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Valvular Calcification and Risk of Peripheral Artery Disease: The Multi-Ethnic Study of Atherosclerosis (MESA)

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Introduction

Over 20% of men and women seen in primary care medical practices aged 70 years or older or aged 50 through 69 years with history of cigarette smoking or diabetes have a low ankle-brachial index (ABI) consistent with peripheral artery disease (PAD). These individuals have higher mortality rates than people without PAD. Symptomatic PAD requiring hospitalization is associated with a five-year mortality rate that approaches 25%. Since PAD remains asymptomatic until advanced stages, investigation of risk markers that better identify at-risk individuals is important.

The presence of valvular calcification seen on routine computed tomography (CT) imaging in asymptomatic subjects may be one such marker. Aortic valvular calcification (AVC) and mitral annular calcification (MAC) can be readily visualized and quantified on chest or cardiac CT without additional cost, radiation, or time. These calcified deposits can also be seen on various other modalities including plain radiography, dual-energy X-ray absorptiometry or echocardiography.

Atherosclerosis in the vasculature is a systemic inflammatory process characterized by calcification of a lipid core with eventual progression to endochondral bone formation similar to that found in skeletal bone. In a study of 650 asymptomatic subjects undergoing whole body CT significant correlations were observed for the presence of calcified atherosclerosis across different vascular beds including the
carotid, coronary, aortic, and iliac vessels. Valvular calcification, either at aortic leaflets or the mitral annular level, is considered to be a biological process that shares many histopathological similarities with atherosclerosis.

The presence of AVC and MAC have each been associated with incident coronary heart and other cardiovascular diseases. While these measures been associated with prevalent PAD, prospective relationships between valvular calcification and incident PAD are unknown. In this study, we investigate whether AVC and MAC are also associated with an increased risk of developing PAD.

Methods

Cohort

The Multi-Ethnic Study of Atherosclerosis (MESA) is a National Heart, Lung, and Blood Institute funded multicenter longitudinal community-based study. The study recruited 6814 adults aged 45 to 84 years and free of clinically recognized cardiovascular disease from 6 field centers (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles, California; New York, New York; and St Paul, Minnesota) to undergo baseline examination between 2000 and 2002. The study participants self-identified with 1 of 4 race/ethnic groups: non-Hispanic white (38%), black (28%), Hispanic (22%), and Chinese (12%). Follow-up visits 2, 3, 4, and 5 were done in 2002–2004,
2004–2005, 2005–2007, and 2010–2012, respectively. Institutional review boards at each site approved the study, and all participants gave informed consent.

**Aortic valvular and Mitral annular calcification**

All participants underwent a cardiac CT scan at baseline. CT scanning and interpretation methods in MESA were previously reported. AVC and MAC were assessed by cardiac CT using either cardiac-gated electron-beam CT (Chicago, Los Angeles County, and New York City field centers) or multi-detector CT systems (Baltimore, Forsyth County, and St Paul field centers), depending on the study site, and the average effective radiation dose per scan in millisieverts (mSv) ranged from 0.6 to 5.6 mSv. Certified technologists scanned all participants twice over phantoms of known physical calcium concentration. Images were analyzed independently at a central reading center (Los Angeles Biomedical Research Institute). Scores for each of these two variables were computed using the phantom-adjusted Agatston score for 2 consecutive scans for each participant, and the mean value was used. Each scan was independently interpreted by separate analysts.

Aortic valve calcification was defined as any calcified lesion within the aortic valve leaflets according to a previously described method. MAC was defined by the presence of calcium on the mitral valve annulus. MAC and AVC scores were classified as present or
absent, and according to pre-specified cut-points (0, 1-100, >100).
Inter-observer (κ-statistic, 0.94) and intra-observer (κ-statistic, 0.94)
agreement between different CT image analysts who measured AVC on
the same cardiac CT image was excellent.\textsuperscript{19} Inter-observer (κ-statistic,
0.86) and intra-observer (κ-statistic, 0.95) agreement between
different CT image analysts who measured MAC on the same cardiac
CT image were also excellent.\textsuperscript{19}

**Peripheral artery disease – Clinical PAD and Low ABI**

During follow-up, clinical PAD was identified by self-report of a
hospitalized PAD diagnosis by the participant at (1) MESA clinic visits,
(2) follow-up phone call, or (3) participant notification. A PAD diagnosis
was also found during review of medical records for other events.
Follow-up for this analysis extended through 2015. Two physician
members of the MESA mortality and morbidity review committee
independently classified events. The full committee made final
classifications if there were disagreements. “Definite” PAD required
more than a physician diagnosis as follows.

