Serum Calcification Propensity and Clinical Events in CKD

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Abstract

Background and objectives Patients with CKD are at high risk for cardiovascular disease, ESKD, and mortality. Vascular calcification is one pathway through which cardiovascular disease risks are increased. We hypothesized that a novel measure of serum calcification propensity is associated with cardiovascular disease events, ESKD, and all-cause mortality among patients with CKD stages 2–4.

Design, setting, participants, & measurements Among 3404 participants from the prospective, longitudinal Chronic Renal Insufficiency Cohort Study, we quantified calcification propensity as the transformation time (T_{50}) from primary to secondary calciprotein particles, with lower T_{50} corresponding to higher calcification propensity. We used multivariable-adjusted Cox proportional hazards regression models to assess the associations of T_{50} with risks of adjudicated atherosclerotic cardiovascular disease events (myocardial infarction, stroke, and peripheral artery disease), adjudicated heart failure, ESKD, and mortality.

Results The mean T_{50} was 313 (SD 79) minutes. Over an average 7.1 (SD 3.1) years of follow-up, we observed 571 atherosclerotic cardiovascular disease events, 633 heart failure events, 887 ESKD events, and 924 deaths. With adjustment for traditional cardiovascular disease risk factors, lower T_{50} was significantly associated with higher risk of atherosclerotic cardiovascular disease (hazard ratio [HR] per SD lower T_{50}, 1.14; 95% confidence interval [95% CI], 1.05 to 1.25), ESKD within 3 years from baseline (HR per SD lower T_{50}, 1.68; 95% CI, 1.52 to 1.86), and all-cause mortality (HR per SD lower T_{50}, 1.16; 95% CI, 1.09 to 1.24), but not heart failure (HR per SD lower T_{50}, 1.06; 95% CI, 0.97 to 1.15). After adjustment for eGFR and 24-hour urinary protein, T_{50} was not associated with risks of atherosclerotic cardiovascular disease, ESKD, and mortality.

Conclusions Among patients with CKD stages 2–4, higher serum calcification propensity is associated with atherosclerotic cardiovascular disease events, ESKD, and all-cause mortality, but this association was not independent of kidney function.

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Introduction

CKD affects more than 10% of the population globally and is strongly associated with adverse health outcomes, including cardiovascular disease, ESKD, and death (1–4). In 2017, CKD accounted for 1.23 million deaths, representing a 33.7% increase from 2007 (5). Vascular calcification is prevalent in patients with CKD and ESKD, and is one mechanism by which risks for cardiovascular disease and death are increased (6,7).

The serum calcification propensity test (T_{50}) is a novel, *in vitro* assay that quantifies the transformation time from primary to secondary calciprotein particles in serum when challenged with exogenous calcium and phosphate (8). The T_{50} test incorporates summary information about vascular calcification promoters (*e.g.*, calcium and phosphate) and inhibitors (*e.g.*, albumin, fetuin-A, magnesium, and pyrophosphate), and may signify the status of the humoral calcification-regulating system. T_{50} was associated with cardiovascular and all-cause mortality among patients with advanced CKD in several small studies (9–12). However, its associations with clinical events, including cardiovascular disease, ESKD, and mortality, among larger patient populations with earlier stages of CKD are unknown. T_{50} can be measured inexpensively and noninvasively, and quantifying its associations with these outcomes could provide insights into the effect of promoter-inhibitor imbalance on prognosis and aid in the early identification of high-risk patients.

The Chronic Renal Insufficiency Cohort (CRIC) Study provides the opportunity to quantify the associations of the T_{50} test with clinical events among a large, diverse sample of adults with CKD stages 2–4. We hypothesized that lower T_{50} (*i.e.*, higher

calcification propensity) is associated with higher risks of atherosclerotic cardiovascular disease, heart failure, ESKD, and mortality.

Materials and Methods

Study Design and Participants

The CRIC study is a prospective, longitudinal cohort study of participants with mild-to-moderate CKD on the basis of an eGFR entry criteria of 20–70 ml/min per 1.73 m^2. A racially and ethnically diverse group of 3939 men and women aged 21–74 years was enrolled from seven clinical centers in the United States (Ann Arbor, MI; Baltimore, MD; Chicago, IL; Cleveland, OH; New Orleans, LA; Philadelphia, PA; and San Francisco, CA) in phase 1, between May 2003 and August 2008 (13). Patients with severe heart failure, cirrhosis, HIV infection, polycystic kidney disease, renal cell carcinoma, those receiving maintenance dialysis or an organ transplant, and those taking immunosuppressive medications were excluded from the CRIC study. The study was approved by the institutional review board at each institution and all participants provided written informed consent.

