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Serum Phosphate is Associated with Aortic Valve Calcification in the Multi-Ethnic Study of Atherosclerosis (MESA)

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Abstract

Objectives—This study sought to investigate associations of phosphate metabolism biomarkers with aortic valve calcification (AVC).

Background—Calcific aortic valve disease (CAVD) is a common progressive condition that involves inflammatory and calcification mediators. Currently there are no effective medical treatments, but mineral metabolism pathways may be important in the development and progression of disease.

Methods—We examined associations of phosphate metabolism biomarkers, including serum phosphate, urine phosphate, parathyroid hormone (PTH) and serum fibroblast growth factor (FGF)-23, with CT-assessed AVC at study baseline and in short-term follow-up in 6,814 participants of the Multi-Ethnic Study of Atherosclerosis (MESA).

Results—At baseline, AVC prevalence was 13.2%. Higher serum phosphate levels were associated with significantly greater AVC prevalence (relative risk 1.3 per 1mg/dL increment, 95% confidence incidence: 1.1 to 1.5, $p < 0.001$). Serum FGF-23, serum PTH, and urine

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phosphate were not associated with prevalent AVC. Average follow-up CT evaluation was 2.4 years (range 0.9–4.9 years) with an AVC incidence of 4.1%. Overall, phosphate metabolism biomarkers were not associated with incident AVC except in the top FGF-23 quartile.

Conclusions—Serum phosphate levels are significantly associated with AVC prevalence. Further study of phosphate metabolism as a modifiable risk factor for AVC is warranted.

Keywords

Phosphate; Aortic Valve; Calcification

Background

Calcific aortic valve disease (CAVD) is a common condition that affects more than a quarter of adults over the age of 65.(1) The presence of aortic valve calcification (AVC) is predictive of future cardiovascular events and mortality,(2,3) and is associated with the severity of hemodynamic stenosis.(4,5) Currently there is no medical treatment for CAVD, and valve replacement remains the only therapy for advanced symptomatic disease.

CAVD is now well understood to be an active metabolic process involving mediators of both inflammation and calcification.(6) Histopathologic studies of early CAVD demonstrate a mixture of lipid deposition, inflammatory infiltrates, and calcification.(7) Despite evidence for an atherosclerotic-like process in CAVD development, studies have failed to show benefit of statin therapy among people with mild to moderate aortic stenosis.(8–10) Uncovering new pathways involved in the pathogenesis of CAVD is a critical next step toward developing effective therapeutic interventions.

Osteogenic pathways are likely important in the development and progression of CAVD, (11–14) and could represent novel, potentially-modifiable, treatment targets. Phosphate has been shown *in vitro* to activate osteogenic mediators in vascular smooth muscle cells.(15) We recently demonstrated a graded association of higher serum phosphate concentrations with aortic sclerosis among older adults in the Cardiovascular Health Study.(16) This intriguing result was based on qualitative measures of aortic sclerosis by echocardiography, assessment of serum phosphate levels as a single biomarker, and focus on mostly older, Caucasian individuals. Herein we expand our investigation of phosphate metabolism and CAVD by assessing quantitative measurements of aortic valve calcium by computed tomography (CT) and evaluating multiple markers of phosphate metabolism previously associated with cardiovascular outcomes, including fibroblast growth factor (FGF)-23, parathyroid hormone (PTH), and urine phosphate, in a large, multi-ethnic cohort.

Methods

Study Population

We evaluated participants from the Multi-Ethnic Study of Atherosclerosis (MESA), a prospective cohort study of 6,814 people who were free from clinical cardiovascular disease aged 45–84 years. Individuals were recruited between July 2000 and August 2002 from six U.S. communities (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina;

Los Angeles, California; New York, New York; and St. Paul, Minnesota) and were composed of four different ethnic groups (Black, Chinese, Hispanic and White). By design, the MESA recruited a final study population that was 38% White, 28% African American, 22% Hispanic, and 12% Asian, primarily of Chinese descent. A complete description of the study design has been published elsewhere.(17) Institutional review boards at each participating center approved the study, and all participants provided informed consent. Exclusions from participation in MESA included self-reporting of physician-diagnosed heart attack, angina or use of nitroglycerin, stroke, transient ischemic attack, heart failure, atrial fibrillation, or any history of previous cardiovascular procedures, including valve replacement.

