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Independent Study Project

"Differentiating Risk Factors for Coronary Artery Calcium Volume and Density: The Multi-Ethnic Study of Atherosclerosis"

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Title: Relative Association of Markers of Mineral Metabolism and Kidney Function with Coronary Artery Calcium Volume and Density: The Multi-Ethnic Study of Atherosclerosis

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Abstract:

Introduction: Coronary artery calcium (CAC) is a prognostic marker for incident cardiovascular disease (CVD), but its calculation involves measures of both CAC volume and density. Whereas CAC volume is directly associated with incident CVD, CAC density is inversely associated with incident CVD yet higher CAC density increases the CAC score. We compare the relative strength of association of markers of mineral metabolism and kidney function with CAC volume vs. density in a large population of community-living individuals free of CVD.

Methods: The Multi-Ethnic Study of Atherosclerosis (MESA) is an observational cohort study of 6814 adults free from CVD at baseline. Among the subset with detectable CAC (N=3398) we used multivariate analysis to determine association between markers of mineral metabolism and kidney function with CAC volume and density. Models evaluating CAC volume were adjusted for density, and vice versa, to understand the independent association of risk factors with each component of the CAC score. Standardized beta coefficients (β) were calculated to facilitate comparison of strengths of association.

Results: Higher serum phosphorus (β= 0.032, 95% CI [0.009, 0.055]), 25-hydroxyvitamin D (β= 0.024 95% CI [0.002, 0.047]), and bicarbonate (β= 0.025 95% CI [0.004, 0.046]) concentrations were directly associated with CAC density independently of CAC volume. Higher eGFR (β= 0.084 95% CI [0.034, 0.133]), urinary albumin to creatinine ratio (β= 0.071 95% CI [0.021, 0.120]) and fibroblast growth factor-23 (β=0.054, 95% CI [0.007, 0.101]) were directly associated with CAC volume independently of density, while parathyroid hormone (β= -0.052, CI 95% [-0.101, -0.002]), and bicarbonate (β= -0.060, 95% CI [-0.105, -0.015])were inversely associated with CAC volume independently of density.

Conclusion: Factors known to promote mineralization including phosphate, vitamin D, and higher serum bicarbonate are more strongly associated with the density of CAC than with its volume. These findings support the hypothesis that these factors promote calcium deposition, but may not promote atherosclerotic plaque progression per se.

Introduction:

Coronary artery calcification (CAC) is a strong prognostic marker for incident cardiovascular disease (CVD).[1-3] The most common method of quantifying CAC has been through the use of the Agatston scoring method. To obtain an Agatston score, areas of calcified plaque within the coronary arteries are identified on cardiac-gated computed tomography (CT). Each calcified plaque is weighted according to its density by multiplying each plaque area by its maximum attenuation category, ranging from 1 (low density) to 4 (high density).[4] Thus, higher CAC density and higher CAC volume both contribute to a higher Agatston Score.

A recent study in the Multi-Ethnic Study of Atherosclerosis (MESA) evaluated the components of CAC—volume and density—with risk of CVD and found that they had opposite directions of association. Whereas higher CAC volume was associated with greater risk of incident CVD, higher CAC density at any given amount of volume was associated with a lower risk of incident CVD.[5] These findings suggest that some factors that promote CAC density may be associated with a higher Agatston CAC score, but may not be associated with CVD events.

Investigating how CVD risk factors differentially contribute to CAC density and volume may provide insights into the mechanisms linking these factors with calcification, and may provide insight into their respective relationships with incident CVD. Given the influence of hyperphosphatemia[6,7] and other abnormalities in kidney function on the development of arterial calcification,[8] we investigated the associations of variables related to mineral metabolism and kidney function with CAC density and volume in the MESA cohort. *A priori*, we hypothesized that higher phosphate, calcium, and bicarbonate concentrations, and lower estimated glomerular filtration rate (eGFR) would be more strongly associated with CAC density than with CAC volume.

Methods:

Study population:

The MESA is a community-based cohort study designed to study subclinical atherosclerosis. MESA enrolled 6,814 participants between 2000 and 2002. All participants were between the ages of 45 and 84 and free of clinical CVD. Participants were recruited at 6 field centers: New York, NY; Baltimore and Baltimore County, MD; Forsyth County, NC; Chicago, IL; and Los Angeles, CA. Details about the study population, recruitment methods used, and measurements obtained for the MESA study have previously been published.[6] Participant data, including demographics, medical history, medication use, and tobacco use, were obtained using a questionnaire. Height and weight were also measured during, and seated blood pressure was measured three times, with the average of the 2nd and 3rd readings recorded.

