±0.21</td>
<td>28.3±0.17</td>
<td>27.1±0.17</td>
<td>27.0±0.18</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Education ≥ high school, %</td>
<td>69.8</td>
<td>84</td>
<td>84.1</td>
<td>87.1</td>
<td>&lt;.0001</td>
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<tr>
<td>Income, annual, %&lt;$50k</td>
<td>72.5</td>
<td>61.6</td>
<td>56</td>
<td>50.1</td>
<td>&lt;.0001</td>
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<tr>
<td>Cigarette Smoking</td>
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<td></td>
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<td>Pack-years, mean±SD</td>
<td>13.1±1.4</td>
<td>10.1±0.6</td>
<td>8.9±0.6</td>
<td>9.5±0.8</td>
<td>0.004</td>
</tr>
<tr>
<td>Current, %</td>
<td>15.3</td>
<td>12.1</td>
<td>9.9</td>
<td>9.4</td>
<td>0.002</td>
</tr>
<tr>
<td>Phys activity, mod-heavy</td>
<td>6155±239</td>
<td>6020±196</td>
<td>5111±165</td>
<td>5277±192</td>
<td>0.0001</td>
</tr>
<tr>
<td>mean MET-h/wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SBP, mean±SD mmHg</td>
<td>126±0.76</td>
<td>125±0.65</td>
<td>123±0.67</td>
<td>125±0.77</td>
<td>0.04</td>
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<tr>
<td>DBP, mean±SD mmHg</td>
<td>72±0.37</td>
<td>71±0.34</td>
<td>72±0.25</td>
<td>72±0.36</td>
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<td>Hypertension medication, %</td>
<td>29.6</td>
<td>36.4</td>
<td>35.9</td>
<td>36.3</td>
<td>0.02</td>
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<tr>
<td>Lipid levels, mean±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Total:HDL</td>
<td>4.3±0.05</td>
<td>4.1±0.04</td>
<td>4.1±0.04</td>
<td>4.0±0.04</td>
<td>&lt;.0001</td>
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<td>Triglyceride level</td>
<td>146±3.4</td>
<td>135±3.4</td>
<td>128±2.5</td>
<td>126±2.9</td>
<td>&lt;.0001</td>
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<tr>
<td>Cholesterol medication, %</td>
<td>14.6</td>
<td>14.6</td>
<td>15.6</td>
<td>18.1</td>
<td>0.22</td>
</tr>
<tr>
<td>Diabetic, %</td>
<td>17.1</td>
<td>11.6</td>
<td>12.1</td>
<td>11.9</td>
<td>0.01</td>
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<tr>
<td>Hormone replacement therapy, % (women)</td>
<td>27</td>
<td>35.1</td>
<td>35.6</td>
<td>30.9</td>
<td>0.07</td>
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<tr>
<td>Total energy, kcal/day</td>
<td>1623±30</td>
<td>1624±26</td>
<td>1599±27</td>
<td>1734±32</td>
<td>0.005</td>
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<tr>
<td>Alcohol, avg drinks/wk</td>
<td>5.8±0.53</td>
<td>4.8±0.32</td>
<td>4.7±0.33</td>
<td>4.5±0.28</td>
<td>0.08</td>
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<tr>
<td>Carbohydrate intake, g/day</td>
<td>199±3.8</td>
<td>201±3.2</td>
<td>199±3.3</td>
<td>210±3.8</td>
<td>0.1</td>
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<tr>
<td>Saturated fat, %kcal</td>
<td>11.2</td>
<td>11</td>
<td>10</td>
<td>9.5</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Linoleic acid, %kcal</td>
<td>6.8</td>
<td>6.4</td>
<td>6.7</td>
<td>6.6</td>
<td>0.007</td>
</tr>
<tr>
<td>Fruits, servings/day</td>
<td>1.5</td>
<td>1.7</td>
<td>1.8</td>
<td>2.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cruciferous veggies, serv/day</td>
<td>0.27</td>
<td>0.3</td>
<td>0.42</td>
<td>0.56</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

1 BMI, body mass index (kg/m²); SBP, systolic blood pressure; DBP, diastolic blood pressure; kcal, kilocalorie.

2 Test for trend across quartile ranks of non-fried fish intake.
Table 2. Brachial artery measures and non-fried fish intake, by gender.\textsuperscript{1}

