METHODS AND MATERIALS

Participants

We studied participants from the Framingham Offspring and Third Generation Cohorts. [1-3] We performed vascular testing in the Offspring cohort at the 7th examination cycle (1998-2001) and in the Third Generation at the 1st examination cycle (2002-2005). In the Offspring cohort, 796 participants underwent a 6-minute walk prior to vascular testing. Of the 7634 participants at these examinations, we excluded in a hierarchical manner a total of 1926 for the following reasons: 205 for offsite visit (205), 1146 for vascular examination not performed or incomplete as the Doppler studies were initiated partway through the Offspring 7th examination, 540 for technically inadequate vascular studies, and 35 for missing clinical covariates; yielding a final sample of 5708 individuals for the present analyses. Each examination included risk factor ascertainment through routine medical history, physical examination, and laboratory assessment. The Boston University Medical Center Institutional Review Board approved the research and all participants provided written informed consent.

Measurement of Brachial Flow Patterns

Brachial artery flow was assessed using pulsed Doppler at rest and for 15 seconds following cuff release. Doppler recordings were analyzed using a semi-automated signal-averaging method with correction for insonation angle. [4] Sonographers measured flows separately from arterial diameters blinded to corresponding flow-mediated dilation. Resulting flow waveforms were integrated to assess mean resting and mean hyperemic flow velocities. We have previously reported the clinical correlates of brachial flow velocity in the Offspring cohort. [4, 5] Diastole was defined from the timing of the dicrotic notch on a brachial pressure waveform obtained by tonometry and mean diastolic flow velocity at rest was determined by integrating the flow waveform during diastole. Absolute minimum flow velocity during diastole was not measured. Therefore, we defined the presence of diastolic flow reversal as mean diastolic flow velocity≤0cm/s as individuals with an average diastolic flow velocity of 0cm/s or less display flow reversal during diastole. FVR was calculated by dividing mean arterial pressure (MAP) by mean volume flow at baseline [5].

Measurement of Brachial Vasodilator Function

Participants were asked to not eat or drink (except for water or decaffeinated coffee or tea) after 8 pm the night prior to the vascular test. We have described in prior reports the methodology and reproducibility for measuring flow-mediated dilation of the brachial artery. [4, 6, 7] Briefly, the subject was positioned supine with the arm in a comfortable position for imaging the brachial artery. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall in the longitudinal plane was selected for continuous 2D gray-scale imaging. We imaged brachial artery diameter using high resolution ultrasound (Toshiba #SSH-140A, 7.5 MHz linear array transducer in Offspring and Philips Sonos 5500, 11-3L linear array transducer in the Third Generation) at rest and 1 minute after a 5-minute cuff occlusion (200 mm Hg or 50 mm Hg + SBP) on the forearm used to induce reactive hyperemia. Arterial diameters were measured off-line using commercially available software (Brachial Analyzer, Medical Imaging Applications, Iowa City, Iowa, version3.2.3.sp2). Flow-mediated dilation was calculated as the percent change in brachial diameter from the resting state (100*[hyperemic diameter at 60 seconds-resting diameter]/resting diameter). Reactive hyperemic, a measure of forearm microvessel dilation, was assessed from post-cuff occlusion hyperemic flow velocity measured by Doppler as described above. [4]

Measurement of Arterial Stiffness

As previously described, we performed noninvasive hemodynamic assessment after 5 minutes of rest. [8, 9] Radial, femoral and carotid arterial tonometry were performed along with

simultaneous electrocardiographic recording. Transit distances were evaluated by measuring from the suprasternal notch to each recording site. Digital signals were used to calculate carotid-femoral (aortic) and carotid-radial (muscular artery) pulse wave velocities as previously described. [8, 9]

Statistical Analyses

We tabulated clinical characteristics by cohort. First, we evaluated the association of diastolic flow reversal, defined as mean diastolic flow velocity ≤0, with clinical variables in logistic regression models: 1. Adjusted for age, sex, and cohort and 2. Adjusted for age, sex, cohort and step-wise selection from the following eligible covariates: mean arterial pressure, heart rate, body mass index, total/HDL cholesterol ratio, triglycerides, glucose, diabetes, current smoking, hypertension treatment, walk test before vascular testing, and prevalent CVD.

Next, we compared vascular function measures in participants with diastolic flow reversal and without flow reversal in multivariable linear regression models: 1. Adjusted for age, sex and cohort, and 2. Addition adjustment for all the clinical covariates described above. We hypothesized that the relation of vascular function measures with diastolic flow velocity would be non-linear with a greater decrement in endothelial function in the presence of flow reversal. We tested for a non-linear association with restricted cubic spline models with 3 knots to evaluate the association of the continuous exposure (independent variable; mean diastolic flow velocity) and continuous outcomes (dependent variables: flow-mediated dilation, hyperemic flow velocity, and stiffness measures) in models adjusting for the clinical covariates described above.[10] We placed knots based at 20^{th}, 50^{th} (median), and 80^{th} percentile values of mean diastolic flow velocity. Additionally we compared the association (beta coefficients) of diastolic flow velocity with the vascular function measures in subsets of participants with or without flow reversal. Finally, we evaluated whether there was effect modification by the presence of flow reversal on the association of hyperemic flow velocity with flow-mediated dilation.

All analyses were performed using SAS 9.3.[10] Two-sided $P<0.05$ were considered statistically significant. The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

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