MATERIAL AND METHODS

Population

Between 2000 and 2002, the Multi-Ethnic Study of Atherosclerosis (MESA) enrolled 6814 men and women of four racial/ethnic groups (White, Black, Hispanic and Asian), aged 45-84 years. Participants, who were free of clinical cardiovascular disease,[1] were recruited from the population near six field centers (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; New York, New York; St Paul, Minnesota; Los Angeles, California). They gave written informed consent and local institutional review boards approved the study protocol.

From the 6814 MESA subjects, 3447 participants were selected for a follow-up ultrasound scan at exam 5 (2010-2011), among those who had valid baseline carotid IMT measurements and had consented to be included in MESA Air. The latter includes a subcohort of MESA to investigate associations of long-term air pollution exposure with subclinical atherosclerosis and cardiovascular disease.[2] All images from the baseline ultrasound scan of these 3447 participants were reviewed and carotid IMT and plaques were re-measured using a strict quality protocol. We excluded 192 subjects with missing 25-OHD or PTH and 4 subjects with 25-OHD >100ng/ml (suggesting supplementation with high-dose vitamin D), leaving 3251 subjects for the main analysis (47.7% of the entire MESA cohort).

In sensitivity analyses, we also evaluated cross-sectional associations in 6737 subjects with available original IMT measurements at baseline, of whom we excluded 347 subjects with missing 25-OHD or PTH and 6 subjects with 25-OHD >100ng/ml, leaving 6393 subjects (93.4% of the cohort).

Measurements

Vitamin D / PTH

Serum 25-OHD concentrations were measured by mass spectrometry, with 25-OHD calibrated to NIST standards (interassay CV <3.4%).[3] Serum PTH was measured by two-site immunoassay on a Beckman Access 2 automated immunoassay platform (inter-assay CV 6.1% at 30.1 pg/ml and 3.4% at 94.5 pg/ml).[4] As season-specific thresholds for 25-OHD may be most relevant,[5] 25-OHD was adjusted for seasonal variation using a cosinor model derived and internally validated in MESA.[6]

Carotid artery measurements

At baseline, trained and certified sonographers from each field center performed B-mode ultrasonography of the near and far walls of the left and right internal carotid and common carotid arteries (ICA / CCA) using a Logiq 700 ultrasound system (General Electric Medical Systems) with a linear array vascular ultrasound transducer (M12L). Images were recorded on super-VHS videotapes at high resolution and frame rates using a Medical Digital Recording (MDR) device (PACSGEAR, Pleasanton, CA) and converted into DICOM compatible digital records. At exam 5, a similar protocol was performed using the same ultrasound and digitizing equipment, however images focused on the far walls of the ICA and CCA. For exam 5, the ultrasound reading center core lab at the University of Wisconsin selected reference images from exam 1

for sonographers to try to match the scanning characteristics of the initial study, including the carotid artery display depth on the screen, angle of approach, surrounding tissues and internal landmarks, degree of jugular venous distension, and ultrasound system settings. The video output was directly digitized using the same MDR settings without use of videotape.

Carotid IMT measurements were performed in triplicate at the distal 1cm of the CCA and the proximal ICA on both sides, regardless of the presence of plaque. Carotid plaque was defined as the presence of focal wall thickening at least 50% greater than that of the surrounding vessel wall or as a focal region with CIMT greater than 1.5mm that protrudes into the lumen distinctly from the adjacent boundary.[7] Carotid plaques were evaluated from transverse and longitudinal views of the ICA, CCA and the bulb. Three trained readers from the ultrasound reading center reviewed all images at both examinations and performed all measurements. They also assessed the image quality and the matching of carotid images between the two examinations. Reader reproducibility was assessed by having all 4 readers blindly read 24 scans, chosen as 4 per field center and half each from exams 1and 5. Intra-reader reproducibility was excellent for maximum CCA-IMT (total error of the mean [TEM] 3-4%, intra-class correlation coefficients [ICCs] 0.93-0.99) and very good for maximum ICA-IMT (TEM 8-9%; ICC 0.86-0.96). Inter-reader reproducibility was excellent for maximum CCA-IMT (TEM 2-4%, ICC 0.96) and very good for maximum ICA-IMT (TEM 5-10%, ICCs 0.86-0.88). Scan-rescan reproducibility was evaluated by 44 repeated scans from 3 sonographers, using a single reader. Pearson correlations for matched segments ranged from 0.979-0.996. Mean (SD) differences were <0.01 (<0.05) mm with no outliers on limit of agreement (Bland-Altman) analysis for matched segments.

We computed a plaque score as the total number of segments with at least one plaque present in the near or far wall of all 6 segments of the right and left carotid arteries (common, bulb, and internal carotid), ranging from 0-12.

Covariates

At baseline, demographic data, smoking status, medical conditions, physical activity and the use of medications, were recorded in a questionnaire. Body-mass index (BMI) and resting blood pressure were measured. Blood samples were taken from all participants at baseline for measurements of all biomarkers. Plasma HDL cholesterol was measured using CDC-standardized methods and LDL cholesterol was estimated using the Friedewald equation. We calculated the glomerular filtration rate (GFR) with the combined creatinine-cystatin C equation.[8]

Analyses

For IMT, we evaluated cross-sectional and longitudinal associations of 25-OHD and PTH with CCA-IMT, ICA-IMT, and changes in IMT using linear regression. Change in IMT was restricted to participants with matching carotid segments in the two ultrasound exams (CCA: 2583/3182, ICA: 1408/2419) and the dependent variable was the difference in IMT measurements. For carotid plaque, we evaluated the prevalence of any carotid plaque at baseline and the incidence of a new plaque at the second ultrasound exam (among those free of plaques at baseline) using logistic regression. Change in the additive plaque score was evaluated using ordered logistic regression.[9]

25-OHD and PTH were modeled as categorical exposures using commonly used thresholds: 20/30ng/ml (25-OHD) [10]; 65pg/ml and tertiles (PTH)[4]. The presence of non-linear trends was excluded graphically. Two-sided P values from the Wald test with robust standard errors were obtained for continuous exposures. A level <0.05 determined statistical significance.

The primary analysis was adjusted for confounders chosen prior to analyses: demographic variables, physical activity (total intentional exercise per week, in tertiles), BMI, LDL cholesterol, HDL cholesterol, GFR (continuous terms) and the use of statins at baseline. Analyses evaluating PTH were further adjusted for 25-OHD. Diabetes, hypertension and CRP were considered to be potential mediators and not included as covariates.[11-13] All models were time-adjusted to account for possible differences in the time between the 2 ultrasounds.

In a subgroup analysis, we evaluated the presence of heterogeneity of the associations between race/ethnicity by adding multiplicative interaction terms of race/ethnicity with 25-OHD or PTH.

Missing covariate data were infrequent in covariates (≤2.5%) and were multiple-imputed with 5 imputed datasets using imputation by chained-equations.[14] Carotid measurements, 25-OHD and PTH were not imputed.

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