Laboratory Measurements:

Venous blood samples were collected from participants after a 12-hour overnight fast by trained phlebotomists using a standardized protocol. Serum phosphorus was measured using the Vitros 950 IRC instrument by reflectance spectrophotometry. Analytic coefficients of variation were <5%. Bicarbonate was measured using a pH rate-of-change method on a Beckman DxC automated clinical chemistry analyzer, with intra-assay imprecision of <5%. Calcium levels were measured using a Siemens Dimensions Vista 500 system, with coefficient of variance <1%. . Total and HDL cholesterol were measured using a cholesterol oxidase method with a Roche COBAS FARA centrifugal analyzer. HDL cholesterol was measured in ethylenediaminetetraacetic acid plasma with the cholesterol oxidase method (Roche Diagnostics) after precipitating non-high density lipoprotein cholesterol with magnesium/dextran. Serum C-reative protein concentration was measured using the BN II nephelometer (Dade Behring Inc., Deerfield, IL). Serum interleukin-6 (IL-6) concentrations were

measured using ultrasensitive ELISA (Quantikine HS Human interleukin-6 Immunoassay, R& D Systems, Minneapolis, MN). Serum FGF-23 was measured using an immunoassay targeted to both mid-molecule and distal epitopes (Kainos Laboratories, Japan). Serum intact PTH was measured using the Beckman-Coulter DxI automated two-site immunoassay (Beckman-Coulter, Brea, CA). Serum 25-hydroxyvitamin D levels were measured using high-performance HPLC–tandem mass spectrometry with internal standards. Urine was also collected for measurement of urinary albumin and creatinine. Levels of total and HDL cholesterol and serum glucose were also measured. Diabetes was defined as a fasting blood glucose level ≥126 mg/dL or the use of insulin or glucose-lowering medications.[9] Glomerular filtration rate was estimated (eGFR) using serum creatinine and demographic variables using the Chronic Kidney Disease Epidemiology Collaboration creatinine-based equation.[10]

Coronary artery calcium density and volume:

CAC was assessed using either cardiac-gated ultrafast axial CT scanners at the Chicago, Los Angeles, and New York field centers (Imatron C-150; Imatron, San Francisco, California),[11, 12] or prospectively electrocardiogram gated helical CT at the Baltimore, Forsyth County, and St. Paul field centers (Lightspeed, General Electric Medical Systems, Waukesha, Wisconsin or Volume Zoom, Siemens, Erlanger, Germany)[11, 13] and calibrated using phantoms of known calcium density.[1] The nominal CT slice thickness was 3.0 mm for electron-beam CT and 2.5 mm for four-detector row CT. CAC was identified as having a Hounsfield unit (HU) attenuation of >130, and scored using the Agatston method. An Agatston score is calculated by finding the area of each calcified plaque in each coronary CT slice, and weighting the area of that plaque by a multiplicative factor depending on the densest point of calcification within that plaque, measured in HU. Plaques with a point of maximum density of 130-199 HU were multiplied by 1, plaques with a maximum density of 200-299 HU were

multiplied by 2, plaques with a maximum density of 300-399 HU were multiplied by 3, and plaques with a maximum density of 400 HU or greater were multiplied by 4.[4]

The MESA dataset does not contain data on CAC density for each unique coronary plaque. Thus, for this study, the CAC density score was determined by first determining the plaque area score (in square millimeters) for each participant by dividing CAC volume (in cubic millimeters) by the CT scan slice thickness (2.5mm or 3mm). The Agatston score was then divided by plaque area score to obtain an average plaque density score for each participant, which is a unitless score that ranges from 1 to 4. By necessity, the evaluation of CAC density requires the presence of CAC, and therefore participants with an Agatston score of zero were excluded from analysis (N=3416, 50%)

Statistical analysis:

Standard summary statistics are presented for baseline characteristics stratified by quartile of CAC density. For normally distributed variables the mean and standard deviation are presented. Variables with a skewed distribution (serum C-reactive protein, interleukin 6, urinary albumin to creatinine ratio, parathyroid hormone, fibroblast growth factor 23) the median and interquartile range of the variable are presented. Statistical significance was calculated using the chi-squared test for categorical variables, analysis of variance for normally distributed variables, and the Kruskal-Wallis test for skewed variables.

