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ORIGINAL RESEARCH

Vitamin D Metabolites and Risk of Cardiovascular Disease in Chronic Kidney Disease: The CRIC Study

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BACKGROUND: The ratio of 24,25-dihydroxyvitamin D_3/25-hydroxyvitamin D_3 (vitamin D metabolite ratio [VDMR]) may reflect functional vitamin D activity. We examined associations of the VDMR, 25-hydroxyvitamin D (25[OH]D), and 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) with cardiovascular disease (CVD) in patients with chronic kidney disease.

METHODS AND RESULTS: This study included longitudinal and cross-sectional analyses of 1786 participants from the CRIC (Chronic Renal Insufficiency Cohort) Study. Serum 24,25-dihydroxyvitamin D_3, 25(OH)D, and $1,25(OH)_2D$ were measured by liquid chromatography–tandem mass spectrometry 1 year after enrollment. The primary outcome was composite CVD (heart failure, myocardial infarction, stroke, and peripheral arterial disease). We used Cox regression with regression-calibrated weights to test associations of the VDMR, 25(OH)D, and $1,25(OH)_2D$ with incident CVD. We examined cross-sectional associations of these metabolites with left ventricular mass index using linear regression. Analytic models adjusted for demographics, comorbidity, medications, estimated glomerular filtration rate, and proteinuria. The cohort was 42% non-Hispanic White race and ethnicity, 42% non-Hispanic Black race and ethnicity, and 12% Hispanic ethnicity. Mean age was 59 years, and 43% were women. Among 1066 participants without prevalent CVD, there were 298 composite first CVD events over a mean follow-up of 8.6 years. Lower VDMR and $1,25(OH)_2D$ were associated with incident CVD before, but not after, adjustment for estimated glomerular filtration rate and proteinuria (hazard ratio, 1.11 per 1 SD lower VDMR [95% CI, 0.95–1.31]). Only 25(OH)D was associated with left ventricular mass index after full covariate adjustment (0.6 $g/m^{2.7}$ per 10 ng/mL lower [95% CI, 0.0–1.3]).

CONCLUSIONS: Despite modest associations of 25(OH)D with left ventricular mass index, 25(OH)D, the VDMR, and $1,25(OH)_2D$ were not associated with incident CVD in chronic kidney disease.

Key Words: cardiovascular disease ■ chronic kidney disease ■ vitamin D

Vitamin D is an essential nutrient for human health. Beyond its role in calcium and phosphate homeostasis, vitamin D may also maintain cardiovascular health through effects including the regulation of cardiac myocyte hypertrophy, secretion of natriuretic peptides, and renin-angiotensin system signaling.[1] Impaired vitamin D metabolism is ubiquitous in chronic kidney disease (CKD) and routinely assessed and treated in this population. Given the well-described cardiovascular pleiotropic effects of vitamin D, abnormalities of vitamin D metabolism in CKD may contribute to the development of cardiovascular disease (CVD), the prevailing cause of death

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CLINICAL PERSPECTIVE

What Is New?

- In a cohort of 1786 adults with chronic kidney disease, serum concentrations of 25-hydroxyvitamin D and 1,25-dihdroxyvitamin D and the ratio of 24,25-dihydroxyvitamin D_3/25-hydroxyvitamin D_3 were not associated with risk of cardiovascular disease.

What Are the Clinical Implications?

- The traditional and functional biomarkers of vitamin D status tested in this study do not reflect the markedly elevated risk of cardiovascular disease experienced by patients with chronic kidney disease.
- Testing and treatment to these biomarker targets in the population with chronic kidney disease to assess and manage cardiovascular disease risk are currently not well supported.

Nonstandard Abbreviations and Acronyms

CRIC	Chronic Renal Insufficiency Cohort
FGF-23	fibroblast growth factor-23
LVMI	left ventricular mass index
1,25(OH)$_2$D	1,25-dihydroxyvitamin D
PTH	parathyroid hormone
25(OH)D	25-hydroxyvitamin D
24,25(OH)$_2$D	24,25-dihydroxyvitamin D
VDBP	vitamin D binding protein
VDMR	vitamin D metabolite ratio

in CKD.[2–5] The 2 biomarkers most widely used to assess vitamin D status in CKD are circulating concentrations of 25-hydroxyvitamin D (25[OH]D) and parathyroid hormone (PTH); however, both are imperfect measures to guide vitamin D treatment.[6–8] 25(OH)D is a relatively inactive metabolite of vitamin D and correlates inconsistently with biological responses of vitamin D receptor activation by 1,25-dihydroxyvitamin D (1,25[OH]$_2$D, the biologically active vitamin D metabolite).[9,10] PTH reflects vitamin D activity at only one of many target organs, and its secretion is influenced by additional factors, including serum calcium, phosphate, and fibroblast growth factor-23 (FGF-23).[11]

The ratio of 24,25-dihydroxyvitamin D_3/25(OH)D_3 (vitamin D metabolite ratio [VDMR]) is a measure of functional vitamin D activity that may better define vitamin D status. Vitamin D receptor activation by 1,25(OH)$_2$D induces the metabolic clearance of 25(OH)D into 24,25-dihydroxyvitamin D (24,25[OH]$_2$D),[12,13] and our group has previously validated the VDMR as a surrogate measure of 25(OH)D_3 clearance.[14] We have shown that lower estimated glomerular filtration rate (eGFR) is associated with lower VDMR[15] and that lower VDMR is associated with increased risk of mortality in CKD.[16,17]

We tested associations of the VDMR, total 25(OH)D, and total 1,25(OH)$_2$D with risk of CVD events and left ventricular mass index (LVMI) in a prospective multicenter cohort of adults with CKD. This knowledge may improve our understanding of how impaired vitamin D metabolism increases CVD risk and whether the VDMR is a novel therapeutic target in this population.

METHODS

Because of the sensitive nature of the data collected for this study, requests to access the data set from qualified researchers trained in human subject confidentiality protocols may be sent to the CRIC (Chronic Renal Insufficiency Cohort) Study at www.cristudy.org.

Study Population

The CRIC Study is an ongoing prospective cohort study of adults with CKD.[18] Between 2003 and 2008, the CRIC Study recruited 3939 adults with eGFR ranging from 20 to 70 mL/min per 1.73 m^2 using the Modification of Diet in Renal Disease study equation from 7 clinical centers located across the United States: Ann Arbor and Detroit, MI; Baltimore, MD; Chicago, IL; Cleveland, OH; New Orleans, LA; Philadelphia, PA; and Oakland, CA. Exclusion criteria included dialysis for at least 1 month, prior kidney transplant, and New York Heart Association class III and IV heart failure. Institutional review boards at all participating centers approved the study, and all participants gave written informed consent.

This ancillary study used vitamin D metabolite measurements originally obtained for a prior case-cohort study in the CRIC Study population.[16] As this other study oversampled participants based on progression to kidney failure or death rather than CVD, the current study used a 2-phase design with weight adjustment to account for the probability of participant selection (Figure 1). The current study included a total of 1786 participants who attended the 1-year CRIC Study visit (baseline for the current analysis): 1103 participants were randomly selected, whereas an additional 683 were oversampled for progression to kidney failure or death (as part of the design of the other case-cohort study).

