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The relationship between endothelial function and aortic valve calcification: Multi-Ethnic Study of Atherosclerosis

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HIGHLIGHTS

- Endothelial dysfunction (ED) is a systemic disorder and a key variable in the pathogenesis of atherosclerosis.
- We investigated whether MESA participants with ED were more likely to have aortic valve calcification (AVC).
- ED measured by biomarkers and flow mediated dilationwas not significantly associated with prevalence or progression of AVC.

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ABSTRACT

Background and aims: Aortic valve calcification (AVC) may be associated with atherogenic processes arising from endothelial dysfunction (ED). Limited data is available about the relationship between ED, defined by flow mediated dilation (FMD%) and biomarkers, and the prevalence and progression of AVC in a multiethnic population.
Methods: A sample of 3475 individuals from the Multi-Ethnic Study of Atherosclerosis (MESA), with both initial and repeat CT scans at a mean of 2.65 ± 0.84 years and FMD% and serologic markers of ED [C-reactive protein (CRP), Von Willebrand factor (vWF), Plasminogen Activator Inhibitor (PAI), fibrinogen, Interleukin 6 (IL6), E-selectin and ICAM-1 (Intercellular Adhesion Molecule 1)], were analyzed. Multivariate modeling evaluated the association between ED and the prevalent AVC and AVC progression.
Results: The median levels of FMD% was lower and vWF%, fibrinogen, IL6 and ICAM-1 were significantly higher in the AVC prevalence group *versus* no AVC prevalence (all $p < 0.001$). In the fully adjusted model for established risk factors, decreasing FMD% or increasing biomarkers was not independently associated with AVC prevalence [OR FMD% 1.028 (0.786, 1.346), CRP 0.981 (0.825, 1.168), vWF 1.132 (0.559, 2.292), PAI 1.124 (0.960, 1.316), fibrinogen 1.116 (0.424, 2.940), IL6 1.065 (0.779, 1.456), E-selectin 0.876 (0.479, 1.602) and ICAM-1 1.766 (0.834, 3.743)]. In the AVC progression group, FMD%, vWF%, fibrinogen and IL6 were significantly different ($p < 0.05$). After adjusting for cardiac risk factors, AVC progression was not independently associated with decreasing FMD% or increasing biomarkers [OR FMD% 1.105 (0.835, 1.463), CRP 1.014 (0.849, 1.210), vWF% 1.132 (0.559, 2.292), PAI 1.124 (0.960, 1.316), fibrinogen 0.909 (0.338, 2.443), IL6 1.061 (0.772, 1.459), E-selectin 0.794 (0.426, 1.480) and ICAM-1 0.998 (0.476, 2.092)].
Conclusions: Endothelial dysfunction by FMD% and biomarkers is not significantly associated with the prevalence or progression of aortic valve calcification after adjustment for cardiac risk factors.

1. Introduction

AVC represents a degenerative disease process contributing to valve leaflet dysfunction, stenosis and significant morbidity in the population [1]. ED has been implicated as an early insult to vascular integrity contributing to hypertension, atherosclerosis as well as AVC.

The growing understanding of endothelial dysfunction's role in vascular damage has led to an evolution of the understanding of AVC

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from a passive disease process to an active and regulated process [2,3]. Lipoprotein depositions combined with inflammatory cell infiltration lead to endothelial deposition of microscopic collections of calcification [4,5]. Histologically, this process is very similar to atherosclerosis, leading to a continuum of bone generation in the valve and degradation of valve function [6,7]. Risk factors for AVC such as diabetes, hypertension and elevated BMI appear to have a similar, related role in other diseases mediated by endothelial dysfunction. Like analogous vascular disease, AVC represents a significant health burden, with as many as 25% of adults aged 65 or greater displaying aortic sclerosis [4].

Endothelial dysfunction has been quantified using soluble biomarkers as well as flow mediated dilation (FMD%) to assess systemic vascular reactive hyperemia. Vasomotor reactivity was assessed following occlusion of the brachial artery and the subsequent vascular response to endothelial nitric oxide release [8,9]. Ultrasound helped qualify the extent of vasodilatation, which is a measure of endothelial function. Loss of reactivity indicates systemic endothelial dysfunction and degradation of athero-protective mechanisms [8–11]. Furthermore, biomarkers such as CRP, vWF%, PAI, fibrinogen, IL6, E-selectin and ICAM-1 can provide mechanistic information and indication of disease severity. These biomarkers also serve to compliment FMD% measurements of endothelial dysfunction [12–23].

Limited data is available about the relationship between ED by measurement of FMD% and biomarkers and the prevalence, incidence and progression of AVC in a multiethnic population, free of CVD at baseline. Prior studies of AVC and endothelial dysfunction have examined correlative relationships among people already displaying the disease in a cross section analysis and small sample size. In addition, these studies use echocardiography as the main imaging modality to identify the AVC with associated poor diagnostic accuracy. The purpose of this study is to determine whether endothelial dysfunction, as defined by FMD% and biomarkers, is associated with the prevalence of AVC and to determine whether measures of endothelial dysfunction at baseline predict the progression of AVC detected by cardiac CT in a diverse population free of recognized cardiovascular disease.

2. Materials and methods

This study utilized data gathered during the Multi-Ethnic Study on Atherosclerosis (MESA study) which has been characterized elsewhere [24]. Briefly, this study enrolled 6814 men and women free of clinical CVD at baseline examination, identified as Black, White, Hispanic or Chinese between the ages of 45 and 84 years from 6 communities across the United States (Baltimore City and County, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; New York, NY; and St. Paul, MN) between July 2000 and August 2002. This study aims to investigate the characteristics of subclinical cardiovascular disease. Patients were recruited using telephone customer listings, residency and home registry and later Medicare beneficiary listings. Exclusion criteria included self-reported CVD, including angina or prior cardiovascular procedures such as percutaneous coronary interventions, coronary bypass or valve surgery, or pacemaker/defibrillator implantation. Written informed consent was obtained from each patient included in the study, the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and the study protocol has been priorly approved by the Institution's ethics committee on research on humans.

2.1. Aortic valve calcification measurement

Participants underwent initial computed tomographic (CT) scanning utilizing either electron-beam CT or multi-detector CT systems to characterize coronary calcium scores. Exposure control and intra and inter operator variability were standardized by utilizing a single central reading facility as well as radiographic phantoms, which had an identical and known quantity of calcium placed beneath the thorax of participants at each imaging site. Two scans of each participant were performed with the use of mean calcium score in Agatston units in data analyses. Of the initial 6814 enrollees, 5142 were further categorized by the presence (Agatston score > 0) or absence (Agatston score = 0) of AVC on baseline imaging with CT. Follow-up testing, including repeat chest CT examination, was performed in two stages.