In evaluating the associations of kidney function and mineral metabolism with CAC volume and density, we subdivided eGFR, urinary albumin/creatinine ratio, serum calcium, phosphorous, 25-hydroxyvitamin D, parathyroid hormone, fibroblast growth factor-23, and bicarbonate into two to four categories based on clinically significant cut-points. For each category, we calculated mean CAC density and volume scores adjusted for demographics and major cardiovascular risk factors (age, sex, race/ethnicity, diabetes, hypertension medication use, systolic blood pressure, body mass index, smoking, total and HDL cholesterol, and lipid-lowering medication use) as well as eGFR and urine

albumin to creatinine ratio. Linear regression was used to determine change in CAC density and volume per one standard deviation change in each variable of interest. For skewed variables (urinary albumin to creatinine ratio, paraythyroid hormone, fibroblast growth factor 23, and CAC volume), natural logarithms of the variables were used, and we evaluated per SD changes of the natural logged variable. In this way, the beta coefficients each represent a one SD increase and are comparable to one another. Models evaluating CAC density were additionally adjusted for CAC volume, and vice versa; models evaluating CAC volume were additionally adjusted for CAC density. We then performed analyses with additional mutual adjustment for all of the kidney function and mineral metabolism exposure variables. We also present the results of this analysis restricted to participants with an eGFR <60mL/min/1.73m^2. All analyses were performed using Stata SE version 13.0 (Statacorp, College Station, TX).

Results:

Among the 3,398 study participants, the mean age was 66, 42% were female, and 44% were white, 24% were black, 20% were Hispanic, and 12% were Chinese. The mean eGFR was 77 ml/min/1.73m^2 and 18% had diabetes. By inclusion criteria, all participants had Agataston CAC scores > 0. Relative to the lowest CAC density quartile, persons with higher density were older, more frequently male, had higher HDL, urine albumin to creatinine ratio, and 25-hydroxyvitamin D levels. Persons in the highest density quartile also had lower body mass index, c-reactive protein concentrations, and eGFR (**Table 1**).

Table 2 summarizes the associations of kidney and mineral metabolism markers with CAC density with adjustment for CAC volume, CVD risk factors, eGFR, and urinary albumin to creatinine ratio. Lower eGFR was associated with greater CAC density (β= -0.022, 95% CI [-0.043, -0.002]), while higher serum phosphorus (β=0.023, 95% CI [0.003, 0.044}) and bicarbonate (β= 0.022, 95% CI [0.003, 0.041]) concentrations were associated with greater CAC density.

Table 2 also shows associations of these markers with CAC volume, adjusted for CAC density, CVD risk factors, eGFR and urine albumin to creatinine ratio. Higher eGFR (β= 0.067, 95% CI [0.022, 0.111]), urinary albumin to creatinine ratio (β= 0.073, 95% CI [0.028, 0.118]), and phosphorus (β= 0.054, 95% CI [0.009, 0.100]) were associated with greater CAC volume. In contrast, lower serum bicarbonate (β= -0.046, 95% CI [-0.089, -0.004]) and parathyroid hormone (β= -0.043, 95% CI [-0.087, 0.00]) were associated with greater CAC volume.

Table 3 summarizes the associations of each measure with CAC volume and density in a multivariable model that not only included CVD risk factors and kidney function measures, but also simultaneously adjusted for the other mineral metabolism markers. In this model, higher serum phosphorus (β= 0.032, 95% CI [0.009, 0.055]), 25-hydroxyvitamin D (β= 0.024, 95% CI [0.002, 0.047]), and serum bicarbonate (β= 0.025, 95% CI [0.004, 0.046]) were all independently associated

with greater CAC density. Higher eGFR (β= 0.084, 95% CI [0.034, 0.133]), urinary albumin to creatinine ratio (β= 0.071, 95% CI [0.021, 0.120]) and fibroblast growth factor-23 (β=0.054, 95% CI [0.007, 0.101]) were independently associated with greater CAC volume, while lower parathyroid hormone (β= -0.052, CI 95% [-0.101, -0.002]), and serum bicarbonate concentrations (β= -0.060, 95% CI [-0.105, -0.015]) were independently associated with greater CAC volume. **Figure 1** depicts the relative strength of association of each marker on a per standard deviation greater scale. In order, higher phosphate, 25-hydroxyvitamin D, and bicarbonate concentrations had the strongest associations with CAC density. In contrast, there was no independent association of eGFR, urine albumin to creatinine ratio or PTH with CAC density.

Supplemental Table 1 summarizes the associations of the kidney and mineral metabolism markers with CAC density after adjustment for CVD risk factors, eGFR, urine ACR, and mineral metabolism variables in the subset of participants with eGFR < 60 ml/min/1.73m^2. Higher serum phosphorus (p=0.05) and bicarbonate (p=0.04) concentrations were associated with greater CAC density. None of the variables showed significant independent associations with CAC volume in the low eGFR subset.