Physician adjudicators subclassified clinical PAD as (1) lower
extremity claudication, (2) atherosclerosis of arteries of the lower
extremities, or (3) arterial embolism and/or thrombosis of the lower
extremities. Criteria for clinical PAD were met by a)
Ultrasonographically- or angiographically-demonstrated obstruction or
ulcerated plaque (>50\% of the diameter or >75 of the cross-sectional
area) demonstrated on ultrasound or angiogram of the iliac arteries or below, b) Absence of pulse by Doppler in any major vessel of the lower extremities, c) Exercise test that is positive for lower extremity claudication, d) Surgery, angioplasty, or thrombolysis for peripheral vascular disease, e) Amputation of one or more toes or part of the lower extremity because of ischemia or gangrene, or f) Exertional leg pain relieved by rest in combination with either physician-diagnosed claudication diagnosed or an ankle-arm blood pressure ratio ≤0.8.

As for subclinical PAD, the ABI was performed at baseline examination, as well as clinic exam 3, performed between September 2002 and February 2004, and clinic exam 5, performed between April 2010 and December 2011. To obtain the measurements used to calculate the ABI, participants rested supine for 5 minutes, and then systolic blood pressures were measured in both arms and legs with the appropriate-sized cuffs. For each leg, the systolic blood pressure in each posterior tibial and dorsalis pedis artery was measured using a continuous-wave Doppler ultrasound 5-mHz probe. The leg-specific ABI was calculated as the higher systolic blood pressure in the posterior tibial or dorsalis pedis divided by the average of the left and right brachial pressures. In the event that left and right brachial pressures differed by 10 mmHg or more, the higher of the brachial pressures was chosen, since subclavian stenosis could be present. The lower of the two leg-specific ABIs was used for analysis.
Individuals with a history of lower extremity revascularization and those with an ABI ≤0.90 at the baseline visit were excluded from the clinical PAD and low ABI analyses respectively. Participants with evidence of non-compressible vessels (ABI>1.4) at baseline were also excluded from the low ABI analyses. Incident clinical PAD required a physician-adjudicated diagnosis of “definite” PAD as defined above. Incident low ABI was defined as a decline in ABI of at least 0.15 and to 0.90 or less in either leg. The approach for ABI decline was used to limit the impact of regression to the mean and measurement error and avoids small clinically insignificant changes being included in the incident low ABI definition.\textsuperscript{20} If only one follow-up ABI was available, then that was used for the analysis. If both follow-up ABIs were available, then exam 5 was used unless the participant already met criteria for ABI decline at V3.

**Measurement of covariates**

Standardized questionnaires were used at baseline to obtain age, sex, race/ethnicity, level of education, annual household income, physical activity, alcohol consumption, smoking history, and medication usage, including statin, antihypertensive, and antidiabetic use. Education was categorized into “high school or less,” “some college,” or “college or more.” Annual household income was dichotomized at < $20,000, ≥ $20,000 but less than $50,000, or ≥ $50,000. Body mass index (BMI) was calculated as weight in kilograms
divided by height in meters squared. Three separate systolic and diastolic resting blood pressure measurements were taken in seated participants, with the last two measurements being averaged for analysis. Physical activity was recorded as participant-reported number of intentional exercise metabolic equivalent (MET)-minutes per week. Alcohol consumption was categorized as current, former, or never and also by self-reported number of drinks per week. Cigarette smoking was calculated in pack-years and also defined as current, former, or never. Aspirin use was defined as a self-reported use of at least 3 days per week. Hypertension was defined as a self-report of physician diagnosis and use of an anti-hypertensive medication, or systolic blood pressure ≥140, or diastolic blood pressure ≥90 mmHg.

