Plasma n-3 and n-6 Fatty Acids Are Differentially Related to Carotid Plaque and Its Progression

MESA (the Multi-Ethnic Study of Atherosclerosis)

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Objective—ω-3 (n-3) fatty acids (FAs) have long been considered healthful dietary components, yet recent clinical trials have questioned their cardiovascular benefits. By contrast, the ω-6 (n-6) FAs have been considered harmful, proatherogenic macronutrients, despite an absence of empirical evidence supporting this hypothesis. We aimed to determine whether plasma n-3 and n-6 FAs are related to risk of carotid plaque and its progression in 3327 participants of the MESA (Multi-Ethnic Study of Atherosclerosis).

Approach and Results—Carotid plaque was assessed using ultrasonography at baseline and after a median period of 9.5 years. Plasma phospholipid n-3 and n-6 FAs were determined using gas chromatography-flame ionization detection. Relative risk regression analyses assessed the relations of FAs with the presence or progression of carotid plaque adjusted for typical cardiovascular disease risk factors. At baseline, it was found that participants in the fourth quartile of n-3 docosahexaenoic acid showed a 9% lower risk of carotid plaque (P=0.05), whereas those in the second quartile of n-3 α-linolenic acid showed an 11% greater risk compared with respective referent quartiles (P=0.02). In prospective analyses, individuals in the top quartile of docosahexaenoic acid showed a 12% lower risk of carotid plaque progression during 9.5 years compared with those in the referent quartile (P=0.002). No significant relations were observed among n-6 FAs and plaque outcomes. No significant race/ethnicity interactions were found.

Conclusions—These findings support docosahexaenoic acid as an atheroprotective macronutrient, whereas null findings for n-6 FAs challenge the view that they promote atherosclerosis. **(*Arterioscler Thromb Vasc Biol*. 2018;38:00-00. DOI: 10.1161/ATVBAHA.117.310366.)**

Key Words: atherosclerosis ■ fatty acids ■ humans ■ prospective studies ■ risk factors

Atherosclerosis is a pathophysiological process involving an insult to the vascular endothelium followed by inflammation, endothelial activation, oxidative stress, and lipid accumulation in the arterial wall resulting in an atherosclerotic plaque and vessel occlusion. Atherogenesis has been shown to occur as early as adolescence,[1] and it is, therefore, imperative that modifiable risk factors be identified and controlled at a young age. Among these, long-chain ω-3 (n-3) and ω-6 (n-6) polyunsaturated fatty acids (FAs) have been, respectively, characterized as anti- and proatherogenic dietary components, yet findings among studies are inconsistent, and no large multiethnic prospective study has examined these FAs in relation to subclinical atherosclerosis outcomes.

The n-3 FAs are a well-studied class of macronutrients considered to be atheroprotective and include the plant-derived α-linolenic acid (ALA) and fish oil eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Their plasma concentrations have been shown to be inversely associated with inflammation and endothelial activation[2–7] and have been associated with lower risk of cardiovascular outcomes in cohort studies.[8,9] And yet, null results from recent meta-analyses and large randomized controlled trials[10–12] have cast uncertainty as to the cardiovascular health benefits of n-3 FAs.

In contrast to the n-3 FAs, the n-6 FAs are not as well studied but have previously been suggested to be proinflammatory, proatherogenic compounds.[13] This view likely originated with the observation that the essential n-6 FA, linoleic acid (LA) can be converted to arachidonic acid (AA)—a substrate for eicosanoid lipid mediators that promote vascular disease.[13,14] However, this presumption has been shifting,[15] and it has since been shown that both LA and AA may have cardiovascular benefits.[5,16–18] By contrast, the n-6 FAs γ-linolenic acid and

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Nonstandard Abbreviations and Acronyms	
ω-3	n-3
ω-6	n-6
AA	arachidonic acid
ALA	α-linolenic acid
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acid
LA	linoleic acid
MESA	Multi-Ethnic Study of Atherosclerosis

dihomo-γ-linolenic acid—primarily synthesized denovo from LA—have been associated with inflammation and endothelial activation,[5] but it is unknown whether higher levels have implications for atherosclerosis. No large prospective cohort study has examined plasma n-6 FAs and atherosclerosis outcomes to date.