Mineral Metabolism Measurements

Fasting morning blood and urine samples were obtained in all MESA participants at their baseline examination. Specimens were stored at -80°C at the University of Vermont Laboratory for Clinical Biochemistry using established methods from previous population cohort cardiovascular studies that maintain long-term stability of samples.(18) First use (no freeze thaw) samples were shipped on dry ice to the University of Washington, where all mineral metabolism measurements were performed. Samples were measured in random order and blinded to AVC status. Measurements were made in singlicate due to limited serum availability. Serum and urine phosphate concentrations were measured using a timed-rate colorimetry method on a Beckman-Coulter UniCel DxC instrument, with interassay coefficients of variation of 2.5% and 4.2%, respectively. Intact serum FGF-23 was measured using a sandwich immunoassay that detects the full length FGF-23 molecule (Kainos ELISA 96-well plate). The coefficient of variation for high and low control samples were 6.7% and 12.4%, respectively. Intact PTH was measured by automated immunoassay using a Beckman-Coulter UniCel DxI instrument (low and high coefficients of variation 6.1% and 3.4%, respectively).

Covariate Data

Demographics, medical history, smoking status and medication history were obtained using standardized questionnaires. Blood pressure (BP) was measured three times in a seated position using a Dinamap model Pro 100 automated oscillometric sphygmomanometer. The averages of the last two systolic and diastolic measurements were used for these analyses. Diabetes was defined as a fasting blood glucose level $\geq 126\text{mg/dL}$ or use of a diabetic medication. Estimated glomerular filtration rate (eGFR) was determined using cystatin C measurements according to the equation:(19) $\text{eGFR} = 76.7 \times (\text{cystatin C})^{-1.19}$. Chronic kidney disease (CKD) was defined as $\text{eGFR} < 60 \text{ ml/min/1.73m}^2$. Serum 25-hydroxyvitamin D (25-OHD) concentrations were measured using liquid chromatography tandem mass spectrometry. Urine albumin to creatinine ratio (ACR) was measured from single-voided fasting spot morning collections using nephelometry and the rate Jaffe reaction, respectively. Total and high-density lipoprotein (HDL) cholesterol were measured at a central lipid laboratory using a cholesterol oxidase method (Roche Diagnostics), the low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.(20)

Computed Tomography

ECG-triggered chest CT scans were performed on all participants during the baseline examination using either electron beam or multi-detector scanners and following standardized protocols. Descriptions of methodology, equipment, quality control, reproducibility and equivalency across scanner type have been previously published.(21–23) Follow-up CT scans were performed for a random half of the cohort between September 2002 and January 2004, and the other half between March 2004 and July 2005. Measurements of AVC scores were summated from individual lesions quantified using the method by Agatston et al.(24) Calcified lesions of the aortic annulus, aortic sinuses and proximal aorta were excluded from measurement. Absence of AVC was assigned a score of zero. All studies were evaluated at the MESA CT reading center (Harbor-UCLA Research and Education Institute) by a single blinded reader.

Statistical Analysis

Baseline characteristics stratified by presence of AVC were tabulated. Kendall tau rank correlation coefficients were calculated to examine relationships between different phosphate metabolism biomarkers. Prevalent AVC was defined as an AVC score > 0 at the baseline exam. Incident AVC was defined as having an AVC score > 0 on follow-up evaluation when AVC was absent (AVC score of 0) at baseline. Associations of prevalent and incident AVC with phosphate metabolism markers were examined continuously and categorically using *a priori* defined, nested, multivariable regression models. Flexible spline regression plots were used to assess the functional forms of relationships between exposures and the risk of AVC; no obvious departure from linearity was detected for any marker. Multivariable models included covariates selected prior to analyses based on the biologic plausibility that they may confound the associations of mineral metabolism markers with AVC. Relative risks for prevalent and incident AVC were estimated using a Poisson working model and inference was performed with robust variance estimates. The initial model contained demographic covariates including age, sex, ethnicity and study site. A second model included the demographic covariates, as well as other potential confounders including eFGR, systolic BP, diastolic BP, diabetes, smoking status, LDL cholesterol, HDL cholesterol, 25-OHD, calcium, and the log urine albumin to creatinine ratio. All models include scanner type, and incident and progression models adjusted for time between CT scans. Parallel models were created for the other phosphate metabolism exposure variables, FGF-23, PTH, and urine phosphate concentration. FGF-23 and urine phosphate concentrations were analyzed both continuously and as quartiles. PTH groups were dichotomized at a previously used cutpoint of 65 pg/mL. Extreme FGF-23 outlier values (top 0.25%) were excluded from the continuous analysis to avoid excessive influence in the models. The distribution of urine phosphate levels is skewed; thus, values were log transformed to yield more interpretable coefficients. Models of urine phosphate include the log of urine creatinine to account for spot urinary concentration. A final, mutually-adjusted model contained all covariates and the three phosphate axis exposure variables.