Measurement of Vitamin D Metabolites

Concentrations of 24,25(OH)$_2D_3$, 25-hydroxyvitamin D_2 (25[OH]D_2), 25-hydroxyvitamin D_3 (25[OH]D_3), 1,25-dihydroxyvitamin D_2 (1,25[OH]$_2D_2$), and 1,25-dihydroxyvitamin D_3 (1,25[OH]$_2D_3$) were measured using

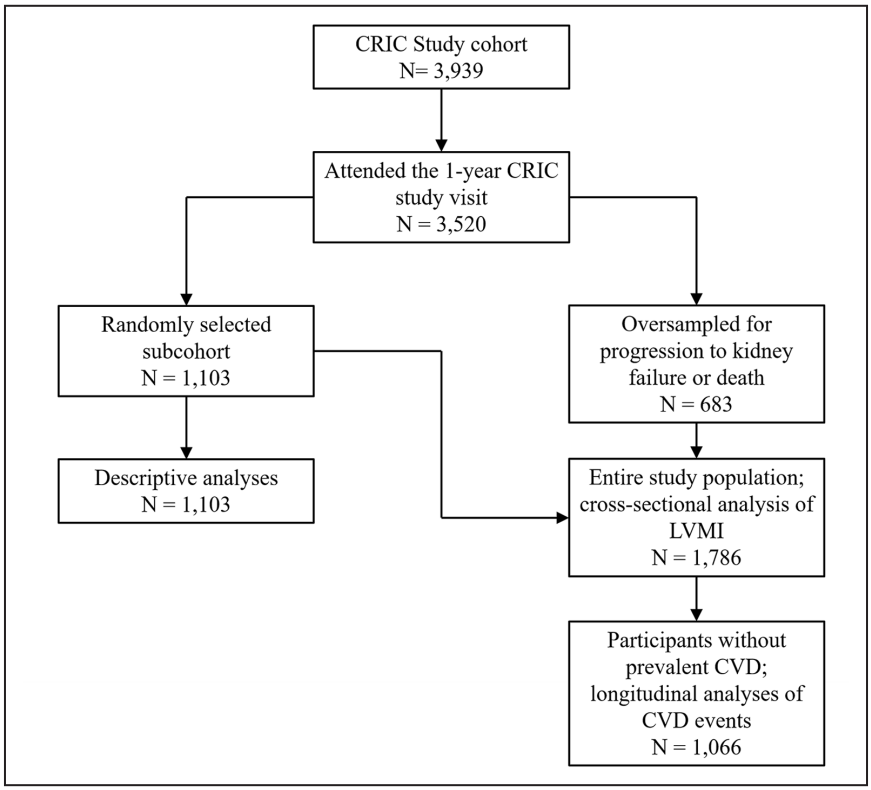

Figure 1. Cohort assembly.
CRIC indicates Chronic Renal Insufficiency Cohort; CVD, cardiovascular disease; and LVMI, left ventricular mass index.

immunoaffinity extraction and liquid chromatography–tandem mass spectrometry on a Xevo TQ spectrometer (Waters Corp, Milford, MA) at the University of Washington from serum collected at the 1-year study visit.[15] Interassay coefficients of variation calculated using repeated measurements of quality control specimens ranged from 3.9% to 16.1% for the 5 vitamin D metabolites. Total 25(OH)D and 1,25$(OH)_2$D were calculated by the sum of their respective D_2 and D_3 concentrations. As there was no spectrometric evidence of 24,25$(OH)_2D_2$, the VDMR was calculated by dividing 24,25$(OH)_2D_3$ by 25$(OH)D_3$, and then multiplying by 1000 such that its units are in pg/ng.[14,16] Our laboratory has been an active participant in the Vitamin D Standardization Program, and our method for 25(OH)D is traceable to the relevant National Institute of Standards and Technology and Centers for Disease Control and Prevention reference measurement procedures.[19]

Outcomes

The primary outcome was time to first composite CVD, defined as the first instance of definite or probable heart failure, myocardial infarction, ischemic stroke, or peripheral arterial disease, as previously defined.[20–22] Hospitalizations possibly related to CVD events were assessed using standard event questionnaires administered at semiannual participant interviews and queried through electronic health records from select health care systems. *International Classification of Diseases, Ninth Revision* (*ICD-9*), codes were subsequently obtained for all hospitalizations, and CVD events were adjudicated from medical records by at least 2 physicians. Consistent with prior CRIC Study publications,[22,23] criteria for heart failure were adapted from the FHS (Framingham Heart Study) and included a combination of clinical symptoms, physical examination, radiographic evidence of pulmonary edema, echocardiographic abnormalities, and central venous hemodynamic monitoring data;[24] criteria for myocardial infarction included a combination of clinical symptoms, elevated cardiac biomarkers, and electrocardiographic abnormalities;[25] stroke was defined as a fixed neurologic deficit lasting >24 hours attributable to a presumed vascular cause and adjudicated by 2 vascular neurologists;[26] and peripheral arterial disease was defined as amputation attributable to vascular disease or peripheral surgical or percutaneous revascularization.[27] The secondary outcome was LVMI. LVMI was calculated from 2-dimensional echocardiographic images of the left ventricular short-axis muscle area and apical left ventricular length obtained at the 1-year study visit, defined using the Cornell Criteria, and indexed to height (in meters) raised to the power of 2.7.[28] Exploratory outcomes were composite atherosclerotic

CVD (first instance of definite or probable myocardial infarction, ischemic stroke, or peripheral arterial disease; it excludes heart failure) and individual components of the primary outcome. Follow-up for this study was through May 2020.

Covariates

Covariates were ascertained concurrently with vitamin D metabolites. Sociodemographic characteristics, smoking status, comorbidities, and medication use were ascertained using standardized questionnaires. Participants identified themselves as belonging to 1 of 4 racial or ethnic groups: non-Hispanic White, non-Hispanic Black, Hispanic, or some other race or ethnicity. Diabetes was defined as a fasting blood glucose ≥126 mg/dL, nonfasting blood glucose ≥200 mg/dL, or use of insulin or any other antidiabetic medication. Anthropometric measurements and blood pressure were assessed using standardized protocols.[29] Serum creatinine was measured using an enzymatic method on a Vitros 950 Chemistry Analyzer (Ortho-Clinical Diagnostics, Raritan, NJ) at the CRIC Study Central Laboratory and standardized to isotope dilution mass spectrometry–traceable values.[30–32] eGFR was calculated from serum creatinine and cystatin C using an internally validated study equation for the CRIC Study population.[31] Serum calcium, phosphate, C-terminal FGF-23, PTH, and 24-hour urine protein were also measured as previously described.[33,34]

Statistical Analysis

We described participant characteristics across tertiles of VDMR among randomly selected participants. We reported the incidence rate of composite incident CVD among randomly selected and all study participants. All subsequent analyses included participants who were oversampled and were weight adjusted using Borgan II weights to account for the 2-phase design.[35] Specifically, weights were calculated as one over the probability of being sampled, where the probability of being sampled for participants who had kidney failure or death was 1, whereas the probability of being sampled for participants in the random subcohort who did not have kidney failure or death was the number of participants without kidney failure or death in the subcohort divided by the number of participants without kidney failure or death in the overall cohort. Among participants without prevalent CVD in the entire study population, we generated Kaplan-Meier curves with inverse probability weighting[36,37] to evaluate CVD-free survival across categories of vitamin D metabolites. We used Cox regression models with regression-calibrated weights and Huber-White sandwich estimator of variance[38] to estimate longitudinal associations between each vitamin D metabolite (the VDMR, total 25[OH]D, and total $1,25[OH]_2D$) with risk of incident CVD (ie, primary and exploratory outcomes). Regression calibration may gain extra efficiency by using available covariate data observed in the entire study population.[39–41] Our initial model adjusted for age, sex, race, and ethnicity; a second model additionally adjusted for diabetes, smoking status, systolic blood pressure, body mass index, medication use (calciferols, vitamin D receptor agonist, renin-angiotensin system inhibitors, β-blockers, diuretics, and statins); and a third model additionally adjusted for eGFR and proteinuria. As vitamin D metabolism is regulated through complex endocrine feedback loops, a final fourth model was a model that additionally adjusted for potential mediators: serum concentrations of calcium, phosphate, PTH, and FGF-23. This model also adjusted for hs-CRP (high-sensitivity C-reactive protein), given that inflammation may mediate associations between vitamin D and CVD. Given that seasonal variability in 25(OH)D has been reported in other cohorts,[42] we performed a sensitivity analysis using 25(OH)D as the exposure that also adjusted for season of blood draw. We did not do this for the VDMR and $1,25(OH)_2D$ as there was no substantial seasonal variability in these exposures. Finally, we examined the functional forms of the associations between each vitamin D metabolite and LVMI in all study participants (including those with prevalent CVD) using generalized additive models with inverse probability weighting. We used a series of linear regression models with regression-calibrated weights and Huber-White sandwich estimator of variance to describe cross-sectional relationships of vitamin D metabolites with LVMI, adjusting for the same covariates as the Cox models above. Two-sided $P<0.05$ was considered statistically significant. All analyses were conducted with *R* version 3.6.1 (*R* Foundation for Statistical Computing), and the following *R* packages were used: the "survival" package for survival models, the "survey" package for calibration, the "mice" package for imputation, and the "mgcy" package for generalized additive models.

