MATERIALS AND METHODS

The Relationship of Cigarette Smoking with Inflammation and Subclinical Vascular Disease: The Multi-Ethnic Study of Atherosclerosis.

McEvoy. Smoking, inflammation, and atherosclerosis

Study Population

MESA is an ethnically diverse, community-based, prospective cohort study. The study design and participant recruitment have been previously described (1). MESA recruited 6,814 men and women, aged 45 to 84 years, from four different ethnic groups (Caucasian, Chinese-American, African-American, and Hispanic). Participants were enrolled from six centers in the United States: Forsyth County, North Carolina; New York City, New York; Baltimore, Maryland; St. Paul, Minnesota; Chicago, Illinois; and Los Angeles, California. Exclusion criteria included known clinical CVD at recruitment. All participants gave written informed-consent, and the study was approved by the institutional review boards from all field-centers.

At the baseline visit (July 2000-August 2002), participants completed self-administered questionnaires, standardized interviews, and in-person examinations of medical history, anthropometric measurements, and laboratory data. At baseline, all 6,814 study participants also underwent lab-testing for inflammatory biomarkers, non-contrast cardiac computed-tomography (CT) scans, and 6,725 had a baseline carotid ultrasound. In addition, based on MESA study protocol, a representative subset of participants had baseline FMD (n=3,026) and baseline cardiac MRI that included

measurement of aortic distensibility (n=3,530). Full details of the MESA methods are available at the MESA website (http://www.mesa-nhlbi.org).

Smoking Status

For the present analysis we excluded 18 participants without baseline smoking information (leaving 6,796 subjects for the analysis sample). The primary smoking variables were smoking status, smoking dose (pack-years), and time since cessation in former smokers. Smoking status categories were; current-smokers, former-smokers, or never-smokers. Participants with a lifetime smoking history of fewer than 100 cigarettes, and/or who denied ever smoking were defined as never-smokers. Participants who smoked in the last 30 days were defined as current-smokers. Smokers were stratified by cumulative exposure into quartiles of pack-years of smoking. Pack-years were calculated by multiplying the reported average number of packs of cigarettes smoked per day (duration over which this average was estimated was not defined in the protocol) by the number of years of smoking. Finally, time since cessation was assessed as time elapsed since last cigarette use in former-smokers.

In a subgroup of MESA participants enrolled in the MESA-Lung Sub-study (3,965 of 4,484 randomly sampled MESA participants), smoking was confirmed by urinary cotinine levels collected at visit 1 (Immulite 2000 Nicotine Metabolite Assay; Diagnostic Products Corp., Los Angeles, CA)(2). Self-reported former-smokers and never-smokers who had cotinine levels >500ng/mL were reclassified as current-smokers (N=84 [1.2%], Supplementary E-Table 1). (3) Similarly, per the MESA-Lung protocol, pack-years of cigarettes were increased by 25% among former-smokers with cotinine levels >100 ng/mL (2). Otherwise, all other participants (those without cotinine or those with

measured cotinine who did not meet the above reclassification criteria) were classified based on self-report. Finally, never smokers at the baseline visit who reported being former smokers at MESA visit 2 were reclassified as baseline former smokers.

Additional Study Covariates

Family history of CVD was obtained by asking the participants whether any immediate family member had a prior myocardial infarction, coronary angioplasty, or coronary-artery bypass surgery. We obtained self-reported information on alcohol consumption, recent fever (within 2 weeks), aspirin, non-steroidal anti-inflammatory drug (NSAID), and steroid use. All medication use was verified by study staff. Body mass index (BMI) was calculated as measured weight in kilograms divided by height in meters squared. Diabetes was defined as a fasting blood glucose concentration of ≥126 mg/dL or the use of insulin or oral hypoglycemic medications. Blood pressure was recorded as the mean of the last 2 of 3 seated measurements. Hypertension was defined as systolic blood pressure ≥140mmHg, diastolic blood pressure ≥90mmHg, or the use of medications prescribed for hypertension. The ankle blood pressure index (ABI) was computed separately for each leg, with the numerator being the higher of the posterior tibial or dorsalis pedis systolic pressures in each leg and the denominator the average of the right and left brachial systolic pressures.

