Serum Calcification Propensity and Coronary Artery Calcification Among Patients With CKD: The CRIC (Chronic Renal Insufficiency Cohort) Study

Joshua D. Bundy, Xuan Cai, Julia J. Scialla, Mirela A. Dobre, Jing Chen, Chi-yuan Hsu, Mary B. Leonard, Alan S. Go, Panduranga S. Rao, James P. Lash, Raymond R. Townsend, Harold I. Feldman, Ian H. de Boer, Geoffrey A. Block, Myles Wolf, Edward R. Smith, Andreas Pasch, and Tamara Isakova, on behalf of the CRIC Study Investigators

Rationale & Objective: Coronary artery calcification (CAC) is prevalent among patients with chronic kidney disease (CKD) and increases risks for cardiovascular disease events and mortality. We hypothesized that a novel serum measure of calcification propensity is associated with CAC among patients with CKD stages 2 to 4.

Study Design: Prospective cohort study.

Setting & Participants: Participants from the Chronic Renal Insufficiency Cohort (CRIC) Study with baseline (n = 1,274) and follow-up (n = 780) CAC measurements.

Predictors: Calcification propensity, quantified as transformation time (T_{50}) from primary to secondary calciprotein particles, with lower T_{50} corresponding to higher calcification propensity. Covariates included age, sex, race/ethnicity, clinical site, estimated glomerular filtration rate, proteinuria, diabetes, systolic blood pressure, number of antihypertensive medications, current smoking, history of cardiovascular disease, total cholesterol level, and use of statin medications.

Outcomes: CAC prevalence, severity, incidence, and progression.

Analytical Approach: Multivariable-adjusted generalized linear models.

Results: At baseline, 824 (65%) participants had prevalent CAC. After multivariable adjustment, T_{50} was not associated with CAC prevalence but was significantly associated with greater CAC severity among participants with prevalent CAC: 1-SD lower T_{50} was associated with 21% (95% CI, 6%-38%) greater CAC severity. Among 780 participants followed up an average of 3 years later, 65 (20%) without baseline CAC developed incident CAC, while 89 (19%) with baseline CAC had progression, defined as annual increase ≥ 100 Agatston units. After multivariable adjustment, T_{50} was not associated with incident CAC but was significantly associated with CAC progression: 1-SD lower T_{50} was associated with 28% (95% CI, 7%-53%) higher risk for CAC progression.

Limitations: Potential selection bias in follow-up analyses; inability to distinguish intimal from medial calcification.

Conclusions: Among patients with CKD stages 2 to 4, higher serum calcification propensity is associated with more severe CAC and CAC progression.

Complete author and article information (including a list of the CRIC Study Principal Investigators) appears at end of article text.

Correspondence to J.D. Bundy (jdbundy@northwestern.edu) or T. Isakova (tamara.isakova@northwestern.edu)

Am J Kidney Dis. XX(XX):1-9. Published online Month X, XXXX.

doi: 10.1053/j.ajkd.2019.01.024

Cardiovascular disease (CVD) is the leading cause of death among patients with chronic kidney disease (CKD) and is a major public health challenge.[1,2] Vascular calcification is common in CKD and is one mechanism by which CVD risk is increased in patients with CKD.[3] In addition to developing medial calcification, which is associated with increased arterial stiffness[4] and heart failure,[5] patients with CKD also develop intimal calcification, indicative of atherosclerosis. Both types of calcification can contribute to the coronary artery calcium (CAC) score. The presence[6,7] and progression[8,9] of CAC are strongly associated with CVD in the general population and previous studies have shown that reduced kidney function is associated with more severe calcification[10] and more rapid CAC progression.[3,11] Among patients with CKD stages 2 to 4, CAC score independently predicts risks for CVD and all-cause mortality.[12]

The gold standard for quantifying vascular calcification is computed tomography (CT), but radiation exposure limits the utility of longitudinal computed tomographic measurements of calcification in clinical practice. A novel in vitro assay quantifies the propensity for calcification in serum by evaluating the transformation time (T_{50}) from primary to secondary calciprotein particles when challenged with additional calcium and phosphate.[13] Primary calciprotein particles are amorphous accumulations of calcium and phosphate. Their transformation to secondary calciprotein particles, composed of crystalline calcium phosphate, may provide information about the status of the humoral calcification-regulating system.[14] Under normal homeostatic conditions, calcification promoters (eg, calcium and phosphate) and inhibitors (eg, albumin, fetuin-A, magnesium, and pyrophosphate) are balanced such that vascular calcification does not occur. The T_{50} test represents a composite functional measure of this promoter-inhibitor balance. Higher calcification propensity (denoted by lower T_{50}) may reflect decreased inhibitory capacity to remove excess mineral from the

circulation and has previously been associated with cardiovascular and all-cause mortality among individuals with advanced CKD, kidney transplant recipients, and patients with kidney failure undergoing hemodialysis.[15-18] However, associations with CVD, including CAC, in patients with mild to moderate CKD are unknown.

The Chronic Renal Insufficiency Cohort (CRIC) Study provides a unique opportunity to examine the associations of T_{50} with the presence and progression of CAC among a diverse sample of patients with CKD stages 2 to 4. We tested the hypothesis that low T_{50} values would be associated with prevalent and incident CAC among patients with CKD stages 2 to 4.

Methods

Study Design and Participants

The CRIC Study is a prospective cohort study of a racially and ethnically diverse group of men and women aged 21 to 74 years with mild to moderate CKD (estimated glomerular filtration rate [eGFR] entry criteria 20-70 mL/min/1.73 m^2). A total of 3,939 participants were enrolled from 7 centers in the United States between May 2003 and August 2008.[19] Patients with cirrhosis, human immunodeficiency virus (HIV) infection, polycystic kidney disease, or renal cell carcinoma; those receiving dialysis or an organ transplant; or those taking immunosuppressive medications were excluded. Participants with a history of coronary artery revascularization did not undergo CT. The study was approved by the institutional review boards from each clinical center, and all participants provided written informed consent.