RESULTS

Participant Characteristics of the Random Subcohort

Among the 1103 randomly selected participants, 42% were non-Hispanic White race and ethnicity, 42% were non-Hispanic Black race and ethnicity, and 12% were Hispanic ethnicity. The mean (SD) age was 59 (11) years, and 43% were women. The mean (SD) VDMR, total 25(OH)D, and total $1,25(OH)_2D$ were 37.4 (19.6) pg/ng, 20.4 (10.7) ng/mL, and 31.7 (15.2) pg/mL, respectively. Participants with higher VDMR were more likely to be White race, less likely to be Black race or

Table 1. Characteristics of the Random Subcohort of Participants in the CRIC Study (N=1103) by Tertiles of the VDMR

Characteristic	All participants (N=1103)	Tertile 1 (N=368)	Tertile 2 (N=367)	Tertile 3 (N=368)
VDMR range, pg/ng	0–137	0–26.5	26.5–43.2	43.2–137
Demographics				
Age, mean (SD), y	59 (11)	58 (11)	61 (10)	58 (10)
Female sex, n (%)	470 (43)	157 (43)	152 (41)	161 (44)
Race or ethnicity, n (%)				
Non-Hispanic White	467 (42)	102 (28)	148 (40)	217 (59)
Non-Hispanic Black	465 (42)	204 (55)	153 (42)	108 (29)
Hispanic	135 (12)	54 (15)	52 (14)	29 (8)
Some other race or ethnicity	36 (3)	8 (2)	14 (4)	14 (4)
Medical history and lifestyle, n (%)				
Hypertension	983 (89)	345 (94)	342 (93)	296 (80)
Diabetes	554 (50)	220 (60)	208 (57)	126 (34)
Prevalent CVD	376 (34)	135 (37)	144 (39)	97 (26)
Prevalent heart failure	105 (10)	40 (11)	37 (10)	28 (8)
Prevalent myocardial infarction	248 (23)	90 (24)	86 (23)	72 (20)
Prevalent stroke	109 (10)	39 (11)	43 (12)	27 (7)
Prevalent peripheral arterial disease	74 (7)	25 (7)	36 (10)	13 (4)
Current smoker	115 (10)	55 (15)	30 (8)	30 (8)
Medication use, n (%)				
Calciferol use	135 (12)	30 (8)	38 (10)	67 (18)
Vitamin D receptor agonist use	78 (7)	43 (12)	31 (8)	4 (1)
Calcimimetic use	2 (1)	2 (1)	0 (0)	0 (0)
Phosphate binder use				
Calcium based	75 (7)	41 (11)	11 (3)	23 (6)
Non–calcium based	10 (1)	8 (2)	1 (0)	1 (0)
RAS inhibitor use	766 (69)	256 (70)	273 (74)	237 (64)
β-Blocker use	563 (51)	208 (57)	190 (52)	165 (45)
Diuretic use	645 (59)	237 (64)	232 (63)	176 (48)
Statin use	662 (60)	216 (59)	238 (65)	208 (57)
Physical examination data, mean (SD)				
BMI, kg/m^2	32.2 (8.1)	33.6 (9.4)	32.7 (7.7)	30.2 (6.5)
Systolic BP, mm Hg	127 (22)	129 (23)	129 (22)	121 (19)
Diastolic BP, mm Hg	70 (14)	71 (13)	69 (14)	70 (13)
Laboratory data				
eGFR, mean (SD), mL/min per 1.73 m^2	43 (17)	33 (14)	41 (14)	53 (14)
24-h Urine protein, median (IQR), g	0.1 (0–0.7)	0.3 (0.1–1.3)	0.1 (0.1–0.7)	0.1 (0.0–0.3)
Calcium, mean (SD), mg/dL	9.3 (0.5)	9.1 (0.6)	9.3 (0.5)	9.4 (0.4)
Phosphate, mean (SD), mg/dL	4 (1.2)	4.2 (1.4)	4.0 (1.1)	3.8 (1.0)
FGF-23, median (IQR), pg/mL	131 (86–224)	175 (109–337)	139 (87–219)	101 (69–154)
PTH, median (IQR), pg/mL	63 (41–102)	96 (66–164)	66 (45–96)	44 (29–62)
24,25(OH)$_2$D$_3$, mean (SD), ng/mL	0.77 (0.69)	0.26 (0.19)	0.64 (0.37)	1.41 (0.74)
25(OH)$_2$D$_3$, mean (SD), ng/mL	18.5 (10.4)	13.4 (7.9)	18.5 (9.9)	23.5 (10.6)
Total 25(OH)D, mean (SD), ng/mL	20.4 (10.7)	14.8 (8.6)	20.7 (10.3)	25.7 (10.2)
Total 1,25(OH)$_2$D, mean (SD), pg/mL	31.7 (15.2)	31.8 (17.0)	31.0 (13.8)	32.3 (14.6)
hs-CRP, median (IQR), mg/L	2.5 (1.1–6.3)	2.6 (1.2–6.7)	2.7 (1.1–6.9)	2.1 (1.0–5.0)
Left ventricular mass index, mean (SD), g/m$^{2.7}$	51 (14)	54 (14)	52 (14)	47 (12)

SI conversion factors: To convert VDMR to pmol/nmol, multiply by 0.962; calcium to mmol/L, multiply by 0.25; phosphate to mmol/L, multiply by 0.323; 24,25(OH)$_2$D$_3$ to mmol/L, multiply by 2.4; 25(OH)D to nmol/L, multiply by 2.496; 1,25(OH)$_2$D to pmol/L, multiply by 2.496; hs-CRP to nmol/L, multiply by 9.524.1,25(OH)$_2$D indicates 1,25-dihydroxyvitamin D; 24,25(OH)$_2$D$_3$, 24,25-dihydroxyvitamin D$_3$; 25(OH)$_2$D$_3$, 25-dihydroxyvitamin D$_3$; 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; BP, blood pressure; CRIC, Chronic Renal Insufficiency Cohort; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; PTH, parathyroid hormone; RAS, renin-angiotensin system; and VDMR, vitamin D metabolite ratio.

Hispanic ethnicity, less likely to have hypertension, diabetes, and prevalent CVD, more likely to use calciferols, had higher eGFR, had lower concentrations of FGF-23 and PTH, and had higher concentrations of $24,25(OH)_2D_3$, $25(OH)D_3$, and total 25(OH)D (Table 1).