The current study of 3283 MESA (Multi-Ethnic Study of Atherosclerosis) participants without apparent cardiovascular disease at baseline examined whether objectively measured plasma levels of n-3 or n-6 FAs were associated with the presence of carotid plaque or occurrence of plaque progression during a median 9.5-year study period and whether race/ethnicity modified any observed associations.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Demographic and clinical characteristics of 3283 MESA participants are presented in Table 1, stratified by the presence of carotid plaque at baseline. Compared with individuals with carotid plaque at baseline, those without carotid plaque had a lower mean age (P<0.001), were more likely to be women (P=0.003), and more likely to have never smoked (P<0.001). Those without carotid plaque additionally had lower mean systolic blood pressure (P<0.001), were less likely to have diabetes mellitus (P<0.001), had lower levels of total cholesterol (P<0.001), and had greater levels of plasma LA (P<0.001) and DHA (P=0.03).

Characteristics of MESA participants were stratified by the occurrence of carotid plaque progression in Table 2. Significant differences were found between those who exhibited plaque progression and those who did not during the median 9.5-year study period. Individuals in whom plaque progression did not occur had a lower mean age (P<0.001), body mass index (P=0.016), systolic blood pressure (P<0.001), total cholesterol (P=0.04), had fewer current and former smokers (P<0.001), and fewer individuals taking medication for hypertension (P<0.001) or lipids (P<0.001). In addition, this group was found to have greater levels of HDL-C (P=0.002), plasma DHA (P=0.005), and plasma LA (P=0.003) compared with those who exhibited plaque progression.

Risk of carotid plaque at baseline was evaluated in 3314 MESA participants and is presented across quartiles of plasma n-3 and n-6 FAs in Table 3. Individuals in the second quartile of the n-3 ALA were found to have a significantly 11% greater risk of having plaque than those in the referent (P=0.02), although no significant relations were observed in third or

Table 1. Demographic and Clinical Characteristics of 3327 Multi-Ethnic Study of Atherosclerosis Participants by the Presence of Carotid Plaque at Baseline

	All	Presence of Plaque (+)	Absence of Plaque (–)	*P* Value
n	3327	1566	1761	
Age, y (SD)	60.3 (9.4)	63.0 (9.2)	57.9 (8.9)	<0.001
Sex, n (women, %)	1565 (47.0)	780 (49.8)	785 (44.6)	0.003
Race/ethnicity, n (%)				<0.001
White	1303 (39.2)	684 (43.7)	619 (35.2)	
Chinese	439 (13.2)	159 (10.2)	280 (15.9)	
Black	870 (26.1)	411 (26.2)	459 (26.1)	
Hispanic	715 (21.5)	312 (19.9)	403 (22.9)	
Smoking, n (%)				<0.001
Never	1727 (52.0)	720 (46.0)	1007 (57.3)	
Former	1216 (36.6)	633 (40.5)	583 (33.2)	
Current	379 (11.4)	211 (13.5)	168 (9.6)	
BMI, kg/m^2; mean (SD)	28.3 (5.3)	28.4 (5.2)	28.1 (5.3)	0.11
SBP, mm Hg; mean (SD)	124.3 (20.2)	128.0 (20.7)	121.0 (19.1)	<0.001
Hypertension medication, n (%)	1163 (35.0)	656 (41.9)	507 (28.8)	<0.001
Lipid-lowering medication, n (%)*	542 (16.3)	338 (21.6)	204 (11.6)	<0.001
Diabetes mellitus (treated or untreated), n (%)	330 (9.9)	213 (13.6)	117 (6.7)	<0.001
Total cholesterol, mmol/L; mean (SD)	5.03 (0.91)	5.10 (0.95)	4.96 (0.85)	<0.001
HDL-C, mmol/L; mean (SD)	1.32 (0.39)	1.31 (0.39)	1.33 (0.38)	0.32
ω-3 PUFAs, mean (SD)†				
ALA	0.17 (0.074)	0.17 (0.080)	0.17 (0.068)	0.57
EPA	0.93 (0.84)	0.93 (0.87)	0.93 (0.81)	0.84
DHA	3.88 (1.49)	3.82 (1.46)	3.94 (1.51)	0.030
ω-6 PUFAs, mean (SD)				
LA	20.24 (3.27)	19.96 (3.18)	20.50 (3.33)	<0.001
GLA	0.11 (0.055)	0.12 (0.054)	0.11 (0.056)	0.16
DGLA	3.18 (0.83)	3.20 (0.81)	3.17 (0.84)	0.33
AA	11.8 (2.54)	11.9 (2.54)	11.7 (2.53)	0.06