Computed Tomographic Measurements

Of the entire cohort, 1,142 participants were randomly selected and stratified by age, sex, race/ethnicity, diabetes status, and eGFR for electron-beam or multidetector CT. In addition, all eligible participants from 3 centers were scanned as part of an ancillary study, yielding 1,964 total participants scanned within the first 3 years of the original baseline examination (Fig 1). Of these participants, 1,274 had T_{50} measured at the same study visit as their first computed tomographic scan (ie, "baseline" for the present study) as part of an ancillary study. Repeated CT was performed among 1,123 participants an average of 3.2 ± 0.6 (standard deviation [SD]) years later, 780 of whom had T_{50} data.

Trained and certified technologists scanned participants twice using phantoms of known physical calcium concentrations. A cardiologist read all scans at a central reading center (Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center) to quantify calcification according to the Agatston score.[20] Total CAC score was calculated as the sum of scores from the left main, left anterior descending, left circumflex, and right coronary arteries. Final scores are the mean of 2 scans.[21]

Exposure Assessment

We quantified calcification propensity as the T_{50} from primary to secondary calciprotein particles in vitro, with lower T_{50} corresponding to higher calcification propensity.[13] Serum samples, stored at −80°C and shipped with sufficient dry ice, were used for the test, which was performed using a Nephelostar nephelometer at the Calciscon Laboratory in Switzerland.[13] Mean intra-assay coefficient of variation is 2.2%, and mean interassay coefficient of variation is 3.4%. The reference range is 270 to 460 minutes, as determined in 253 healthy Swiss adults. T_{50} values were reported in other populations, including 184 patients with CKD stages 3 to 4 (mean, 329 ± 95 minutes)[15] and 2,785 patients undergoing hemodialysis (median, 212 [10th-90th percentiles, 109-328] minutes).[18]

Covariate Assessment

We obtained covariate data from the same study visit as the first computed tomographic scan or the most recent previous annual visit if missing (<2% missing for all covariates except 24-hour urinary protein [8%]). Self-reported sociodemographic characteristics, medical history, and current medications were obtained using a questionnaire. Body weight, height, and blood pressure were measured using standard protocols.[19] Diabetes was defined as fasting glucose level ≥ 126 mg/dL, nonfasting glucose level ≥ 200 mg/dL, and/or use of antidiabetic medications. History of CVD was defined as self-reported prior coronary artery disease, heart failure, stroke, or peripheral vascular disease.

Glucose, cholesterol, bicarbonate, phosphate, calcium, magnesium, serum albumin, and total parathyroid hormone (PTH) were measured using standard laboratory methods. The 24-hour urinary protein was measured using the turbidimetric method with benzethonium chloride. Fibroblast growth factor 23 (FGF-23) was measured using a second-generation carboxy-terminal assay (Immutopics). High-sensitivity C-reactive protein (hsCRP)

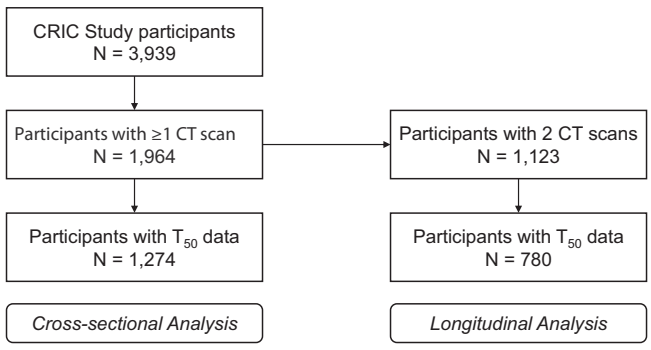

Figure 1. Flow chart describes the study sample selection for cross-sectional and longitudinal analyses. Abbreviations: CRIC, Chronic Renal Insufficiency Cohort; CT, computed tomography; T_{50}, transformation time.

and interleukin 6 (IL-6) were measured at the original baseline examination using the particle-enhanced immunonephelometry method. Fetuin-A was measured at the original baseline examination using the quantitative sandwich enzyme immunoassay technique. We calculated eGFR using the equation derived in CRIC.[22]

Statistical Analysis

We summarized baseline characteristics of study participants as mean ± SD or median with interquartile range for continuous variables and percentages for categorical variables, by T_{50} quartile. We evaluated the cross-sectional association of T_{50} with CAC score using a 2-part model.[21] First, we modeled the prevalence of CAC score > 0 among all participants using Poisson regression with robust variance estimation. Second, among those with CAC scores > 0, we modeled the severity of CAC using linear regression and natural log-transformed CAC score. We exponentiated regression coefficients and expressed them as percent difference in CAC per 1-SD lower T_{50} or between quartiles of T_{50} compared to the highest quartile (reference). Additionally, we modeled the prevalence of moderate (≥200 units) and severe (≥400 units) CAC.

We evaluated the longitudinal association of T_{50} with CAC stratifying by the presence of baseline CAC.[23] Among those with no baseline CAC, we defined incidence as CAC score > 0 at follow-up. Among those with baseline CAC, we defined progression as an annual increase in CAC score ≥ 100 units, which is significantly associated with higher risk for coronary heart disease.[9] Additionally, we assessed progression defined as an annual increase in CAC score ≥ 200 units. We evaluated incidence and progression using Poisson regression with robust variance estimation, using an offset to account for the time between computed tomographic scans.

We included covariates in sequential regression models based on prior clinical knowledge. In addition to unadjusted analyses, 2 multivariable-adjusted models were used: (1) adjusted for age, sex, race/ethnicity, and clinical site; and (2) adjusted for variables in model 1 plus eGFR, proteinuria, diabetes, systolic blood pressure, number of antihypertensive medications, current smoking, history of CVD, total cholesterol level, and use of statin medications. We included baseline CAC score in models analyzing participants with baseline CAC. In additional analyses, we evaluated the impact of adjusting for variables potentially affecting T_{50} (calcium, phosphate, bicarbonate, magnesium, serum albumin, fetuin-A, FGF-23, and PTH values and use of medications including warfarin, active vitamin D, phosphate binders, and calciferols) and inflammatory variables (IL-6 and hsCRP levels) on associations of T_{50} with CAC score. Magnesium, IL-6, hsCRP, and fetuin-A were measured at the original baseline examination. Because the onset of end-stage kidney disease may increase the risk for calcification,[24] we conducted sensitivity analyses excluding those with end-stage kidney disease at baseline (ie, at the time of the scan; cross-sectional analyses) and during follow-up (longitudinal analyses).

