MATERIALS AND METHODS

Study Sample

The Framingham Heart Study (FHS) is a longitudinal observational study, whose Offspring and Third Generation Cohorts have been previously described.\textsuperscript{1,2} Participants who attended Third Generation examination 1 (2002-2005, n=4095) and Offspring examination 8 (2005-2008, n=3021) and who participated in the multi-detector computed tomography (MDCT) sub-study (2002-2005) were eligible for this study. Exclusion criteria for MDCT were weight $\geq$160 kilograms, pregnancy, and $<35$ years of age for men or $<40$ years of age for women. A total of 2093 Third Generation and 1422 Offspring participants underwent MDCT. For both Third Generation and Offspring Cohorts, we excluded individuals with prevalent CVD (coronary heart disease, congestive heart failure, stroke, or transient ischemic attack; n=28 and n=107, respectively), incomplete tonometry records (n= 238 and n=363, respectively), incomplete calcification measures (n=1924 and n=1521, respectively), and missing covariates (n=0 and n=15, respectively). Our final sample size thus included n=1905 Third Generation and n=1015 Offspring Cohort participants. Clinical covariates were collected during the examination visit at which tonometry was performed. Systolic blood pressure (SBP) was recorded as the average of two physician measurements during the examination. The study protocol was approved by the Institutional Review Board of the Boston University Medical Center and all participants gave written informed consent.

Arterial Tonometry Data Acquisition and Analysis

Tonometric measurements were obtained in the morning in the fasting state. After 5 minutes of rest, supine auscultatory brachial systolic and diastolic blood pressures were obtained by using a computer-controlled device. Skilled technicians obtained arterial tonometry measures with electrocardiogram from the right brachial, radial, femoral and carotid arteries using a custom transducer (Cardiovascular Engineering, Inc., Norwood, MA). Next, trained sonographers obtained 2-dimensional echocardiographic images of the left ventricular outflow tract from the parasternal long axis view followed by pulsed Doppler of the left ventricular outflow tract from the apical 5-chamber view. Technicians obtained body surface measurements from suprasternal notch to pulse recording sites using a fiberglass tape measure for carotid, brachial and radial sites and a caliper for the femoral site. Tonometry, echocardiographic and ECG data were digitized during the primary acquisition (1000 Hz), transferred to the core lab (Cardiovascular Engineering, Inc., Norwood, MA) and analyzed by operators blinded to clinical data.

Details of the tonometry data analysis have been reported.\textsuperscript{3} Tonometry waveforms were signal-averaged using the ECG R-wave as a fiducial point. The brachial waveform was calibrated using cuff systolic and diastolic pressure. Diastolic and integrated mean brachial pressures were then used to calibrate carotid pressure tracings. CPP was derived from calibrated carotid pressure waveforms. CFPWV was calculated from tonometry waveforms and body surface measurements, which were adjusted for parallel transmission in the brachiocephalic artery and aortic arch, using the suprasternal notch as a fiducial point. We used the carotid pressure waveform to measure forward wave amplitude, augmentation index (AI), and the timing of the reflected wave. AI was calculated as the augmented pressure divided by CPP. Forward and reflected pressure waves were separated and the amplitude (peak minus
trough) of each wave was assessed.\textsuperscript{3,4} The global reflection factor was computed as backward wave amplitude divided by forward wave amplitude.\textsuperscript{3} Tonometry measures have been highly reproducible.\textsuperscript{5}

**Computed Tomography Imaging and Analysis**

Each participant was imaged using an eight-slice MDCT scanner (LightSpeed Ultra, General Electric, Milwaukee, WI) with prospective ECG triggering during a single breath hold in mid-inspiration (~18 seconds) using sequential data acquisition. Scans were prospectively initiated at 50% of the RR interval.\textsuperscript{6} In the chest, 48 contiguous 2.5-mm thick slices were acquired from the carina to the diaphragm in a single breath hold (120kVp, 320 mA for <220 pounds and 400 mA >220 pounds of body weight, gantry rotation time 500 ms, temporal resolution 330 ms). Abdominal imaging encompassed 125 mm, with the upper edge of the S1 vertebral body delineating the lower imaging border. Images were reconstructed using a field of view of 35 cm.\textsuperscript{7} Each participant underwent thoracic scans twice.

CT scans were downloaded onto a dedicated offline workstation (Aquarius, Terarecon, San Mateo, CA) and were assessed for the presence and quantity of TAC, AAC, and CAC by an experienced technician. A calcified lesion was identified as an area of 3 or more connected pixels with a CT attenuation >130 Hounsfield units. The Agatston score was calculated as previously described.\textsuperscript{8} If a particular lesion was observed in multiple CT cross-sections, the Agatston score was defined as the sum of the score from each individual cross-section. The healthy referent sample was used to define 90th percentile cut points for arterial calcification measures which were used for sensitivity analyses.\textsuperscript{9} Reproducibility of these measures has been excellent.\textsuperscript{10}

**Statistical Methods**

All exposure (tonometry) variables were analyzed as continuous variables. Primary tonometry variables of interest were CFPWV and CPP and secondary tonometry variables were forward wave amplitude and AI. Raw CFPWV was inverse transformed to reduce heteroscedasticity, standardized to a normal distribution, and multiplied by -1 to correct for the direction of effect. CPP was standardized to a normal distribution. TAC, AAC, and CAC measures were also natural log-transformed after adding 1 to the Agatston (calcification) score to account for cases with no calcification. The primary calcification measure (dependent variable) of interest was TAC. Calcification measures were analyzed both as continuous variables and as dichotomous variables (present/absent). We constructed multivariable-adjusted Tobit regression models and logistic regression models to analyze the association of the tonometry variables with continuous and prevalent arterial calcification, respectively. For each analysis, Model 1 included adjustment for age, sex, cohort type (Third Generation vs. Offspring), body mass index, height, heart rate, history of antihypertensive and lipid-lowering medications, total cholesterol/HDL, diabetes, and current smoking. Model 2 included additional adjustment for examination SBP.

We constructed multivariable-adjusted restrictive logistic regression cubic splines to demonstrate the continuous relations between CFPWV and prevalent arterial calcification. Knots were placed at the 5\textsuperscript{th}, 50\textsuperscript{th}, and 95\textsuperscript{th} percentiles. The choice of these knots takes into account potential non-linearity at the extreme values of CFPWV. Wald tests for non-linear associations were conducted.\textsuperscript{11} We examined for effect modification by cohort status and by sex.
in our primary and secondary analyses. Multivariable-adjusted stepwise logistic regression models also were conducted to determine the associations of CFPWV, CPP, forward wave amplitude, and AI with prevalent arterial calcification. In addition, in sensitivity analyses, we defined alternative dependent variables as the presence of regional arterial calcification >90th percentile in a healthy referent sample. Finally, we conducted logistic regression on a subset of participants with prevalent CAC, but no TAC or AAC, (n=1446) in order to examine the separate associations of primary tonometry measures with CAC in the absence of aortic calcification. All analyses were performed using SAS v.9.3 (SAS Institute, Cary, NC).
REFERENCES