Discussion:

In a community-living population free of CVD, we found that higher serum phosphate, 25-(OH) vitamin D, and bicarbonate concentrations were more strongly associated with the density of CAC than with the volume of CAC. These findings support mechanisms identified in animal models suggesting that these factors primarily promote arterial calcium deposition rather than atherosclerotic plaque progression. Conversely, albuminuria and higher FGF23 were more strongly associated with CAC volume than with CAC density. Thus, assessment of independent associations of CAC density vs. volume provides unique opportunities for evaluating potential mechanisms contributing to CAC. By extension, evaluating factors that promote these separate components of CAC may also provide new insights to mechanisms contributing to CVD.

In vitro studies have demonstrated that treatment of vascular smooth muscle cells with higher extracellular phosphorus concentrations induce vascular smooth muscle cells to transform into osteoblast-like cells and to deposit calcium in the extracellular matrix.[6,7] To our knowledge, no known mechanisms have been identified by which phosphate may induce greater atherosclerotic plaque development. Thus, the findings demonstrating stronger associations of phosphate with CAC density than with volume are consistent with biological mechanisms.

Similarly, acidosis, reflected by low serum bicarbonate concentrations, has been associated with decreased bone mineralization, both through acidic effects on calcium solubility and through direct effects on osteoblasts and osteoclasts.[14] These in vitro findings are supported by both in-vivo study of chronic metabolic acidosis in rodents and some observational studies in humans.[15,16] Conversely, exposing rodent arteries to alkalotic fluid induces arterial calcification.[17] To our knowledge, there are no known mechanisms through which higher bicarbonate would promote atherosclerotic plaque burden.

We found that higher serum bicarbonate is more strongly associated with CAC density than CAC volume are consistent with in vitro and animal studies once more.

The associations of higher 25-hydroxyvitamin D with CAC density and volume are more complicated. Classic actions of vitamin D are known to promote intestinal calcium and phosphate absorption. In animal models of kidney disease, high dose vitamin D is commonly used as a model of vascular calcium deposition.[18] These effects may be responsible for the association of 25 hydroxyvitamin D with CAC density observed here. However, previous studies have also shown associations between lower 25-hydroxyvitamin D and factors known to promote atherosclerosis including obesity, hypertension, and inflammation.[19-21] While there was a trend of lower 25-hydroxyvitamin D and greater CAC volume, this result did not reach statistical significance. Future studies in other settings will be required to confirm and compare relative strengths of association of 25-hydroxyvitamin D with CAC volume and density.

We found that greater ACR and FGF23 were associated with greater CAC volume, but not CAC density. The mechanisms responsible for these findings are less certain. High-grade albuminuria is associated with marked lipid abnormalities,[22] which could promote greater atherosclerotic disease burden and microalbuminuria is a known risk factor for incident CVD.[23] It is unclear, however if this is the primary mechanism explaining the association between albuminuria and CAC volume in our study population since we adjusted for total and HDL cholesterol concentrations, and since the degree of albuminuria was quite modest. Others have suggested that albuminuria is a sensitive marker of systemic endothelial dysfunction and injury, which in turn may promote atherosclerosis.[24]

Recent studies have consistently demonstrated that higher FGF23 concentrations are particularly strongly associated with development of left ventricular hypertrophy and heart failure risk.[25,26] In contrast, basic science studies have demonstrated that FGF23 does not directly promote vascular

calcium deposition and clinical studies have found week to altogether absent associations of FGF23 with calcification.[27] While mechanisms linking higher FGF23 with atherosclerotic plaque volume observed here are uncertain, it is intriguing that this marker, among all the mineral markers, was not associated with CAC density, consistent with in vitro studies.

Collectively, the findings presented here provide a relatively consistent message regarding pathways contributing to CAC development. With the exception of FGF23, markers of mineral metabolism and alkalosis appear to be more strongly associated with the density of CAC than the volume of CAC. These factors may stimulate greater calcium deposition with less influence on atherosclerosis per se. In contrast, albuminuria and FGF23 appear more strongly associated with the volume of CAC than with its density. We evaluated multiple risk factors in parallel, and findings will need to be confirmed in future studies. However, this study is among the first to evaluate associations of risk factors with CAC volume vs. density. By extension, the mechanisms linking these risk factors with CVD may also be distinct.