We tested for effect modification by including T_{50}-by-subgroup interaction terms (defined by age, sex, race/ethnicity, and diabetes) in regression models. All analyses were conducted using SAS, version 9.4 (SAS Institute, Inc), and R, version 3.4.2 (The R Foundation). All tests were 2 sided and statistical significance was defined as $P < 0.05$.

Results

Among 1,274 participants with data for CT and T_{50}, mean age was 57.5 ± 11.7 years, 46.9% were women, 44.3% had diabetes, 27.2% had a history of CVD, and mean eGFR was 44.5 ± 17.8 mL/min/1.73 m^2. Participants included in the current analyses were on average healthier compared with those not included in the current analyses (Table S1). Median T_{50} was 321 (interquartile range, 270-366) minutes. Those with low T_{50} were more likely to be non-Hispanic black ($P < 0.001$), have a history of CVD ($P = 0.004$) and diabetes ($P < 0.001$), and be taking antihypertensive ($P < 0.001$), statin ($P = 0.01$), and active vitamin D medications ($P < 0.001$; Table 1). On average, those with low T_{50} had higher systolic blood pressures ($P = 0.006$), 24-hour urine protein excretion ($P < 0.001$), and phosphate ($P < 0.001$), FGF-23 ($P < 0.001$), PTH ($P < 0.001$), IL-6 ($P = 0.001$), and hsCRP ($P = 0.02$) levels, and lower eGFR ($P < 0.001$), bicarbonate ($P < 0.001$), calcium ($P = 0.006$), magnesium ($P = 0.005$), serum albumin ($P < 0.001$), and fetuin-A ($P < 0.001$) values. Of the 1,274 participants, 824 (64.7%) had baseline CAC. Figure 2 shows the distribution of baseline CAC by T_{50} quartiles. Mild CAC severity (<100 Agatston units) was similar among T_{50} quartiles, while lower T_{50} quartiles were more likely to have severe CAC (>400 Agatston units).

Table 2 shows cross-sectional associations of T_{50} with the prevalence and severity of CAC. T_{50} was not associated with the prevalence of CAC score > 0 after multivariable adjustment ($P = 0.6$ for linear trend across quartiles). However, lower T_{50} was associated with greater CAC severity among participants with baseline CAC. After multivariable adjustment, 1-SD lower T_{50} value was associated with 21% (95% confidence interval [CI], 6%-38%) greater CAC severity, and we observed graded associations across T_{50} quartiles. Additionally, lower T_{50} was significantly associated with greater prevalence of moderate and severe CAC (Table S2).

Table 3 shows the longitudinal associations of T_{50} with the incidence and progression of CAC among 780 participants with follow-up CT an average of 3.2 ± 0.6 years later. Among 320 participants without baseline CAC, 65 developed incident CAC during follow-up; T_{50} was not associated with incident CAC. Among 460 participants with baseline CAC, 89 had an annual increase of ≥100 Agatston units and 37 had an annual increase of ≥200

Table 1. Baseline Characteristics of 1,274 CRIC Participants by Quartiles of T_{50}

Variables	T_{50} Q4	T_{50} Q3	T_{50} Q2	T_{50} Q1
No. of patients	316	320	322	316
T_{50} range, min	367-600	322-366	270-321	72-269
Age, y	57 ± 12	58 ± 12	57 ± 12	58 ± 11
Female sex	146 (46%)	145 (45%)	158 (49%)	148 (47%)
Race/ethnicity				
Non-Hispanic white	183 (58%)	150 (47%)	150 (47%)	127 (40%)
Non-Hispanic black	97 (31%)	124 (39%)	134 (42%)	161 (51%)
Hispanic	9 (3%)	22 (7%)	10 (3%)	16 (5%)
Other	27 (9%)	24 (8%)	28 (9%)	12 (4%)
Body mass index, kg/m^2	30 ± 7	31 ± 7	31 ± 7	31 ± 7
Current cigarette smoking	32 (10%)	24 (8%)	36 (11%)	45 (14%)
History of cardiovascular disease	77 (24%)	78 (24%)	80 (25%)	111 (35%)
Diabetes mellitus	120 (38%)	134 (42%)	139 (43%)	171 (54%)
Systolic blood pressure, mm Hg	122 ± 19	125 ± 20	126 ± 21	128 ± 21
No. of antihypertensive medications	2 [1-3]	3 [2-4]	2 [1-3]	3 [2-4]
Total cholesterol, mg/dL	186 ± 40	181 ± 39	186 ± 42	182 ± 45
Statin medication use	158 (50%)	194 (61%)	173 (54%)	192 (61%)
Warfarin medication use	16 (5%)	16 (5%)	18 (6%)	12 (4%)
eGFR, mL/min/1.73 m^2	50 ± 16	45 ± 16	45 ± 18	38 ± 18
Urinary protein, g/24 h	0.1 [0.1-0.4]	0.2 [0.1-0.8]	0.2 [0.1-1.0]	0.2 [0.1-1.5]
Bicarbonate, mmol/L	25 ± 3	24 ± 3	24 ± 3	23 ± 4
Active vitamin D medication use	6 (2%)	18 (6%)	24 (8%)	33 (10%)
Phosphate-binder medication use	20 (6%)	13 (4%)	28 (9%)	26 (8%)
Calciferol medication use	43 (14%)	40 (13%)	48 (15%)	40 (13%)
Calcium, mg/dL	9.4 ± 0.4	9.3 ± 0.5	9.3 ± 0.5	9.3 ± 0.6
Phosphate, mg/dL	3.6 ± 0.9	3.8 ± 1.1	3.8 ± 0.7	4.2 ± 1.0
Magnesium, mg/dL	2.0 ± 0.3	2.0 ± 0.3	1.9 ± 0.2	1.9 ± 0.3
Serum albumin, g/dL	4.2 ± 0.4	4.1 ± 0.4	4.1 ± 0.4	4.0 ± 0.5
Fetuin-A, mg/L	582 ± 116	529 ± 104	517 ± 97	492 ± 111
FGF-23, RU/mL	115 [77-175]	135 [84-246]	139 [97-276]	170 [101-358]
Parathyroid hormone, pg/mL	55 [36-85]	64 [43-93]	56 [36-90]	65 [42-122]
Interleukin 6, pg/mL	1.4 [0.8-2.5]	1.7 [1.1-2.6]	1.7 [1.1-2.8]	1.8 [1.1-2.9]
hsCRP, mg/L	1.6 [0.8-4.3]	2.4 [0.9-5.7]	2.3 [1.1-5.3]	2.4 [0.9-5.5]