AA indicates arachidonic acid; ALA, α-linolenic acid; BMI, body mass index; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; and PUFA, polyunsaturated fatty acid.

*At baseline.

†PUFAs are expressed as percentage of total plasma phospholipid fatty acids.

Table 2. Demographic and Clinical Characteristics of 3296 Multi-Ethnic Study of Atherosclerosis Participants by the Occurrence of Carotid Plaque Progression During the 9.5-y Study Period

	All	Plaque Progression (+)	No Plaque Progression (–)	*P* Value
n	3296	1854	1442	
Age, y (SD)	60.3 (9.4)	62.3 (9.2)	57.7 (8.9)	<0.001
Sex, n (women, %)	1547 (46.9)	917 (49.5)	630 (43.7)	<0.001
Race/ethnicity, n (%)				0.002
White	1292 (39.2%)	775 (41.8%)	517 (35.9%)	
Chinese	435 (13.2%)	218 (11.8%)	217 (15.0%)	
Black	862 (26.2%)	471 (25.4%)	391 (27.1%)	
Hispanic	707 (21.5%)	390 (21.0%)	317 (22.0%)	
Smoking, n (%)				<0.001
Never	1707 (51.9%)	876 (47.3%)	831 (57.7%)	
Former	1208 (36.7%)	727 (39.3%)	481 (33.4%)	
Current	376 (11.4%)	248 (13.4%)	128 (8.9%)	
BMI, kg/m^2; mean (SD)	28.3 (5.3)	28.4 (5.2)	28.0 (5.3)	0.016
SBP, mm Hg; mean (SD)	124.3 (20.2)	127.1 (20.4)	120.6 (19.3)	<0.001
Hypertension medication, n (%)	1150 (34.9%)	743 (40.1%)	407 (28.2%)	<0.001
Lipid-lowering medication, n (%)*	1297 (39.4%)	816 (44.0%)	481 (33.4%)	<0.001
Diabetes mellitus, n (%)	328 (10.0%)	236 (12.7%)	92 (6.4%)	<0.001
Total cholesterol, mmol/L; mean (SD)	5.02 (0.91)	5.05 (0.92)	4.99 (0.89)	0.04
HDL-C, mmol/L; mean (SD)	1.32 (0.39)	1.30 (0.38)	1.34 (0.39)	0.002
ω-3 PUFAs, mean (SD)†				
ALA	0.17 (0.07)	0.17 (0.08)	0.17 (0.07)	0.9
EPA	0.93 (0.84)	0.92 (0.83)	0.94 (0.86)	0.5
DHA	3.88 (1.49)	3.82 (1.46)	3.97 (1.52)	0.005
ω-6 PUFAs, mean (SD)				
LA	20.2 (3.27)	20.1 (3.24)	20.4 (3.30)	0.003
GLA	0.11 (0.06)	0.16 (0.05)	0.11 (0.06)	0.20
DGLA	3.18 (0.83)	3.20 (0.82)	3.16 (0.84)	0.13
AA	11.8 (2.54)	11.8 (2.56)	11.7 (2.51)	0.36

AA indicates arachidonic acid; ALA, α-linolenic acid; BMI, body mass index; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; HDL-C, high-density lipoprotein cholesterol; LA, linoleic acid; PUFA, polyunsaturated fatty acid; and SBP=systolic blood pressure.