Among the strengths of this study are its evaluation of relatively large cohort of patients from a wide range of ages and multiple race/ethnic groups. The availability of multiple markers of mineral metabolism and kidney function, allowing assessment of independent associations of each, is an additional key strength. This study also has important limitations. The cross-sectional design does not allow for evaluation of direction of associations or temporality. Future studies will be required to examine relationships of mineral markers with longitudinal change in CAC volume and density over time. While the main findings mirror discoveries in experimental animals and in vitro experiments, the observational nature of the study precludes assessment of mechanisms. Laboratory measurements used here were conducted in fasting morning blood samples. We and others have shown circadian changes in serum phosphate.[28] Diet may also influence serum bicarbonate concentrations. Whether temporal

changes in these markers, or assessment at later times of the day or after meals may have influenced the results is uncertain.

In conclusion, in this cross-sectional study among community-living adults without clinical CVD, we found that higher serum phosphorus, 25-hydroxyvitamin D, and bicarbonate concentrations are more strongly associated with CAC density than with CAC volume. In contrast, higher albuminuria and FGF23 are more strongly associated with CAC volume than density. These findings are consistent with known vascular effects of these measures in animal and in vitro studies. We conclude that evaluation of associations of risk factors with CAC density and volume independently, has potential to provide novel insights into mechanisms contributing to CAC in community-living individuals.

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Table 1. Distribution of Baseline Characteristics by CAC Density Score

	Q1	Q2	Q3	Q4	P-value
Number of Participants	**850**	**849**	**850**	**849**	
Range	**< 2.23**	**2.23-2.79**	**2.80-3.17**	**≥3.18**	
Age (years), ± SD	63±10	67±9	68±9	68±9	< 0.001
Female, n (%)	409 (48%)	351 (41%)	338 (40%)	336 (40%)	0.001
Race, n (%)					< 0.001
White	373 (44%)	407 (48%)	437 (51%)	280 (33%)	
Chinese	88 (10%)	53 (6%)	71 (8%)	192 (23%)	
Black	203 (24%)	223 (27%)	203 (24%)	183 (22%)	
Hispanic	186 (22%)	157 (18%)	139 (16%)	194 (23%)	
Diabetes, n (%)	137 (16%)	163 (19%)	152 (18%)	155 (18%)	0.41
Hypertension medication use, n (%)	340 (40%)	399 (47%)	415 (49%)	398 (47%)	0.001
Systolic blood pressure (mmHg) ± SD	128±21	131±21	133±21	131±23	< 0.001
Diastolic blood pressure (mmHg) ± SD	72±10	73±10	73±10	72±10	0.88
Body mass index (kg/m^2) ± SD	28.8±5.5	28.9±5.5	28.4±5.1	27.3±5.0	< 0.001
Smoking status, n (%)					0.02
Never	399 (47%)	369 (44%)	351 (41%)	394 (46%)	
Former	325 (38%)	373 (44%)	382 (45%)	362 (43%)	
Current	125 (15%)	105 (12%)	114 (13%)	92 (11%)	
Total cholesterol (mg/dL) ± SD	196±36	196±37	194±36	193±37	0.44
HDL cholesterol (mg/dL) ± SD	49±14	49±15	49±14	51±15	0.01
Use lipid-lowering meds, n (%)	179 (21%)	163 (19%)	200 (24%)	198 (23%)	0.10
C-reactive protein (mg/L)*	1.97 [0.87-4.50]	2.17 [0.99-4.48]	1.79 [0.85-3.96]	1.63 [0.70-3.80]	<0.001
Interleukin 6 (pg/ml)*	1.26 [0.84-1.96]	1.37 [0.90-2.12]	1.33 [0.89-2.12]	1.28 [0.86-1.95]	0.05
Urinary albumin/creatinine (mg/g)*	5.4 [3.4-11.8]	6.0 [3.6-13.7]	6.5 [3.6-14.4]	7.0 [4.0-15.6]	<0.001
eGFR (mL/min/1.73 m^2) ± SD	79±18	78±17	76±23	76±17	0.006
Calcium (mg/dL) ± SD	9.6±0.4	9.6±0.4	9.7±0.4	9.6±0.4	0.69
Phosphorus (mg/dL) ± SD	3.6±0.5	3.7±0.5	3.7±0.5	3.7±0.5	0.12
25-hydroxyvitamin D (ng/mL) ± SD	25.6±11.0	25.2±10.5	27.1±13.0	26.8±11.9	0.004
Parathyroid hormone (pg/mL)*	40 [31-53]	41 [31-53]	41 [31-54]	39 [31-53]	0.38

Fibroblast growth factor-23 (pg/mL)*	38 [31-48]	39 [31-48]	40 [33-50]	38 [32-48]	0.007
Bicarbonate (mmol/L) ± SD	23.0±1.8	23.2±1.8	23.2±1.8	23.2±1.8	0.16

* Median (Interquartile range), p-value by Kruskal-Wallis test.