One-half of the cohort returned between September 2002 and January 2004, while the other half returned between March 2004 and July 2005, with average between-scan intervals of 1.6 and 3.2 years, respectively. Data was included for all patients with baseline and follow up imaging at a mean of 2.65 ± 0.84 years and whose FMD% and biomarkers measurements were available, resulting in the 3475 participants included in this study.

AVC occurrence among participants was delineated between new occurrence or progression of disease. All participants with incident AVC and those with AVC at baseline who progressed were included in a single progression analyses regardless of magnitude or direction of Agatston scores on CT. This combined outcome of AVC incidence and progression was used to increase the statistical power.

Full details regarding the equipment, scanning methods, and quality control in MESA, have been published elsewhere [25]. Moreover, accurate quantification of calcification of the aortic valve, mitral annulus (MAC), and thoracic aortic (TAC) by using cardiac CT have been published [26].

2.2. Endothelial dysfunction measurement

FMD% was measured in 3280 MESA participants at baseline. Brachial artery FMD% was assessed on patients who had fasted for 6 h, rested for 15 min by occluding the artery of the right arm below the antecubital fossa. A 9 MHz linear-array multifrequency transducer measured brachial artery dilatation at baseline and with cuff inflation to 50 mmHg above systolic blood pressure. Images were analyzed at a central facility with previously validated semi automated processes, which determined baseline and maximal dilatation [27]. Biomarkers implicated in endothelial dysfunction including CRP, vWF%, PAI, fibrinogen, IL6, E-selectin and ICAM-1 were measured in 3454, 872, 3458, 3458, 3384, 873 and 1531in selected random participants, respectively. Plasma ICAM-1 was measured at baseline among 2621 MESA participants using an ELISA assay (Parameter Human ICAM-1; R &D Systems, Minneapolis, MN). The coefficient of variation was 5.0%. Serum E-selectin was measured using Parameter Human sE-selectin Immunoassay; R&D Systems; coefficient of variation 5.7–8.8%) [28]. vWF activity was measured in the Laboratory for Clinical Biochemistry Research at University of Vermont (Burlington, VT, USA). vWF activity was measured by the Liatest (latex immunoturbidometric) assay on the STA analyzer (Diagnostica Stago, Parsippany, NJ). In this assay the degree of agglutination (measured by light absorption) between vWF in the sample and vWF monoclonal antibody in the assay is directly proportional to vWF activity. The intra-assay and inter-class coefficient of variation was 3.7 and 4.5% respectively [29]. Fibrinogen and CRP were measured using the BNII nephelometer (N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL). IL-6 was measured by ultrasensitive enzyme-linked immunosorbent assay (ELISA) (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN) [30]. Since there is no agreement on the cutoff points defining the ED, we avoid classifying the participants based on their FMD% or biomarkers levels. Instead, the log transformed FMD% and biomarkers were used as continuous variables in the statistical models.

2.3. Risk factors

Risk factors and demographics were obtained during enrollment utilizing multiple instruments more fully described previously [24,31]. Information relating to tobacco exposure, alcohol use, education level, medical conditions including diabetes, hypertension and dyslipidemia as well as participant activity levels and family history were gathered to

assess risk factor contribution to AVC. Continuous variables including body mass index, blood pressure, lipid parameters, and fibrinogen were normalized by their standard deviations. Age and race/ethnicity were self-reported. Diabetes and impaired fasting glucose were categorized according to the 2003 American Diabetic Association fasting criteria algorithm [32]. Hypertension was defined as systolic blood pressure > 139 mm Hg at baseline visit or diastolic blood pressure > 89 mm Hg, or by a history of physician diagnosed hypertension and taking a medication for hypertension. Assessment of personal habits such as alcohol and tobacco use was also performed. The MESA Typical Week Physical Activity Survey (TWPAS) was used to estimate the amount and frequency of physical activity (PA) for study participants. This survey was adapted from a previous study activity survey. PA patterns were broken down by the TWPAS into 28 items, divided into 10 categories: household chores, lawn/yard/garden/farm, care of children/adults, transportation, walking (not at work), dancing, sport activities, conditioning activities, leisure activities, and occupational and volunteer activities. Participants were asked if they engaged in any of the activity categories. If they did participate in the activity category, they were asked how often during the week they engaged in that activity, for how long and at what intensity (light, moderate or vigorous) [33]. Lipids, including total and high-density lipoprotein cholesterol, triglycerides, inflammatory markers, and glucose levels, were measured from fasting plasma samples in a central laboratory (University of Vermont, Burlington, VT). Venous blood samples were collected after a 12 h fast by certified technicians using standardized venipuncture procedures. Samples were then centrifuged at 2000 for 15 min at 4 °C within 30 min of collection. EDTA plasma samples were aliquoted on ice, stored at −70 °C, and then shipped on dry ice to the MESA central laboratory at the University of Vermont. HDL cholesterol was measured in EDTA plasma using the cholesterol oxidase cholesterol method after precipitation of non-HDL-cholesterol with magnesium/dextran (Roche Diagnostics, Indianapolis, IN). Triglyceride was measured in EDTA plasma using Triglyceride GB reagent on the Roche COBAS FARA centrifugal analyzer (Roche Diagnostics). LDL cholesterol was estimated by the Friedewald equation in individuals with triglyceride values < 400 mg/dL [27]. Log transformation was used for skewed variables such as C reactive protein, with no change in the final results.

2.4. Statistical analysis

Three outcomes of AVC were examined in this analysis. Prevalent AVC was defined as the presence of AVC (Agatston score > 0) at exam 1 (baseline). Incident AVC was defined as participants who had Agatston score of zero but developed AVC at follow-ups. AVC Progression analysis included all participants with baseline AVC, regardless of the magnitude of, or direction of, change in, Agatston scores, as well as those with zero Agatston scores at baseline who developed incident AVC during the follow up. We utilized this combined outcome to increase the statistical power for AVC progression analysis. FMD% and biomarkers were used as proxy for endothelial dysfunction. The T test was used to compare the differences of continuous variables by prevalent AVC and AVC progression. Medians and interquartile ranges were reported for FMD% and biomarkers due to non-normality, and the Wilcoxon rank-sum test was applied to compare the differences of FMD % and biomarkers by prevalent, incident AVC and AVC progression of AVC. FMD% and biomarkers were log-transformed due to non-normality to improve the model fit. Logistic regression was applied to examine the association of endothelial dysfunction and outcomes. Odds ratio (OR) and 95% confidence interval (CI) were reported. For each outcome, three logistic regression models were applied, the first model only included FMD% or biomarkers; age and gender were additionally adjusted in the second model. Race, BMI, FPG, systolic blood pressure, smoking, alcohol drinking, LDL–C, TGs and lipid lowering medications were additionally adjusted in the final model. In the AVC progression analysis, we adjusted for the baseline Agatston score. Since follow-up CTs were performed at Exam 2 or Exam 3, the difference in the follow-up time was taken into account in additional analysis by including a dummy variable for exams in the model. However, the magnitudes of the change of associations were minimal compared to the original models using the cumulative incidence without time to events and the conclusion remained the same. In secondary analysis, we examined an outcome of absolute change in AVC score among those with detectable AVC at exam 1. The absolute change was calculated as the difference between AVC score at follow-up and baseline. We used robust linear regression with additional adjustment for baseline AVC score. All *p*-values were two tailed, and a significance level of 0.05 was used. Data was analyzed using SAS version 9.3 (SAS Institute, Cary, NC).