*At the time of Examination 5.

†PUFAs are expressed as percentage of total plasma phospholipid fatty acids.

Table 3. Risk of Carotid Plaque (Absent or Present) at Baseline Is Presented Across Quartiles of Plasma n-3 and n-6 Fatty Acids in 3314 Multi-Ethnic Study of Atherosclerosis Participants

	Fatty Acid Quartile			
	1	2	3	4
ω-3 FAs				
ALA (18:3)	Ref	1.11 (1.02–1.21)*	1.05 (0.96–1.16)	1.06 (0.97–1.17)
EPA (20:5)	Ref	1.02 (0.93–1.12)	1.00 (0.91–1.10)	0.99 (0.90–1.09)
DHA (22:6)	Ref	0.95 (0.87–1.04)	0.96 (0.88–1.05)	0.91 (0.82–0.99)*
ω-6 FAs				
LA (18:2)	Ref	1.06 (0.97–1.15)	1.03 (0.94–1.12)	0.99 (0.89–1.09)
GLA (18:3)	Ref	1.02 (0.92–1.12)	1.03 (0.94–1.13)	1.00 (0.91–1.10)
DGLA (20:3)	Ref	1.04 (0.95–1.14)	0.97 (0.89–1.07)	1.04 (0.94–1.14)
AA (20:4)	Ref	1.08 (0.98–1.18)	1.02 (0.93–1.12)	1.06 (0.96–1.17)

First quartiles serve as referents. Adjustments were made for age, sex, race/ethnicity, BMI, smoking status, systolic blood pressure, hypertension medication use, diabetes mellitus, lipid-lowering medication use, HDL-C, and total cholesterol. Values are expressed as relative risk (95% confidence intervals). AA indicates arachidonic acid; ALA, α-linolenic acid; BMI, body mass index; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; GLA, γ-linolenic acid; HDL-C, high-density lipoprotein cholesterol; and LA, linoleic acid.

*P<0.05.

fourth quartiles. Participants in the fourth quartile of DHA and in n-3/n-6 ratio were found to have a 9% and 17% lower risk of carotid plaque compared with referent quartiles. Additional analyses of highly unsaturated n-6 FAs docosapentaenoic acid (22:5) and adrenic acid (22:4) were conducted, and no relations with carotid plaque at baseline were found. Interaction analysis revealed no modification effect of race/ethnicity on associations between FAs and baseline carotid plaque.

Risk of carotid plaque progression in 3283 MESA participants is presented across quartiles of plasma n-3 and n-6 FAs in Table 4. Carotid plaque progression was treated as a logistical variable (0 or 1). Individuals in the top quartile of DHA showed a 12% lower risk of carotid plaque progression than those in the referent (P=0.002). No significant relations of n-6 FAs docosapentaenoic acid and adrenic acid with carotid plaque progression were observed. Interaction analysis revealed no modification effect of race/ethnicity on associations between FAs and carotid plaque progression.

Secondary analyses of n-3 and n-6 exposure variables with carotid plaque outcomes were conducted (Table I in the online-only Data Supplement). Those in the top quartile of the n-3/n-6 ratio were found to be at a significantly lower risk of having carotid plaque at baseline (P=0.03), whereas those in the top quartiles of EPA+DHA and total n-3 FAs were found to

Table 4. Risk of Carotid Plaque Progression Is Presented Across Quartiles of Plasma ω-3 and ω-6 Fatty Acids in 3283 Multi-Ethnic Study of Atherosclerosis Participants