Total and high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose were measured from fasting blood samples. Low-density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation in those with triglycerides <400 mg/dl. Diabetes was defined as a fasting glucose >125 mg/dl or use of anti-diabetic medications. High-sensitivity C-reactive protein (hsCRP) and serum cystatin C were determined with a BNII nephelometer (N Latex Cystatin C & N High Sensitivity CRP; Dade Behring Inc., Deerfield, IL). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equations.21

Statistical methods
Descriptive characteristics were provided by prevalent MAC and prevalent AVC. Both exposures, MAC and AVC scores, were used in analysis as binary (presence vs. absence), categorical with pre-specified cut-points of 0 and 100, and continuous (log2 (score+1)). Incidence rate of clinical PAD was computed with Poisson regression per 100 person-years of follow-up. Kaplan-Meier plots were drawn to present the survival curves by exposure categories. Log rank tests were used to compare the survival distributions.

Cox hazard models were used to estimate the hazard ratio (HR) of incident clinical PAD associated with MAC and AVC scores. For the analysis of low ABI, we used Poisson regression with offset to accommodate differential time to exposure, estimating the rate ratios (RR) of low ABI associated with MAC and AVC scores. Several nested models were used for both analyses: M1 adjusting for age, gender, and race; M2 further adjusting for diabetes, hypertension, smoking, alcohol use, lipid lowering therapy, physical activity, body mass index, eGFR, C-reactive protein, ABI, high-density lipoprotein cholesterol, and total cholesterol. We used generalized additive models (GAMs) with splines for continuous exposures to address the functional form for both analyses of clinical PAD and low ABI; we found no meaningful departures from linearity. P-values and confidence intervals are not adjusted for multiple testing. Analyses were conducted using R environment for statistical computing.22
Results

A total of 6778 participants were included in the prospective incident clinical PAD analysis (mean age=62 years; 53% female; 38% white, 28% African-American 22% Hispanic, and 12% Chinese) (Figure 1). Of these participants, 907 (13.3%) had baseline AVC and 640 (9.4%) had baseline MAC. Participants with baseline MAC were older, less likely to be physically active or a current smoker, and more likely to be female, white, have higher BMI, have higher SBP, higher CRP, lower DBP, have diabetes, and report using both anti-hypertensive and lipid lowering medications (Table 1). While similar findings were observed when baseline characteristics were compared according to baseline AVC, these participants had more traditional cardiovascular risk factors present (Supplemental Table 1). They were more likely to be male, have a positive smoking history, and a lower HDL.

The Kaplan Meier clinical PAD-free survival curves according to baseline MAC scores are shown in Figure 2. The log rank test p values were <0.01, suggesting that the survival distribution differed by MAC categories. The clinical PAD incidence rates per 100-person years of follow-up for MAC=0 and MAC>0 were 0.117 (95% confidence interval (CI) 0.075, 0.182) and 0.403 (95% CI 0.189, 0.858) respectively. When positive MAC scores were stratified according to cut-point of 100, the
clinical PAD incidence rates were similar, 0.396 (95% CI 0.145, 1.082) for 0<MAC<100 and 0.411 (95% CI 0.130, 1.294) for MAC≥100.

After full adjustment, individuals with baseline MAC had a significantly higher risk of developing clinical PAD compared to those without MAC (hazard ratio (HR) 1.79, 95% confidence interval (CI) 1.04, 3.05) (Table 2). When the MAC classified into 3 groups was used, associations for 0<MAC<100 and MAC≥100 with incident PAD were both of borderline significance and similar in magnitude. When the continuous transformation of the MAC score was used, the association was borderline significant for each unit increase in log2(MAC+1) (HR 1.07, 95% CI 1.00, 1.15, p-value=0.06) without any departures from linearity in the fully adjusted model (Figure 3).

The presence of baseline MAC was not associated with a significantly higher risk of incident low ABI in fully adjusted analyses (RR 1.28, 95% CI 0.75, 2.19) (Table 2). Similarly, no significant adjusted associations were observed for each logarithmic increase in MAC score and risk of incident low ABI (Table 2). However, all risk ratios in these analyses were greater than one.