A central laboratory (University of Vermont, Burlington, VT) measured levels of total and high-density lipoprotein cholesterol (HDL-C), triglycerides, and plasma glucose in blood samples obtained after a 12-hour fast. Low-density lipoprotein cholesterol was calculated using the Friedewald equation. HsCRP and fibrinogen were measured using

the BNII nephelometer (N-High-Sensitivity CRP and N-Antiserum to Human Fibrinogen; Dade Behring). Intra-assay coefficients of variation (CVs) for hsCRP ranged from 2.3 to 4.4% and inter-assay CVs ranged from 2.1 to 5.7%. The intra-assay and inter-assay CVs for fibrinogen were 2.7 and 2.6%, respectively. Finally, IL-6 was measured by ultrasensitive enzyme-linked immunosorbent assay (Quantikine HS Human IL-6 Immunoassay; R&D Systems). The analytic CV for IL-6 was 6.3%(4).

Arterial Distensibility (Pulsatile Function)

Using ultrasonography, common carotid arterial distensibility was assessed in 6,530 subjects as a ratio of diameter change over the cardiac cycle to brachial artery pulse pressure: distensibility = [2(*D*s − *D*d)/*D*s]/(*P*s − *P*d). *D*s is defined as systolic carotid artery diameter; *D*d the diastolic carotid diameter; *P*s the systolic blood pressure; *P*d the diastolic blood pressure(5). Reproducibility studies were performed in 221 participants; 211 were intraobserver repeated-image analyses, and 10 were interobserver correlations. For distensibility, the intraobserver correlation was 0.71. The interobserver correlation was 0.85. Variability in reading exams was assessed in 204 patients, revealing an intraobserver class correlation coefficient of 0.68 (5).

Aortic distensibility was assessed using cardiac MRI. The cardiac MRI protocol at the baseline visit and the characteristics of MESA participants with and without MRI measurements have been described previously (6). Using automated contour with FLOW software (Medis, Leiden, The Netherlands), aortic distensibility (AD) was calculated in 3,530 subjects as; *AD = (Maximum area — Minimum area) / [(Minimum area)* × Δ *P]* × *1000*, where Δ P is the pulse pressure in mmHg(7). Blood pressure was

measured immediately before and after both the carotid ultrasound and aortic MRI measurements. Quality control measures included intra- and inter-reader evaluations. Aortic MRIs from 87 participants were read separately by two readers with an inter-reader intra-class correlation for distensibility of 0.82 (95% CI; 0.74-0.88). Those MRIs were also re-read by one reader with an intra-reader intra-class correlation of 0.91 (95% CI; 0.86-0.94). The MRI analysts were blinded to other study data.

Flow-Mediated Dilation (FMD)

As detailed elsewhere (8), assessment of endothelial function using brachial FMD was performed at the baseline visit in a subset of MESA participants (N=3,027). A linear-array multi-frequency 9-MHz transducer (GE Logiq 700, General Electric Medical Systems, Wisconsin) was used to image the right brachial artery. A blood pressure cuff was inflated to 50 mmHg above the participant's systolic blood pressure for 5 minutes. Images of the right brachial artery were captured continuously for 30 seconds before cuff inflation and for 2 minutes immediately on cuff deflation to document the vasodilator response. FMD was computed as follows: % FMD= [(maximum diameter- baseline diameter)/baseline diameter] X 100%.