Exposure Assessment

T_{50} was measured among 3404 participants with stored samples from the first annual follow-up visit (*i.e.*, baseline for the current analysis) and 96% of participants reported fasting. We quantified calcification propensity as the transformation time (T_{50}), in minutes, from primary to secondary calciprotein particles *in vitro*, with lower T_{50} corresponding to higher calcification propensity (8). The T_{50} test was performed using a Nephelostar nephelometer at the Calciscon Laboratory in Switzerland, using serum samples stored at −80°C and shipped with sufficient dry ice. The mean intraassay and interassay coefficients of variation are 2.2% and 3.4%, respectively. T_{50} values have been reported in other populations, including 184 patients with CKD stages 3–4 (mean, 329 [SD 95] minutes) (14) and 2785 patients undergoing hemodialysis (median, 212 [10th–90th percentiles, 109–328] minutes) (11).

Outcome Assessment

The outcomes for this analysis are time to (*1*) a composite of atherosclerotic cardiovascular disease events (myocardial infarction, stroke, or peripheral artery disease events) (15), (*2*) heart failure, (*3*) all-cause and cause-specific mortality, and (*4*) ESKD. Cardiovascular disease events are reported every 6 months and confirmed by medical record adjudication as possible, probable, or definite events (15). All deaths were confirmed by death certificate. We used a superlearning algorithm to determine if a death was cardiovascular-related on the basis of causes of death listed on the death certificate, using the 233 adjudicated cardiovascular-related deaths as a gold standard. ESKD was defined as the receipt of dialysis or kidney transplantation ascertained every 6 months and confirmed by medical record review, supplemented by information from the US Renal Data System. Follow-up time was censored at the earliest occurrence of death, loss to follow-up (n=176; 5%), or end of the follow-up period through September 2015, for a maximum duration of 11.2 years.

Covariate Assessment

Sociodemographic characteristics, medical history, and medication use were obtained *via* self-reported questionnaire. Body weight, height, and BP were measured using standard protocols (13). We defined diabetes as fasting glucose ≥126 mg/dl, nonfasting glucose ≥200 mg/dl, and/or the use of antidiabetic medications. We defined history of cardiovascular disease as self-reported prior coronary artery disease, heart failure, stroke, or peripheral artery disease. Glucose, cholesterol, phosphate, calcium, bicarbonate, magnesium, and serum albumin were measured using standard laboratory methods. Twenty-four hour urinary protein was measured using the turbidimetric method, with benzethonium chloride. Total parathyroid hormone (PTH) was measured using the total PTH assay, which includes the 1–84 PTH molecule and 7–84 fragments assay (Scantibodies, Santee, CA). Fibroblast growth factor-23 (FGF23) was measured by a second-generation C-terminal assay (Immutopics). High-sensitivity C-reactive protein (hsCRP) and IL-6 were measured at the original baseline examination using the particle enhanced immunonephelometry method. Fetuin-A concentration was measured at the original baseline examination using the quantitative sandwich enzyme immunoassay technique. We calculated eGFR using the equation derived in the CRIC cohort (16). We obtained covariate data from the same study visit as T_{50} or, if missing, the original baseline visit 1 year prior (<5% missing for all covariates except 24-hour urinary protein [10%]).

Statistical Analyses

We summarized the baseline characteristics of the study participants, stratified by quartiles of T_{50}, as the mean±SD or median (25th–75th percentile) for continuous variables or the number (percentage) for categorical variables. We transformed variables with skewed distributions by taking the natural log of their raw values. We visually assessed departures from linearity in the associations of T_{50} with events using plots of Martingale residuals and, given no apparent departures, retained a linear functional form for T_{50}. Kaplan–Meier plots were used to characterize the unadjusted associations of T_{50} with events.

Cox proportional hazards regression models, stratified by study site, were used to estimate the multivariable-adjusted associations of T_{50} with time to events. Covariates included in regression models were selected on the basis of prior clinical knowledge. In addition to unadjusted analyses, we used three sequentially adjusted models: (*1*) adjusted for age, sex, and race/ethnicity; (*2*) adjusted for variables in model 1 plus history of cardiovascular disease and traditional cardiovascular disease risk factors (history of diabetes, systolic BP, use of antihypertensive medications, total cholesterol, HDL cholesterol, and current smoking) (17); and (*3*) adjusted for variables in model 2 plus eGFR and 24-hour urinary protein. We tested for effect modification by baseline age, sex, race/ethnicity, diabetes, and eGFR, by including interaction terms with T_{50} in the models. Additionally, we used linear regression to assess the strength of multivariable-adjusted associations of individual variables in model 3 with T_{50}.