Table 2: Association of Kidney and Mineral Markers with CAC Density and Volume, Adjusted for Traditional CVD Risk Factors

Variable	Frequency (Percentage)	Adjusted[$] mean density(95% CI)	Continuous Δ density score[#]	Adjusted[$] mean volume (95% CI)	Continuous Δ Ln(volume)[#]
eGFR (mL/min/1.73 m^2)					
≥ 90	680 (20%)	2.658 (2.614, 2.701)		90.14 (81.89, 99.22)	
60-89	2248 (66%)	2.700 (2.677, 2.722)		79.03 (75.20, 83.04)	
< 60	460 (14%)	2.712 (2.660, 2.763)		80.04 (71.43, 89.69)	
Per 19 mL/min/1.73 m^2* greater			-0.022 (-0.043, -0.002) 0.03		0.067 (0.022, 0.111) 0.003
Urinary albumin/creatinine ratio (mg/g)					
≤10	2285 (68%)	2.694 (2.672, 2.717)		78.80 (74.95, 82.84)	
11-30	686 (20%)	2.701 (2.660, 2.742)		83.36 (76.16, 91.24)	
>30	409 (12%)	2.669 (2.614, 2.725)		92.57 (81.94, 104.58)	
Per 1.22 natural log units* greater			-0.007 (-0.028, 0.013) 0.48		0.073 (0.028, 0.118) 0.002
Calcium (mg/dL)					
<9.0	67 (2%)	2.706 (2.574, 2.838)		64.24 (48.10, 85.81)	
9.0-9.4	946 (29%)	2.665 (2.630, 2.700)		82.50 (76.37, 89.12)	
9.5-9.9	1596 (49%)	2.705 (2.678, 2.731)		80.08 (75.54, 84.90)	
≥10.0	629 (19%)	2.707 (2.664, 2.751)		81.17 (73.84, 89.22)	
Per 0.4 mg/dL* greater			0.007 (-0.012, 0.027) 0.46		0.024 (-0.019, 0.067) 0.27
Phosphorus (mg/dL)					
<3.0	256 (8%)	2.674 (2.606, 2.742)		65.44 (56.42, 75.90)	
3.0-3.4	886 (27%)	2.674 (2.637, 2.710)		80.90 (74.68, 87.63)	
3.5-3.9	1193 (37%)	2.705 (2.674, 2.736)		80.66 (75.39, 86.29)	
≥4.0	924 (28%)	2.702 (2.665, 2.739)		85.38 (78.76, 92.57)	
Per 0.5 mg/dL* greater			0.023 (0.003, 0.044) 0.03		0.054 (0.009, 0.100) 0.02
25-hydroxyvitamin D (ng/mL)					
<20.0	920 (31%)	2.664 (2.627, 2.702)		84.00 (77.44, 91.11)	
20.0-29.9	993 (34%)	2.705 (2.671, 2.740)		78.58 (72.94, 84.66)	
≥30.0	1049 (35%)	2.703 (2.668, 2.738)		79.65 (73.83, 85.92)	
Per 11.6 ng/mL* greater			0.020 (-0.001, 0.042) 0.07		-0.007 (-0.054, 0.040) 0.77
Parathyroid hormone (pg/mL)					
<65	2886 (88%)	2.695 (2.675, 2.715)		81.58 (78.10, 85.21)	
≥65	377 (12%)	2.679 (2.621, 2.736)		73.36 (64.73, 83.15)	

Per 0.43 natural log units* greater			0.005 (-0.015, 0.025) 0.65		-0.043 (-0.087, 0.00) 0.05
Fibroblast growth factor-23 (pg/mL)					
<30.0	669 (21%)	2.693 (2.651, 2.735)		75.57 (68.94, 82.84)	
30.0-39.9	1066 (33%)	2.712 (2.679, 2.744)		78.50 (73.06, 84.34)	
40.0-49.9	809 (25%)	2.687 (2.649, 2.724)		81.18 (74.76, 88.17)	
≥50	716 (22%)	2.673 (2.632, 2.714)		88.19 (80.63, 96.47)	
Per 0.35 natural log units* greater			-0.010 (-0.030, 0.009) 0.30		0.055 (0.012, 0.098) 0.01
Bicarbonate (mEq/L)					
<23.0	1137 (35%)	2.663 (2.631, 2.695)		84.70 (78.96, 90.87)	
23.0-23.9	721 (22%)	2.693 (2.653, 2.732)		84.02 (77.01, 91.66)	
24.0-24.9	675 (21%)	2.720 (2.679, 2.761)		78.32 (71.57, 85.70)	
≥25.0	680 (21%)	2.711 (2.670, 2.752)		72.99 (66.67, 79.92)	
Per 3.6 mEq/L* greater			0.022 (0.003, 0.041) 0.03		-0.046 (-0.089, -0.004) 0.03