	Fatty Acid Quartile			
	1	2	3	4
ω-3 FAs				
ALA (18:3)	Ref	1.08 (1.00–1.17)	1.05 (0.97–1.13)	1.08 (1.00–1.17)
EPA (20:5)	Ref	1.03 (0.95–1.11)	1.03 (0.95–1.11)	0.98 (0.91–1.07)
DHA (22:6)	Ref	0.94 (0.88–1.01)	0.93 (0.86–1.00)	0.88 (0.81–0.95)*
ω-6 FAs				
LA (18:2)	Ref	1.01 (0.93–1.09)	1.01 (0.93–1.08)	0.98 (0.91–1.07)
GLA (18:3)	Ref	1.01 (0.93–1.09)	1.01 (0.93–1.09)	1.00 (0.93–1.09)
DGLA (20:3)	Ref	1.05 (0.97–1.13)	0.98 (0.90–1.06)	1.05 (0.97–1.14)
AA (20:4)	Ref	1.00 (0.92–1.08)	1.05 (0.97–1.13)	1.04 (0.96–1.13)

First quartiles serve as referents. Adjustments were made for age, sex, race/ethnicity, BMI, hypertension medication use, smoking status, systolic blood pressure, diabetes mellitus, lipid-lowering medication use (as a time-dependent variable), HDL-C, and total cholesterol. Carotid plaque progression was treated as a logistical variable, and values are expressed as relative risk (95% confidence intervals). AA indicates arachidonic acid; ALA, α-linolenic acid; BMI, body mass index; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; GLA, γ-linolenic acid; HDL-C, high-density lipoprotein cholesterol; and LA, linoleic acid.

*P<0.01.

be at a lower risk of plaque progression (P=0.01 and P=0.02, respectively). No significant relations between total n-6 FAs and either the presence or progression of carotid plaque were observed.

Discussion

In this prospective study of 3314 MESA participants, individuals in the top quartile of plasma DHA were found to have a 9% lower risk of carotid plaque at baseline and 12% lower risk of carotid plaque progression during the 9.5-year follow-up period compared with referent quartiles. These findings support previous evidence that DHA promotes cardiovascular health. By contrast, individuals in the second quartile of ALA were found to have an 11% greater risk for carotid plaque compared with the referent quartile at baseline, but no significant risk was observed for those in subsequent quartiles, and no associations were observed in prospective analyses. The null findings for plasma n-6 FAs with carotid atherosclerosis outcomes dispute the hypothesis that they are proatherogenic macronutrients. Additional secondary analyses of plasma n-3 and n-6 exposure variables were conducted. Plasma EPA+DHA, total n-3 FAs, and the ratio of n-3 to n-6 FAs showed inverse associations with plaque outcomes similar to those of DHA alone (although incrementally weaker). Total n-6 FA levels were not associated with the presence or progression of carotid plaque.

Fish Oil n-3 FAs

Fish oil FAs have been shown to have numerous cardiovascular benefits that would be expected to constrain atherogenesis, including, but not limited to, reducing elevated triglyceride levels, modestly raising HDL-C,[19] enhancing endothelial function,[20] and lowering blood pressure.[21] More recently, EPA and DHA have been shown to actively resolve inflammation through metabolism in to potent lipid signaling compounds[22–24] and may also facilitate efferocytosis[25,26]—that is, the process by which apoptotic cells are removed by phagocytic cell, critical in suppressing necrotic plaque formation.[27] Consistent with these antiatherogenic effects, observational studies have shown that higher levels of EPA or DHA are associated with lower prevalence of coronary and carotid atherosclerosis as determined by angiography[28] and ultrasound,[29,30] although null findings have also been reported.[31–33]

Our results indicate that individuals in the top quartiles of plasma DHA (>4.8% of total phospholipids), but not EPA, have lower risks of showing atherosclerotic plaque or experiencing plaque progression during a ≈10-year period. The magnitude of associations for DHA (9% lower risk for plaque prevalence and 12% for plaque progression) suggests a modest-to-moderate preventive influence on plaque outcomes when compared with other well-documented risk factors. For example, in this sample, current cigarette smokers showed a 38% greater risk of plaque progression compared with nonsmokers (P<0.001; data not shown). It may be speculated that the comparatively lower strength of associations for DHA may have contributed to the mixed findings among previous studies, particularly in those with shorter follow-up periods.