3. Results

Of the 3474 participants who met the study inclusion criteria, 3056 had no prevalent AVC on baseline CT examination, while 418 participants had prevalent AVC. As shown in Fig. 1, the former group comprised the population at risk for incident AVC, while the latter group defined the population at risk for AVC progression. To increase our statistical power, we combined participants who developed AVC at follow up (119) and those who had higher AVC score at follow up than baseline (233), with a resultant total sample of 352 included in the progression analysis.

3.1. ED and AVC prevalence

Baseline characteristics of participants by AVC prevalence are depicted in Table 1. 3474 participants aged 45–84 years, including 1708 (49.17%) men and 1766 (50.83%) women, were included in the prevalence analysis. Of 3280 participants in whom FMD% was measured at baseline, 815 (24.85%) were in the lowest quartile. The number of participants in the upper quartile of biomarkers was: CRP 865/3454, vWF 221/872, fibrinogen 865/3458, PAI 217/857, IL6 847/3384, E-selectin 219/783 and ICAM-1383/1531. The prevalent AVC at baseline was 12% (418 out of 3474), participants with prevalent AVC at baseline were older, had higher proportion of males, smokers, alcohol drinkers and higher BMI, FPG and TG. In addition, they had a higher proportion of hypertension, use of lipid lowering medications and lower HDL compared with those with no AVC at baseline ($p < 0.001$). No statistically significant difference by sites, and exercise between the two groups were observed ($p > 0.05$) (Table 1). FMD% and other biomarkers of endothelial dysfunction by baseline AVC prevalence are described in Table 2. FMD% was lower and the levels of vWF, fibrinogen, IL6 and ICAM-1 were statistically significantly higher among participants with prevalent AVC compared to those who did not have AVC at baseline ($p < 0.01$) (Table 2). There was no statistically significant difference for CRP, PAI, fibrinogen and E-selectin among participants with and without AVC at baseline. The Results of logistic regression models examining association of endothelial dysfunction and prevalent AVC at baseline are presented in Table 3A. Decreasing FMD% and increasing levels of vWF, fibrinogen, IL6 and ICAM-1 were associated with increased prevalence of AVC. In Model 2, (adjusted for age and gender), only increasing fibrinogen and IL6 showed significant OR of AVC, whereas FMD% and the rest of the biomarkers became non-significant. Inversely, AVC was not significantly associated with decreasing FMD% or increasing levels of all biomarkers in Model 3 (Model 2 + other risk factors) (Table 3A). We conducted a sensitivity analysis only among patients with complete data on all biomarkers (N = 640). Our results from the sensitivity analysis between subgroups (N = 640) *versus* full cohort analysis (N = 3742) were consistent, except for VWF and ICAM. Results of the sensitivity analysis of association of AVC prevalence and endothelial dysfunction biomarkers showed log transformed VWF (OR = 3.08, 95% CI: 1.32–7.18; $p = 0.009$) and ICAM-1 (OR = 4.91, 95% CI: 1.19–20.2; $p = 0.027$) were statistically significantly associated with higher odds of endothelial dysfunction

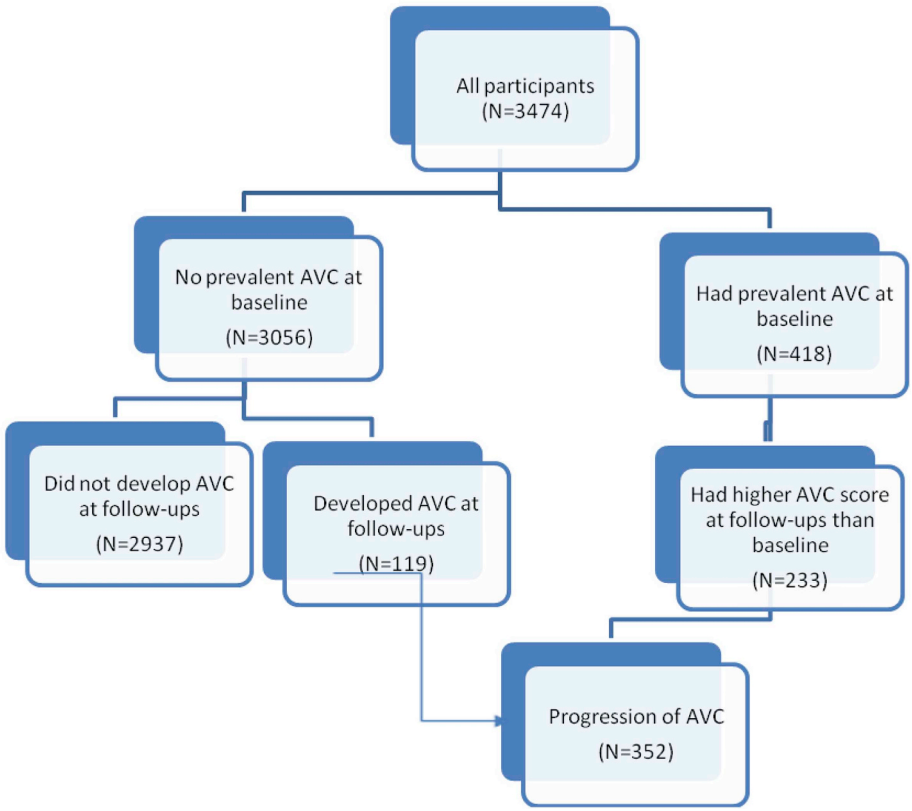

Figure 1. Flow diagram of MESA participants, categorized by aortic valve calcification status.
AVC; aortic valve calcification.

(Table 3B).