CVD Events

In the randomly selected subcohort (N=1103), 727 participants did not have prevalent CVD and experienced 187 first CVD events (99 heart failure, 56 myocardial infarction, 29 stroke, and 15 peripheral arterial disease; the sum of the individual events does not match because of simultaneous first events) over a mean (SD) follow-up of 9.6 (4.6) years. In the entire study population (N=1786), 1066 participants did not have prevalent CVD and experienced 298 first CVD events (167 heart failure, 87 myocardial infarction, 38 stroke, and 27 peripheral arterial disease) over 8.6 (4.8) years. Continuing with analyses using the entire study population without prevalent CVD, the unadjusted incidence rate of CVD was lowest among participants in the highest tertile of VDMR (Figure 2). Lower VDMR was associated with a greater risk of CVD in models adjusted for demographics, comorbidity, smoking status, systolic blood pressure, body mass index, and medication use (Table 2). However, with additional adjustment for eGFR and proteinuria, the association was attenuated and no longer statistically significant (hazard ratio [HR], 1.11 per 1 SD lower VDMR [95% CI, 0.95–1.31]). Similarly, neither 25(OH)D nor $1,25(OH)_2D$ was associated with CVD after full covariate adjustment that included eGFR and proteinuria. In exploratory analyses that used composite atherosclerotic CVD and individual components of CVD as outcomes, VDMR was associated with heart failure (HR, 1.23 per 1 SD lower VDMR [95% CI, 1.01–1.51]) and 25(OH)D was associated with stroke (HR, 1.47 per 10 ng/mL lower $1,25(OH)_2D$ [95% CI, 1.06–2.04]) after full covariate adjustment (Table 3). Results from the sensitivity analysis of 25(OH)D that additionally adjusted for season of blood draw were like that of the primary analysis (HR, 1.04 per 10 ng/mL lower 25(OH)D [95% CI, 0.90–1.20]).

Left Ventricular Mass Index

Among all 1786 study participants, the mean (SD) LVMI was 51 (14) $g/m^{2.7}$. Generalized additive models suggested generally linear and inverse unadjusted associations between each vitamin D metabolite and LVMI (Figure 3). When modeled continuously, lower VDMR, 25(OH)D, and $1,25(OH)_2D$ were all significantly associated with greater LVMI in models that adjusted for demographics, comorbidity, smoking status, systolic blood pressure, body mass index, and medication use (Table 4). With additional adjustment for eGFR and proteinuria, 25(OH)D was the only metabolite that remained significantly associated with LVMI (0.6 $g/m^{2.7}$ greater LVMI per 10 ng/mL lower 25[OH]D [95% CI, 0.0–1.3]). This association was attenuated and no longer statistically significant after adjusting for serum calcium, phosphate, PTH, and FGF-23 (0.5 $g/m^{2.7}$ greater LVMI per 10 ng/mL lower 25[OH]D [95% CI, −0.1 to 1.1]).

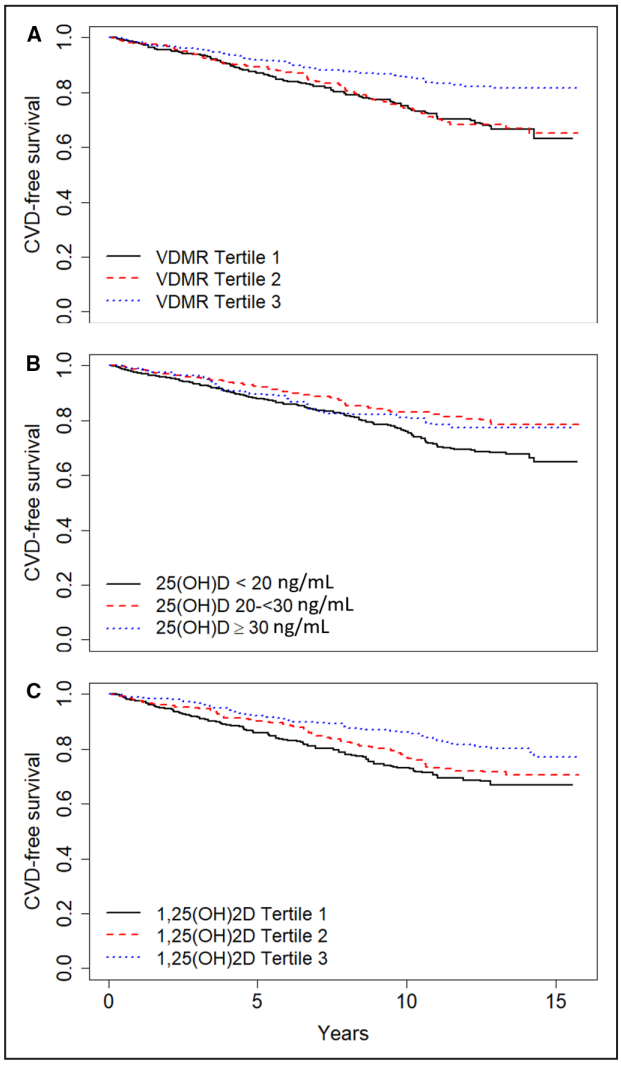

Figure 2. Kaplan-Meier curves for composite CVD events among study participants without prevalent CVD (N=1066) across categories of VDMR (A), 25[OH]D (B), and 1, 25[OH]$_2$D (C). 1,25(OH)2D indicates 1, 25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; CVD, cardiovascular disease; and VDMR, vitamin D metabolite ratio.

DISCUSSION

In a large, multicenter prospective cohort of adults with CKD, we assessed associations of the VDMR, 25(OH)D, and $1,25(OH)_2D$ with CVD. Although lower 25(OH)D was significantly associated with greater LVMI, none of the vitamin D metabolites was associated with risk of composite

Table 2. Associations of Vitamin D Metabolites With CVD Events Among Participants Without Prevalent CVD (N=1066)

Variable	No. of events	Incidence rate, events/100 person-years (95% CI)	Hazard ratio (95% CI)			
			Model 1	Model 2	Model 3	Model 4
VDMR						
Tertile 1	125	3.6 (2.7–4.4)	1.92 (1.36–2.71)*	1.48 (1.03–2.13)*	1.06 (0.72–1.57)	1.02 (0.68–1.54)
Tertile 2	102	3.4 (2.6–4.1)	1.79 (1.26–2.54)*	1.38 (0.95–2.01)	1.30 (0.89–1.89)	1.33 (0.91–1.95)
Tertile 3	71	1.6 (1.1–2.0)	Reference	Reference	Reference	Reference
Per 1-SD decrement			1.41 (1.22–1.62)*	1.28 (1.10–1.50)*	1.11 (0.95–1.31)	1.09 (0.92–1.29)
P value			<0.0001	0.002	0.20	0.34
25(OH)D, ng/mL						
<20	191	3.3 (2.7–3.9)	1.48 (1.01–2.15)*	1.23 (0.82–1.84)	1.08 (0.71–1.65)	1.07 (0.69–1.66)
20 to <30	65	2.0 (1.4–2.5)	0.89 (0.57–1.39)	0.90 (0.58–1.39)	0.89 (0.57–1.39)	0.85 (0.55–1.33)
≥30	42	2.3 (1.5–3.1)	Reference	Reference	Reference	Reference
Per 10-ng/mL decrement			1.24 (1.08–1.42)*	1.12 (0.97–1.30)	1.05 (0.91–1.21)	1.04 (0.88–1.21)
P value			0.003	0.11	0.52	0.66
$1,25(OH)_2D$						
Tertile 1	116	3.4 (2.5–4.2)	2.21 (1.57–3.12)*	1.78 (1.25–2.54)*	1.35 (0.92–1.98)	1.28 (0.87–1.89)
Tertile 2	110	3.0 (2.3–3.7)	1.56 (1.11–2.21)*	1.44 (1.03–2.03)*	1.28 (0.91–1.80)	1.30 (0.93–1.82)
Tertile 3	72	1.9 (1.4–2.5)	Reference	Reference	Reference	Reference
Per 10-pg/mL decrement			1.18 (1.05–1.34)*	1.11 (0.99–1.24)	1.02 (0.92–1.14)	1.02 (0.92–1.13)
P value			0.007	0.08	0.66	0.69