The null findings for EPA were unexpected and counter to the above observational studies and intervention studies that have shown that EPA supplementation reduces atherosclerosis.[34–37] It may be speculated that the relatively low EPA levels in MESA participants may have contributed to the null results. This is supported by a cross-sectional study that examined associations of serum EPA with carotid atherosclerosis in white Americans, Japanese Americans, and native Japanese.[33] High serum EPA levels were found to be related to lower carotid intima media thickness (P=0.004) but only in native Japanese. It was concluded that higher intakes of fish in Japanese living in Japan resulted in higher serum EPA concentrations (EPA=2.5%) and accounted for the lower carotid atherosclerosis—a relation not observed in white (EPA=0.8%) or Japanese-American (EPA=1.0%) participants.[33] Given that the mean EPA level in MESA participants was 0.9%, low levels may have resulted in the null finding and may also have contributed to the above modest findings for DHA. Study samples with greater fish intakes would provide a more comprehensive assessment of the antiatherogenic potential of the fish oil n-3 FAs.

Plant-Derived n-3 ALA

Compared with the fish oil FAs, the plant-derived n-3 ALA is less well-researched but has also been proposed to be antiatherogenic. And yet, plasma ALA concentrations have previously been shown to be unrelated to coronary atherosclerosis,[28] and randomized controlled trials and intervention studies have

shown that ALA has no effect on markers of inflammation, endothelial activation, lipid levels,[38,39] or carotid atherosclerosis.[7] In the present analysis, the greater risk of carotid plaque observed in the second quartile of ALA at baseline was unexpected, but null findings in subsequent quartiles and for the plaque progression outcome suggest that ALA levels are not a risk factor for carotid plaque.

n-6 FAs

In contrast with the n-3 FAs, the n-6 FAs have been characterized, and potentially mischaracterized, as proinflammatory and proatherogenic FAs that are overrepresented in Western diets.[13,14] And although it has been suggested that dietary LA metabolizes to AA and subsequently to inflammatory eicosanoids (which may then promote atherogenesis), there is little empirical evidence to support this. First, these processes are tightly regulated and not driven by principles of mass action. Higher dietary LA intakes do not increase plasma or cell membrane AA levels,[40] and intervention studies in humans have failed to demonstrate that LA or AA supplementation results in greater leukotriene synthesis or inflammation.[41–44]

To date, no large cohort studies have examined n-6 FAs and atherosclerosis outcomes, but studies of cardiovascular events have been conducted. A meta-analysis of 25 case–control and prospective studies showed that plasma LA was associated with lower risk of incident nonfatal CHD (P<0.01) but not fatal CHD (P=0.51).[17] Similar to LA, higher plasma phospholipid AA levels have been shown to be associated with a 35% and 14% lower risk of incident CHD in 2 cohort studies,[18,45] although a previous meta-analysis of 18 prospective studies found no relation of plasma phospholipid AA with CHD events.[17] Our results for AA are similar to the meta-analysis findings because no relations between n-6 FAs and carotid atherosclerosis were observed. Overall, our findings suggest n-6 FAs have no influence on atherosclerosis, but given the limited research in this area, further studies of n-6 FAs and atherosclerosis outcomes are warranted.

Future Research

Previous n-3 and n-6 research has largely focused on typical atherogenic mechanisms, including inflammation, endothelial function, and blood lipids. Yet growing evidence suggests that n-3 FAs also affect plaque phenotype. For example, it has been shown that patients administered short-term n-3 FA treatment have atherosclerotic plaques with greater proportions of n-3 FAs and thicker fibrous caps, indicating greater plaque stability.[46] Additional studies in cell culture and animal models have found that n-3 FAs stimulate efferocytosis,[25,47] which would, in turn, promote plaque stability. Whether these experimental findings translate to reduced clinical events and whether n-6 FAs may affect plaque phenotype requires further research.