We tested the proportional hazards assumption visually using plots of Schoenfeld residuals and statistically using

Table 1. Baseline characteristics of 3404 CRIC participants by quartiles of T_{50}

Variables	Quartiles of T_{50}, min			
	Quartile 4 (*n*=850), T_{50}=364–600 min	Quartile 3 (*n*=849), T_{50}=318–363 min	Quartile 2 (*n*=854), T_{50}=264–317 min	Quartile 1 (*n*=851), T_{50}=72–263 min
Age, yr	59±11	59±11	59±11	59±11
Women	351 (41)	371 (44)	411 (48)	397 (47)
Race/ethnicity				
Non-Hispanic White	466 (55)	382 (45)	350 (41)	265 (31)
Non-Hispanic Black	274 (32)	342 (40)	368 (43)	424 (50)
Hispanic	62 (7)	93 (11)	97 (11)	144 (17)
Other	48 (6)	32 (4)	39 (5)	18 (2)
Body mass index, kg/m^2	31±7	32±8	33±8	32±8
Current cigarette smoking	84 (10)	90 (11)	103 (12)	134 (16)
History of cardiovascular disease	266 (31)	305 (36)	289 (34)	359 (42)
Diabetes mellitus	349 (41)	388 (46)	436 (51)	511 (60)
Systolic BP, mm Hg	124±20	126±21	127±22	130±23
Antihypertensive medication use	761 (90)	787 (93)	782 (92)	804 (94)
Total cholesterol, mg/dl	186±41	180±43	183±46	179±46
HDL cholesterol, mg/dl	49±15	48±15	49±17	48±16
LDL cholesterol, mg/dl	104±35	98±33	99±35	98±36
Statin medication use	466 (55)	505 (59)	511 (60)	531 (62)
Warfarin medication use	47 (6)	54 (6)	56 (7)	45 (5)
eGFR, ml/min per 1.73 m^2	48±16	43±16	42±17	36±17
Urinary protein, g/24 h	0.1 [0.1–0.5]	0.2 [0.1–0.8]	0.2 [0.1–1.0]	0.3 [0.1–1.7]
Bicarbonate, mmol/L	26±3	25±3	24±3	23±4
Active vitamin D medication use	30 (4)	53 (6)	57 (7)	90 (11)
Phosphate binder medication use	50 (6)	65 (8)	67 (8)	94 (11)
Calciferol medication use	113 (13)	96 (11)	104 (12)	103 (12)
Calcium, mg/dl	9.4±0.5	9.3±0.5	9.3±0.5	9.2±0.6
Phosphate, mg/dl	3.6±1.0	3.8±1.1	3.8±0.8	4.2±1.1
Magnesium, mg/dl	2.0±0.3	2.0±0.3	1.9±0.3	1.9±0.3
Serum albumin, g/dl	4.2±0.4	4.1±0.4	4.0±0.4	3.9±0.5
Fetuin-A, mg/L	579±110	538±105	519±102	487±108
Fibroblast growth factor-23, RU/ml	124 [82–208]	146 [93–264]	150 [97–283]	197 [113–392]
Parathyroid hormone, pg/ml	55 [39–85]	62 [39–94]	61 [40–95]	74 [44–135]
IL-6, pg/ml	1.5 [0.9–2.6]	1.8 [1.1–2.9]	1.9 [1.3–3.0]	2.2 [1.3–3.7]
High-sensitivity C-reactive protein, mg/L	2.0 [0.9–5.0]	2.4 [0.9–5.9]	2.8 [1.2–6.7]	2.7 [1.1–7.7]

Values are presented as mean±SD, median [interquartile range], or *n* (%).

time-by-variable interaction terms in the models. The proportional hazards assumption was met for all variables in analyses of atherosclerotic cardiovascular disease, heart failure, and mortality. However, we detected violations of the assumption in analyses of ESKD for T_{50} and eGFR. Thus, as recommended by the CRIC study (18), we included a time interaction in the Cox models, reporting associations of T_{50} with early- (0 to <3 years) and late-onset (≥3 years) ESKD, time periods in which hazards were proportional across quartiles of T_{50}.

We additionally conducted two supplementary analyses: the first excluding those with self-reported history of cardiovascular disease from analyses of atherosclerotic cardiovascular disease and heart failure, and the second assessing the effect of additional adjustment for variables potentially affecting T_{50} (calcium, phosphate, bicarbonate, magnesium, serum albumin, fetuin-A, FGF23, PTH, and use of medications, including warfarin, active vitamin D, phosphate binders, and calciferols) and inflammatory markers (hsCRP and IL-6). Analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC) and R version 3.4.2 (R Project for Statistical Computing). Two-sided *P* values <0.05 were considered statistically significant for all analyses.

Results

Among 3404 participants included in the analyses, the mean age was 59 (SD 11) years, 45% were women, 49% had diabetes, 36% had a history of cardiovascular disease, and the mean eGFR was 42 (SD 17) ml/min per 1.73 m^2. The mean T_{50} was 313 (SD 79) minutes. Participants with lower T_{50} were more likely to be women, non-Hispanic black, and current smokers; have a history of cardiovascular disease and diabetes; and be taking antihypertensive, statin, active vitamin D, and phosphate