Note: Values are presented as mean ± standard deviation, median [interquartile range], or number (percent).
Abbreviations: CRIC, Chronic Renal Insufficiency Cohort; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor 23; hsCRP, high-sensitivity C-reactive protein; Q, quartile; T_{50}, transformation time.

Agatston units. T_{50} was significantly associated with both definitions of progression. After multivariable adjustment, 1-SD lower T_{50} value was associated with 28% (95% CI, 7%-53%) higher risk for progressing ≥100 Agatston units per year and we observed graded associations across T_{50} quartiles. Corresponding associations for CAC progression defined as an increase ≥ 200 Agatston units per year were similar, but stronger and larger in magnitude.

We did not detect significant interactions between T_{50} and age, sex, race/ethnicity, or diabetes. Results were similar to the primary analyses following adjustment for variables potentially affecting T_{50} (calcium, phosphate, bicarbonate, magnesium, serum albumin, fetuin-A, FGF-23, and PTH levels and use of medications including warfarin, active vitamin D, phosphate binders, and calciferols) and inflammatory variables (IL-6 and hsCRP levels; Table S3). Sensitivity analyses excluding participants with end-stage kidney disease did not substantively affect results (Table S4).

Discussion

High serum calcification propensity, denoted by low T_{50}, was independently associated with greater CAC severity and progression among patients with CKD stages 2 to 4 and established CAC at baseline. Conversely, T_{50} was not associated with CAC prevalence or the incidence of CAC among patients without CAC. These findings implicate T_{50} as a measure of the severity of calcification and risk for progression. Given the strength of associations and consistency of results in several additional analyses, T_{50} and its components may be involved in the calcification pathway, identify patients with CKD who are at high risk for CVD, and offer insights into mechanisms of calcification and targets for therapy to reduce the burden of CVD among patients with CKD.

There are several possible mechanisms that could explain our findings. First, information gained from the T_{50} test could relate to medial vascular calcification and

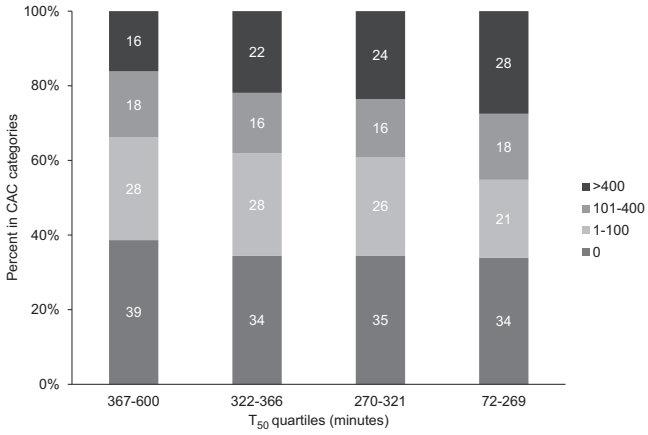

Figure 2. Bar chart describes proportions of participants in coronary artery calcium (CAC) categories, by quartile of transformation time (T_{50}).

its determinants. Disordered mineral metabolism is common in CKD,[25] marked by abnormal levels of the calcification promoters phosphate and calcium, which are themselves associated with the presence[26] and progression[23] of CAC. However, vascular calcification is a tightly regulated and dynamic process that begins with the deposition of amorphous calcium phosphates on an organic template. Although the mechanisms of formation of the initial nidus are complex, the subsequent ripening and transformation of the nidus to more crystalline forms are likely governed by calcification promoters and inhibitors.[27,28] Given that the T_{50} test represents a composite measure that captures summary information about calcification promoters and inhibitors in the serum,[13] we surmise that T_{50} may not reflect the initiation of vascular calcification but may provide information on the terminal step of transformation when the nidus is already formed. Thus, the T_{50} test may identify individuals prone to propagation, but not necessarily initiation, of vascular calcification.

It is likely that when calcification is established, it progresses rapidly, especially among patients with high calcification propensity (ie, low T_{50}). Our findings support this hypothesis, showing that although a low T_{50} value was not associated with prevalence of CAC score > 0 or incidence, it was strongly associated with severity and progression, with larger magnitudes of association observed for larger increases in CAC. Components of the calcification process, particularly fetuin-A, a potent inhibitor of calcification, have previously shown a similar association with CAC severity among patients with established calcification.[29]

The T_{50} test could also reflect factors that promote atherosclerotic intimal calcification, which is characterized by endothelial damage, lipid deposition, macrophage accumulation, and inflammation.[30] Prior in vitro studies suggest that secondary calciprotein particles, the maturation of which is captured in part by T_{50}, stimulate inflammation and apoptosis of macrophages, which may promote ectopic calcification.[31,32] Furthermore, secondary calciprotein particles may trigger atherosclerosis through endothelial damage and increase local inflammation through the production of proatherosclerotic cytokines, including IL-6.[33] In our analysis, additional adjustment for the inflammatory variables hsCRP and IL-6 did not significantly change the associations we observed despite being associated with both T_{50} and CAC. While these measurements were not collected concurrently with T_{50}, the T_{50} test may capture information inherent in inflammatory variables. Although we could not distinguish intimal versus medial calcification, the relationships of T_{50} with both types of calcification warrant further mechanistic and translational research approaches.