Incident rates are based on participants without prevalent CVD in the randomly selected subcohort (N=727). The number of events and hazard ratios are regression-calibrated estimates among all participants without prevalent CVD (N=1066). The incidence rate for composite CVD among all participants without prevalent CVD in the randomly selected subcohort (N=727) was 2.45 (95% CI, 2.12–2.84) events per 100 person-years. The SD for VDMR is 19.7 pg/ng. Model 1 adjusted for age, sex, race, and ethnicity. Model 2 additionally adjusted for diabetes, smoking status, systolic blood pressure, body mass index, and medication use (calciferols, vitamin D receptor agonists, renin-angiotensin system inhibitors, β-blockers, diuretics, and statins). Model 3 additionally adjusted for estimated glomerular filtration rate and proteinuria. Model 4 additionally adjusted for the following potential mediators: serum calcium, phosphate, parathyroid hormone, fibroblast growth factor-23, and hs-CRP (high-sensitivity C-reactive protein). SI conversion factors: To convert VDMR to pmol/nmol, multiply by 0.962; 25(OH)D to nmol/L, multiply by 2.496; $1,25(OH)_2D$ to pmol/L, multiply by 2.496. $1,25(OH)_2D$ indicates 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; CVD, cardiovascular disease; and VDMR, vitamin D metabolite ratio.

*$P<0.05$.

CVD events. Findings from exploratory outcomes raise the possibility of associations between VDMR and heart failure, and between 25(OH)D and stroke.

The VDMR has recently emerged as a promising biomarker of vitamin D status. It has been validated as a biomarker of $25(OH)D_3$ clearance (which may reflect

Table 3. Associations of Vitamin D Metabolites With Individual CVD Components Among Participants Without Prevalent CVD (N=1066)

Variable		Hazard ratio (95% CI)					
	No. of events	VDMR, per 1-SD decrement	*P* value	25(OH)D, per 10-ng/mL decrement	*P* value	$1,25(OH)_2D$, per 10-pg/mL decrement	*P* value
Composite CVD	298	1.11 (0.95–1.31)	0.20	1.05 (0.91–1.21)	0.52	1.02 (0.92–1.14)	0.66
Heart failure	196	1.23 (1.01–1.51)	0.04	0.96 (0.81–1.14)	0.66	0.99 (0.88–1.11)	0.87
Atherosclerotic CVD	190	1.11 (0.91–1.36)	0.29	1.13 (0.95–1.34)	0.18	1.12 (0.98–1.29)	0.09
Myocardial infarction	117	1.24 (0.96–1.60)	0.10	1.08 (0.87–1.35)	0.47	1.17 (0.97–1.42)	0.11
Stroke	51	0.97 (0.68–1.40)	0.89	1.47 (1.06–2.04)	0.02	0.95 (0.80–1.11)	0.51
Peripheral arterial disease	53	0.79 (0.59–1.06)	0.11	1.06 (0.78–1.43)	0.72	1.06 (0.88–1.26)	0.55

Hazard ratios are regression-calibrated estimates among all participants without prevalent CVD adjusted for age, sex, race and ethnicity, diabetes, smoking status, systolic blood pressure, body mass index, medication use (calciferols, vitamin D receptor agonists, renin-angiotensin system inhibitors, β-blockers, diuretics, and statins), estimated glomerular filtration rate, and proteinuria. The SD for VDMR is 19.7 pg/ng.

SI conversion factors: To convert VDMR to pmol/nmol, multiply by 0.962; 25(OH)D to nmol/L, multiply by 2.496; $1,25(OH)_2D$ to pmol/L, multiply by 2.496. $1,25(OH)_2D$ indicates 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; CVD, cardiovascular disease; and VDMR, vitamin D metabolite ratio.

tissue-level 1,25[OH]$_2$D activity),[14] is not associated with VDBP (vitamin D binding protein) concentration[43] (reducing likelihood of confounding by VDBP, unlike individual vitamin D metabolites), and is associated with adverse outcomes in other cohorts.[44,45] In a 2019 study within the CRIC Study population, we observed associations of the VDMR with mortality (HR, 1.18 per 1 SD lower VDMR [95% CI, 1.02–1.36]).[16] As CVD is the leading cause of death in CKD, we hypothesized associations between the VDMR and CVD but observed none in the current study. The estimated HR of CVD risk (1.11 per 1 SD lower VDMR [95% CI, 0.95–1.31]) is nonetheless comparable to the HR of mortality in the 2019 CRIC Study, and their CIs overlap. Thus, the current study may have lacked power to detect small associations between the VDMR and CVD. Another potential explanation for the discrepancy between studies is that associations of VDMR with mortality are driven more by non-CVD causes of death. Altogether, the utility of the VDMR to assess CVD risk in CKD remains uncertain, yet it may be useful for other outcomes. We previously reported associations of the VDMR with fracture risk in 2 populations with normal eGFR.[44,45] Given the ubiquity of mineral and bone disorders in CKD, the relationship between VDMR and fractures in CKD remains an important knowledge gap for future studies.

Leading up to the recent large trials of vitamin D supplementation,[7] years of epidemiological and animals studies linked 25(OH)D deficiency with risks of CVD and other chronic illnesses.[46] The 2017 Kidney Disease: Improving Global Outcomes clinical practice guidelines correspondingly suggested 25(OH)D deficiency in CKD might be corrected to targets recommended for the general population.[47] Since then, randomized controlled trials in the general population, like VITAL (Vitamin D and Omega-3 Trial) and the ViDA (Vitamin D Assessment) study, have failed to demonstrate clinical benefits of vitamin D supplementation on CVD and other outcomes.[7] Although definitive conclusions about 25(OH)D treatment goals in CKD are ideally supported by randomized trials in this population, such trials in CKD with clinical outcomes are rare to nonexistent. Looking at observational studies, 2 prior studies in cohorts with CKD did not find associations between 25(OH)D and CVD, and neither did the current study.[2,48] Taken together, the preponderance of current evidence suggests testing and treatment to 25(OH)D targets in CKD are poorly supported and should be deemphasized. We found associations of 25(OH)D with LVMI, but not, like others, with CVD. Given the evidence linking left ventricular hypertrophy with CVD, these results suggest that associations between 25(OH)D and LVMI may be confounded in a manner that they are not with CVD events.