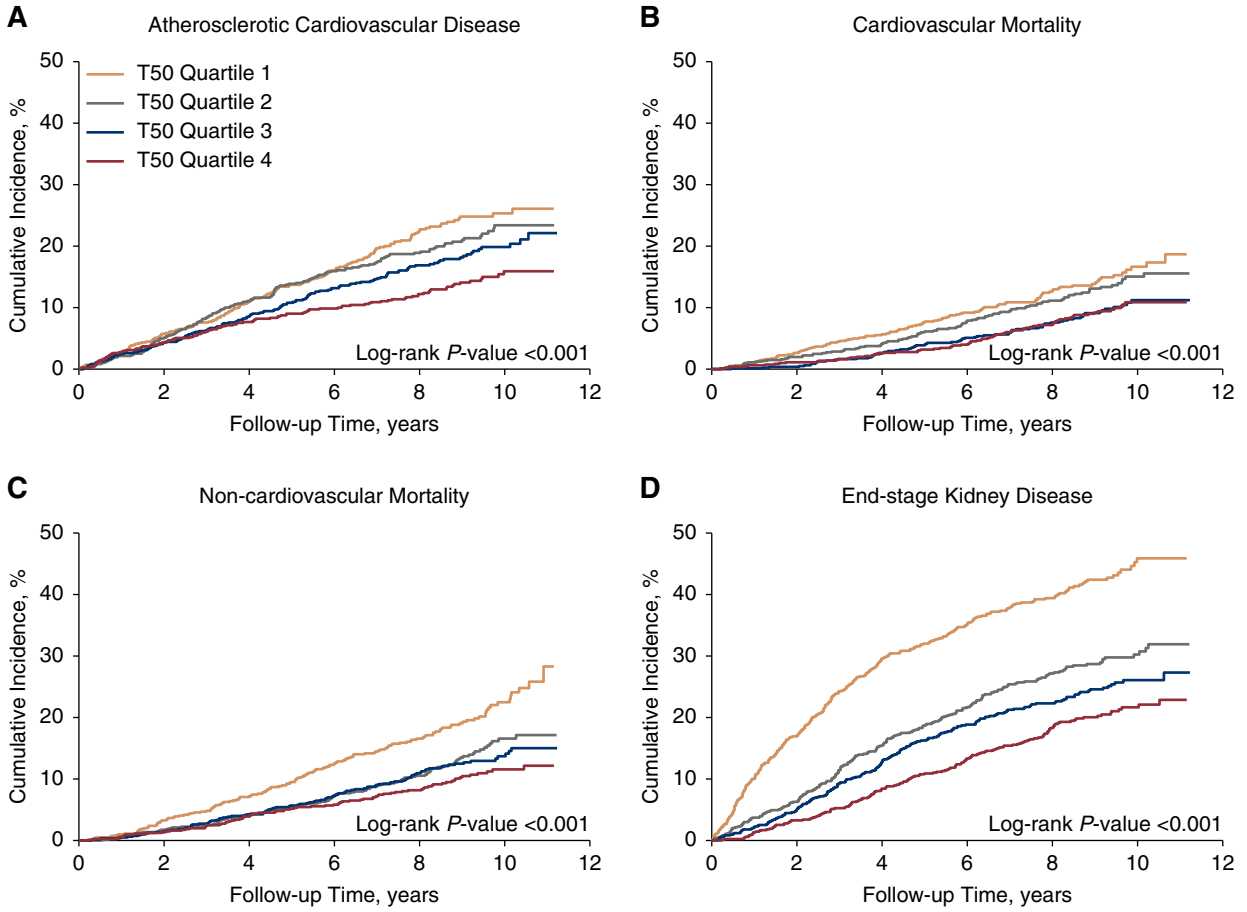

Figure 1. | Lower T_{50} is associated with higher cumulative incidence of atherosclerotic cardiovascular disease, cardiovascular mortality, noncardiovascular mortality, and ESKD. (A) Cumulative incidence of atherosclerotic cardiovascular disease. (B) Cumulative incidence of cardiovascular mortality. (C) Cumulative incidence of noncardiovascular mortality. (D) Cumulative incidence of ESKD.

binder medications (Table 1). On average, participants with lower T_{50} had higher systolic BP, 24-hour urinary protein, phosphate, FGF23, PTH, hsCRP, and IL-6; and lower eGFR, bicarbonate, calcium, magnesium, serum albumin, and fetuin-A. T_{50} was significantly correlated with eGFR (rho=0.26; Supplemental Figure 1). After multivariable adjustment, eGFR was the variable most strongly associated with T_{50} (Supplemental Table 1). In unadjusted Kaplan–Meier analyses, lower T_{50} was significantly associated with higher risk of all events, particularly atherosclerotic cardiovascular disease, cardiovascular mortality, noncardiovascular mortality, and ESKD (Figure 1).

Table 2 shows the numbers of events and hazard ratios (HRs) of atherosclerotic cardiovascular disease and heart failure associated with T_{50} quartiles and per one SD lower T_{50}. During 24,022 person-years of follow-up for cardiovascular disease events, we documented 571 atherosclerotic cardiovascular disease (312 myocardial infarction, 120 stroke, and 139 peripheral artery disease) events (23.8 per 1000 person-years) and 633 heart failure events (26.6 per 1000 person-years). T_{50} was significantly associated with both outcomes after adjustment for age, sex, and race/ethnicity. After additional adjustment for traditional cardiovascular disease risk factors, T_{50} remained associated with higher risk of atherosclerotic cardiovascular disease (HR per SD lower T_{50}, 1.14; 95% confidence interval [95% CI], 1.05 to 1.25), but not heart failure (HR per SD lower T_{50}, 1.06; 95% CI, 0.97 to 1.15). Further adjustment for eGFR and proteinuria attenuated the association between T_{50} and atherosclerotic cardiovascular disease, and the association was no longer significant (HR per SD lower T_{50}, 1.07; 95% CI, 0.98 to 1.17). In a supplemental analysis excluding those with a self-reported history of cardiovascular disease, point estimates were similar to the main analyses, although 95% CIs were wider (Supplemental Table 2).