<table>
<thead>
<tr>
<th>Brachial Measure</th>
<th>Overall (n = 3045)</th>
<th>Men (n = 1500)</th>
<th>Women (n = 1377)\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age-Adjusted</td>
<td>Fully-adjusted\textsuperscript{3}</td>
<td>Age-Adjusted</td>
</tr>
<tr>
<td></td>
<td>mean±SD</td>
<td>P</td>
<td>mean±SD</td>
</tr>
<tr>
<td>Baseline diameter\textsuperscript{5}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/Rare</td>
<td>4.43±0.03</td>
<td>&lt;.0001</td>
<td>4.51±0.04</td>
</tr>
<tr>
<td>1-3x/month</td>
<td>4.32±0.03</td>
<td>0.45</td>
<td>4.53±0.04</td>
</tr>
<tr>
<td>1-2x/week</td>
<td>4.27±0.03</td>
<td>0.34</td>
<td>4.77±0.03</td>
</tr>
<tr>
<td>&gt;2x/week</td>
<td>4.26±0.03</td>
<td>0.84</td>
<td>4.52±0.01</td>
</tr>
<tr>
<td></td>
<td>Maximum diameter\textsuperscript{6}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/Rare</td>
<td>4.50±0.004</td>
<td>0.48</td>
<td>4.45±0.01</td>
</tr>
<tr>
<td>1-3x/month</td>
<td>4.50±0.003</td>
<td>0.23</td>
<td>4.51±0.01</td>
</tr>
<tr>
<td>1-2x/week</td>
<td>4.49±0.003</td>
<td>0.17±0.01</td>
<td>4.98±0.01</td>
</tr>
<tr>
<td>&gt;2x/week</td>
<td>4.50±0.004</td>
<td>0.18±0.01</td>
<td>4.99±0.01</td>
</tr>
<tr>
<td></td>
<td>FMD, mm change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never/Rare</td>
<td>0.18±0.004</td>
<td>0.84</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>1-3x/month</td>
<td>0.18±0.003</td>
<td>0.14±0.01</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>1-2x/week</td>
<td>0.18±0.003</td>
<td>0.16±0.01</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>&gt;2x/week</td>
<td>0.18±0.004</td>
<td>0.17±0.01</td>
<td>0.19±0.01</td>
</tr>
<tr>
<td></td>
<td>FMD, % change\textsuperscript{7}</td>
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<td></td>
</tr>
<tr>
<td>Never/Rare</td>
<td>4.28±0.11</td>
<td>0.51</td>
<td>4.04±0.16</td>
</tr>
<tr>
<td>1-3x/month</td>
<td>4.52±0.09</td>
<td>0.20</td>
<td>3.78±0.11</td>
</tr>
<tr>
<td>1-2x/week</td>
<td>4.33±0.09</td>
<td>0.20</td>
<td>3.92±0.12</td>
</tr>
<tr>
<td>&gt;2x/week</td>
<td>4.47±0.10</td>
<td>0.21</td>
<td>3.94±0.17</td>
</tr>
</tbody>
</table>

\textsuperscript{1}FMD, brachial artery flow-mediated dilation; FMD % change = [(Maximum diameter – Baseline diameter)/ Baseline diameter] x 100%.
\textsuperscript{2}Adjustment for current hormone replacement therapy resulted in sample size loss of 168 women.
\textsuperscript{3}Multivariate linear regression analysis with adjustment for age, sex, race/ethnicity, body mass index, current smoking status, education level, and income level.
\textsuperscript{4}Multivariate linear regression analysis with adjustment for age, race/ethnicity, body mass index, current smoking status, education level, income level, and (in women) current hormone replacement therapy.
\textsuperscript{5}P value for [non-fried fish x sex] interaction = 0.0001.
\textsuperscript{6}Adjusted for baseline artery diameter.
\textsuperscript{7}P value for [non-fried fish x sex] interaction = 0.09.
Table 3. Brachial artery measures and non-fried fish intake, by racial/ethnic group.¹