The association between baseline AVC assessed as (1) presence vs. absence, (2) a 3-categorical exposure, or (3) per logarithmic increase in score, and risk of both clinical PAD and incident low ABI were not significantly increased in fully adjusted analyses (Table 3).
Discussion

The presence of MAC is associated with a higher risk of developing clinical PAD but not a low ABI. Although a trend was observed for a continuous increase in MAC scores and incident clinical PAD, when MAC scores were stratified, the risk of clinical PAD was similarly increased for higher (≥100) or lower (1-100) amounts. No significant associations were noted for the presence of AVC and risk of either clinical PAD or low ABI.

MAC has a reported prevalence between 8% and 15% and is associated with an increased risk of coronary heart disease, stroke, and cardiovascular death.\textsuperscript{4,11-13, 23-26} We now report, for the first time, a prospective association between MAC and incident PAD as well. Atherosclerosis development is not uniform throughout the vascular tree and PAD is a distinct form of atherosclerosis and, therefore, findings presented here are important. In the Reduction of Atherothrombosis for Continued Health registry, 40% of people with PAD had no concomitant coronary or cerebrovascular disease.\textsuperscript{27} Prior reports are cross-sectional, single-center studies in highly selected populations that relied on echocardiography for MAC detection, a method that lacks adequate specificity in distinguishing between calcification and dense collagen. In one study of individuals referred for echocardiogram who also underwent ABI within a similar time period, mean ABI was significantly lower in the MAC group compared with the
control group and those with MAC had a higher prevalence of PAD, defined as an ABI<0.9. In another study of 151 individuals who recently experienced acute coronary syndrome, valvular calcification was also associated with prevalent peripheral atherosclerosis.

Although there is no clear mechanism linking MAC to PAD, growing evidence to suggests that MAC develops in a similar manner to atherosclerosis. The process is often initiated by endothelial injury at foci of increased mechanical stress and this, in combination with the resulting inflammation, are the primary stimuli for valvular calcification. Over 80% of patients with evidence of MAC on autopsy also had calcified sediments in at least one coronary artery. Animal studies of rabbits fed a high cholesterol diet found that a tendency for fatty plaque, composed of foam cells, to form in the posterior leaflet of the mitral valve and analogous to that seen in the early stages of atherosclerotic plaques. Many studies have shown also shown a strong association between MAC and traditional cardiovascular risk factors.

While the development of clinical PAD has a more meaningful impact on approaches to secondary prevention compared to an incident low ABI, which can be largely asymptomatic, both represent atherosclerotic progression so it does not logically follow that MAC would only be associated with incident clinical PAD in this study. Prior studies have also not been able to demonstrate an association
between MAC and asymptomatic lower extremity atherosclerotic burden. In a study of over 1200 consecutive asymptomatic patients free of clinical coronary heart disease who underwent electron-beam computed tomography, standardized increases in calcium in the aorto-iliac vessels was associated with aortic annular calcification, but not MAC, after multivariate adjustment. Although Adler and colleagues reported a cross-sectional association between presence of MAC and a low ABI, this was only true for moderate-to-severe PAD, defined as an ABI<0.7, which is more likely to occur in the presence of symptomatic disease. In that study, an ABI between 0.7 and 0.9 was actually more prevalent in individuals without MAC. It is possible, therefore, that MAC may more closely represent active, advanced clinical disease rather than the actual lower extremity atherosclerotic burden. MAC burden and progression is characterized by a self-perpetuating cycle of inflammation. Recent observations have demonstrated that inflammatory activity, as measured by 18F-Fluorodeoxyglucose positron emission tomography, is increased in individuals who have MAC as well as those who experience more rapid MAC progression. Underlying inflammation may be more active in individuals with MAC and this may correlate more strongly with unstable, active disease at the endothelial level.