Finally, we acknowledge the possibility that T_{50} and its constituents may not be directly on the causal pathway to calcification. Given that T_{50} was not associated with the incidence of CAC, it is possible that T_{50} captures a disease state that is already in progress and that is mediated through different causal mechanisms. T_{50} may also reflect another component that itself upsets promoter-inhibitor homeostasis. Our findings point to a threshold effect characterized by severe calcification in those with low T_{50} and established CAC. However, in the current analysis, it is impossible to distinguish whether such a threshold effect directly involves T_{50} and its components or other mechanisms. Nevertheless, our findings point to the utility of T_{50} in providing information about calcification severity and progression. Further analyses are necessary in broader patient populations and larger sample sizes to determine whether T_{50} can predict future calcification and whether its components are potential therapeutic targets. Additionally, evaluation of complementary assays of promoter-inhibitor balance and calciprotein particles, such as the hydrodynamic radius,[34] may offer additional insights.

The present study has several strengths. First, it is the first to estimate the associations of the T_{50} test, a novel measure of calcification propensity, with CAC score among patients with mild to moderate CKD. One previous analysis among 73 patients with diabetes and CKD identified an association between fetuin-A–mineral complex and CAC score,[35] but for the first time we show associations of a composite calcification propensity measure with CAC score in a larger longitudinal analysis. Second, the CRIC Study uses standardization of methods and measurements across clinical sites, which minimizes bias. Third, our results were robust to adjustment for several covariates, including variables related to T_{50} (ie, mineral metabolism variables) and inflammatory variables and various sensitivity analyses.

However, the present study has potential limitations. First, the T_{50} test is conducted in vitro with supersaturation of calcium and phosphate, which results in synthetic calciprotein particles. Because physiochemical properties and predicted pathogenic effects of synthetic

Table 2. Association of T_{50} With Prevalence and Severity of Coronary Artery Calcification at Baseline

	Continuous: Per 1-SD[a] ↓ T_{50}	Categorical				*P* for Linear Trend
		T_{50} Q4 (≥367 min)	T_{50} Q3 (322-366 min)	T_{50} Q2 (271-321 min)	T_{50} Q1 (≤270 min)	
All Participants (N = 1274): Prevalence of CAC > 0, Prevalence Ratio (95% CI)						
n/N[b]		194/316	210/320	211/322	209/316	
Unadjusted	1.01 (0.97-1.05)	1.00 (reference)	1.07 (0.95-1.20)	1.07 (0.95-1.20)	1.08 (0.96-1.21)	0.2
Model 1[c]	1.03 (0.99-1.06)	1.00 (reference)	1.05 (0.95-1.17)	1.10 (0.99-1.22)	1.11 (1.00-1.23)	0.04
Model 2[d]	0.99 (0.96-1.03)	1.00 (reference)	1.03 (0.93-1.14)	1.09 (0.98-1.21)	1.01 (0.91-1.13)	0.6
Participants With Baseline CAC > 0 (n = 824): CAC Severity, % Difference (95% CI)						
Unadjusted	29% (12% to 49%)	0% (reference)	21% (−20% to 82%)	23% (−18% to 84%)	88% (25% to 183%)	0.004
Model 1[c]	38% (21% to 58%)	0% (reference)	21% (−17% to 77%)	39% (−5% to 104%)	118% (48% to 221%)	<0.001
Model 2[d]	21% (6% to 38%)	0% (reference)	19% (−17% to 71%)	36% (−5% to 96%)	58% (8% to 129%)	0.01

Abbreviations: CAC, coronary artery calcium; CI, confidence interval; Q, quartile; SD, standard deviation; T_{50}, transformation time.
[a]One SD is 77 minutes.
[b]Events/total number.
[c]Model 1 adjusted for age, sex, race/ethnicity, and clinical site.
[d]Model 2 adjusted for model 1 + estimated glomerular filtration rate, proteinuria, diabetes, systolic blood pressure, number of antihypertensive medications, current smoking, history of cardiovascular disease, total cholesterol level, and use of statin medications.

and endogenous calciprotein particles appear to be comparable,[36] it is reasonable to investigate T_{50} as a functional read-out of the calciprotein transformation process.

Second, we cannot rule out the possibility of selection bias, especially for progression analyses that required participants to survive long enough for a follow-up scan. Although participants included in the present analysis were on average healthier compared to the full cohort, the population under study is still of clinical and public health relevance.

Third, the present study represents a relatively small sample size with short follow-up but are the first data of its kind and importantly, included longitudinal analyses. Fourth, while we considered many covariates in our analyses, we were unable to evaluate some additional variables important to mineral metabolism and calcification in CKD, including vitamin K and pH. Fifth, we were unable to distinguish between intimal and medial calcification owing to limitations in CT technology. Although each may represent different developmental pathways, both are

Table 3. Association of T_{50} With Incidence and Progression of Coronary Artery Calcification