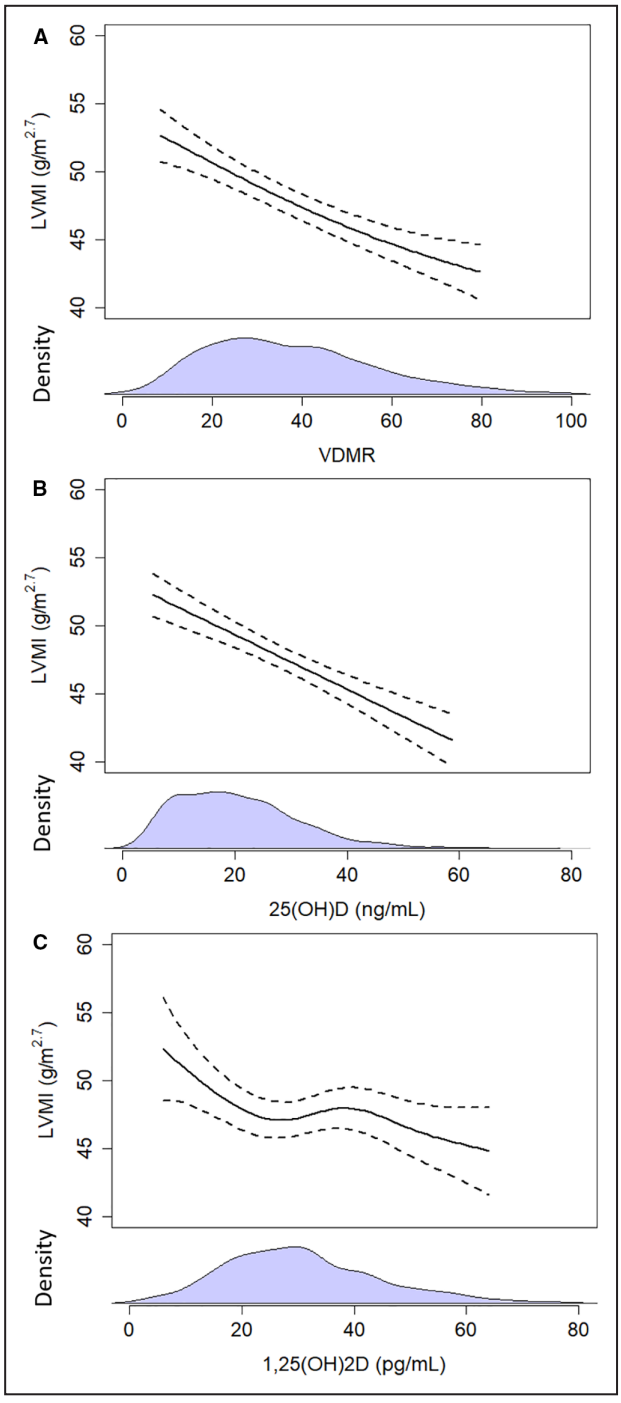

Figure 3. Generalized additive models of VDMR (A), 25[OH]D (B), and 1, 25[OH]$_2$D (C) and left ventricular mass index (LVMI) among all study participants (N=1786).
Dotted lines represent 95% CIs. Below each model is a density curve of the distribution of the corresponding vitamin D metabolite.1, 25(OH)$_2$D indicates 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; LVMI, left ventricular mass index; and VDMR, vitamin D metabolite ratio

Although testing of 1,25(OH)$_2$D concentrations is not routinely recommended in the general population, its utility may be different in CKD, as 1,25(OH)$_2$D is mainly

Table 4. Associations of Vitamin D Metabolites With LVMI in the Entire Study Population (N=1786)

Variable	Difference in LVMI, mean (95% CI), g/m$^{2.7}$			
	Model 1	Model 2	Model 3	Model 4
VDMR				
Tertile 1	5.4 (3.5 to 7.2)*	1.6 (–0.1 to 3.3)	–0.2 (–2.0 to 1.6)	–0.3 (–2.1 to 1.5)
Tertile 2	3.3 (1.5 to 5.1)*	0.4 (–1.2 to 2.0)	–0.3 (–1.9 to 1.3)	–0.4 (–2.0 to 1.3)
Tertile 3	0 (Reference)	0 (Reference)	0 (Reference)	Reference
Per 1-SD decrement	2.3 (1.6 to 3.0)*	0.7 (0.1 to 1.4)*	–0.0 (–0.7 to 0.7)	–0.0 (–0.8 to 0.7)
P value	<0.0001	0.03	0.97	0.91
25(OH)D, ng/mL				
<20	6.5 (4.7 to 8.3)*	1.3 (–0.4 to 3.1)	0.8 (–1.0 to 2.5)	0.4 (–1.3 to 2.1)
20 to <30	3.2 (1.3 to 5.1)*	0.5 (–1.2 to 2.3)	0.5 (–1.3 to 2.2)	0.3 (–1.4 to 2.0)
≥30	0 (Reference)	0 (Reference)	0 (Reference)	Reference
Per 10-ng/mL decrement	2.8 (2.2 to 3.5)*	0.9 (0.3 to 1.6)*	0.6 (0.0 to 1.3)*	0.5 (–0.1 to 1.1)
P value	<0.0001	0.003	0.04	0.13
1,25(OH)$_2$D				
Tertile 1	4.1 (2.3 to 5.9)*	2.1 (0.5 to 3.8)*	0.7 (–1.1 to 2.4)	0.0 (–1.7 to 1.1)
Tertile 2	0.5 (–1.3 to 2.4)	0.1 (–1.4 to 1.7)	–0.5 (–2.0 to 1.1)	–0.4 (–2.0 to 1.2)
Tertile 3	0 (Reference)	0 (Reference)	0 (Reference)	Reference
Per 10-pg/mL decrement	1.1 (0.5 to 1.7)*	0.6 (0.1 to 1.1)*	0.3 (–0.3 to 0.8)	0.1 (–0.4 to 0.6)
P value	0.0002	0.01	0.34	0.68

The SD for VDMR is 19.7 pg/ng. Model 1 adjusted for age, sex, race, and ethnicity. Model 2 additionally adjusted for diabetes, smoking status, systolic blood pressure, body mass index, and medication use (calciferols, vitamin D receptor agonists, renin-angiotensin system inhibitors, β-blockers, diuretics, and statins). Model 3 additionally adjusted for estimated glomerular filtration rate and proteinuria. Model 4 additionally adjusted for the following potential mediators: serum calcium, phosphate, parathyroid hormone, fibroblast growth factor-23, and hs-CRP, (high-sensitivity C-reactive protein).

SI conversion factors: To convert VDMR to pmol/nmol, multiply by 0.962; 25(OH)D to nmol/L, multiply by 2.496; 1,25(OH)$_2$D to pmol/L, multiply by 2.496. 1,25(OH)$_2$D indicates 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; LVMI, left ventricular mass index; and VDMR, vitamin D metabolite ratio.

*P<0.05.

produced by the kidneys[9,10] and associated with mortality in CKD.[3,49,50] Still, the current state of evidence makes it difficult to draw definitive conclusions on the relationship between 1,25(OH)$_2$D and CVD in CKD, and whether treatment with 1,25(OH)$_2$D or its analogs improves CVD outcomes. Like the current study, Kendrick et al did not find associations between 1,25(OH)$_2$D concentrations and CVD events in participants with advanced CKD,[48] whereas Kim et al recently reported an inverse relationship between 1,25(OH)$_2$D and cardiac valve calcification in a population with CKD.[51] The PRIMO (Paricalcitol Capsule Benefits in Renal Failure-Induced Cardiac Morbidity) and OPERA (Oral Paritcalcitol on Stage 3-5 Chronic Kidney Disease) trials did not find activated vitamin D treatment to alter LVMI or other measures of cardiac function over 1 year of therapy in patients with CKD.[52,53] However, patients treated with activated vitamin D had fewer CVD-related hospitalizations in both trials. Future trials that use clinical outcomes are needed to better define the role of 1,25(OH)$_2$D testing and treatment in CKD.

To our knowledge, this is the first longitudinal study to examine associations of the VDMR with CVD. We did so in a well-characterized cohort of adults with a wide range of CKD severity. Other strengths include accurate measurements of vitamin D metabolites using liquid chromatography–tandem mass spectrometry. This study also has important limitations. First, observational designs are limited in causal inference and need to be supported by clinical trials for the reasons outlined above. Next, our study had only a modest number of events and is likely underpowered to detect significant associations with individual CVD outcomes and did not account for multiple testing. Last, we lacked data on vitamin D supplement dose. We could not adjust for this, nor could we test whether vitamin D supplementation, which has likely increased since the measurement of vitamin D metabolites at baseline,[54,55] modified the associations of interest.

In conclusion, although lower 25(OH)D was associated with greater LVMI, the VDMR, 25(OH)D, and 1,25(OH)$_2$D do not appear to be risk factors for clinical CVD events in adults with nondialysis CKD. Future research should evaluate associations of the VDMR with risks of other pertinent outcomes in CKD and elucidate the role of 1,25(OH)$_2$D testing and treatment in CKD.

APPENDIX

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Disclosures

None.