Strengths and Limitations

The present study represents one of the largest prospective analyses of plasma phospholipid FAs and carotid atherosclerosis in a multiethnic cohort and is the first to examine plasma n-6 FAs and atherosclerotic plaque outcomes. The prospective design and approximate 10-year follow-up period allowed for temporality of associations to be evaluated. In addition, ultrasound measurement precision was strong with interreader and intrareader reproducibility of 0.95 and 0.99, respectively. In terms of limitations, all study participants in this subcohort completed Examination 5, and a selection bias for healthier individuals with less carotid atherosclerosis than those in the entire cohort is, therefore, possible. Plasma phospholipids were measured at Examination 1, and the possibility of subsequent changes in plasma FA levels cannot be evaluated. In addition, null results for γ-linolenic acid, n-6 docosapentaenoic acid, and adrenic acid should be interpreted with caution because of their low plasma concentrations (mean levels <0.5%) and relatively higher coefficients of variation compared with other long-chain FA measurements. Adjustments were made for demographic and clinical risk factors in the statistical model, but residual confounding remains possible. Finally, MESA is a US cohort study, and findings may not be generalizable to other populations.

Conclusions

Our findings support a protective influence of DHA on atherosclerotic plaque burden, whereas null findings for the n-6 FAs challenge the notion that they promote atherosclerosis. Continued research is necessary to further scrutinize the roles of n-3 and particularly the n-6 FAs in atherosclerotic plaque development, progression and stability, as well as hard cardiovascular end points.

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Disclosures

None.

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Highlights

- Plasma ω-3 and ω-6 fatty acids were differentially related to carotid plaque prevalence and progression during an approximate 10-year period in 3327 participants of a multiethnic US cohort.
- High levels of the fish oil ω-3, docosahexaenoic acid, were related to lower risk of atherosclerotic plaque prevalence and progression.
- No relations were found among ω-6 fatty acids and carotid plaque.
- No influence of race was observed.
- The results support the narrative that fish oil has cardiovascular health benefits independent of race/ethnicity, whereas ω-6 fatty acids did not appear to be proatherogenic in this sample.

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Plasma n-3 and n-6 Fatty Acids Are Differentially Related to Carotid Plaque and Its Progression: MESA (the Multi-Ethnic Study of Atherosclerosis)

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Supplemental Material

Supplemental Table I. Secondary analyses of plasma omega-3 and -6 variables with carotid plaque outcomes were conducted in MESA participants. First quartiles serve as referents. Adjustments were made for age, sex, race/ethnicity, BMI, hypertension medication use, smoking status, systolic blood pressure, diabetes, lipid lowering medication use (as a time dependent variable), HDL-C, and total cholesterol. Values are expressed as relative risk (95% confidence intervals). Carotid plaque outcomes were treated as logistical variables (0 or 1).

	Fatty acid variable quartile			
	1	2	3	4
Baseline carotid plaque (n=3327)				
EPA + DHA	Ref	0.97 (0.89 - 1.06)	0.95 (0.87 - 1.05)	0.93 (0.85 - 1.02)
Total n-3 FAs	Ref	0.97 (0.89 – 1.06)	0.96 (0.88 – 1.05)	0.92 (0.84 – 1.01)
N3/N6 ratio	Ref	0.94 (0.86 – 1.02)	0.96 (0.87 – 1.05)	**0.90 (0.82 – 0.99)***
Total n-6 FAs	Ref	1.03 (0.92 – 1.16)	1.05 (0.94 – 1.18)	1.05 (0.93 – 1.18)
Carotid plaque progression (n=3296)				
EPA + DHA	Ref	0.94 (0.88 - 1.02)	0.93 (0.86 - 1.01)	**0.90 (0.83 - 0.98)†**
Total n-3 FAs	Ref	0.96 (0.89 - 1.03)	0.95 (0.88 - 1.03)	**0.90 (0.83 - 0.98)†**
N3/N6 ratio	Ref	0.97 (0.90 - 1.05)	0.98 (0.91 - 1.07)	0.93 (0.86 - 1.01)
Total n-6 FAs	Ref	1.00 (0.93 - 1.08)	1.01 (0.93 - 1.09)	1.01 (0.94 - 1.10)

*$p<0.05$

†<0.02

MATERIALS AND METHODS

Study Population

MESA study design and methods have been previously described (1), and information regarding study protocol is available online (www.mesa-nhlbi.org). Briefly, 6,814 men and women aged 45 to 84 years without clinical evidence of CV disease were recruited from six communities in the United States (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; New York, NY; and St. Paul, MN). All participants gave informed consent, and Institutional Review Board approval was obtained at all MESA sites.