3.2. ED and AVC progression

A total of 3474 individuals who had both initial and repeat CT scans as well as FMD% and/or serologic markers of endothelial dysfunction were included in the analysis. Table 4 shows the baseline demographic and cardiac risk factor distribution between those with and without AVC progression. Among 3056 participants without AVC at baseline, 119 (3.9%) developed AVC, 48 out of 1623 (3%) females and 71 out of 1433 (5%) males ($p < 0.0001$). 3419 participants had follow up CT exam. The average time between follow-up CT scans was 2.65 ± 0.84 years. For those with incident AVC, the mean Agatston score at follow up scan was 38.72 with ± SD 61.84, range 1.2–416. 418 subjects (12%) had evidence of AVC on initial scan and were included in the AVC progression category. Among 418 patients who had AVC at baseline, 233 (55.7%) had AVC progression at follow up. Among the entire cohort (N = 3474), 352 (10%) patients progressed in terms of AVC at follow-up and were all included in the progression analysis.

Factors significantly associated with AVC progression included older age and greater FPG, male gender, hypertension, cigarette smoking and use of lipid lowering medications ($p < 0.05$) (Table 4). There were no statistically significant differences for TC, HDL, TG, and LDL–C level, BMI, alcohol drinking and moderate exercise between the two groups ($p > 0.05$) (Table 4). The median and interquartile range for FMD%, CRP, vWF%, PAI, fibrinogen, IL6, E-selectin and ICAM-1 for those with and without AVC progression is shown in Table 5. FMD% was lower and levels of vWF%, fibrinogen and IL6 were higher in the AVC progression group than in no AVC progression ($p < 0.05$) (Table 5). In contrast, there was no statistically significant differences for PAI, CRP, E-selectin and ICAM-1 between the two groups ($p > 0.05$) (Table 5). The OR of AVC progression associated with decreasing FMD% and increasing biomarkers of endothelial dysfunction are presented in Table 6. Unadjusted univariate logistic regression analysis (model 1) showed that decreasing FMD% and increasing vWF %, fibrinogen and IL6 were associated with progression of AVC (Table 6). After adjustment for demographics and cardiac risk factors, Model 2 and Model 3 showed no significant association between decreasing FMD% and increasing biomarkers and AVC progression (Table 6). We conducted a sensitivity analysis only among patients with complete data on all biomarkers (N = 640). Our Results between subgroup (N = 640) and full cohort analysis (N = 3742) were consistent. Results of sensitivity analysis of association of AVC progression and endothelial dysfunction biomarkers showed non-statistically significant patterns (Table 7). In a secondary analysis, the absolute change in AVC score among those with detectable AVC at exam 1 was modeled after adjusting for baseline Agatston score. The absolute change was calculated as the difference between AVC score at follow-up and baseline. The results were similar to the prior logistic regression using binary outcome of AVC progression with no evidence of significant association between ED and the absolute change in the AVC absolute change (data not shown). Additionally, AVC progression was associated with former smoking status, and HTN. The completion of high school education or higher, as well Chinese and Black race/ethnicity appeared to be protective factors with respect to AVC progression. The MESA site had no association with AVC progression.

4. Discussion

This manuscript represents the first population based study on the relationship between endothelial function and aortic valve calcification in a multiethnic cohort without known cardiovascular disease at baseline. While previous studies have examined the relationship of FMD % and ED to AVC occurrence retrospectively, this is the first study to examine the potential of biomarkers and FMD% to predict new onset AVC or progression of existing AVC.

Table 1
Demographic characteristics and cardiac risk factors among participants with and without AVC at baseline (N = 3474).

Characteristics	No prevalent AVC n = 3056	Prevalent AVC n = 418	*p*-value
	Mean ± SD or N (%)	Mean ± SD or N (%)	
Age	60.16 ± 9.66	69.32 ± 8.27	< 0.0001
Male gender	1433 (46.8)	275 (65.7)	< 0.001
Race/ethnicity			< 0.001
White	1019 (33.4)	181 (43.3)	
Chinese	563 (18.4)	47 (11.2)	
Black	716 (23.4)	84 (20.1)	
Hispanic	758 (24.8)	106 (25.3)	
Site			0.1599
Wake Forest University	528 (17.2)	93 (22.2)	
Columbia University	610 (19.9)	85 (20.3)	
Johns Hopkins University	83 (2.7)	9 (2.1)	
University of Minnesota	480 (15.7)	62 (14.8)	
Northwestern University	616 (23.6)	70 (16.7)	
UCLA	739(24.1)	99 (23.6)	
Body Mass Index	27.97 ± 5.35	28.51 ± 4.85	0.01
Pack of cigarettes/year			< 0.0001
Smoking status	9.50 ± 18.94	15.85 ± 26.71	< 0.0001
Never	1661 (54.3)	176 (42.1)	
Former	1013 (33.1)	203 (48.5)	
Current	380 (12.4)	39 (9.3)	
Systolic blood pressure	123.87 ± 19.66	132.57 ± 19.68	< 0.0001
Diastolic blood pressure	71.81 ± 10.05	72.33 ± 9.50	
FPG, mg/dl	95.91 ± 28.00	100.93 ± 32.16	< 0.0001
TC, mg/dl	194.34 ± 34.89	194.20 ± 36.85	0.13
LDL-C, mg/dl	117.2 ± 30.22	118.1 ± 33.13	0.61
HDL, mg/dL	50.784 ± 14.50	47.77 ± 13.87	< 0.0001
TG, mg/dl	133.03 ± 94.83	140.86 ± 72.06	< 0.0001
Use of lipid-lowering medication	393 (12.86)	94 (22.49)	< 0.0001
Alcohol use			0.0493
Never	703 (23.1)	74 (17.7)	
Former	670 (22.0)	99 (23.7)	
Current	1672 (54.9)	244 (58.5)	
Completion of high school education or higher	2530 (82.8)	319 (76.3)	0.0011
Moderate exercise	2362 (77.3)	332 (79.4)	0.3377

FPG, fasting plasma glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.
Numerical values reported as mean ± SD and categorical values reported as n (%).
Percentages may not add up to 100% in all instances due to rounding. Counts may not be equal to the total cohort sample size due to missing data.
p value from T-test for continuous variables and Chi-squared test for categorical variables.

Endothelial dysfunction is recognized to occur in many inflammatory disease states, and has been shown to be predictive of coronary artery disease in certain populations [11,34–36]. A blunted brachial vasoreactive response has been shown to increase risk of CAD, unstable angina and stroke in those with peripheral arterial disease [34,35]. Individuals with endothelial dysfunction have been shown to be at elevated risk for hypertension, and there is some suspicion that this risk may begin early in life [37,38]. Biomarkers of endothelial dysfunction have been recognized in other diverse disease states such as chronic kidney disease and rheumatoid arthritis; conditions with strong correlations to cardiovascular disease [36,39,40]. While prediction of disease incidence or progression is lacking from these studies, some evidence suggests that therapies that lower biomarkers of ED may reduce end organ risk as well [36]. In their study, Nozaki et al. found the potential to predict coronary heart disease using a battery of tests, which included biomarkers of endothelial dysfunction showing that there is clinical cardiovascular relevance to the assessment of ED [41].