Participants with prevalent AVC were included in the severity and progression analyses. AVC progression was defined as the difference in AVC score between scans. AVC scores were log transformed due to a skewed distribution. Associations of phosphate axis variables

with AVC severity were represented using geometric mean ratios from multivariable linear models with the same covariates as above. Interactions of phosphate axis exposures with predefined covariates, including age, ethnicity, sex, LDL, and CKD, were explored. The threshold for statistical significance was defined as $p < 0.05$.

Due to non-response and inadequate sample volume, approximately 5% of the study subjects were missing covariate data on one or more of the following variables: 25-OHD, cystatin C, smoking, and cholesterol. In regression analyses, these subjects' values were multiply imputed using chained equations.⁽²⁵⁾ The five analyses over the imputations were combined using Rubin's rules to account for the variability in the imputation procedure.⁽²⁶⁾ Missing covariate data accounted for less than 1% of the variation in the coefficient estimates of interest. All statistical analyses were performed using R version 2.13.

Results

Baseline characteristics

There were 6,814 participants in MESA with 2 individuals missing baseline AVC scores. Participants who had prevalent AVC were older, more likely to be male, and had more comorbidities than those who were free of AVC (Table 1). Phosphate metabolism biomarkers were adequately measurable in serum samples of 6,544 individuals. Serum phosphate levels were normally distributed with a mean of 3.67 ± 0.52 mg/dL. The majority of phosphate levels were in the normal range; only 52 (0.8%) of participants had phosphate levels above 5 mg/dL. The respective mean FGF-23 and PTH levels were 39.8 ± 14.2 pg/dL and 44.7 ± 21.8 pg/mL, and the median urine phosphate level was 44.1 mg/dL (IQR: 24.9 to 67.7 mg/dL). Kidney function was predominately normal in the study sample (mean eGFR 98.3 mL/min/m², CKD present in 5.4%). There was low correlation among the phosphate metabolism biomarkers (Table 2).

Associations of phosphate metabolism markers with prevalent AVC

The prevalence of baseline AVC was 13.2%. There was a statistically significant association between higher serum phosphate concentrations and greater AVC prevalence in all models for the continuous and categorical evaluation of serum phosphate (Table 3). The association of serum phosphate with prevalent AVC was similar in magnitude to other previously identified modifiable risk factors, such as LDL cholesterol (Figure 1). Interactions were not statistically significant in the predetermined subgroups, including those with CKD (Figure 2). FGF-23, PTH, and urine phosphate concentrations were not associated with prevalent AVC in any of these models.

Associations of phosphate markers with incident AVC

Mean follow-up time between CT scans was 2.4 years with a range of 0.9 to 4.9 years. Among the 5,145 participants who were free of baseline AVC and underwent CT scanning during follow-up, 211 (4.1%) developed incident AVC. There were no significant associations of serum phosphate, PTH or urine phosphate concentration with incident AVC (Table 4). There was also no association of serum FGF-23 concentrations with incident AVC when FGF-23 was analyzed as a continuous variable. In contrast, the highest FGF-23

quartile was associated with a greater incidence of AVC in fully adjusted models (RR: 1.52, 95% CI 1.01–2.28).

Lack of associations of phosphate markers with AVC severity or progression

Among the 913 participants who had non-zero baseline AVC scores, the median AVC score was 58 (IQR 19–145). In this subgroup there was no relationship of any of the phosphate metabolism markers with AVC score severity or with progression of AVC score (Supplementary Table).

Discussion

In this large, multi-ethnic population that was free of clinical cardiovascular disease, higher serum phosphate levels were associated with a greater prevalence of AVC independent of demographics, atherosclerotic risk factors, kidney function, and other mineral metabolism markers. This result validates previous epidemiologic associations of serum phosphate with early CAVD in a larger, more diverse study population that includes gold-standard measurements of aortic valve calcium and adjustment for a comprehensive set of traditional risk factors for aortic valve disease.(16) Furthermore, the current study provides a more complete evaluation of phosphate metabolism by simultaneously evaluating FGF-23, PTH, and urinary phosphate concentrations, as related markers of phosphate metabolism.

The size of the association for serum phosphate concentrations is comparable to that of established modifiable risk factors such as cholesterol and hypertension. To date, studies using statins and angiotensin-converting enzyme inhibitors have not shown benefit at reducing progression in more advanced CAVD, when hemodynamic impairment has manifested. It has been postulated that risk factors governing disease progression in more advanced stages may differ from those involved in CAVD initiation.(27,28) This study did not find an association with phosphate in progression or severity of AVC. However, the population studied was a relatively young and cardiovascular disease-free cohort, and follow-up was relatively short for a disease process that develops over decades in later life. Future studies with longer follow-up are needed to further elucidate the relationship of mineral metabolism to disease progression.