Recruitment and the baseline examination of MESA were conducted between the years of 2000-2002. Information regarding age, sex, ethnicity, education, and lifestyle factors including smoking status, physical activity, and dietary intake were obtained through questionnaires. Height and weight were measured by trained staff according to standard procedures. Body mass index (BMI) was calculated as weight (kg)/ height (m^2). Fasting blood was drawn and serum and EDTA-anticoagulant tubes were collected and processed using a standardized protocol (2) and samples were aliquoted and stored at -70^{o}C until time of use.

Carotid Ultrasonography

B-mode ultrasonography was conducted at Exam 1 (2000-2002) and Exam 5 (2010-2012) on 3,441 MESA participants as described by Tattersall et al. (3). Images of the right and left common, bifurcation, and internal carotid arterial segments were obtained and converted into digital records. Using reference images from baseline, certified sonographers matched the scanning conditions of the initial study including display depth, internal landmarks, angle of approach, degree of jugular venous distension, and ultrasound system settings. Images were interpreted by the University of Wisconsin MESA Carotid Ultrasound Reading Center. Carotid

plaque was defined as a discrete, focal wall thickening ≥1.5 cm or focal thickening at least 50% greater than the surrounding intima media. Kappa values for intra- and inter-reader reproducibility of carotid plaque were 0.83 (95% confidence interval [CI] 0.70–0.96) and 0.89 (95% CI 0.72–1.00), respectively.

Plasma fatty acid profile

Phospholipid fatty acids were measured in EDTA plasma using the method described by Cao et al (4). Lipids were extracted from the plasma using a chloroform/methanol method, and cholesterol esters, triglyceride, phospholipids and free fatty acids were separated by thin layer chromatography. Fatty acids from the phospholipids were derivatized to methyl esters and measured by gas chromatography-flame ionization detection. Values were expressed as a percent of the total phospholipid fraction, and the total was comprised of the following fatty acids: tetradecanoic, pentadecanoic, two isomers of hexadecanoic, heptadecanoic, five isomers of octadecenoic, six isomers of octadecadienoic, eicosanoic (20:0), stearodonic, eicosenoic (20:1n9), eicosadienoic, eicosatrienoic, eicosatetraenoic, docosanoic, eicosapentaenoic, docosatetraenoic, tetracosanoic, docosapentaenoic (22:5n6), tetracosanoic, docosapentaenoic (22:5n3), and docosahexaenoic acids. Coefficients of variation were obtained from intra-laboratory quality control (n=20): LA 2.6%; GLA 16.4% ; DGLA 9.2%; AA, 2.4%; n-6 DPA 44%; adrenic acid 22%; ALA, 2.4%; EPA, 3.3%; and DHA, 2.7%.

Methods/Analysis plan

Statistical analysis was conducted using Stata (version 14.1, Stata Corp, College Station, TX). Baseline characteristics are presented as means (SD) for continuous variables and frequencies (%) for categorical variables. A relative risk model was used to assess association

between each fatty acid and plaque presence (>0 vs =0) at baseline (visit 1), adjusting for age, gender, race, BMI, smoking, hypertension medication, systolic blood pressure, diabetes, lipid lowering medication use, total cholesterol, and HDL-C. Fatty acids were transformed into quartiles to obtain robust associations in case of nonlinear relationships. The total n-3 fatty acid variable included ALA, EPA, DPA, and DHA; the total n-6 fatty acid variable included LA, GLA, DGLA, and AA. The n-3/n-6 ratio is the quotient of these two variables. Progression of carotid plaque was examined as a binary variable (plaque score increased vs unchanged between visits 1 and 5). Risk of carotid plaque progression was analyzed using a similar relative risk regression model with each fatty acid separately, adjusting for the same covariates as above. Interactions with race/ethnicity were additionally tested.

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