<table>
<thead>
<tr>
<th>Brachial Measure</th>
<th>White (n = 1059)</th>
<th>Black (n = 619)</th>
<th>Chinese (n = 606)</th>
<th>Hispanic (n = 761)</th>
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</thead>
<tbody>
<tr>
<td>x fish frequency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age-Adjusted</td>
<td>Fully-Adjusted</td>
<td>Age-Adjusted</td>
<td>Fully-Adjusted</td>
</tr>
<tr>
<td></td>
<td>mean±SD</td>
<td>P</td>
<td>mean±SD</td>
<td>P</td>
</tr>
<tr>
<td>Baseline diameter³</td>
<td>3.47±0.06</td>
<td>0.14</td>
<td>4.40±0.10</td>
<td>0.48</td>
</tr>
<tr>
<td>Never/Rare</td>
<td>4.51±0.08</td>
<td>0.43</td>
<td>4.52±0.11</td>
<td>0.38</td>
</tr>
<tr>
<td>1-3x/month</td>
<td>4.33±0.08</td>
<td>0.36</td>
<td>4.53±0.09</td>
<td>0.53</td>
</tr>
<tr>
<td>1-2x/week</td>
<td>4.25±0.05</td>
<td>0.43</td>
<td>4.43±0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>&gt;2x/week</td>
<td>4.20±0.05</td>
<td>0.44</td>
<td>4.42±0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>Maximum diameter⁴</td>
<td>4.37±0.01</td>
<td>0.44</td>
<td>4.37±0.02</td>
<td>0.35</td>
</tr>
<tr>
<td>Never/Rare</td>
<td>4.59±0.01</td>
<td>0.16</td>
<td>4.60±0.02</td>
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<tr>
<td>1-3x/month</td>
<td>4.46±0.01</td>
<td>0.51</td>
<td>4.44±0.02</td>
<td>0.57</td>
</tr>
<tr>
<td>1-2x/week</td>
<td>4.46±0.01</td>
<td>0.44</td>
<td>4.44±0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>&gt;2x/week</td>
<td>4.38±0.01</td>
<td>0.40</td>
<td>4.38±0.02</td>
<td>0.40</td>
</tr>
<tr>
<td>FMD, mm change</td>
<td>0.18±0.01</td>
<td>0.39</td>
<td>0.17±0.02</td>
<td>0.40</td>
</tr>
<tr>
<td>Never/Rare</td>
<td>0.16±0.01</td>
<td>0.20</td>
<td>0.18±0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>1-3x/month</td>
<td>0.19±0.01</td>
<td>0.21</td>
<td>0.17±0.02</td>
<td>0.21</td>
</tr>
<tr>
<td>1-2x/week</td>
<td>0.19±0.01</td>
<td>0.19</td>
<td>0.16±0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>&gt;2x/week</td>
<td>0.19±0.01</td>
<td>0.19</td>
<td>0.16±0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>FMD, % change</td>
<td>4.38±0.22</td>
<td>0.34</td>
<td>4.13±0.49</td>
<td>0.83</td>
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<tr>
<td>Never/Rare</td>
<td>3.66±0.23</td>
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<td>4.04±0.42</td>
<td>0.13</td>
</tr>
<tr>
<td>1-3x/month</td>
<td>4.59±0.31</td>
<td>0.60</td>
<td>3.55±0.42</td>
<td>0.57</td>
</tr>
<tr>
<td>1-2x/week</td>
<td>4.70±0.18</td>
<td>0.73</td>
<td>3.73±0.34</td>
<td>0.48</td>
</tr>
<tr>
<td>&gt;2x/week</td>
<td>4.75±0.17</td>
<td>0.53</td>
<td>4.29±0.53</td>
<td>0.32</td>
</tr>
</tbody>
</table>