Table 3 shows the numbers of events and HRs of all-cause, cardiovascular, and noncardiovascular mortality associated with T_{50}. During 27,188 person-years of follow-up for mortality, we documented 924 all-cause deaths (34.0 per 1000 person-years), 349 of which were from cardiovascular causes and 433 from noncardiovascular causes; cause of death could not be determined in 142 cases. T_{50} was significantly associated with higher risk of all-cause mortality after adjustment for traditional cardiovascular disease risk factors (HR per SD lower T_{50}, 1.16; 95% CI, 1.09 to 1.24), but this association was no longer significant after adjustment for eGFR and proteinuria (HR per SD lower T_{50}, 1.05; 95% CI, 0.98 to 1.12). Only the association between

Table 2. Associations of T_{50} with atherosclerotic cardiovascular disease and heart failure events

Cardiovascular Disease Outcomes	Quartiles of T_{50}, min				Per 1-SD Lower T_{50} (79 min)	*P* Value
	Quartile 4 (≥364 min)	Quartile 3 (318–363 min)	Quartile 2 (264–317 min)	Quartile 1 (≤263 min)		
Atherosclerotic cardiovascular disease (*n*=3397)						
Events/total number	107/847	142/847	155/852	167/851		
Hazard ratio (95% CI)						
Unadjusted	Reference	1.34 (1.05 to 1.73)	1.56 (1.22 to 2.00)	1.76 (1.38 to 2.24)	1.25 (1.15 to 1.36)	<0.001
Model 1[a]	Reference	1.30 (1.01 to 1.68)	1.53 (1.19 to 1.96)	1.65 (1.28 to 2.11)	1.23 (1.13 to 1.35)	<0.001
Model 2[b]	Reference	1.28 (0.99 to 1.65)	1.42 (1.10 to 1.82)	1.34 (1.04 to 1.73)	1.14 (1.05 to 1.25)	0.002
Model 3[c]	Reference	1.22 (0.94 to 1.57)	1.30 (1.01 to 1.68)	1.14 (0.88 to 1.48)	1.07 (0.98 to 1.17)	0.14
Congestive heart failure (*n*=3396)						
Events/total number	132/ 847	142/ 847	166/ 852	193/ 850		
Hazard ratio (95% CI)						
Unadjusted	Reference	1.07 (0.84 to 1.35)	1.32 (1.05 to 1.65)	1.66 (1.33 to 2.07)	1.21 (1.12 to 1.31)	<0.001
Model 1[a]	Reference	0.97 (0.76 to 1.23)	1.17 (0.93 to 1.47)	1.39 (1.10 to 1.74)	1.14 (1.05 to 1.24)	0.002
Model 2[b]	Reference	0.91 (0.71 to 1.15)	1.06 (0.84 to 1.34)	1.12 (0.89 to 1.41)	1.06 (0.97 to 1.15)	0.18
Model 3[c]	Reference	0.83 (0.65 to 1.06)	0.95 (0.75 to 1.20)	0.85 (0.67 to 1.07)	0.95 (0.88 to 1.03)	0.25

95% CI, 95% confidence interval.
[a]Model 1: adjusted for age, sex, and race/ethnicity.
[b]Model 2: adjusted for variables in model 1 plus history of cardiovascular disease, history of diabetes, systolic BP, use of antihypertensive medications, total cholesterol, HDL cholesterol, and current smoking.
[c]Model 3: adjusted for variables in model 2 plus eGFR and 24-hour urinary protein.

T_{50} and noncardiovascular mortality remained statistically significant after adjustment for eGFR and proteinuria (HR per SD lower T_{50}, 1.12; 95% CI, 1.01 to 1.24).

Table 4 shows the numbers of events and HRs of ESKD associated with T_{50}. During 23,146 person-years of follow-up for ESKD, we documented 887 ESKD events (38.3 per 1000 person-years), 405 of which occurred during the first 3 years of follow-up. After adjustment for traditional cardiovascular disease risk factors, T_{50} was significantly associated with ESKD, although more strongly in the early time period (HR per SD lower T_{50}, 1.68; 95% CI, 1.52 to 1.86) versus the late time period (HR per SD lower T_{50}, 1.14; 95% CI, 1.04 to 1.26). The associations between T_{50} and ESKD were attenuated and no longer significant after adjustment for eGFR and proteinuria.