Aortic valve calcification shares risk factors with coronary artery disease. Han et al. investigated the relationship between coronary endothelial function and coronary calcification in forty-six patients: 17 men [37%]; age, 47.4 ± 11.4 years. These patients prospectively underwent testing for coronary endothelial function and measurement of coronary artery calcification. The study demonstrated that in patients without significant coronary artery disease, coronary endothelial dysfunction showed no apparent association with coronary calcification [42]. Our findings complement these Results and indicate that ED and extravascular calcification may represent separate, independent processes in the progression of atherosclerosis. Towler et al. used low-

Table 2
Median (IQR) of FMD% and endothelial biomarkers by AVC prevalence at baseline.

Biomarkers	Participants with no AVC at baseline (N = 3056)		Participants with AVC at baseline (N = 418)		*p*-value
	N	Median (IQR)	N	Median (IQR)	
FMD%	2882	4 (2.4–6.1)	398	2.9 (1.7–4.8)	< 0.0001
CRP (mg/L)	3038	1.74 (0.77–4.08)	416	1.86 (0.88–3.95)	0.3994
vWF%	793	129.00 (96.00–168.00)	79	150.0 (109.00–187.00)	0.0037
PAI (ng/ml)	780	19.00 (9.00–36.00)	77	23.0 (11.0–37.00)	0.3403
Fibrinogen (μmol/L)	3041	334.00 (292.00–384.00)	417	347.0 (309.00–395.00)	0.0003
IL6 (pg/ml)	2978	1.10 (0.713–1.74)	406	1.35 (0.95–2.13)	< .0001
E-selectin (ng/ml)	794	51.50 (37.64–66.22)	79	50.32 (34.53–63.74)	0.6935
ICAM-1 (ng/ml)	1369	264.07 (226.12–307.98)	162	278.96 (240.68–317.20)	0.0065

FMD%, flow mediated dilation; CRP, C-reactive protein; vWF, Von Willebrand factor; PAI, plasminogen activator inhibitor; IL6, interleukin 6; ICAM-1, soluble intercellular adhesion molecule-1; IQR, interquartile range.
Values are presented as medians (IQR), and *p*-values were computed using Wilcoxon test for the difference in medians.
p value from Wilcoxon rank-sum test to compare the medians.

Table 3A
Results of logistic regression models of AVC prevalence and log-transformed FMD% and endothelial dysfunction biomarkers (N = 3472).

	ORs and 95% CI	*p*-value
FMDFMD%		
Model 1	0.656 (0.581, 0.741)	< 0.0001
Model 2	0.912 (0.787, 1.057)	0.223
Model 3	1.028(0.786, 1.346)	0.839
CRP		
Model 1	1.044 (0.955, 1.140)	0.345
Model 2	1.096 (0.988, 1.215)	0.084
Model 3	0.981 (0.825, 1.168)	0.831
vWF		
Model 1	2.446 (1.361, 4.396)	0.003
Model 2	1.543 (0.793, 3.003)	0.201
Model 3	1.478 (0.740, 2.953)	0.269
PAI		
Model 1	0.913 (0.695, 1.199)	0.245
Model 2	1.110 (0.958, 1.286)	0.081
Model 3	1.124 (0.960, 1.316)	0.713
Fibrinogen		
Model 1	2.539 (1.534, 4.204)	0.000
Model 2	1.958 (1.118, 3.522)	0.081
Model 3	1.116 (0.424, 2.940)	0.713
IL6		
Model 1	1.636 (1.401, 1.911)	< 0.0001
Model 2	1.324 (1.106, 1.586)	0.002
Model 3	1.065 (0.779, 1.456)	0.649
E-selectin		
Model 1	0.911 (0.566, 1.468)	0.703
Model 2	1.086 (0.607, 1.943)	0.780
Model 3	0.876 (0.479, 1.602)	0.667
ICAM-1		
Model 1	2.572 (1.431, 4.622)	0.002
Model 2	2.127 (1.034, 4.375)	0.040
Model 3	1.766 (0.834, 3.743)	0.138

density lipoprotein receptor-deficient (*LDLR* $^{-/-}$) mice to study vascular calcification in the ascending aorta. They demonstrated that spatial pattern of genes encoding homeodomain transcription factors regulate mineralization and osseous differentiation programs in the developing skull. Osteopontin, an osteoblast matrix protein gene also expressed by activated macrophages, strongly suggests that vascular calcification, thought to be limited to the media, is an active process that can originate from an osteoprogenitor cell population in the adventitia [43].

Table 3B
Results of logistic regression models of AVC prevalence and log-transformed FMD% and endothelial dysfunction biomarkers, among patients with complete biomarker measurements (N = 640).

	ORs and 95% CI	*p* values
FMDFMD%		
Model 1	0.772 (0.552, 1.078)	0.129
Model 2	1.333 (0.871, 2.039)	0.185
Model 3	1.424 (0.901, 2.252)	0.130
CRP		
Model 1	1.008 (0.800, 1.269)	0.949
Model 2	1.004 (0.768, 1.313)	0.977
Model 3	0.924 (0.684, 1.248)	0.606
vWF		
Model 1	4.062 (1.942, 8.494)	< .0001
Model 2	2.824 (1.254, 6.357)	0.012
Model 3	3.077 (1.318, 7.181)	0.009
PAI		
Model 1	1.223 (0.902, 1.659)	0.196
Model 2	1.372 (0.967, 1.946)	0.076
Model 3	1.143 (0.773, 1.691)	0.504
Fibrinogen		
Model 1	1.573 (0.409, 6.044)	0.510
Model 2	1.214 (0.247, 5.976)	0.812
Model 3	0.695 (0.121, 4.012)	0.685
IL6		
Model 1	1.795 (1.166, 2.764)	0.008
Model 2	1.541 (0.931, 2.551)	0.093
Model 3	1.312 (0.755, 2.282)	0.336
E-selectin		
Model 1	0.785 (0.444, 1.389)	0.406
Model 2	0.807 (0.398, 1.640)	0.554
Model 3	0.623 (0.301, 1.292)	0.204
ICAM-1		
Model 1	3.978 (1.374, 11.516)	0.011
Model 2	4.618 (1.282, 16.630)	0.019
Model 3	4.913 (1.195, 20.204)	0.027

FMD%, flow mediated dilation; CRP, C-reactive protein; PAI, plasminogen activator inhibitor; IL6, interleukin 6; ICAM-1, soluble intercellular adhesion molecule-1; CI, confidence interval; OR, odds ratio; vWF, Von Willebrand factor.
Model 1 only FMD% or biomarkers were included as independent variables.
Model 2 additionally adjusted for age and gender.
Model 3 additionally adjusted for age, gender, race, BMI, FPG, systolic blood pressure, smoking, alcohol drinking, LDL–C, TC, TGs and lipid lowering medications.
Odds ratio represents the odds of having AVC prevalence per one-standard deviation decrement in FMD% or one-standard deviation increment in plasma biomarkers levels.

