MATERIALS AND METHODS

Population

The primary aim of MESA is to investigate subclinical atherosclerosis, its progression, and clinical cardiovascular outcomes. Information regarding study design and exclusion criteria have been described previously (1) and is available at www.mesa-nhlbi.org. Briefly, men and women (N=6,814) without clinical evidence of CHD and between the ages of 45 and 84 years 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). MESA is composed of 38.6% white, 27.6% black, 11.8% Chinese and 22.0% Hispanic men and women. All MESA sites received Institutional Review Board approval, and all participants gave informed consent.

In the present analysis, individuals taking lipid-lowering medication at baseline were excluded (N= 1187). An additional 948 participants of the original MESA 1000, a subcohort of randomly selected individuals enrolled prior to February 2002, were also excluded due to the unavailability of sample. Additional exclusion criteria are found in CHD outcomes below. The remaining population (N= 4,679) was representative of the MESA cohort, composed of the following races/ethnicities: 28.8% Black, 12.0% Chinese American, 22.8% Hispanic, and 36.6% White participants.

Lipid and Lipoprotein Measurements

Fasting blood was drawn into serum and EDTA-anticoagulant tubes, and samples were processed then stored at -70^{o}C using a standardized protocol (1). Lipids and glucose were measured at a central laboratory (Collaborative Studies Clinical Laboratory at Fairview-University Medical Center, Minneapolis, MN) using CDC standardized methods. HDL-C was determined using the cholesterol oxidase method (Roche Diagnostics, Indianapolis, IN), and

LDL-C was calculated using the Friedewald equation. Quartile ranges for lipid analytes are as follows: LDL-C, mg/dL: 1st (12-99), 2nd (100-119), 3rd (120-139), 4th (140-315); non-HDL-C, mg/dL: 1st (23-122); 2nd (123-143), 3rd (144-166), 4th (167-491); TC/HDL-C ratio: 1st (1.28-3.20) 2nd (3.20-3.96), 3rd (3.97-4.86), 4th (4.87-21.46).

ApoB was measured at Health Diagnostics Laboratory Inc (Richmond, Virginia) using Roche reagents and a Roche modular-P analyzer (Roche Diagnostics; Indianapolis, IN). ApoA-I levels were measured in the laboratory of Dr. Alan Remaley (Bethesda, MD) using Diazyme reagents and quantified using a Siemens Dimension analyzer. Quartile ranges for apolipoprotein analytes are as follows: ApoB, mg/dL: 1st (0-91.2), 2nd (91.3-107), 3rd (107-124), 4th (125-542); ApoB/ApoA-I ratio: 1st (0-0.69) 2nd (0.7-0.89), 3rd (0.9-1.07), 4th (1.08-10.9).

NMR Spectroscopy

Lipoproteins were measured at LipoScience, Inc. (Raleigh, N.C.) by NMR spectroscopy using the LipoProfile-3 algorithm as described previously (2, 3). Briefly, lipoprotein particle concentrations were measured on plasma specimens frozen at −70 °C. As each lipoprotein particle subclass has a distinct lipid methyl group, concentrations were determined from the amplitude of their unique NMR signals. Quartile ranges for lipoprotein analytes are as follows: total LDL-P, nmol/L: 1st (200-1023), 2nd (1024-1235), 3rd (1236-1473), 4th (1474-3446); LDL-P/HDL-P ratio: 1st (6.57-28.3) 2nd (28.3-36.8), 3rd (36.8-47.8), 4th (47.8-186.2).

Anthropometric, demographic, and clinical variables

Information regarding age, sex, race/ethnicity, and lifestyle factors was obtained by questionnaires. Height (m), weight (kg), and blood pressure were measured according to standard procedures (1). Hypertension was defined as a mean systolic blood pressure of ≥140 or a diastolic blood pressure of ≥90 mmHg, or the use of anti-hypertensive medication.

Coronary Heart Disease Classification

Incident CHD was defined as the first occurrence of any of the following: myocardial infarction (n=101), resuscitated cardiac arrest (n=17), CHD death (n=45), or definite angina (n=109). Definite angina was defined as symptoms of typical chest pain and physician diagnosis of angina followed by coronary artery bypass grafting and percutaneous coronary intervention (PTCA), evidence of ischemia by stress tests or resting ECG, or ≥70% obstruction on coronary angiography. Fifty-one individuals initially presented with 'probable' angina, and nine of these cases were considered CHD as individuals showed symptoms of typical chest or atypical symptoms and physician diagnosis of angina *followed by* coronary artery bypass grafting. Four cases of probable angina followed by PTCA were excluded (n=4) as obstruction did not reach 70%. An additional 14 individuals that did not experience angina and underwent PTCA without evidence of obstruction ≥70% were also excluded. Notably, some individuals suffered multiple events.

Statistical Analysis

Statistical analysis was conducted using Stata (version 12.1, Stata Corp, College Station, TX). Baseline characteristics are presented as means (SD) for continuous variables and frequencies (%) for categorical variables. Cox regression analysis was performed to test for association between lipid and lipoprotein measures and the primary outcome of CHD, adjusting for sex, race/ethnicity, hypertension medication use, systolic blood pressure, smoking, and stratified by age category and diabetes. Residual analysis based on the martingale residuals suggested a non-linear relationship between risk of CHD and lipid and lipoprotein measures. LDL-C, non-HDL-C, TC/HDL-C, ApoB, ApoB/ApoA-I, as well as NMR-derived measures of total LDL-P and LDL-P/HDL-P were therefore divided into quartiles in the analysis. Each of the

lipid and lipoprotein measures was analyzed separately. To determine whether ApoB, ApoB/ApoA-I, LDL-P, or LDL-P/HDL-P were independently associated with the CHD risk in addition to LDL-C, non-HDL-C, or TC/HDL-C, a separate Cox regression analysis was performed for each of the three measures with a dichotomized LDL-C (<100 mg/dl vs. ≥100 mg/dl), TC/HDL-C ratio (<3.5, ≥3.5) or non-HDL-C (<130 mg/dl vs. ≥130 mg/dl) term included as an additional covariate. Though individuals using lipid-lowering medications at baseline were excluded, a number of participants in the remaining subcohort began using medications at later dates. We therefore evaluated lipid-lowering medication use as a discrete time-dependent variable—no differences in results were observed.

Regression analysis of risk classification was conducted using the 2013 American Heart Association/American College of Cardiology risk calculator (4) as a baseline prediction model derived from age, sex, race/ethnicity (Black vs other), TC, HDL-C, smoking, the presence of diabetes or hypertension, and systolic blood pressure. It was determined whether the addition of individual lipid and lipoprotein markers may improve on this model in estimating future CHD events defined as myocardial infarction, resuscitated cardiac arrest, or CHD death. Continuous net reclassification improvement (NRI) score, or 1/2NRI (>0), was calculated based on risk of CHD event at 5-years as defined by Pencina et al. (5). M_{event} and $M_{nonevent}$ were calculated as the proportions of individuals in which predicted CHD risk in the new model (lipid or lipoprotein + AHA/ACC risk model) was higher than in the baseline model (AHA/ACC only) among "event" and "non-event" groups, respectively. NRI was then calculated as M_{event}, $-M_{nonevent}$ as done previously (6). We additionally assessed discrimination of the baseline and baseline+lipoprotein models by calculating the C-statistic as done by Harrell et al. (7), which is defined as the proportion of all subject pairs in which the predicted and observed survival times are concordant.

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