	Continuous: Per 1-SD[a] ↓ T_{50}	Categorical				*P* for Linear Trend
		T_{50} Q4 (≥367 min)	T_{50} Q3 (322-366 min)	T_{50} Q2 (271-321 min)	T_{50} Q1 (≤270 min)	
Participants With Baseline CAC = 0 (n = 320): Incident CAC, RR (95% CI)						
n/N[b]		13/81	16/81	16/77	20/81	
Unadjusted	1.15 (0.93-1.41)	1.00 (reference)	1.31 (0.68-2.54)	1.31 (0.68-2.52)	1.54 (0.83-2.88)	0.2
Model 1[c]	1.07 (0.84-1.36)	1.00 (reference)	1.34 (0.71-2.53)	1.29 (0.70-2.36)	1.33 (0.70-2.53)	0.4
Model 2[d]	0.95 (0.76-1.18)	1.00 (reference)	1.13 (0.59-2.17)	1.18 (0.63-2.24)	0.93 (0.49-1.76)	0.9
Participants With Baseline CAC > 0 (n = 460): Increase ≥ 100 Agatston U/y, RR (95% CI)						
n/N[b]		12/113	20/114	23/118	34/115	
Unadjusted	1.48 (1.25-1.74)	1.00 (reference)	1.72 (0.88-3.37)	1.86 (0.97-3.57)	2.86 (1.55-5.25)	<0.001
Model 1[c]	1.46 (1.24-1.73)	1.00 (reference)	1.73 (0.92-3.26)	1.94 (1.11-3.39)	2.66 (1.53-4.62)	<0.001
Model 2[d]	1.28 (1.07-1.53)	1.00 (reference)	1.39 (0.72-2.69)	1.81 (1.05-3.12)	1.86 (1.05-3.32)	0.02
Participants With Baseline CAC > 0 (n = 460): Increase ≥ 200 Agatston U/y, RR (95% CI)						
n/N[b]		4/113	4/114	12/118	17/115	
Unadjusted	1.98 (1.48-2.65)	1.00 (reference)	1.03 (0.27-4.03)	2.91 (0.97-8.74)	4.29 (1.49-12.35)	<0.001
Model 1[c]	1.92 (1.39-2.66)	1.00 (reference)	1.03 (0.31-3.35)	3.15 (1.34-7.38)	3.08 (1.27-7.47)	0.003
Model 2[d]	1.81 (1.36-2.41)	1.00 (reference)	1.19 (0.33-4.32)	3.30 (1.53-7.13)	2.95 (1.25-6.97)	0.005

Abbreviations: CAC, coronary artery calcium; CI, confidence interval; Q, quartile; RR, relative risk; SD, standard deviation; T_{50}, transformation time.
[a]One SD is 77 minutes.
[b]Events/total number.
[c]Model 1 adjusted for age, sex, race/ethnicity, clinical site, and baseline CAC score (among those with CAC score > 0 only).
[d]Model 2 adjusted for model 1 + estimated glomerular filtration rate, proteinuria, diabetes, systolic blood pressure, number of antihypertensive medications, current smoking, history of cardiovascular disease, total cholesterol level, and use of statin medications.

associated with higher risk for CVD and mortality in patients with CKD.[37] Finally, one computed tomographic measurement during follow-up does not allow us to pinpoint the exact time point of calcification incidence or progression.

The findings presented here have important clinical and research implications. Calcification is a dynamic process involving complex pathophysiology. Our results suggest that when subclinical disease is established, inherent characteristics of a patient's serum, captured using the T_{50} test, may be useful in determining both the extent of calcification and risk for significant progression. However, given that we did not observe associations with CAC prevalence or incidence, it is unclear in the current study whether low T_{50} precedes calcification or vice versa. One possibility is that the ongoing calcification process consumes inhibitors, such as fetuin-A, resulting in a lower T_{50} value.[38] Alternatively, it is possible that consequences of vascular disease, including inflammation, suppress the synthesis of inhibitors, also prompting a lower T_{50} value.[39] Regardless, a previous analysis in the CRIC Study found that severe CAC was significantly associated with CVD.[12] Thus, in patients with low T_{50}, increased vigilance may be warranted to mitigate the potential for adverse cardiovascular health outcomes. Future research is warranted in those with high calcification propensity to determine whether promoter-inhibitor homeostasis can be improved using novel drug interventions.

In conclusion, higher serum calcification propensity, denoted by lower T_{50}, was significantly associated with the severity and progression of CAC among patients with CKD. However, T_{50} was not associated with the incidence of CAC. These findings provide valuable insights into the development of calcification and atherosclerosis in patients with CKD and highlight potential pathways for risk stratification and therapeutic intervention. Future research should evaluate these associations in other CKD populations and the general population, and clinical trials may be warranted to establish causality.

Supplementary Material

Supplementary File (PDF)

Table S1: Comparison of CRIC participants included and not included in analyses of T_{50} and CAC.

Table S2: Associations of T_{50} with prevalence of moderate and severe CAC at baseline.

Table S3: Impact of adjustment for additional variables on associations of T_{50} with CAC.

Table S4: Impact of excluding participants with ESRD on associations of T_{50} with CAC.

Article Information

CRIC Study Principal Investigators: Lawrence J. Appel, MD, MPH, Harold I. Feldman, MD, MSCE, Alan S. Go, MD, Jiang He, MD, PhD, James P. Lash, MD, Panduranga S. Rao, MD, Mahboob Rahman, MD, and Raymond R. Townsend, MD.

Authors' Full Names and Academic Degrees: Joshua D. Bundy, PhD, MPH, Xuan Cai, MS, Julia J. Scialla, MD, MHS, Mirela A. Dobre, MD, MPH, Jing Chen, MD, Chi-yuan Hsu, MD, MS, Mary B. Leonard, MD, MSCE, Alan S. Go, MD, Panduranga S. Rao, MD, MS, James P. Lash, MD, Raymond R. Townsend, MD, Harold I. Feldman, MD, MSCE, Ian H. de Boer, MD, MS, Geoffrey A. Block, MD, Myles Wolf, MD, MMSc, Edward R. Smith, BSc, MSc, PhD, Andreas Pasch, MD, and Tamara Isakova, MD, MMSc.

Authors' Affiliations: Department of Preventive Medicine (JDB) and Center for Translational Metabolism and Health, Institute for Public Health and Medicine (JDB, XC, TI), Northwestern University Feinberg School of Medicine, Chicago, IL; Department of Medicine, Duke Clinical Research Institute, Duke University, Durham, NC (JJS, MW); Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH (MAD); Department of Medicine, Tulane University School of Medicine, New Orleans, LA (JC); Department of Medicine, University of California San Francisco School of Medicine, San Francisco, CA (C-yH); Department of Pediatrics, Stanford University School of Medicine, Palo Alto (MBL); Comprehensive Clinical Research Unit, Kaiser Permanente Northern California Division of Research, Oakland, CA (ASG); Department of Medicine, University of Michigan Health System, Ann Arbor, MI (PSR); Department of Medicine, University of Illinois College of Medicine at Chicago, Chicago, IL (JPL); Departments of Medicine (RRT, HIF) and Biostatistics, Epidemiology, and Informatics (HIF) and Center for Clinical Epidemiology and Biostatistics (HIF), Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; Department of Medicine, University of Washington, Seattle, WA (IHdB); Colorado Kidney Care, Denver, CO (GAB*); Department of Nephrology, The Royal Melbourne Hospital, Melbourne, Victoria, Australia (ERS); Calciscon AG, Biel-Nidau, Switzerland (AP); and Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL (TI). *Current affiliation: Reata Pharmaceuticals, Inc.