Table 4
Demographic characteristics and cardiac risk factors among participants by progression of AVC.

Characteristics	No AVC progression (N = 3122)	With AVC progression (N = 352)	*p*-value
Age	61.10 ± 9.96	65.97 ± 8.77	< 0.0001
Male gender	1637 (48.79)	71 (59.66)	0.02
Race/ethnicity			0.13
White	1159 (34.55)	14 (34.45)	
Chinese	598 (17.82)	12 (10.08)	
Black	770 (22.95)	30 (25.21)	
Hispanic	828 (24.68)	36 (30.25)	
Site			0.0020
Wake Forest University	605 (97.42)	16 (2.58)	
Columbia University	653 (93.96)	42 (6.04)	
Johns Hopkins University	90 (97.83)	2 (2.17)	
University of Minnesota	524 (96.68)	18 (3.32)	
Northwestern University	669 (97.52)	17 (2.48)	
UCLA	814 (97.14)	24 (2.86)	
Body Mass Index	28.01 ± 30	28.74 ± 5.04	0.47
Pack of cigarettes/year			< 0.0001
Smoking status	10.29 ± 20.26	9.66 ± 16.13	0.27
Never	1778 (53.03)	59 (49.58)	
Former	1176 (35.07)	40 (33.61)	
Current	399 (11.9)	20 (16.8)	
Systolic blood pressure	124.75 ± 19.86	129.59 ± 19.37	< 0.0001
Diastolic blood pressure	71.82 ± 10.02	73.28 ± 9.09	
FPG, mg/dl	96.35 ± 28.56	101.05 ± 28.73	< 0.0001
TC, mg/dl	194.50 ± 34.97	192.75 ± 36.51	0.37
LDL, mg/dL	117.2 ± 30.10	117.1 ± 33.07	0.95
HDL, mg/dL	50.83 ± 14.47	49.62 ± 15.42	0.4
TG, mg/dl	128.01 ± 93.83	132.9 ± 95.65	0.76
Use of lipid-lowering medication	457 (13.62)	30(25.21)	< 0.0001
Alcohol use			0.342
Never	756 (97.30)	21 (2.70)	
Former	744 (22.00)	25 (3.25)	
Current	1843 (96.19)	73 (3.81)	
Completion of high school education or higher	599 (17.86)	24 (20.17)	0.52
Moderate exercise	2607 (96.77)	87 (3.23)	0.2327

FPG, fasting plasma glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.
Numerical values reported as mean ± SD, categorical values reported as n (%).
Percentages may not add up to 100% in all instances due to rounding. Counts may not be equal to the total cohort sample size due to missing data.
AVC progression is the sum of participants who developed incident AVC and those in whom the AVC progressed on follow up compared to baseline.

Table 5
Median (IQR) of FMD% and endothelial biomarkers according to AVC progression.

Biomarkers	N	No AVC progression	N	AVC progression	*p*-value
FMD%	2945	4.1% (2.40%–6.20%)	335	3.3% (2.10%–5.20%)	0.02
CRP (mg/L)	3104	1.74 (0.77–4.08)	350	1.68 (0.94–4.65)	0.6592
vWF%	803	129.00 (96.00–168.00)	69	145.0 (109.00–185.00)	0.0203
PAI (ng/ml)	790	19.00 (9.00–36.00)	67	18.0 (10.00–34.00)	0.9668
Fibrinogen (μmol/L)	3107	335.00 (292.00–384.00)	351	347.0 (310.00–393.00)	0.0013
IL6 (pg/ml)	3045	1.10 (0.713–1.74)	339	1.32 (0.76–2.17)	0.027
E-selectin (ng/ml)	804	51.63 (37.50–66.14)	69	34.60 (38.37–72.37)	0.9681
ICAM-1 (ng/ml)	1387	263.44 (225.73–307.68)	144	284.05 (238.81–326.11)	0.2824

FMD%, flow mediated dilation; CRP, C-reactive protein; vWF, Von Willebrand factor; PAI, plasminogen activator inhibitor; IL6, interleukin 6; ICAM-1, soluble intercellular adhesion molecule-1; IQR, interquartile range.
Values are presented as medians (IQR), and *p*-values were computed using Wilcoxon test for the difference in medians.
AVC progression is the sum of participants who developed incident AVC and those in whom the AVC progressed on follow up compared to baseline.

Table 6
Odds ratio and 95% CIs of AVC progression associated with FMD% and biomarkers of endothelial dysfunction.

	ORs and 95% CI	*p*-value
FMD%		
Model 1	0.687 (0.603, 0.0.783)	< 0.0001
Model 2	0.916 (0.785, 1.068)	0.264
Model 3	1.105(0.835, 1.463)	0.486
CRP		
Model 1	1.064 (0.967, 1.170)	0.203
Model 2	1.074 (0.893, 1.292)	0.042
Model 3	1.014 (0.849, 1.210)	0.881
vWF		
Model 1	2.118 (1.142, 3.930)	0.017
Model 2	1.291 (0.650, 2.566)	0.466
Model 3	1.132 (0.559, 2.292)	0.730
PAI		
Model 1	1.229 (1.068, 1.415)	0.657
Model 2	1.110 (0.958, 1.286)	0.391
Model 3	1.124 (0.960, 1.316)	0.783
Fibrinogen		
Model 1	2.152 (1.251, 3.702)	0.006
Model 2	1.773 (0.973, 3.231)	0.061
Model 3	0.909 (0.338, 2.443)	0.850
IL6		
Model 1	1.505 (1.273, 1.779)	< 0.0001
Model 2	1.255 (1.040, 1.515)	0.018
Model 3	1.061 (0.772, 1.459)	0.715
E-selectin		
Model 1	0.873 (0.527, 1.445)	0.597
Model 2	0.906 (0.504, 1.628)	0.742
Model 3	0.794 (0.426, 1.480)	0.468
ICAM-1		
Model 1	1.662 (0.902, 3.062)	0.103
Model 2	1.223 (0.596, 2.511)	0.583
Model 3	0.998 (0.476, 2.092)	0.997

Table 7
Odds ratio and 95% CIs of AVC progression associated with FMD% and biomarkers of endothelial dysfunction among patients with complete biomarker measurements (N = 640).