We did not detect effect modification of T_{50} by age, sex, race/ethnicity, diabetes, or baseline eGFR for any of the outcomes. In secondary analyses with additional adjustment for variables potentially affecting T_{50} (model 4) and inflammation variables (model 5), results were similar to the main analyses (model 3; Supplemental Table 3). However, the statistically significant association of T_{50} with noncardiovascular mortality observed in model 3 was attenuated after adjustment for either set of variables, and was no longer significant.

Discussion

In this analysis of 3404 participants with CKD stages 2–4, higher serum calcification propensity, denoted by lower T_{50}, was significantly associated with higher risks of atherosclerotic cardiovascular disease, all-cause mortality, and ESKD, independent of traditional cardiovascular disease risk factors. However, the associations between T_{50} and clinical events were attenuated and no longer statistically significant after further adjustment for eGFR and proteinuria. Although T_{50} and its components may be associated with the risk for adverse health outcomes among patients with CKD, modest magnitudes of association in multivariable-adjusted models do not overcome a strong correlation between T_{50} and eGFR. Still, T_{50} and its determinants, namely calcification promoters and inhibitors, may warrant further research as potential therapeutic targets.

Previous research on the associations of T_{50} with clinical events has mostly been conducted among patients with advanced CKD (9–12). In three separate analyses of kidney transplant recipients (*n*=699, *n*=1435, and *n*=685), lower T_{50} was associated with higher risks of all-cause and cardiovascular mortality (9,10), and cardiovascular disease events (12), independent of eGFR. T_{50} was similarly associated with all-cause mortality, myocardial infarction, and peripheral artery disease among 2785 patients undergoing hemodialysis from the Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events (EVOLVE) trial (11). In our analysis including patients with CKD stages 2–4, T_{50} was not associated with most outcomes after adjustment for eGFR and proteinuria, except for noncardiovascular mortality, although this association is likely due to residual confounding, as it disappeared with additional adjustment for either inflammation or mineral metabolism variables. Conversely, Smith *et al.* (14) observed significant associations with all-cause mortality in 184 patients with CKD stages 3–4, even after adjustment for eGFR and proteinuria. There are several possible explanations for the divergent findings in our study. The CRIC study included a population that was younger and, on average, healthier with respect to baseline characteristics. Additionally, it is

Table 3. Associations of T_{50} with all-cause, cardiovascular, and noncardiovascular mortality

Mortality Outcomes	Quartiles of T_{50}, min				Per 1-SD Lower T_{50} (79 min)	*P* Value
	Quartile 4 (≥364 min)	Quartile 3 (318–363 min)	Quartile 2 (264–317 min)	Quartile 1 (≤263 min)		
All-cause mortality (*n*=3404)						
Events/total number	185/850	204/849	240/854	295/851		
Hazard ratio (95% CI)						
Unadjusted	Reference	1.13 (0.93 to 1.38)	1.38 (1.14 to 1.67)	1.83 (1.52 to 2.20)	1.27 (1.19 to 1.36)	<0.001
Model 1[a]	Reference	1.07 (0.87 to 1.30)	1.30 (1.07 to 1.58)	1.63 (1.34 to 1.97)	1.23 (1.15 to 1.32)	<0.001
Model 2[b]	Reference	1.04 (0.85 to 1.27)	1.22 (1.00 to 1.48)	1.40 (1.15 to 1.69)	1.16 (1.09 to 1.24)	<0.001
Model 3[c]	Reference	0.96 (0.79 to 1.18)	1.13 (0.93 to 1.37)	1.09 (0.89 to 1.32)	1.05 (0.98 to 1.12)	0.17
Cardiovascular mortality (*n*=3262)						
Events/total number	72/818	71/815	100/818	106/811		
Hazard ratio (95% CI)						
Unadjusted	Reference	1.01 (0.73 to 1.40)	1.48 (1.09 to 2.00)	1.70 (1.26 to 2.29)	1.24 (1.12 to 1.38)	<0.001
Model 1[a]	Reference	0.98 (0.70 to 1.36)	1.46 (1.08 to 1.99)	1.59 (1.16 to 2.17)	1.22 (1.09 to 1.36)	<0.001
Model 2[b]	Reference	0.94 (0.67 to 1.31)	1.34 (0.98 to 1.83)	1.30 (0.95 to 1.78)	1.13 (1.01 to 1.26)	0.03
Model 3[c]	Reference	0.85 (0.61 to 1.18)	1.22 (0.89 to 1.67)	0.99 (0.72 to 1.36)	1.01 (0.91 to 1.13)	0.81
Noncardiovascular mortality (*n*=3262)						
Events/total number	81/818	99/815	104/818	149/811		
Hazard ratio (95% CI)						
Unadjusted	Reference	1.25 (0.93 to 1.68)	1.37 (1.02 to 1.83)	2.12 (1.62 to 2.78)	1.33 (1.21 to 1.46)	<0.001
Model 1[a]	Reference	1.15 (0.86 to 1.54)	1.26 (0.94 to 1.69)	1.89 (1.43 to 2.50)	1.30 (1.17 to 1.43)	<0.001
Model 2[b]	Reference	1.15 (0.85 to 1.54)	1.19 (0.88 to 1.60)	1.67 (1.26 to 2.21)	1.23 (1.12 to 1.36)	<0.001
Model 3[c]	Reference	1.04 (0.77 to 1.41)	1.09 (0.81 to 1.47)	1.30 (0.97 to 1.74)	1.12 (1.01 to 1.24)	0.03