In vitro studies have shown that osteogenic and apoptotic pathways are important for the development of AVC.(29,30) Transformation of aortic valvular interstitial cells into osteoblast-like phenotypes that mineralize valve tissue is likely a key component.(12,31,32) Uptake of phosphate via the Pit-1 receptor in multiple cell lines, including aortic valve interstitial cells (VIC), has been shown to upregulate bone-specific signaling cascades involving Cbfa1/Runx2 and bone morphogenic proteins.(15,33,34) Additionally, it has been demonstrated that phosphate uptake via Pit-1 induces apoptosis and mineralization of VICs via the mitochondrial pathway(35). The same authors found that explanted human aortic valves with CAVD show significantly higher Pit-1 expression near calcified nodules compared to control valves without CAVD. Finally, phosphate loading of aortic endothelial cells inhibits nitric oxide production through uncoupling of nitric oxide synthase, leading to increased levels of reactive oxygen species.(36) This dysregulation of antioxidant mechanisms may lead to upregulation of pro-osteogenic signaling cascades and the

development of AVC.(37–39) Despite multiple mechanisms phosphate may have on mineralization of the aortic valve it remains unknown if serum phosphate levels are causative or only a biomarker for AVC. Future in vivo studies examining effects on AVC mechanisms by elevating or reducing serum phosphate concentrations within the normal range could be insightful. Moreover, the reasons for differences in serum phosphate levels in individuals without kidney impairment need to be elucidated.

To our knowledge, this is the first study examining the phosphate regulating hormone, FGF-23, with AVC in a population without kidney disease. A previous, unadjusted cross-sectional analysis in hemodialysis patients found that dialysis patients with prevalent AVC by echocardiography had, on average, higher FGF-23 values when compared to individuals without AVC.(40) In mouse models with moderate CKD, high-phosphate diets have been shown to increase FGF-23 levels and to lead to ectopic calcification without producing overt hyperphosphatemia.(41) It is possible that FGF-23 may be a marker of exposure to a chronic phosphate loading. However, this has not been confirmed in studies with normal kidney function, and mechanisms of phosphate effects on osteocyte production of FGF-23 remain unknown. The present study found an association of the highest quartile of FGF-23 levels with incident AVC. While there may be a threshold effect with FGF-23, it is possible that high FGF-23 identifies a sicker population with unmeasured confounders for which we did not adjust. We believe this result deserves further investigation in other populations, but should be interpreted cautiously here, given the lack of a dose-response relationship and the lack of association in the other AVC outcome measures.

We found no association of PTH or urinary phosphate concentration with any AVC outcome. A previous study reported higher age-adjusted 24-hour urinary phosphate levels, which reflect dietary phosphate intake, in patients with aortic stenosis as compared to those with aortic sclerosis. There is a strong linear correlation between spot urine phosphate measurements and 24-hour collections.(42) However, our urinary phosphate measurements exhibited wide variance, perhaps making associations more difficult to detect. Our null results for urine phosphorus could therefore be due to true lack of association or to misclassification of urine phosphorus excretion.

Our study has limitations. First, this is an observational study and remains subject to unmeasured confounders. The population studied was relatively healthy overall, and had a low prevalence of CAVD that was, on average, very mild. The lack of disease severity and short follow-up time limits the ability to detect associations with incident and progressive disease.

In conclusion, this study demonstrates that serum phosphate levels are independently associated with AVC prevalence in a healthy multi-ethnic population. Whether serum phosphate levels are a biomarker or directly implicated in AVC should be evaluated in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We investigated phosphate metabolism biomarkers with aortic valve calcification.
- Serum phosphate levels are significantly associated with AVC prevalence.
- Phosphate metabolism was not associated with AVC severity or progression.