	ORs and 95% CI	*p* values
FMD%		
Model 1	0.713 (0.505, 1.008)	0.055
Model 2	1.128 (0.739, 1.723)	0.576
Model 3	1.170 (0.744, 1.839)	0.496
CRP		
Model 1	1.148 (0.897, 1.467)	0.272
Model 2	1.205 (0.908, 1.598)	0.196
Model 3	1.220 (0.899, 1.656)	0.201
vWF		
Model 1	2.940 (1.368, 6.321)	0.006
Model 2	1.799 (0.791, 4.092)	0.161
Model 3	1.714 (0.739, 3.975)	0.209
PAI		
Model 1	1.117 (0.809, 1.541)	0.502
Model 2	1.227 (0.857, 1.758)	0.264
Model 3	1.129 (0.752, 1.696)	0.559
Fibrinogen		
Model 1	2.957 (0.701, 12.469)	0.140
Model 2	2.808 (0.529, 14.890)	0.225
Model 3	1.485 (0.243, 9.067)	0.669
IL6		
Model 1	1.518 (0.959, 2.402)	0.075
Model 2	1.274 (0.755, 2.149)	0.364
Model 3	1.337 (0.752, 2.378)	0.322
E-selectin		
Model 1	0.784 (0.428, 1.435)	0.430
Model 2	0.782 (0.381, 1.605)	0.503
Model 3	0.691 (0.314, 1.521)	0.358
ICAM-1		
Model 1	1.863 (0.615, 5.645)	0.271
Model 2	1.652 (0.465, 5.867)	0.438
Model 3	1.496 (0.376, 5.961)	0.568

FMD%, flow mediated dilation; CRP, C-reactive protein; PAI, plasminogen activator inhibitor; IL6, Interleukin 6; ICAM-1, soluble intercellular adhesion molecule-1; CI, confidence interval; OR, odds ratio; vWF, Von Willebrand factor.
Model 1 only FMD% or biomarkers were included as independent variables.
Model 2 adjusted for age and gender.
Model 3 adjusted for age, gender, race, baseline Agatston score, BMI, FPG, systolic blood pressure, smoking, alcohol drinking, LDL–C, TGs and lipid lowering medications.
Note: AVC progression is the sum of participants who developed incident AVC and those in whom the AVC progressed on follow up compared to baseline.
Odds ratios represent the odds of AVC progression per one-standard deviation decrement in FMD% or one-standard deviation increment in plasma biomarkers levels.

Kalil et al. randomized 13 hemodialysis patients comparing lanthanum carbonate (LC) with a non-LC phosphorus binders control group (non-LC) at a 1:1 randomization. CAC was obtained at baseline, 6, and 12 months, and endothelial function (brachial artery flow-mediated dilation - FMD%) at baseline and 6 months. The authors concluded that a slower rate of progression of CAC occurred in the LC group, independent of changes in FMD% [44]. Furthermore, the biomarkers of ED (IL6 and asymmetric dimethylarginine) did not differ between the two study groups at follow up, despite the difference in CAC progression [44]. Although the study participants were obviously at higher risk compared to the MESA population, the Results are clearly compatible with the current findings showing dissociation between progression of AVC and ED, as defined by FMD% and biomarkers.

Contrary to the aforementioned studies, Huang et al. studied 124 subjects with suspected CAD in whom coronary calcification and endothelial dysfunction were detected by electron beam CT and FMD%, respectively. There was an inverse association between the degree of coronary artery calcification and endothelium-dependent FMD% in the coronary calcium score tertiles (6.9 ± 0.6% *vs.* 5.3 ± 0.3% *vs.* 3.7 ± 0.3%, respectively; $p < 0.001$) [45]. By multivariate analysis, enhanced coronary calcification was a strong independent predictor of endothelial dysfunction ($p < 0.001$). The cross sectional design of this study provides limited causal inference because exposure and outcome were assessed concurrently. In other words, the study may represent a reverse causation bias rather than a true causal relationship between ED and vascular calcification.

Although the events leading to coronary and aortic valve calcification share some similarities with bone mineralization [5,46], their molecular mechanisms remain under investigated. It has been hypothesized that the valvular and vascular endothelial cells respond differently to the local shear stress [47]. While the coronary artery is exposed to sustained laminar blood flow [48], aortic valve is characterized by pulsatile shear stress on the ventricular side and low and reciprocating shear stress on the aortic side [49]. In addition, a study comparing the transcriptional profiles of both cell types under static and shear stress conditions found up to 10% of the genes considered in that study were significantly different [50], suggesting clear phenotypic differences between these two cell types in response to shear stress. Furthermore, the activation of circulating latent TGF-β1 under high shear stress may be associated with the development of aortic calcification. Despite those differences, it has been shown that the pathological inflammatory responses of the coronary and aortic value endothelium involve similar mediators such as vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) [51]. In a study of Sucosky et al., the ventricularis or aortic

surface of porcine AV leaflets were exposed for 48 h to unidirectional pulsatile and bidirectional oscillatory shear stresses ex vivo [52]. Four inflammatory markers VCAM-1, ICAM-1, BMP-4, and TGF-β1, were detected by immunohistochemistry. Exposure of the aortic surface to pulsatile shear stress (altered hemodynamics), but not oscillatory shear stress, increased expression of the inflammatory markers. In contrast, neither pulsatile nor oscillatory shear stress affected expression of the inflammatory markers on the ventricularis surface [52].

Aortic valve calcification has been a point of intense interest in the study of cardiovascular disease. Previous studies have patients with known AVC to have a strong association with certain risk factors such as male gender, hypertension, diabetes, smoking and dyslipidemia [4,53]. In their study, Aronow et al. found a significantly elevated risk of coronary artery events in subjects with AVC than those without [54]. Multiple studies have considered that AVC and coronary artery disease may represent outcomes of the same disease spectrum, arising at least in part from endothelial dysfunction [4,37,38,55]. Endothelial dysfunction has been shown to persist following valve replacement surgery as well [56]. While other research has shown that subjects with known AVC have significant rates of ED, they have examined this relationship retrospectively [4]. Despite considerable evidence and study of ED relating to cardiovascular disease including AVC as a part of a continuum of atherogenic cardiovascular disease, no study has shown that non-invasive measurement of ED can predict AVC incidence or progression.

Traditional risk factors for AVC and other cardiovascular disease such as smoking, obesity, hypertension and diabetes were examined within the context of this study as well. While other studies have shown ED as defined by biomarkers or FMD% to be predictive of cardiovascular disease processes in high-risk populations, clinical utility is limited among lower risk groups [36,41].