Another potential explanation may be that MAC might play a causative role in thromboembolism, which can lead to acute arterial
occlusion. Emboli can range from ulcerated calcium deposits to noncalcific thrombus at the valvular level.\textsuperscript{34} Turbulent blood flow at the mitral or aortic position in the presence of calcium can lead to fragmentation of red cells and release of adenosine diphosphate and thromboplastin, with resulting microthrombus formation and noncalcific embolism. Systemic emboli to cerebral, coronary, renal, and retinal arteries and the peripheral circulation was discovered on autopsy in one-third of patients with calcific AV disease.\textsuperscript{35} Additionally, MAC has also been independently associated with the presence of advanced aortic atheromas, characterized by a thickness > 5 mm, protrusion into the lumen, and ulceration.\textsuperscript{36}

We did not expect to observe different associations of MAC and AVC with incident PAD. MAC and AVC share a common initial pathology of lipid infiltration, components of chronic inflammation, and calcification, but are considered to be different entities with distinct mechanistic pathways. AVC is up to three times more prevalent than MAC and is considered to be largely a manifestation of the atherosclerotic process while the mechanisms leading to MAC are undoubtedly multifactorial.\textsuperscript{13} Previous studies in well-characterized population-based cohorts followed for many years have also shown that associations for AVC and development of clinical atherosclerotic disease are markedly attenuated after adjustment for traditional risk factors while associations for MAC remain significant.\textsuperscript{4, 37, 38} In addition
to atherosclerotic pathways, MAC also develops as a result of other conditions. MAC is accelerated by conditions that increase mitral valve stress as the higher MV closing pressures create excess annular tension and subsequently accelerated annulus calcification.\textsuperscript{23, 39} MAC is also accelerated by altered calcium-phosphate metabolism in the setting of poor renal function, which results in metastatic calcification in the mitral annulus.\textsuperscript{23,39,40} Finally, in contrast to the atherosclerosis paradigm, MAC is more prevalent among women and may be a result of ectopic calcium deposits related to the severe bone loss caused by postmenopausal osteoporosis.\textsuperscript{41,42} These non-atherosclerotic pathways, taken together, may provide some basis for how MAC, unlike AVC, may impact clinical PAD risk independent of that captured by traditional CV risk factors.

Our study has some limitations. Although the diagnosis of clinical PAD involved a comprehensive adjudication process, the number of events was low (<2%) and reported confidence intervals were wide. The power for categorical MAC and AVC analyses was likely reduced and limited our ability to perform subgroup analyses. MESA participants were relatively healthy at baseline and AVC incidence in this cohort and may be lower than what has been reported for other similarly aged cohorts. Even though the PAD risk associated with higher (≥100) or lower (1-100) MAC was not statistically significant, the magnitude of the effect was quite large. As such, the lack of
statistical significance was likely due to the small numbers of events in
the different categories. Incident low ABI may have been inadequately
assessed, particularly in diabetics, due to exclusion of participants with
an ABI >1.4 at baseline. Additionally, ABI measurements did not
include a post-exercise value and, therefore, may not have detected
PAD in some individuals. Lastly, causality cannot be inferred on the
basis of this observational study. We cannot exclude the possibility
that associations are simply reflective of underlying atherosclerotic
burden or risk due to either unmeasured or inadequately measured
confounders.

In conclusion, MAC, as detected on Cardiac CT, is associated with
a higher risk of developing clinical PAD while no associations were
observed for AVC and incident PAD. Further clarification into this
discordant association using both CT and echocardiogram source data
will be helpful. Ultimately, studies both corroborating these results in
other prospective cohorts with more representative rates of incident
PAD as well as better determining whether MAC has any potential to be
a clinically useful risk marker of development of PAD are needed.

Acknowledgments: We thank the other investigators, the staff, and
the participants of the MESA study for their valuable contributions. A
full list of participating MESA investigators and institutions can be
found at http://www.mesa-nhlbi.org.
References


32. Kurtoğlu E, Korkmaz H, Aktürk E, Yılmaz M, Altaş Y, Uçkan A. Association of mitral annulus calcification with high-sensitivity C-
reactive protein, which is a marker of inflammation. *Mediators Inflamm* 2012; 2012: 606207.


