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.

References

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