Address for Correspondence: Joshua D. Bundy, PhD, MPH, Department of Preventive Medicine, Northwestern Feinberg School of Medicine, 680 N Lake Shore Dr, Ste 1400, Chicago, IL 60611 (e-mail: jdbundy@northwestern.edu) or Tamara Isakova, MD, MMSc, Department of Medicine, Northwestern Feinberg School of Medicine, 633 N St Clair, Ste 18089, Chicago, IL 60611 (e-mail: tamara.isakova@northwestern.edu).

Authors' Contributions: Designed the study: JDB, TI; collected the data: ASG, PSR, JPL, RRT, HIF, AP, TI; conducted the statistical analyses: JDB, XC; analyzed and interpreted the data: JDB, XC, JJS, MAD, JC, C-yH, MBL, ASG, PSR, JPL, RRT, HIF, IHdB, GAB, MW, ERS, AP, TI. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Support: This work was supported by grants P30DK114857, R01DK102438 (Dr Isakova), R01DK110087 (Dr Isakova), R01DK099199 (Dr de Boer), R01DK081374 (Dr Wolf), R01DK076116 (Dr Wolf), R01DK094796 (Dr Wolf), U01DK099930 (Drs Isakova and Wolf), R01DK111952 (Dr Scialla), and R01HL141846 (Dr Dobre) from the National Institutes of Health (NIH), and a Strategically Focused Research Network Center Grant on Health Disparities from the American Heart Association (Dr Wolf). Funding for the CRIC Study was obtained under a cooperative agreement from the National Institute of Diabetes and Digestive and Kidney Diseases (U01DK060990, U01DK060984, U01DK061022, U01DK061021, U01DK061028, U01DK060980, U01DK060963, and U01DK060902). In addition, this study was supported in part by the Perelman School of Medicine at the University of Pennsylvania Clinical and Translational Science Award NIH/

National Center for Advancing Translational Sciences UL1TR000003, the Johns Hopkins Infstitute for Clinical and Translational Research UL1 TR-000424, University of Maryland General Clinical Research Center M01 RR-16500, Clinical and Translational Science Collaborative of Cleveland, UL1TR000439, Michigan Institute for Clinical and Health Research UL1TR000433, University of Illinois at Chicago Clinical and Translational Science Awards UL1RR029879, Tulane COBRE for Clinical and Translational Research in Cardiometabolic Diseases P20 GM109036, and Kaiser Permanente NIH/National Center for Research Resources UCSF-CTSI UL1 RR-024131. Dr Bundy is supported by the National Heart, Lung, and Blood Institute Cardiovascular Epidemiology training grant T32HL069771. None of the funders of this study had any role in the current study design; collection, analysis, and interpretation of data; writing the report; or the decision to submit the report for publication.

Financial Disclosure: Dr de Boer has received research support, honoraria or consultant fees from Abbott, Boehringer Ingelheim, Ironwood, and Medtronic. Dr Block has received research support, honoraria, or consultant fees from Akebia, Amgen, CARA, Davita, Keryx, and Reata. Dr Wolf has received research support, honoraria, or consultant fees from Akebia, Amag, Amgen, Ardelyx, DiaSorin, Keryx, and Shire. Dr Smith has received research support from Amgen and Sanofi and is a stockholder of Calciscon AG (Nidau, Switzerland), which commercializes the test. Dr Pasch is an inventor of the T_{50} test and a stockholder of Calciscon AG. Dr Isakova has received research support or consultant fees from Bayer, Eli Lilly, and Shire.

Acknowledgements: The authors thank the participants, investigators, and staff of the CRIC Study for their time and commitment.

Priror Presentation: Parts of this manuscript were presented in abstract form at ASN Kidney Week, San Diego, CA, October 23-29, 2018.

Peer Review: Received October 14, 2018. Evaluated by 3 external peer reviewers and a statistician, with editorial input from an Acting Editor-in-Chief (Editorial Board Member Masafumi Fukagawa, MD, PhD). Accepted in revised form January 25, 2019. The involvement of an Acting Editor-in-Chief to handle the peer-review and decision-making processes was to comply with *AJKD*'s procedures for potential conflicts of interest for editors, described in the Information for Authors & Journal Policies.