The level of lipid particles including LDL, HDL and TG's were not different between the AVC progression groups in our study. In MESA study by Owens et al., TC/HDL ratio was not statistically different between the two progression groups [53]. The lack of association of lipid particles with AVC progression in our study likely represents the effect of a higher use of lipid lowering therapy in the AVC progression group (30% *vs.* 12%). Prior studies examining the effect of dyslipidemia on AVC progression have shown variable Results. Smaller CT-based studies of early-stage calcific aortic valve disease either found no associations or associations with LDL cholesterol only [1,57]. Previous echocardiographic studies, performed in hospital-based cohorts involving older subjects with later stage calcific aortic valve disease (i.e., aortic stenosis) and higher rates of renal dysfunction and CVD, have shown variable associations [58–61]. Other community-based studies using echocardiography have demonstrated that lipids were not associated with progression of calcific aortic valve disease [62].

The use of lipid-lowering medication seems to be related to prevalent AVC and AVC progression in the current analysis. Non-invasive and invasive imaging techniques have shown an inverse relationship between statins use and plaque calcification known as “calcium paradox” [63]. Puri et al. performed an analysis of serial IVUS data from 8 large multicenter clinical trials [63]. The use of high-intensity statins was associated with an increase in the amount of coronary calcium in all patients irrespective of statin use. The greatest increase in calcium was observed in patients treated with high-intensity statin and coincided with significant plaque regression. Raber et al. studied 103 STEMI patients who underwent intravascular ultrasonography (IVUS) and radiofrequency ultrasonography (RF-IVUS) of the two non-infarct-related epicardial coronary arteries after successful primary PCI. Patients were treated with high-intensity rosuvastatin throughout 13 months and serial intracoronary imaging of matched segments. The proportion of calcified tissue components increased at follow up compared to baseline (+1.28%; CI: 0.66–1.90%; $p < 0.0001$) [64].

BMI was not associated with the progression of AVC in our study. In another MESA study of the AVC incidence and progression by Owens et al., BMI was associated with the incidence but not progression of AVC [53]. Combining the incident and progressive AVC in our analysis likely resulted in diluting the effect of BMI on incident AVC. Peltier et al. found that BMI > 30 kg/m^2 (odds ratio 2.03, 95% confidence intervals 1.24 to 3.34, $p = 0.005$) is associated with non-rheumatic severe calcific AS [65]. Unlike our study, Peltier et al. included 220 consecutive patients with severe calcific degenerative AS considered for surgery rather than a relatively healthy population free of CVD at baseline. The authors also used coronary angiography and transthoracic echocardiography rather than cardiac CT as gold standard to detect AVC. Furthermore, the study did not measure other confounders such as physical activity level, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol concentrations. Agmon et al. examined 381 subjects, a sample of the Olmsted County (Minnesota) population, by transthoracic and transesophageal echocardiography [66]. The odds of aortic valve stenosis were higher with higher body mass index (odds ratio [OR]: 1.07 for every 1 kg/m^2 increase in body mass index; CI: 1.02 to 1.12; p = 0.006). Lack of racial diversity and use of echocardiography limit the interpretation of this study findings. Larsson et al. used data from the Cohort of Swedish Men and the Swedish Mammography Cohort and found that compared with BMI 18.5–22.5 kg/m^2, the multivariable hazard ratios were 1.24 (95% confidence interval [CI] 1.05–1.48) for overweight (BMI 25.0–29.9 kg/m2) and 1.81 (95% CI 1.47–2.23) for obesity (BMI ≥30 kg/m^2) [67]. Since this study adjusted for less risk factors compared to our study, the observed association between obesity and AVS may be confounded by other AVS risk factors. This study used a single questionnaire to assess BMI and WC, which likely introduced some degree of measurement error in the exposure assessment. Another potential limitation is using the Swedish National Patient Registery, hence, the generalizability of findings to individuals not seeking specialist care is unknown. Therefore, these design and analytic differences could explain the different findings for BMI in our study compared to other studies.

The use of ED as measured by FMD% and biomarkers alone may not represent a useful clinical tool for prediction of AVC occurrence. It is possible that a broader series of tests, such as that employed by Nozaki et al., which include measurements of ED and other parameters would provide the ability to predict onset or progression of AVC, with the ultimate goal being a tool that could risk predict disease occurrence in even low risk groups.

Our study strengths include the largest sample size to date exploring the relationship between ED and AVC. Our study is the first attempt to study this relationship in a longitudinal design in a multiethnic cohort free of CVD at baseline. It is also the first study to use two surrogate markers, FMD% and biomarkers, simultaneously, to identify ED within the cohort. In contrast to prior investigations that used echocardiography to diagnose AVC, we used cardiac CT, which is considered the gold standard to detect valvular calcification and thus enhanced the accuracy of classifying the participants with and without AVC. As the MESA study gathered data from a large, multiethnic population, the Results may be widely generalizable to the same risk population. The imaging and laboratory procedures were standardized at a common institution. However, our study is not without limitations. Biomarkers of endothelium dysfunction may not be too precise, however, even after adding FMD% as a second surrogate maker for ED; our analysis produced the same pattern of the association supporting the conclusion. MESA was not powered specifically for the analysis of the incidence and progression of AVC and ED, however, the sample size (418 and 352 for prevalence and progression analyses, respectively) is much larger than in previous studies, and therefore, provides greater power to detect true associations and to minimize Type 2 errors. MESA included asymptomatic participants who were free of clinical CVD at baseline. This might have skewed the spectrum of the population toward more low risk participants. Therefore, further investigation is required to determine whether the current findings can be extrapolated to a higher risk group. Nonetheless, the same selection criteria allow insights into the relationship between AVC and ED at an early stage. Residual confounding

from misclassification of risk factors that are associated with AVC and ED could not be ruled out. For example, alcohol consumption was collected as never, former and current rather than quantified consumption frequency, which may obscure the U-shaped relationship between alcohol consumption and aortic valve calcification [68]. However, the differences of alcohol consumption between patients with and without AVC at baseline was borderline significantly different (Table 1) and were not statistically significant different between patients with and without AVC progression (Table 4). Furthermore, the aforementioned study by Larsson et al. examined the aortic valve stenosis rather than calcification as outcome.

Endothelial dysfunction clearly represents a significant component of cardiovascular disease including calcific valvular disease and efforts to develop clinically meaningful predictive tools should be a continued focus of research.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

Moshrik Abd alamir, principal investigator. Michael Goyfman, study design and statistics.

Dana Johnson, writing of initial draft. Yangyang Liu, statistical analysis. Firas Dabous, statistical analysis. Adib Chaus, editing of final version. Mathew Budoff, senior author.

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