Intrareader reproducibility for baseline diameter, maximum diameter, and %FMD was evaluated by comparing an original and a blinded quality control reread of brachial ultrasounds from 40 MESA participants (32 male, 18 white, 2 Chinese, 10 black, and 10 Hispanic subjects). The intraclass correlation coefficients were 0.99, 0.99, and 0.93, respectively. Intrasubject variability was evaluated by comparing results from repeated examinations of 19 subjects on 2 days a week apart. The intraclass correlation

coefficients for baseline diameter, maximum diameter, and %FMD were 0.90, 0.90, and 0.54, respectively. Percent technical error of measurement was 1.39% for baseline diameter measurement, 1.47% for maximum diameter measurement, and 28.4% for %FMD measurement. (8)

Carotid Intima-media Thickness (cIMT)

The right and left carotid arteries were imaged by trained technicians according to a scanning protocol using B-mode ultrasonography with a GE Logiq 700 machine. The MESA ultrasound reading center (Tufts Medical Center) measured maximal cIMT of the internal and common carotid artery as the mean of the maximum cIMT of the near and far walls on the right and left sides. As results for common and internal cIMT were quantitatively similar, we chose to present results for the internal given that data suggest an advantage for internal cIMT in predicting CVD (9). The interreader correlation coefficient for cIMT was 0.84 (66 studies), and the interreader correlation coefficient was 0.86 (48 studies). (10)

Cardiac CT Protocol

Cardiac CT was performed at 3 sites using a cardiac-gated electron beam CT scanner (Imatron C-150XL, GE-Imatron, San Francisco, CA) and at 3 sites using a 4-slice multi-detector CT instrument. Images were read at the MESA CT reading center (Harbor–University of California, Los Angeles). The MESA scanning protocol has been previously published (11). For this study, CAC was quantified as two binary measures; 1) present (CAC>0) versus absent; or 2) less than versus greater than the 75th percentile for age, sex and race in those with any CAC present at baseline (n=3,392).

Specific details regarding the generation of these percentiles are provided by McClelland et el. (12) The Kappa statistic for agreement on the presence of CAC was 0.92, and the mean rescan percentage absolute difference in CAC >0 was 20.1%. (13)

Statistical analysis

Characteristics were analyzed according to smoking-status groups. Non-normally distributed variables were log transformed. Frequencies and proportions were reported for categorical variables, means with standard deviations or medians with interquartile ranges were reported for continuous variables. Analysis of variance, Chi-squared and Kruskal-Wallis testing were used for comparison of variables between smoking groups, where appropriate.

All outcomes were analyzed in a cross-sectional fashion, determined at the baseline MESA exam (July 2000-August 2002). When considering CVD domains as categorical outcomes, we calculated prevalence odds ratios with robust logistic regression. When considering domains as continuous outcome variables, we used robust linear regression. The β-coefficient for a continuous variable should be interpreted as the estimated, adjusted, absolute difference, comparing each smoking category to the reference category (never-smokers). All models were adjusted for the following covariates: age, gender, race, MESA site, BMI, hypertension status, diabetes status, heart rate, HDL and LDL cholesterol levels, triglycerides, cholesterol lowering medications, level of education, and family history of myocardial infarction. We also tested for interaction on the association between smoking status and the three subclinical outcomes, based on inflammatory status (hsCRP <2mg/L versus ≥2mg/L).

Sensitivity analyses, further adjusting for 1) alcohol status (drinker versus non-drinker) and quantity of alcohol intake (percent calories from alcohol), 2) anti-inflammatory medication (aspirin, NSAIDs, and steroids), and 3) recent self-reported fever (within 2 weeks) were also conducted. For our pack-years analysis, we modeled pack-years as a categorical exposure by quartile (because the association of pack-years as a continuous variable and our outcomes of interest was non-linear for many of these outcomes and the non-linearity also differed by outcome). For our tobacco cessation analysis, we modeled time since cessation as a continuous variable (therefore, beta-coefficients and ORs are presented for every unit [per year] increase in smoking cessation interval and also rescaled to every 5-year cessation interval). Finally, to test the robustness of our findings, all analyses were also performed based on self-reported smoking variables alone (without any cotinine based reclassification). Analyses were performed with STATA (version 12) and a p-value of <0.05 was considered significant (two-sided). P-values for trend were also calculated for the outcomes of interest using a nonparametric test across ordered groups(14).

Materials and Methods References

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