References

1. Go A, Chertow G, Fan D, Mcculloch CE, Hsu C. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004;351(13):1296-1305.
2. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet*. 2013;382(9889):339-352.
3. Kestenbaum BR, Adeney KL, De Boer IH, et al. Incidence and progression of coronary calcification in chronic kidney disease: the Multi-Ethnic Study of Atherosclerosis. *Kidney Int*. 2009;76(9):991-998.
4. Townsend RR. Arterial stiffness and chronic kidney disease: lessons from the Chronic Renal Insufficiency Cohort study. *Curr Opin Nephrol Hypertens*. 2015;24(1):47-53.
5. Chirinos JA, Khan A, Bansal N, et al. Arterial stiffness, central pressures, and incident hospitalized heart failure in the Chronic Renal Insufficiency Cohort Study. *Circ Heart Fail*. 2014;7(5):709-716.
6. Detrano R, Guerci AD, Carr JJ, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med*. 2008;358(13):1338-1345.
7. Rennenberg RJMW, Kessels AGH, Schurgers LJ, Van Engelshoven JMA, De Leeuw PW, Kroon AA. Vascular calcifications as a marker of increased cardiovascular risk: a meta-analysis. *Vasc Health Risk Manag*. 2009;5(1):185-197.
8. Budoff MJ, Hokanson JE, Nasir K, et al. Progression of coronary artery calcium predicts all-cause mortality. *JACC Cardiovasc Imaging*. 2010;3(12):1229-1236.
9. Budoff MJ, Young R, Lopez VA, et al. Progression of coronary calcium and incident coronary heart disease events: MESA (Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol*. 2013;61(12):1231-1239.
10. Budoff MJ, Rader DJ, Reilly MP, et al. Relationship of estimated GFR and coronary artery calcification in the CRIC (Chronic Renal Insufficiency Cohort) study. *Am J Kidney Dis*. 2011;58(4):519-526.
11. Kronmal RA, McClelland RL, Detrano R, et al. Risk factors for the progression of coronary artery calcification in asymptomatic subjects: results from the Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation*. 2007;115(21):2722-2730.
12. Chen J, Budoff MJ, Reilly MP, et al. Coronary artery calcification and risk of cardiovascular disease and death among patients with chronic kidney disease. *JAMA Cardiol*. 2017;2(6):635-643.
13. Pasch A, Farese S, Graber S, et al. Nanoparticle-based test measures overall propensity for calcification in serum. *J Am Soc Nephrol*. 2012;23(10):1744-1752.
14. Pasch A. Novel assessments of systemic calcification propensity. *Curr Opin Nephrol Hypertens*. 2016;25(4):278-284.
15. Smith ER, Ford ML, Tomlinson LA, et al. Serum calcification propensity predicts all-cause mortality in predialysis CKD. *J Am Soc Nephrol*. 2014;25(2):339-348.
16. Keyzer CA, de Borst MH, van den Berg E, et al. Calcification propensity and survival among renal transplant recipients. *J Am Soc Nephrol*. 2016;27(1):239-248.
17. Dahle DO, Åsberg A, Hartmann A, et al. Serum calcification propensity is a strong and independent determinant of cardiac and all-cause mortality in kidney transplant recipients. *Am J Transplant*. 2016;16(1):204-212.
18. Pasch A, Block GA, Bachtler M, et al. Blood calcification propensity, cardiovascular events, and survival in patients receiving hemodialysis in the EVOLVE trial. *Clin J Am Soc Nephrol*. 2017;12(2):315-322.
19. Lash JP, Go AS, Appel LJ, et al. Chronic Renal Insufficiency Cohort (CRIC) study: baseline characteristics and associations with kidney function. *Clin J Am Soc Nephrol*. 2009;4(8):1302-1311.
20. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M, Detrano R. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15(4):827-832.
21. Scialla JJ, Lau WL, Reilly MP, et al. Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. *Kidney Int*. 2013;83(6):1159-1168.
22. Anderson AH, Yang W, Hsu CY, et al. Estimating GFR among participants in the Chronic Renal Insufficiency Cohort (CRIC) Study. *Am J Kidney Dis*. 2012;60(2):250-261.
23. Bundy JD, Chen J, Yang W, et al. Risk factors for progression of coronary artery calcification in patients with chronic kidney disease: the CRIC study. *Atherosclerosis*. 2018;271:53-60.
24. Goodman W, Goldin J, Kuizon B, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med*. 2000;342(20):1478-1483.
25. Shroff R, Long DA, Shanahan C. Mechanistic insights into vascular calcification in CKD. *J Am Soc Nephrol*. 2013;24(2):179-189.

26. He J, Reilly M, Yang W, et al. Risk factors for coronary artery calcium among patients with chronic kidney disease (from the Chronic Renal Insufficiency Cohort Study). *Am J Cardiol*. 2012;110(12):1735-1741.
27. Lanzer P, Boehm M, Sorribas V, et al. Medial vascular calcification revisited: review and perspectives. *Eur Heart J*. 2014;35(23):1515-1525.
28. Vervloet M, Cozzolino M. Vascular calcification in chronic kidney disease: different bricks in the wall? *Kidney Int*. 2017;91(4):808-817.
29. Ix JH, Katz R, De Boer IH, et al. Fetuin-A is inversely associated with coronary artery calcification in community-living persons: the Multi-Ethnic Study of Atherosclerosis. *Clin Chem*. 2012;58(5):887-895.
30. Demer LL, Tintut Y. Vascular calcification: pathobiology of a multifaceted disease. *Circulation*. 2008;117(22):2938-2948.
31. Smith ER, Hanssen E, McMahon LP, Holt SG. Fetuin-A-containing calciprotein particles reduce mineral stress in the macrophage. *PLoS One*. 2013;8(4): e60904.
32. Paloian NJ, Giachelli CM. A current understanding of vascular calcification in CKD. *Am J Physiol Renal Physiol*. 2014;307(8):F891-F900.
33. Kutikhin AG, Velikanova EA, Mukhamadiyarov RA, et al. Apoptosis-mediated endothelial toxicity but not direct calcification or functional changes in anti-calcification proteins defines pathogenic effects of calcium phosphate bions. *Sci Rep*. 2016;6:27255.
34. Chen W, Anokhina V, Dieudonne G, et al. Patients with advanced chronic kidney disease and vascular calcification have a large hydrodynamic radius of secondary calciprotein particles. *Nephrol Dial Transplant*. 2018:gfy117. https://doi.org/10.1093/ndt/gfy117.
35. Hamano T, Matsui I, Mikami S, et al. Fetuin-mineral complex reflects extraosseous calcification stress in CKD. *J Am Soc Nephrol*. 2010;21(11):1998-2007.
36. Smith ER, Hewitson TD, Hanssen E, Holt SG. Biochemical transformation of calciprotein particles in uraemia. *Bone*. 2018;110:355-367.
37. London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant*. 2003;18(9):1731-1740.
38. Matsui I, Hamano T, Mikami S, et al. Retention of fetuin-A in renal tubular lumen protects the kidney from nephrocalcinosis in rats. *Am J Physiol Renal Physiol*. 2013;304(6):F751-F760.
39. Smith ER, Cai MM, McMahon LP, et al. Serum fetuin-A concentration and fetuin-A-containing calciprotein particles in patients with chronic inflammatory disease and renal failure. *Nephrology*. 2013;18(3):215-221.