95% CI, 95% confidence interval.
[a]Model 1: adjusted for age, sex, and race/ethnicity.
[b]Model 2: adjusted for variables in model 1 plus history of cardiovascular disease, history of diabetes, systolic BP, use of antihypertensive medications, total cholesterol, HDL cholesterol, and current smoking.
[c]Model 3: adjusted for variables in model 2 plus eGFR and 24-hour urinary protein.

possible associations of T_{50} with outcomes differ depending on baseline kidney function, although we did not detect effect modification by eGFR. Research in broader patient populations is warranted to further investigate the role of T_{50}, including among the general population.

In our study, T_{50} did not provide risk information independent of eGFR and proteinuria. This finding may be expected, given that eGFR was the strongest correlate of T_{50} in our primary models. We acknowledge the possibility that associations between T_{50} and events are confounded by kidney function, although alternate explanations are also biologically plausible. As a comprehensive, functional measure, T_{50} may contain unique, but ultimately overlapping, information compared with eGFR (19). Furthermore, disordered promoter-inhibitor balance may be an intermediate on the pathway from reduced kidney function to clinical events. Alternatively, it is possible that a lower T_{50} could precede further kidney function decline, which should be explored further in longitudinal analyses and in other patient populations. Still, the magnitudes of association were modest and advocating for the use of T_{50} in risk prediction may be premature. However, the components of disordered calcification promoter-inhibitor homeostasis, as integrated by the T_{50} test, may be actionable therapeutic targets. In two randomized, controlled trials of 34 patients with CKD stages 3–4 and 57 patients undergoing dialysis, magnesium supplementation increased T_{50} (20,21). Despite these favorable results, it is unknown whether improvements in T_{50} translate to decreased risks of cardiovascular disease, ESKD, or mortality.

Despite the attenuation of associations of T_{50} with clinical events after adjustment for eGFR and proteinuria, our results suggest T_{50} is associated with an atherosclerotic cardiovascular disease pathway. After adjustment for traditional cardiovascular disease risk factors (17), one SD lower T_{50} was associated with a 14% (95% CI, 5% to 25%) higher risk of atherosclerotic cardiovascular disease. Conversely, T_{50} was not associated with the risk of heart failure after adjustment for these risk factors. Our findings complement results from the EVOLVE trial, which showed significant associations of T_{50} with cardiovascular disease, particularly myocardial infarction, but not heart failure (11).

Atherosclerosis, commonly observed as calcification of the vessel intima, is a complex pathophysiologic phenomenon characterized by lipid deposition, endothelial dysfunction, macrophage accumulation, and inflammation (22), and is linked with higher risks of myocardial infarction and peripheral artery disease. Calcification of the vessel media shares some similar pathophysiologic mechanisms (23), but is a distinct phenomenon leading primarily to arterial stiffness (24) and heart failure (25). It is plausible that the calcification promoter-inhibitor balance information captured by the T_{50} test could reflect either type of calcification, but our results imply a stronger association with intimal, atherosclerotic calcification. Intimal calcification

Table 4. Association of T_{50} with ESKD by follow-up time

ESKD Outcomes	Quartiles of T_{50}, min				Per 1-SD Lower T_{50} (79 min)	*P* Value
	Quartile 4 (≥364 min)	Quartile 3 (318–363 min)	Quartile 2 (264–317 min)	Quartile 1 (≤263 min)		
All follow-up (*n*=3345)						
Events/total number	157/843	190/837	224/840	316/825		
Years 0 to <3, hazard ratio (95% CI)						
Unadjusted	Reference	1.80 (1.24 to 2.62)	2.25 (1.56 to 3.22)	5.28 (3.79 to 7.34)	1.89 (1.71 to 2.08)	<0.001
Model 1[a]	Reference	1.61 (1.10 to 2.34)	1.97 (1.37 to 2.83)	4.30 (3.07 to 6.02)	1.76 (1.59 to 1.95)	<0.001
Model 2[b]	Reference	1.54 (1.06 to 2.24)	1.78 (1.24 to 2.57)	3.72 (2.65 to 5.21)	1.68 (1.52 to 1.86)	<0.001
Model 3[c]	Reference	1.08 (0.74 to 1.58)	0.90 (0.62 to 1.31)	1.12 (0.78 to 1.60)	1.05 (0.94 to 1.17)	0.43
Years 3–12, hazard ratio (95% CI)						
Unadjusted	Reference	1.08 (0.84 to 1.41)	1.31 (1.02 to 1.69)	1.63 (1.26 to 2.10)	1.25 (1.13 to 1.37)	<0.001
Model 1[a]	Reference	1.00 (0.77 to 1.30)	1.17 (0.91 to 1.51)	1.36 (1.05 to 1.77)	1.17 (1.06 to 1.29)	0.001
Model 2[b]	Reference	1.00 (0.77 to 1.30)	1.09 (0.84 to 1.40)	1.29 (0.99 to 1.68)	1.14 (1.04 to 1.26)	0.008
Model 3[c]	Reference	0.88 (0.67 to 1.14)	0.79 (0.60 to 1.02)	0.78 (0.60 to 1.03)	0.93 (0.85 to 1.03)	0.17