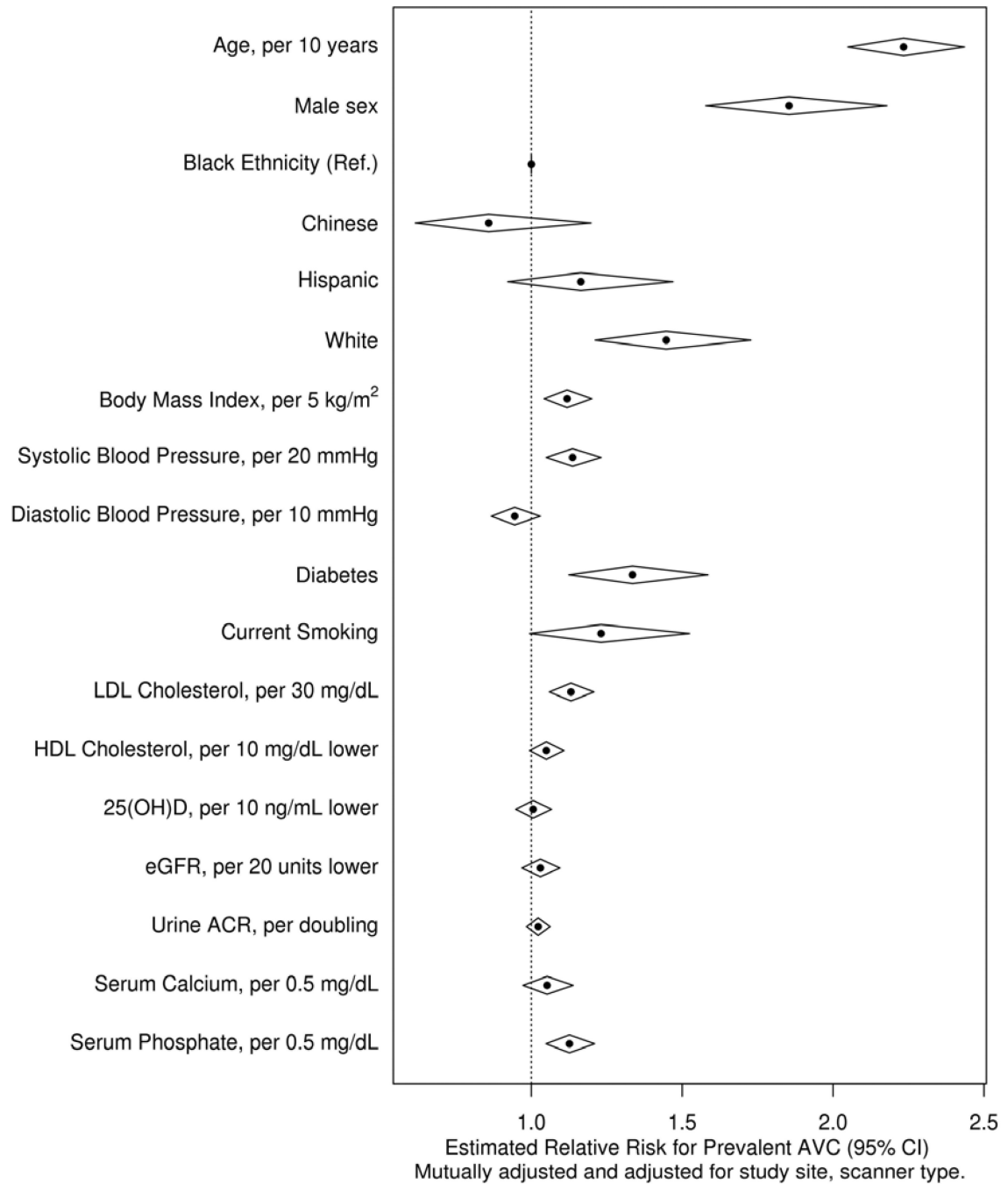


Figure 1. Risk of Aortic Valve Calcification by Demographics and Previously Studied Risk Factors

Relative risk estimates demonstrating comparable effect size of serum phosphate and other modifiable risk factors. Diamond widths represent 95% confidence intervals. The magnitudes of effect for continuous risk factors are standardized to their respective standard deviation.