REFERENCES

1. Gluba-Brzózka A, Franczyk B, Ciałkowska-Rysz A, Olszewski R, Rysz J. Impact of vitamin D on the cardiovascular system in advanced chronic kidney disease (CKD) and dialysis patients. *Nutrients*. 2018;10:10. doi: 10.3390/nu10060709
2. Mehrotra R, Kermah DA, Salusky IB, Wolf MS, Thadhani RI, Chiu YW, Martins D, Adler SG, Norris KC. Chronic kidney disease, hypovitaminosis D, and mortality in the United States. *Kidney Int*. 2009;76:977–983. doi: 10.1038/ki.2009.288
3. Wolf M, Shah A, Gutierrez O, Ankers E, Monroy M, Tamez H, Steele D, Chang Y, Camargo CA, Tonelli M, et al. Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int*. 2007;72:1004–1013. doi: 10.1038/sj.ki.5002451
4. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C-Y. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004;351:1296–1305. doi: 10.1056/NEJMoa041031
5. Kim SM, Lutsey PL, Michos ED. Vitamin D and cardiovascular disease: can novel measures of vitamin D status improve risk prediction and address the vitamin D racial paradox? *Curr Cardiovasc Risk Rep*. 2017;11:3. doi: 10.1007/s12170-017-0528-7
6. Bouillon R. Free or total 25OHD as marker for vitamin D status? *J Bone Miner Res*. 2016;31:1124–1127. doi: 10.1002/jbmr.2871
7. Bouillon R, Manousaki D, Rosen C, Trajanoska K, Rivadeneira F, Richards JB. The health effects of vitamin D supplementation: evidence from human studies. *Nat Rev Endocrinol*. 2022;18:96–110. doi: 10.1038/s41574-021-00593-z
8. Pontoriero G, Cozzolino M, Locatelli F, Brancaccio D. CKD patients: the dilemma of serum PTH levels. *Nephron Clin Pract*. 2010;116:c263–c268. doi: 10.1159/000318787
9. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266–281. doi: 10.1056/NEJMra070553
10. Rosen CJ. Clinical practice. Vitamin D insufficiency. *N Engl J Med*. 2011;364:248–254. doi: 10.1056/NEJMcp1009570
11. Latic N, Erben RG. FGF23 and vitamin D metabolism. *JBMR Plus*. 2021;5:e10558. doi: 10.1002/jbm4.10558
12. Bosworth C, de Boer IH. Impaired vitamin D metabolism in CKD. *Semin Nephrol*. 2013;33:158–168. doi: 10.1016/j.semnephrol.2012.12.016
13. Jones G, Prosser DE, Kaufmann M. 25-hydroxyvitamin D-24-hydroxylase (CYP24A1): its important role in the degradation of vitamin D. *Arch Biochem Biophys*. 2012;523:9–18. doi: 10.1016/j.abb.2011.11.003