95% CI, 95% confidence interval.
[a]Model 1: adjusted for age, sex, and race/ethnicity.
[b]Model 2: adjusted for variables in model 1 plus history of cardiovascular disease, history of diabetes, systolic BP, use of antihypertensive medications, total cholesterol, HDL cholesterol, and current smoking.
[c]Model 3: adjusted for variables in model 2 plus eGFR and 24-hour urinary protein.

is a continuum characterized by initial, unstable microcalcifications, with progression to more stable, dense macrocalcifications (26). The T_{50} test reflects maturation of calciprotein particles, which may directly influence these processes (27–29). We previously reported that lower T_{50} was associated with greater coronary artery calcification severity and progression, but only among those with established calcification (30). Given that T_{50} was not associated with atherosclerotic cardiovascular disease independent of eGFR, it is possible T_{50} provides a readout of progressive, stable intimal macrocalcification, as detected by coronary computed tomography. Progressive macrocalcification is also associated with declining kidney function (31), which may explain the observed attenuation of the association between T_{50} and atherosclerotic cardiovascular disease after adjustment for eGFR. Although it is impossible to substantiate our speculation in this study, further mechanistic and translational research is warranted to explore the role of T_{50} in different types of calcification and cardiovascular disease pathways.

The association between T_{50} and ESKD was complex in our study, characterized by a large magnitude of association for early (<3 years) onset of ESKD and modest, or no, association for later (≥3 years) onset of ESKD. Although associations of T_{50} with ESKD in both time periods were attenuated after adjustment for baseline eGFR and proteinuria, lower T_{50} may be a marker of more severe disease already in progress. Those in the lowest quartile of T_{50} were at very high risk of ESKD compared with those in the highest quartile of T_{50}, and progressed to ESKD much earlier, as illustrated by the Kaplan–Meier analysis. These findings may be expected, considering the strong association between T_{50} and eGFR observed in our study and previous studies (14,32). Lower values of T_{50} indicate impaired calcification promoter-inhibitor homeostasis and calcium-phosphate metabolism, which are associated with progression of CKD (33). Furthermore, impaired calcium-phosphate metabolism strongly predicts the risk of ESKD, as shown in a previous risk prediction analysis (34). Similarly to the analyses of other outcomes, the role of T_{50} in the progression of CKD warrants further exploration to determine if improvement of calcification promoter-inhibitor balance can prevent ESKD.

There are several strengths in this analysis. First, the CRIC study uses rigorous quality control and assurance across clinical sites, which minimizes bias. Additionally, clinical outcomes are adjudicated. Second, this analysis represents the largest sample size to date evaluating the associations of T_{50} with clinical events among patients with CKD stages 2–4. Third, we were able to include many covariates to evaluate the associations of T_{50}, accounting for traditional cardiovascular disease risk factors and variables potentially related to T_{50}, including mineral metabolism and inflammation biomarkers. However, there are several limitations. First, the T_{50} test is conducted *in vitro*, which results in synthetic calciprotein particles. Still, there are data to suggest synthetic particles are physiologically similar to particles *in vivo*, thus T_{50} may be a reasonable indicator of the calciprotein particle transformation process (35). Second, although several potential confounders of the associations between T_{50} and outcomes were included in our analyses, we were unable to evaluate other potentially important variables, including vitamin K and pH. Third, we had only one baseline T_{50} measurement per participant and limited follow-up data did not allow us to assess associations of longitudinal T_{50} trends with outcomes in this sample.

In conclusion, higher serum calcification propensity, denoted by lower T_{50}, was associated with atherosclerotic cardiovascular disease, ESKD, and mortality, independent of traditional cardiovascular disease risk factors. However, these associations were attenuated and were no longer

significant after adjustment for eGFR and proteinuria. Future studies should evaluate T_{50} in other patient populations and investigate therapeutic interventions to improve calcification promoter-inhibitor homeostasis to prevent associated morbidity and mortality.

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Supplemental Material

This article contains the following supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.04710419/-/DCSupplemental.

Supplemental Table 1. Multivariable-adjusted, cross-sectional associations of baseline characteristics with T_{50}.

Supplemental Table 2. Associations of T_{50} with atherosclerotic cardiovascular disease and heart failure events in CRIC study participants without self-reported history of cardiovascular disease.

Supplemental Table 3. Effect of adjustment for additional variables on associations of T_{50} with clinical events.

Supplemental Figure 1. Scatterplot and correlation coefficient of eGFR and T_{50}.

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