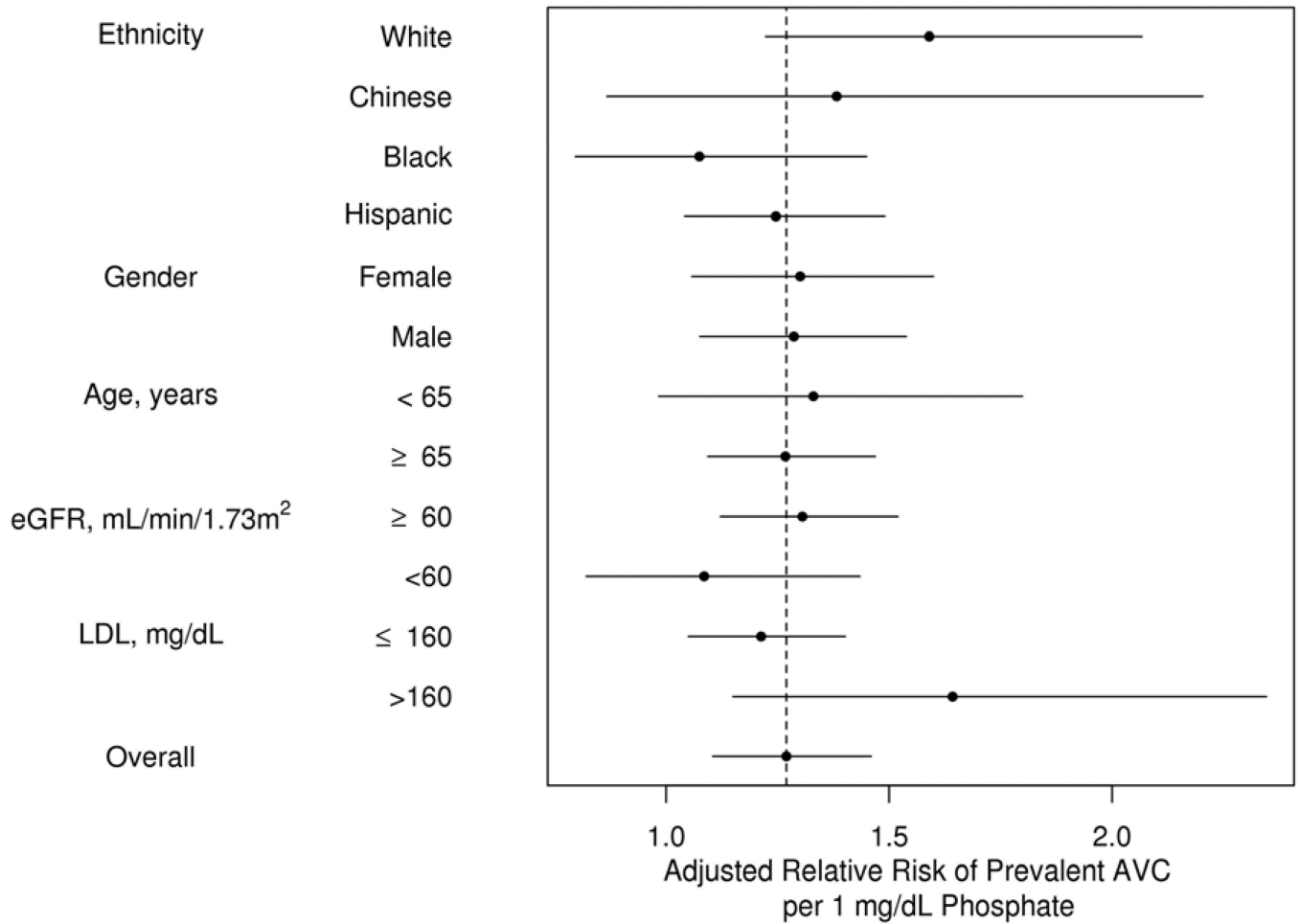


Figure 2. Risk of Aortic Valve Calcification in Predetermined Subgroups

No statistically significant difference in adjusted relative risk for any predefined subgroup.

Horizontal bars represent 95% confidence intervals.

Table 1

Baseline Participant Characteristics

Characteristic	Aortic valve calcium	
	Absent (N=5,899)	Present (N=913)
Age (years)	60.86 ± 9.9	70.49 ± 8.1
Male	2663 (45.1)	549 (60.1)
Ethnicity		
Black	1,660 (28.1)	233 (25.5)
Chinese	736 (12.5)	67 (7.3)
Hispanic	1,296 (22.0)	200 (21.9)
White	2,207 (37.4)	413 (45.2)
Diabetes	677 (11.5)	181 (20.0)
Current smoking	790 (13.4)	96 (10.6)
Education		
High school or less	2,068 (35.2)	392 (43.2)
High school to some college	1,695 (28.8)	241 (26.5)
College degree or higher	2,118 (36.0)	275 (30.3)
Body mass index (kg/m ²)	28.3 ± 5.6	28.49 ± 5.0
Systolic blood pressure (mmHg)	125.3 ± 21.1	135 ± 22.1
Diastolic blood pressure (mmHg)	71.9 ± 10.3	72.2 ± 10.1
Hypertension medication	2,030 (34.42)	505(55.37)
Low-density lipoprotein cholesterol (mg/dL)	117.0 ± 31.0	118.6 ± 34.4
High-density lipoprotein cholesterol (mg/dL)	51.3 ± 14.9	49.0 ± 14.2
Lipid lowering medication	864 (14.65)	235(25.77)
Estimated glomerular filtration rate (ml/min/1.73m ²)	99.81 ± 25.8	86.33 ± 24.5
Chronic kidney disease	262(4.5)	114(12.6)
Urine albumin to creatinine ratio	25.3 ± 160.8	38.17 ± 222.8
<i>Mineral metabolism measurements</i>		
Serum 25-hydroxyvitamin D (ng/mL)	25.2 ± 11.5	26.3 ± 12.0
Serum calcium (mg/dL)	9.7 ± 0.4	9.7 ± 0.4
Serum phosphorus (mg/dL)	3.7 ± 0.5	3.7 ± 0.5
Urine phosphorus (mg/dL)	51.2 ± 36.6	50.2 ± 30.8
Serum fibroblast growth factor-23 (pg/mL)	39.4 ± 14.1	42.4 ± 14.8
Serum parathyroid hormone (pg/mL)	44.3 ± 20.5	47.3 ± 28.7