*One standard deviation

Change (95% CI) p-value

$Adjusted for age, sex, race, diabetes, hypertension medication use, systolic blood pressure, body mass index, smoking, total and HDL cholesterol, lipid-lowering medication use, eGFR, and albumin to creatinine ratio

All density values have been adjusted for the natural log of volume, and all volume values have been adjusted for density

Table 3: Association of Kidney and Mineral Markers with Coronary Artery Density and Volume, Fully Adjusted

Variable	Frequency (Percentage)	Adjusted[$] mean density (95% CI)	Continuous Δ density score[#]	Adjusted[$] mean volume (95% CI)	Continuous Δ Ln(volume)[#]
eGFR (mL/min/1.73 m^2)					
≥ 90	680 (20%)	2.663 (2.615, 2.711)		91.24 (82.23, 101.23)	
60-89	2248 (66%)	2.701 (2.676, 2.726)		78.02 (73.97, 82.29)	
< 60	460 (14%)	2.690 (2.631, 2.749)		79.29 (69.78, 90.11)	
Per 19 mL/min/1.73 m^2* greater			-0.017 (-0.040, 0.006) 0.14		0.084 (0.034, 0.133) 0.001
Urinary albumin/creatinine ratio (mg/g)					
≤10	2285 (68%)	2.686 (2.661, 2.711)		78.76 (74.61, 83.13)	
11-30	686 (20%)	2.714 (2.669, 2.759)		79.67 (72.27, 87.84)	
>30	409 (12%)	2.686 (2.626, 2.746)		93.95 (82.49, 107.01)	
Per 1.22 natural log units* greater			0.003 (-0.020, 0.026) 0.79		0.071 (0.021, 0.120) 0.005
Calcium (mg/dL)					
<9.0	67 (2%)	2.701 (2.562, 2.839)		70.87 (52.51, 95.63)	
9.0-9.4	946 (29%)	2.661 (2.624, 2.698)		83.37 (76.91, 90.38)	
9.5-9.9	1596 (49%)	2.701 (2.673, 2.729)		80.36 (75.57, 85.45)	
≥10.0	629 (19%)	2.716 (2.668, 2.763)		78.21 (70.55, 86.69)	
Per 0.4 mg/dL* greater			0.014 (-0.008, 0.036) 0.20		-0.002 (-0.049, 0.046) 0.95
Phosphorus (mg/dL)					
<3.0	256 (8%)	2.669 (2.596, 2.742)		65.50 (55.95, 76.68)	
3.0-3.4	886 (27%)	2.662 (2.623, 2.702)		83.98 (77.09, 91.48)	
3.5-3.9	1193 (37%)	2.703 (2.670, 2.736)		80.39 (74.89, 86.29)	
≥4.0	924 (28%)	2.712 (2.672, 2.752)		83.07 (76.17, 90.59)	
Per 0.5 mg/dL* greater			0.032 (0.009, 0.055) 0.007		0.035 (-0.015, 0.086) 0.17
25-hydroxyvitamin D (ng/mL)					
<20.0	920 (31%)	2.660 (2.621, 2.698)		85.59 (78.74, 93.05)	
20.0-29.9	993 (34%)	2.705 (2.670, 2.739)		78.66 (72.99, 84.75)	
≥30.0	1049 (35%)	2.708 (2.672, 2.743)		78.49 (72.63, 84.83)	

			0.024 (0.002, 0.047)		-0.021 (-0.070, 0.029)
Per 11.6 ng/mL* greater			0.04		0.41
Parathyroid hormone (pg/mL)					
<65	2886 (88%)	2.690 (2.669, 2.711)		81.88 (78.19, 85.74)	
≥65	377 (12%)			72.12 (63.01, 82.54)	
Per 0.43 natural log units* greater			0.021 (-0.002, 0.044)		-0.052 (-0.101, 0.002)
			0.07		0.04
Fibroblast growth factor-23 (pg/mL)					
<30.0	669 (21%)	2.697 (2.652, 2.742)		75.92 (68.85, 83.72)	
30.0-39.9	1066 (33%)	2.706 (2.671, 2.740)		79.21 (73.46, 85.40)	
40.0-49.9	809 (25%)	2.689 (2.647, 2.728)		80.41 (73.67, 87.77)	
≥50	716 (22%)	2.671 (2.627, 2.715)		88.18 (80.15, 97.02)	
Per 0.35 natural log units* greater			-0.013 (-0.035, 0.009)		0.054 (0.007, 0.101)
			0.24		0.03
Bicarbonate (mEq/L)					
<23.0	1137 (35%)	2.657 (2.623, 2.692)		86.85 (80.53, 93.65)	
23.0-23.9	721 (22%)	2.695 (2.652, 2.737)		83.12 (75.88, 91.06)	
24.0-24.9	675 (21%)	2.724 (2.681, 2.767)		78.73 (71.72, 86.42)	
≥25.0	680 (21%)	2.710 (2.668, 2.753)		71.76 (65.42, 78.71)	
Per 3.6 mEq/L* greater			0.025 (0.004, 0.046)		-0.060 (-0.105, -0.015)
			0.02		0.009