¹FMD, brachial artery flow-mediated dilation; FMD % change = [(Maximum diameter – Baseline diameter)/ Baseline diameter] x 100%.
²Multivariate linear regression analysis with adjustment for age, sex, body mass index, current smoking status, education level, and income level.
³P value for [non-fried fish x race/ethnicity] interaction = 0.24.
⁴Adjusted for baseline artery diameter.
<table>
<thead>
<tr>
<th>Brachial Measure</th>
<th>White (n = 380)</th>
<th>Black (n = 282)</th>
<th>Chinese (n = 558)</th>
<th>Hispanic (n = 384)</th>
</tr>
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<tbody>
<tr>
<td><strong>Age-Adjusted</strong></td>
<td><strong>Fully-Adjusted</strong></td>
<td><strong>Age-Adjusted</strong></td>
<td><strong>Fully-Adjusted</strong></td>
<td><strong>Age-Adjusted</strong></td>
</tr>
<tr>
<td><strong>EPA+DHA quartile</strong></td>
<td><strong>mean±SD</strong></td>
<td><strong>P</strong></td>
<td><strong>mean±SD</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>Baseline diameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>4.30±0.07</td>
<td>0.07</td>
<td>4.54±0.16</td>
<td>0.81</td>
</tr>
<tr>
<td>Q2</td>
<td>4.08±0.08</td>
<td>0.81</td>
<td>4.57±0.17</td>
<td>0.18</td>
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<tr>
<td>Q3</td>
<td>4.18±0.11</td>
<td>0.85</td>
<td>4.53±0.19</td>
<td>0.85</td>
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<tr>
<td>Q4</td>
<td>4.05±0.11</td>
<td>0.85</td>
<td>4.52±0.19</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Maximum diameter</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>4.37±0.01</td>
<td>0.91</td>
<td>4.34±0.03</td>
<td>0.85</td>
</tr>
<tr>
<td>Q2</td>
<td>4.38±0.01</td>
<td>0.91</td>
<td>4.34±0.03</td>
<td>0.85</td>
</tr>
<tr>
<td>Q3</td>
<td>4.36±0.01</td>
<td>0.91</td>
<td>4.32±0.03</td>
<td>0.85</td>
</tr>
<tr>
<td>Q4</td>
<td>4.38±0.01</td>
<td>0.91</td>
<td>4.34±0.03</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>FMD, mm change</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>0.19±0.01</td>
<td>0.99</td>
<td>0.16±0.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Q2</td>
<td>0.20±0.01</td>
<td>0.99</td>
<td>0.17±0.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Q3</td>
<td>0.18±0.01</td>
<td>0.99</td>
<td>0.15±0.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Q4</td>
<td>0.20±0.01</td>
<td>0.99</td>
<td>0.16±0.03</td>
<td>0.87</td>
</tr>
<tr>
<td><strong>FMD, % change</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>4.78±0.24</td>
<td>0.64</td>
<td>3.61±0.76</td>
<td>0.86</td>
</tr>
<tr>
<td>Q2</td>
<td>5.22±0.27</td>
<td>0.86</td>
<td>3.71±0.78</td>
<td>0.86</td>
</tr>
<tr>
<td>Q3</td>
<td>4.64±0.36</td>
<td>0.86</td>
<td>3.29±0.85</td>
<td>0.86</td>
</tr>
<tr>
<td>Q4</td>
<td>5.17±0.39</td>
<td>0.86</td>
<td>3.69±0.88</td>
<td>0.86</td>
</tr>
</tbody>
</table>

1 Concentrations of plasma phospholipid EPA and DHA (expressed as percentages of total fatty acids) were summed and ranked into quartiles from lowest to highest based on sample range.

2 EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FMD, brachial artery flow-mediated dilation; FMD % change = [(Maximum diameter – Baseline diameter)/ Baseline diameter] x 100%.

3 Multivariate linear regression analysis with adjustment for age, sex, body mass index, current smoking status, education level, and income level.

4 Adjusted for baseline artery diameter.
Figure 1

The figure shows the baseline diameter (nm) and flow-mediated dilation (%) change across different quartiles for men and women. The data is divided into two groups: plasma phospholipid EPA+DHA and non-fried fish intake. Symbols indicate statistical significance.

- Men:
  - Baseline diameter: Quartile 1 (4.9) to Quartile 4 (4.7)
  - Flow-mediated dilation: Quartile 1 (4.2) to Quartile 4 (4.4)

- Women:
  - Baseline diameter: Quartile 1 (4.0) to Quartile 4 (3.9)
  - Flow-mediated dilation: Quartile 1 (4.5) to Quartile 4 (4.0)
Figure 1. Brachial artery measures and plasma phospholipid EPA+DHA levels vs. non-fried fish intake, by gender. Results shown are least squares means ± SD after adjustment for age, race/ethnicity, body mass index, current smoking status, education level, income level, and (in women) current hormone replacement therapy. Concentrations of plasma phospholipid eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) levels (expressed as percentages of total fatty acids) were summed and ranked into quartiles from lowest to highest based on sample range. Sample size of each plasma phospholipid EPA+DHA quartile for men was as follows: Q1 = 204, Q2 = 170, Q3 = 196, and Q4 = 174. Sample size of each plasma phospholipid EPA+DHA quartile for women was as follows: Q1 = 159, Q2 = 207, Q3 = 174, and Q4 = 204 (adjustment for current hormone replacement therapy resulted in sample size loss of 106 women.) Non-fried fish intake quartiles were created based on the sample range, and represent frequency of dietary non-fried fish consumption classified as (1) rare/never, (2) 1-3x/month, (2) 1-2x/week, and (4) >2x/week. Sample size of each non-fried fish intake quartile for men was as follows: Q1 = 337, Q2 = 425, Q3 = 408, Q4 = 343. Sample size of each non-fried fish intake quartile for women was as follows: Q1 = 304, Q2 = 488, Q3 = 425, Q4 = 367.