Values are mean ± SD or n(%).

Table 2

Correlation Coefficients Among Phosphorus Metabolism Biomarkers.

	Serum phosphorus	Urine phosphorus	FGF-23	PTH
Serum phosphorus	1.00	-0.03	0.06	-0.15
Urine phosphorus	-0.03	1.00	0.02	0.11
FGF-23	0.06	0.02	1.00	0.03
PTH	-0.15	0.11	0.03	1.00

FGF-23 = fibroblast growth factor-23; PTH = parathyroid hormone

Table 3

Associations of Phosphate Metabolism Markers with Prevalent Aortic Valve Calcium.

	Prevalent Cases N (%)	Demographic adjusted* RR (95% CI)	Fully adjusted† RR (95% CI)	Adds mutual adjustment‡ RR (95% CI)	P-value§
Serum phosphate (mg/dL)					
3.0	103 (13.7%)	<i>reference</i>	<i>reference</i>		
3.01 – 3.5	261 (13.3%)	1.12 (0.91 to 1.38)	1.08 (0.88 to 1.33)	1.10 (0.89 to 1.36)	0.36
3.51 – 4.0	294 (12.5%)	1.16 (0.94 to 1.43)	1.12 (0.91 to 1.38)	1.14 (0.92 to 1.41)	0.22
>4.0	207 (14.2%)	1.64 (1.30 to 2.06)	1.50 (1.18 to 1.89)	1.51 (1.19 to 1.92)	<0.001
Linear per 1.0 mg/dL higher		1.34 (1.18 to 1.53)	1.27 (1.10 to 1.46)	1.27 (1.10 to 1.47)	<0.001
Serum FGF-23 (pg/mL)					
30.5	167 (10.2%)	<i>reference</i>	<i>reference</i>		
30.5 – 37.7	184 (11.3%)	0.98 (0.80 to 1.20)	0.99 (0.81 to 1.20)	0.97 (0.80 to 1.19)	0.79
37.8 – 46.5	249 (15.2%)	1.23 (1.02 to 1.48)	1.17 (0.97 to 1.41)	1.16 (0.97 to 1.40)	0.11
46.5 – 590	262 (16%)	1.19 (0.99 to 1.44)	1.09 (0.90 to 1.32)	1.07 (0.88 to 1.30)	0.51
Linear per 20 pg/mL higher		1.11 (1.03 to 1.20)	1.04 (0.96 to 1.13)	1.03 (0.95 to 1.12)	0.46
Urine phosphate (mg/dL)¶					
24.9	162 (11.3%)	<i>reference</i>	<i>reference</i>		
24.9 – 44.1	245 (14.5%)	1.10 (0.92 to 1.33)	1.00 (0.81 to 1.25)	1.01 (0.81 to 1.25)	0.94
44.2 – 67.8	256 (15.2%)	1.25 (1.04 to 1.50)	1.07 (0.83 to 1.36)	1.07 (0.84 to 1.37)	0.57
67.9 – 398	205 (12.2%)	1.11 (0.91 to 1.35)	0.93 (0.7 to 1.24)	0.93 (0.69 to 1.24)	0.6
Per doubling		1.04 (0.98 to 1.11)	0.96 (0.86 to 1.08)	0.95 (0.85 to 1.06)	0.37
Serum PTH (pg/mL)					
<65	743 (12.9%)	<i>reference</i>	<i>reference</i>		
65	123 (15.8%)	1.09 (0.92 to 1.30)	0.96 (0.80 to 1.16)	1.01 (0.83 to 1.22)	0.95
Linear per 10 pg/mL greater		1.02 (0.99 to 1.04)	0.99 (0.97 to 1.02)	1.00 (0.97 to 1.03)	0.81

* Demographic adjusted models adjusted for age (in 10 year categories), gender, ethnicity, study site, and scanner type.