14. Hsu S, Zelnick LR, Lin YS, Best CM, Kestenbaum BR, Thummel KE, Hoofnagle AN, de Boer IH. Validation of the 24,25-dihydroxyvitamin D3 to 25-hydroxyvitamin D3 ratio as a biomarker of 25-hydroxyvitamin D3 clearance. *J Steroid Biochem Mol Biol*. 2022;217:106047. doi: 10.1016/j.jsbmb.2021.106047
15. de Boer IH, Sachs MC, Chonchol M, Himmelfarb J, Hoofnagle AN, Ix JH, Kremsdorf RA, Lin YS, Mehrotra R, Robinson-Cohen C, et al. Estimated GFR and circulating 24,25-dihydroxyvitamin D3 concentration: a participant-level analysis of 5 cohort studies and clinical trials. *Am J Kidney Dis*. 2014;64:187–197. doi: 10.1053/j.ajkd.2014.02.015
16. Bansal N, Katz R, Appel L, Denburg M, Feldman H, Go AS, He J, Hoofnagle A, Isakova T, Kestenbaum B, et al. Vitamin D metabolic ratio and risks of death and CKD progression. *Kidney Int Rep*. 2019;4:1598–1607. doi: 10.1016/j.ekir.2019.08.014
17. Selamet U, Katz R, Ginsberg C, Rifkin DE, Fried LF, Kritchevsky SB, Hoofnagle AN, Bibbins-Domingo K, Drew D, Harris T, et al. Serum calcitriol concentrations and kidney function decline, heart failure, and mortality in elderly community-living adults: the health, aging, and body composition study. *Am J Kidney Dis*. 2018;72:419–428. doi: 10.1053/j.ajkd.2018.03.026
18. Feldman HI, Appel LJ, Chertow GM, Cifelli D, Cizman B, Daugirdas J, Fink JC, Franklin-Becker ED, Go AS, Hamm LL, et al. The chronic renal insufficiency cohort (CRIC) study: design and methods. *J Am Soc Nephrol*. 2003;14:S148–S153. doi: 10.1097/01.Asn.0000070149.78399.Ce
19. Sempos CT, Betz JM, Camara JE, Carter GD, Cavalier E, Clarke MW, Dowling KG, Durazo-Arvizu RA, Hoofnagle AN, Liu A, et al. General steps to standardize the laboratory measurement of serum total 25-hydroxyvitamin D. *J AOAC Int*. 2017;100:1230–1233. doi: 10.5740/jaoacint.17-0259
20. Orlandi PF, Xie D, Yang W, Cohen JB, Deo R, Ricardo AC, Schrauben S, Wang X, Hamm LL, He J, et al. Slope of kidney function and its association with longitudinal mortality and cardiovascular disease among individuals with CKD. *J Am Soc Nephrol*. 2020;31:2912–2923. doi: 10.1681/asn.2020040476
21. Bansal N, McCulloch CE, Lin F, Alper A, Anderson AH, Cuevas M, Go AS, Kallem R, Kusek JW, Lora CM, et al. Blood pressure and risk of cardiovascular events in patients on chronic hemodialysis. *Hypertension*. 2017;70:435–443. doi: 10.1161/HYPERTENSIONAHA.117.09091
22. Rahman M, Wang X, Bundy JD, Charleston J, Cohen D, Cohen J, Drawz PE, Ghazi L, Horowitz E, Lash JP, et al. Prognostic significance of ambulatory BP monitoring in CKD: a report from the Chronic Renal Insufficiency Cohort (CRIC) study. *J Am Soc Nephrol*. 2020;31:2609–2621. doi: 10.1681/asn.2020030236
23. Toth-Manikowski SM, Yang W, Appel L, Chen J, Deo R, Frydrych A, Krousel-Wood M, Rahman M, Rosas SE, Sha D, et al. Sex differences in cardiovascular outcomes in CKD: findings from the CRIC study. *Am J Kidney Dis*. 2021;78:200–209.e201. doi: 10.1053/j.ajkd.2021.01.020
24. Ho KK, Anderson KM, Kannel WB, Grossman W, Levy D. Survival after the onset of congestive heart failure in Framingham heart study subjects. *Circulation*. 1993;88:107–115. doi: 10.1161/01.CIR.88.1.107
25. Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. *Circulation*. 2007;116:2634–2653. doi: 10.1161/CIRCULATIONAHA.107.187397
26. Adams HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of org 10172 in acute stroke treatment. *Stroke*. 1993;24:35–41. doi: 10.1161/01.STR.24.1.35
27. Wang GJ, Shaw PA, Townsend RR, Anderson AH, Xie D, Wang X, Nessel LC, Mohler ER, Sozio SM, Jaar BG, et al. Sex differences in the incidence of peripheral artery disease in the chronic renal insufficiency cohort. *Circ Cardiovasc Qual Outcomes*. 2016;9:S86–S93. doi: 10.1161/circoutcomes.115.002180
28. Bansal N, Roy J, Chen HY, Deo R, Dobre M, Fischer MJ, Foster E, Go AS, He J, Keane MG, et al. Evolution of echocardiographic measures of cardiac disease from CKD to ESRD and risk of all-cause mortality: findings from the CRIC study. *Am J Kidney Dis*. 2018;72:390–399. doi: 10.1053/j.ajkd.2018.02.363
29. National Center for Health Statistics. *National Health and Nutrition Examination Survey (NHANES) Anthropometry Procedures Manual*. Centers for Disease Control and Prevention: Atlanta; 2000.
30. Joffe M, Hsu CY, Feldman HI, Weir M, Landis JR, Hamm LL. Variability of creatinine measurements in clinical laboratories: results from the CRIC study. *Am J Nephrol*. 2010;31:426–434. doi: 10.1159/000296250
31. Anderson AH, Yang W, Hsu CY, Joffe MM, Leonard MB, Xie D, Chen J, Greene T, Jaar BG, Kao P, et al. Estimating GFR among participants in the Chronic Renal Insufficiency Cohort (CRIC) study. *Am J Kidney Dis*. 2012;60:250–261. doi: 10.1053/j.ajkd.2012.04.012
32. Levey AS, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, Van Lente F. Expressing the modification of diet in renal disease study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem*. 2007;53:766–772. doi: 10.1373/clinchem.2006.077180
33. Isakova T, Cai X, Lee J, Xie D, Wang X, Mehta R, Allen NB, Scialla JJ, Pencina MJ, Anderson AH, et al. Longitudinal FGF23 trajectories and mortality in patients with CKD. *J Am Soc Nephrol*. 2018;29:579–590. doi: 10.1681/asn.2017070772
34. Scialla JJ, Xie H, Rahman M, Anderson AH, Isakova T, Ojo A, Zhang X, Nessel L, Hamano T, Grunwald JE, et al. Fibroblast growth factor-23 and cardiovascular events in CKD. *J Am Soc Nephrol*. 2014;25:349–360. doi: 10.1681/ASN.2013050465
35. Borgan O, Langholz B, Samuelsen SO, Goldstein L, Pogoda J. Exposure stratified case-cohort designs. *Lifetime Data Anal*. 2000;6:39–58. doi: 10.1023/a:1009661900674
36. Lin DY, Ying Z. Cox regression with incomplete covariate measurements. *J Am Stat Assoc*. 1993;88:1341–1349. doi: 10.2307/2291275
37. Breslow NE, Lumley T, Ballantyne CM, Chambless LE, Kulich M. Improved Horvitz-Thompson estimation of model parameters from two-phase stratified samples: applications in epidemiology. *Stat Biosci*. 2009;1:32–49. doi: 10.1007/s12561-009-9001-6
38. White H. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. *Econometrica*. 1980;48:817–838. doi: 10.2307/1912934
39. Baldoni PL, Sotres-Alvarez D, Lumley T, Shaw PA. On the use of regression calibration in a complex sampling design with application to the Hispanic community health study/study of Latinos. *Am J Epidemiol*. 2021;190:1366–1376. doi: 10.1093/aje/kwab008
40. Lumley T. Two-phase sampling. *Complex Surveys*. John Wiley & Sons; 2010:157–183. doi: 10.1002/9780470580066.ch8
41. Borgan Ø, Breslow NE, Chatterjee N, Gail MH, Scott A, Wild CJ. *Handbook of Statistical Methods for Case-Control Studies*. Chapman & Hall/CRC; 2020.
42. Sachs MC, Shoben A, Levin GP, Robinson-Cohen C, Hoofnagle AN, Swords-Jenny N, Ix JH, Budoff M, Lutsey PL, Siscovick DS, et al. Estimating mean annual 25-hydroxyvitamin D concentrations from single measurements: the multi-ethnic study of atherosclerosis. *Am J Clin Nutr*. 2013;97:1243–1251. doi: 10.3945/ajcn.112.054502
43. Ginsberg C, Hoofnagle AN, Katz R, Becker JO, Kritchevsky SB, Shlipak MG, Sarnak MJ, Ix JH. The vitamin D metabolite ratio is independent of vitamin D binding protein concentration. *Clin Chem*. 2021;67:385–393. doi: 10.1093/clinchem/hvaa238
44. Ginsberg C, Hoofnagle AN, Katz R, Hughes-Austin J, Miller LM, Becker JO, Kritchevsky SB, Shlipak MG, Sarnak MJ, Ix JH. The Vitamin D metabolite ratio is associated with changes in bone density and fracture risk in older adults. *J Bone Miner Res*. 2021;36:2343–2350. doi: 10.1002/jbmr.4426
45. Ginsberg C, Katz R, de Boer IH, Kestenbaum BR, Chonchol M, Shlipak MG, Sarnak MJ, Hoofnagle AN, Rifkin DE, Garimella PS, et al. The 24,25 to 25-hydroxyvitamin D ratio and fracture risk in older adults: the cardiovascular health study. *Bone*. 2018;107:124–130. doi: 10.1016/j.bone.2017.11.011
46. Gunta SS, Thadhani RI, Mak RH. The effect of vitamin D status on risk factors for cardiovascular disease. *Nat Rev Nephrol*. 2013;9:337–347. doi: 10.1038/nrneph.2013.74
47. KDIGO 2017 clinical practice guideline update for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). *Kidney Int Suppl (2011)*. 2017;7:1–59. doi: 10.1016/j.kisu.2017.04.001
48. Kendrick J, Cheung AK, Kaufman JS, Greene T, Roberts WL, Smits G, Chonchol M. Associations of plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations with death and progression to maintenance dialysis in patients with advanced kidney disease. *Am J Kidney Dis*. 2012;60:567–575. doi: 10.1053/j.ajkd.2012.04.014
49. Dusso AS. Kidney disease and vitamin D levels: 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and VDR activation. *Kidney Int Suppl (2011)*. 2011;1:136–141. doi: 10.1038/kisup.2011.30
50. Inaguma D, Nagaya H, Hara K, Tatematsu M, Shinjo H, Suzuki S, Mishima T, Kurata K. Relationship between serum 1,25-dihydroxyvitamin D and mortality in patients with pre-dialysis chronic kidney disease. *Clin Exp Nephrol*. 2008;12:126–131. doi: 10.1007/s10157-007-0023-4

51. Kim IY, Ye BM, Kim MJ, Kim SR, Lee DW, Kim HJ, Rhee H, Song SH, Seong EY, Lee SB. 1,25-dihydroxyvitamin D deficiency is independently associated with cardiac valve calcification in patients with chronic kidney disease. *Sci Rep*. 2022;12:915. doi: 10.1038/s41598-022-04981-x
52. Thadhani R, Appelbaum E, Pritchett Y, Chang Y, Wenger J, Tamez H, Bhan I, Agarwal R, Zoccali C, Wanner C, et al. Vitamin D therapy and cardiac structure and function in patients with chronic kidney disease: the PRIMO randomized controlled trial. *JAMA*. 2012;307:674–684. doi: 10.1001/jama.2012.120
53. Wang AY, Fang F, Chan J, Wen YY, Qing S, Chan IH, Lo G, Lai KN, Lo WK, Lam CW, et al. Effect of paricalcitol on left ventricular mass and function in CKD–the OPERA trial. *J Am Soc Nephrol*. 2014;25:175–186. doi: 10.1681/asn.2013010103
54. McKenna MJ, Murray BF, O'Keane M, Kilbane MT. Rising trend in vitamin D status from 1993 to 2013: dual concerns for the future. *Endocr Connect*. 2015;4:163–171. doi: 10.1530/ec-15-0037
55. Lips P, de Jongh RT, van Schoor NM. Trends in vitamin D status around the world. *JBMR Plus*. 2021;5:e10585. doi: 10.1002/jbm4.10585