Figures

Figure 1: Flowchart of participants included in the analysis for developing PAD

Figure 2. Kaplan-Meier plot of clinical PAD-free survival by presence of MAC and by MAC categories

Figure 3. GAM plot for risk of clinical PAD according to logarithmic increase in baseline MAC score
Table 1. Baseline characteristics of MESA participants according to presence or absence of mitral annular calcification (MAC)*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MAC (n=640)</th>
<th>No MAC (n=6138)</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>71.8 (7.7)</td>
<td>61.1 (9.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Male, %</strong></td>
<td>254 (39%)</td>
<td>2942 (48%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Race, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>313 (49%)</td>
<td>2299 (37%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chinese</td>
<td>37 (6%)</td>
<td>763 (12%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>141 (22%)</td>
<td>1738 (28%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>149 (23%)</td>
<td>149 (23%)</td>
<td></td>
</tr>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td>28.9 (5.7)</td>
<td>28.3 (5.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Smoking status, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>308 (48%)</td>
<td>3041 (50%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Current</td>
<td>60 (9%)</td>
<td>825 (13%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Pack-years smoking</strong></td>
<td>12.8 (22.2)</td>
<td>11.1 (20.8)</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Diabetes, %</strong></td>
<td>123 (19%)</td>
<td>723 (12%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>SBP, mm Hg</strong></td>
<td>135 (23)</td>
<td>126 (21)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>DBP, mm Hg</strong></td>
<td>70 (10)</td>
<td>72 (10)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Total cholesterol, mg/dL</strong></td>
<td>193 (38)</td>
<td>194 (35)</td>
<td>0.66</td>
</tr>
<tr>
<td><strong>LDL cholesterol, mg/dL</strong></td>
<td>115 (33)</td>
<td>117 (31)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>HDL cholesterol, mg/dL</strong></td>
<td>52 (15)</td>
<td>51 (15)</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Lipid lowering therapy, %</strong></td>
<td>162 (25%)</td>
<td>930 (15%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Antihypertensive use, %</strong></td>
<td>348 (54%)</td>
<td>2175 (35%)</td>
<td>&lt;0.01</td>
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<td><strong>Physical activity, MET-min/wk</strong></td>
<td>4625 (4203)</td>
<td>5870</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>C-reactive protein, mg/L</strong></td>
<td>1.1 (1.6)</td>
<td>0.9 (1.7)</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Continuous variables are expressed as mean (SD). Categorical variables are N (percent).

Comparisons were made between MAC and no MAC groups using chi-square tests for categorical variables and t-tests for continuous.
<table>
<thead>
<tr>
<th>Presence vs. absence</th>
<th>Clinical PAD (n=6778)</th>
<th>Low ABI (n=5762)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events / # at risk</td>
<td>Model 1† HR (95% CI)</td>
<td>Model 1 HR (95% CI)</td>
</tr>
<tr>
<td>MAC = 0</td>
<td>90/6138</td>
<td>1.00</td>
</tr>
<tr>
<td>MAC &gt; 0</td>
<td>27/640</td>
<td>2.43 (1.52, 5.23)</td>
</tr>
<tr>
<td>Categories</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAC = 0</td>
<td>90/6138</td>
<td>1.00</td>
</tr>
<tr>
<td>0 &lt; MAC &lt; 100</td>
<td>15/341</td>
<td>2.40 (1.35, 4.26)</td>
</tr>
<tr>
<td>MAC ≥100</td>
<td>12/299</td>
<td>2.48 (1.31, 4.67)</td>
</tr>
<tr>
<td>Continuous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per log unit</td>
<td>117/6778</td>
<td>1.12 (1.05, 1.19)</td>
</tr>
</tbody>
</table>

Table 2: Associations of baseline MAC with incident PAD
MAC=mitral annular calcification, PAD=peripheral arterial disease, ABI=ankle-brachial index

*Results of multivariable Cox Proportional Hazards Models (clinical PAD) and poisson regression models (low ABI)

†Model 1 adjusted for age, sex, and race/ethnicity
‡Model 2 adjusted for Model 1 + diabetes, hypertension, smoking, alcohol use, lipid lowering therapy, physical activity, body mass index, estimated glomerular filtration rate, C-reactive protein, ABI, high-density lipoprotein cholesterol, and total cholesterol
Table 3: Associations of baseline AVC with incident PAD