*p trend < 0.05.
APPENDIX B

THE MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS:

FOOD FREQUENCY QUESTIONNAIRE

Example of Sample Questions

<table>
<thead>
<tr>
<th>MEAT AND POULTRY (Continued)</th>
<th>Average Last Year</th>
<th>Your Serving Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of Food</td>
<td>Rare or Never</td>
<td>1 Per Mo.</td>
</tr>
<tr>
<td>Fried chicken</td>
<td>friedchicken1</td>
<td>o</td>
</tr>
<tr>
<td>Liver including chicken livers, other organ meats</td>
<td>liver1</td>
<td>o</td>
</tr>
<tr>
<td>Gravies made with meat or poultry drippings</td>
<td>gravy1</td>
<td>o</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FISH (not including fish in the mixed dishes listed above)</th>
<th>Average Last Year</th>
<th>Your Serving Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fried fish or fish sandwich, fried shrimp, calamari</td>
<td>friedfish1</td>
<td>o</td>
</tr>
<tr>
<td>Shrimp, lobster, crab, oysters, mussels (not fried)</td>
<td>shrimp1</td>
<td>o</td>
</tr>
<tr>
<td>Tuna, salmon, sardines (including sashimi or sushi)</td>
<td>tuna1</td>
<td>o</td>
</tr>
<tr>
<td>Other broiled, steamed, baked or raw fish (trout, sole, halibut, poke, grouper)</td>
<td>poachedfish1</td>
<td>o</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SWEETS</th>
<th>Average Last Year</th>
<th>Your Serving Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar, jelly, jam, molasses on bread or cereal</td>
<td>jelly1</td>
<td>o</td>
</tr>
<tr>
<td>Regular ice cream</td>
<td>regicecream1</td>
<td>o</td>
</tr>
<tr>
<td>Frozen yogurt, low-fat ice cream, ice milk, sherbert</td>
<td>frozenyogurt1</td>
<td>o</td>
</tr>
<tr>
<td>Dessert made with tofu</td>
<td>tofodessert1</td>
<td>o</td>
</tr>
<tr>
<td>White doughnuts, cookies, cakes, pastries, Pop Tarts, Chinese desserts, Mexican desserts</td>
<td>whitedaughnut1</td>
<td>o</td>
</tr>
</tbody>
</table>
CURRICULUM VITAE

Jennifer Shultz Anderson, MD, PhD, MEd

Education and Training


University of Virginia. M.Ed. in Exercise Physiology. May 1998.


University of Washington School of Medicine- M.D. June 2006.

University of Washington Internal Medicine Residency Program, July 2006-June 2009.

Wake Forest University Cardiology Fellow/Research Fellow Program, July 2009-present.

State Licensure

- North Carolina Medical Board, 12/29/09-present
- Washington State Medical Board, 8/29/08-6/25/10

Board Certification/Other Certifications

- American Board of Internal Medicine, certification 8/20/09-present
- Advanced Care Life Support and Basic Life Support/CPR, 4/2007-present

Publications


Nettleton JA et al. Interactions of whole grain intake with select SNPs previously identified as predictive of fasting glucose and fasting insulin concentrations: Results from a 14-cohort meta-analysis. Diabetes Care 2010 (in press). (Anderson JS = first author for Health ABC contribution to this meta-analysis.)

Kanoni S et al. Interactions between zinc intake, fasting glucose, and SNPs associated with fasting glucose: a meta-analysis. In press 2010. (Anderson JS = first author for Health ABC contribution to this meta-analysis.)


**Presentations**


**Grants**


**Professional Memberships/Honors**

- American Heart Association, member 2010-present
- American Society for Nutrition, Nutrient-Gene Interaction Research Interest Group, member 2009-present
- American College of Physicians, member 2007-present
- American Society for Nutrition, member, 2009-present
- American Medical Student Association/American Medical Association, 2002-present
- University of Washington Magnuson Scholar 2000-2001
Extracurricular/Volunteer Work

- **BestHealth** community lecture series on health issues, Winston-Salem, NC, March 24, 2010
- **WXii Television**, news spotlight on Heart Health Month, Winston-Salem, NC, January 21, 2010
- **Boise Veterans Affairs Medical Center Cardiac Health and Fitness Program**, Boise, ID, 2007-2008, *Developer/Director*
- **University of Washington School of Medicine First Year Curriculum Development Committee**, 2002-2003, *Student Representative*
- **Center for Human Development and Disability**, Fall 2000, *Volunteer / Pediatric Nutrition Trainee*
- **University of Washington Interdisciplinary Graduate Program, Curriculum Development Committee**, 1999-2000, *Student Representative*
- **University of Washington Interdisciplinary Graduate Program, Admissions Committee**, 1998-1999 academic year, *Student Representative*
- An avid enthusiast of running/cycling/triathlons, riding/training horses, and spending quality time with my boys in the Great Outdoors