† Additionally adjusts for body mass index, systolic blood pressure, diastolic blood pressure, diabetes, smoking, LDL-cholesterol, HDL-cholesterol, HDL-cholesterol, 25-hydroxyvitamin D, eGFR, log of albumin to creatinine ratio, and serum calcium.

‡ Mutually adjusted models are fully adjusted models with additional adjustment for the other phosphate metabolism markers.

§ P-values are present for the complete mutually adjusted models

//Models for urine phosphate additionally adjust for the log of urine creatinine.

CI = Confidence Interval; FGF = Fibroblast growth factor; PTH = Parathyroid Hormone; RR = Relative Risk

Table 4
Associations of Phosphate Metabolism Markers with Incident Aortic Valve Calcium.

	Incident Cases N (%)	Demographic adjusted* RR (95% CI)	Fully adjusted† RR (95% CI)	Adds mutual adjustment‡ RR (95% CI)	P-value§
Serum phosphate (mg/dL)					
3.0	31 (5.5%)		<i>reference</i>		
3.0 – 3.5	58 (3.9%)	0.80 (0.53 to 1.22)	0.79 (0.51 to 1.20)	0.78 (0.51 to 1.19)	0.25
3.51 – 4.0	67 (3.7%)	0.82 (0.54 to 1.25)	0.81 (0.52 to 1.25)	0.79 (0.51 to 1.23)	0.29
>4.0	47 (4.3%)	1.09 (0.68 to 1.74)	1.11 (0.68 to 1.80)	1.04 (0.64 to 1.69)	0.87
Linear per 1.0 mg/dL higher		1.26 (0.92 to 1.73)	1.25 (0.90 to 1.72)	1.2 (0.87 to 1.64)	0.27
Serum FGF-23 (pg/mL)					
30.5	43 (3.4%)		<i>reference</i>		
30.5 – 37.7	45 (3.6%)	0.99 (0.66 to 1.48)	0.99 (0.66 to 1.48)	1.00 (0.66 to 1.51)	1
37.8 – 46.5	43 (3.5%)	0.96 (0.63 to 1.45)	0.94 (0.62 to 1.44)	0.95 (0.62 to 1.46)	0.81
46.5 – 590	72 (6.0%)	1.52 (1.05 to 2.20)	1.56 (1.05 to 2.33)	1.52 (1.01 to 2.28)	0.04
Linear per 20 pg/mL higher		1.16 (0.99 to 1.37)	1.13 (0.94 to 1.35)	1.10 (0.92 to 1.31)	0.29
Urine phosphate (mg/dL)¶					
24.9	34 (3.1%)		<i>reference</i>		
24.9 – 44.1	57 (4.5%)	1.38 (0.91 to 2.10)	1.28 (0.78 to 2.10)	1.28 (0.78 to 2.09)	0.33
44.2 – 67.8	51 (4.2%)	1.32 (0.86 to 2.02)	1.15 (0.65 to 2.03)	1.14 (0.65 to 2.01)	0.64
67.9 – 398	65 (4.9%)	1.61 (1.06 to 2.44)	1.21 (0.65 to 2.24)	1.18 (0.64 to 2.2)	0.59
Per doubling		1.23 (1.08 to 1.41)	1.18 (0.94 to 1.49)	1.16 (0.92 to 1.46)	0.2
Serum PTH (pg/mL)					
<65	179 (4.1%)		<i>reference</i>		
65	23 (4.2%)	0.87 (0.56 to 1.33)	0.84 (0.54 to 1.32)	0.86 (0.54 to 1.36)	0.51
Linear per 10 pg/mL greater		1.01 (0.94 to 1.09)	1.01 (0.94 to 1.08)	1.01 (0.95 to 1.08)	0.75

* Demographic adjusted models adjusted for age (in 10 year categories), gender, ethnicity, study site, and scanner type.

† Additionally adjusted for body mass index, systolic blood pressure, diastolic blood pressure, diabetes, smoking, LDL-cholesterol, HDL-cholesterol, 25-hydroxyvitamin D, eGFR, log of albumin to creatinine ratio, and serum calcium.

‡ Mutually adjusted models additionally adjust for the other phosphate metabolism markers.

§ P-values are present for the complete mutually adjusted models

//Models for urine phosphate additionally adjust for the log of urine creatinine.

CI = Confidence Interval; FGF = Fibroblast growth factor; PTH = Parathyroid Hormone; RR = Relative Risk