<table>
<thead>
<tr>
<th></th>
<th>Clinical PAD (n=6778)</th>
<th>Low ABI (n=5762)</th>
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</tr>
<tr>
<td>Presence vs. absence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVC = 0</td>
<td>82/5870</td>
<td>1.00</td>
</tr>
<tr>
<td>AVC &gt; 0</td>
<td>35/907</td>
<td>1.99 (1.30, 3.06)</td>
</tr>
<tr>
<td>Categories</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVC = 0</td>
<td>82/5870</td>
<td>1.00</td>
</tr>
<tr>
<td>0 &lt; AVC &lt; 100</td>
<td>20/583</td>
<td>1.81 (1.08, 3.02)</td>
</tr>
<tr>
<td>AVC ≥100</td>
<td>15/324</td>
<td>2.34 (1.30, 4.21)</td>
</tr>
<tr>
<td>Continuous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per log unit</td>
<td>117/6778</td>
<td>1.09 (1.02, 1.02)</td>
</tr>
</tbody>
</table>
AVC=aortic valvular calcification, PAD=peripheral arterial disease, ABI=ankle-brachial index

*Results of multivariable Cox Proportional Hazards Models (clinical PAD) and logistic regression models (low ABI)

†Model 1 adjusted for age, sex, and race/ethnicity

‡Model 2 adjusted for Model 1 + diabetes, hypertension, smoking, alcohol use, lipid lowering therapy, physical activity, body mass index, estimated glomerular filtration rate, C-reactive protein, ABI, high-density lipoprotein cholesterol, and total cholesterol
Figure 1.

MESA cohort (N=6814)

2, missing baseline AVC or MAC
N=6812

79, missing baseline

251, baseline ABI

43, baseline

677, missing follow-up

4, Clinical

30, Missing follow-up PAD

5762 included in follow-up ABI

6778 included in clinical PAD

AVC=aortic valvular calcification, MAC=mitral annular calcification,
PAD=peripheral arterial disease, ABI=ankle-brachial index
Figure 2.

A) Presence vs. Absence

B) Categorical
Generalized additive model: Penalized spline with 4 degrees of freedom for continuous MAC in Model 2 (red curve). Dotted curves show 95% confidence interval. Blue lines show log2 (1) and log2 (101), the cut-offs for the 3 categories exposure. We see no departure from linearity.
**Supplemental Table 1.** Baseline characteristics of MESA participants according to presence or absence of aortic valve calcification (AVC)*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AVC (n=907)</th>
<th>No AVC (n=5870)</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>70.5 (8.1)</td>
<td>60.9 (9.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Male, %</td>
<td>544 (60%)</td>
<td>2652 (45%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Race, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>411 (45%)</td>
<td>2200 (37%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chinese</td>
<td>67 (7%)</td>
<td>733 (12%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>231 (25%)</td>
<td>1641 (28%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>198 (22%)</td>
<td>1289 (22%)</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.5 (5.0)</td>
<td>28.3 (5.5)</td>
<td>0.33</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>506 (56%)</td>
<td>2842 (48%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Current</td>
<td>97 (11%)</td>
<td>787 (13%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Pack-years smoking</td>
<td>16.2 (25.9)</td>
<td>10.5 (19.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>178 (20%)</td>
<td>668 (11%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>135 (22)</td>
<td>125 (21)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>72 (10)</td>
<td>72 (10)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MAC (n=640)</th>
<th>No MAC (n=6138)</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>195 (38)</td>
<td>194 (35)</td>
<td>0.44</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>119 (34)</td>
<td>117 (31)</td>
<td>0.15</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>49 (14)</td>
<td>51 (15)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lipid lowering therapy, %</td>
<td>232 (25%)</td>
<td>860 (15%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Antihypertensive use, %</td>
<td>502 (55%)</td>
<td>2021 (34%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Physical activity, MET-min/wk</td>
<td>4969 (5017)</td>
<td>5874 (6014)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.0 (1.6)</td>
<td>0.9 (1.7)</td>
<td>0.05</td>
</tr>
</tbody>
</table>
*Continuous variables are expressed as mean (SD). Categorical variables are N (percent).

†Comparisons were made between AVC and no AVC groups using chi-square tests for categorical variables and t-tests for continuous