*One standard deviation

[#]Change (95% CI) p-value

[$]Adjusted for all standard cardiac risk factors (age, sex, race, diabetes, hypertension medication use, systolic pressure, body mass index, smoking, total and HDL cholesterol,
and lipid-lowering medication use) and mutually adjusted for all variables shown in
the table.

All density values have been adjusted for the natural log of volume, and all volume values have been adjusted for density

Supplement Table 1: Association of Kidney and Mineral Metabolism Measures with CAC Density and Volume in Participants with CKD[#]

Variable	Frequency (Percentage)	Adjusted[*] Mean Density (95% CI)	Adjusted[*] Mean Volume (95% CI)
Calcium (mg/dL)			
<9.0	11 (3%)	2.706 (2.574, 2.838)	4.163 (3.874, 4.452)
9.0-9.4	94 (22%)	2.665 (2.630, 2.700)	4.413 (4.336, 4.490)
9.5-9.9	211 (49%)	2.705 (2.678, 2.731)	4.383 (4.325, 4.441)
≥10.0	117 (27%)	2.707 (2.664, 2.751)	4.397 (4.302, 4.491)
P-value (trend)		0.71	0.48
Phosphorus (mg/dL)			
<3.0	25 (6%)	2.674 (2.606, 2.742)	4.181 (4.033, 4.329)
3.0-3.4	102 (24%)	2.674 (2.637, 2.710)	4.393 (4.313, 4.473)
3.5-3.9	135 (31%)	2.705 (2.674, 2.736)	4.390 (4.323, 4.458)
≥4.0	171 (39%)	2.702 (2.665, 2.739)	4.447 (4.366, 4.528)
P-value (trend)		0.05	0.77
25-hydroxyvitamin D (ng/mL)			
<20.0	88 (22%)	2.809 (2.696, 2.922)	4.728(4.462, 4.994)
20.0-29.9	124 (32%)	2.725 (2.637, 2.814)	4.846 (4.638, 5.055)
≥30.0	181 (46%)	2.807 (2.729, 2.885)	4.743 (4.558, 4.927)
P-value (trend)		0.82	0.95
Parathyroid hormone (pg/mL)			
<65	343 (79%)	2.781 (2.724, 2.838)	4.819 (4.686, 4.953)
≥65	90 (21%)	2.785 (2.662, 2.908)	4.591 (4.303, 4.879)
P-value		0.95	0.18
Fibroblast growth factor-23 (pg/mL)			
<30.0	34 (8%)	2.641 (2.459, 2.823)	5.027 (4.598, 5.457)
30.0-39.9	90 (21%)	2.823 (2.714, 2.931)	4.766 (4.509, 5.022)
40.0-49.9	110 (25%)	2.863 (2.760, 2.966)	4.699 (4.455, 4.943)
≥50	198 (46%)	2.744 (2.670, 2.819)	4.770 (4.593, 4.947)
P-value (trend)		0.93	0.51
Bicarbonate (mEq/L)			
<23.0	170 (40%)	2.702 (2.620, 2.785)	4.781 (4.586, 4.975)
23.0-23.9	92 (22%)	2.804 (2.697, 2.911)	4.920 (4.668, 5.171)
24.0-24.9	77 (18%)	2.874 (2.755, 2.992)	4.763 (4.483, 5.044)
≥25.0	89 (21%)	2.822 (2.714, 2.929)	4.618 (4.365, 4.871)
P-value (trend)		0.04	0.31

[#]CKD is defined as having an eGFR of <60mL/min/1.73 m^2

*Adjusted for age, sex, race, diabetes, systolic blood pressure, hypertension med use, body mass index, smoking, total chol., HDL chol.,
lipid-lowering med use, eGFR, urine ACR, calcium, phosphate, PTH, FGF23, and serum bicarbonate. Density scores are additionally
adjusted for volume scores, and volume scores are additionally adjusted for density scores.

Figure 1. Relative Strength of Associations of Variables with CAC Density and CAC Volume.