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Vitamin D Metabolic Ratio and Risks of Death and CKD Progression

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Introduction: Assessment of impaired vitamin D metabolism is limited by lack of functional measures. CYP24A1-mediated vitamin D clearance, calculated as the ratio of serum 24,25-dihydroxyvitamin D3 to 25-hydroxyvitamin D3 (the vitamin D metabolic ratio, VDMR), is induced by 1,25-dihydroxyvitamin D and may assess tissue-level activity. We tested associations of the VDMR with risks of death and progression to end-stage renal disease (ESRD) in patients with chronic kidney disease (CKD).

Methods: We studied participants from the Chronic Renal Insufficiency Cohort (CRIC), which included a random subset of 1080 CRIC participants plus additional participants who experienced ESRD or died (case cohort study design). Serum 24,25-dihydroxyvitamin D3 and 25-hydroxyvitamin D3 was measured 1 year after enrollment. The primary outcomes included death and progression to ESRD. Using inverse probability weighting, we tested associations of VDMR ($24,25[OH]_2D_3/25[OH]D_3$) with risks of death and ESRD, adjusting for demographics, comorbidity, and kidney function (estimated glomerular filtration rate [eGFR] and urine protein-to-creatinine ratio [PCR]).

Results: There were a total of 708 ESRD events and 650 deaths events over mean (SD) follow-up periods of 4.9 (2.9) years and 6.5 (2.5) years, respectively. Lower VDMR was associated with increased risk of ESRD prior to adjusting for kidney function (hazard ratio [HR], 1.80 per 20 pg/ng lower VDMR; 95% confidence interval [CI], 1.56–2.08), but not with adjustment for kidney function (HR, 0.94 per 20 pg/ng; 95% CI, 0.81–1.10). Lower VDMR was associated with modestly increased mortality risk, including adjustment for kidney function (HR, 1.18 per 20 pg/ng; 95% CI, 1.02–1.36).

Conclusion: Lower VDMR, a measure of CYP24A1-mediated vitamin D clearance, was significantly associated with all-cause mortality but not with progression to ESRD in patients with CKD.

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In CKD, production of circulating 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$, calcitriol)[1–5] is impaired, resulting in systemic effects such as bone disease and secondary hyperparathyroidism. $1,25(OH)_2D_3$ deficiency also may contribute to progression to ESRD, cardiovascular disease, and premature death. Despite the attention devoted to treating $1,25(OH)_2D_3$ deficiency

and its consequences, clinical decision making is limited by the lack of an effective measurement of functional $1,25(OH)_2D_3$ deficiency; 25-hydroxyvitamin D_3 (25[OH]D_3) is a relatively inactive substrate form of vitamin D, circulating $1,25(OH)_2D_3$ concentration is tightly regulated and poorly reflects tissue levels, and circulating parathyroid hormone reflects functional $1,25(OH)_2D_3$ deficiency at only one of many relevant biological sites.[6–8]

Vitamin D clearance may offer a valuable new tool to guide clinical diagnosis and treatment of vitamin D deficiency in persons with CKD.[9] The CYP24A1 enzyme is normally responsible for the majority of vitamin D clearance. CYP24A1 expression is used as a readout of tissue-level $1,25(OH)_2D_3$ activity in animal studies because $1,25(OH)_2D_3$ potently induces this enzyme,[10] and 24,25-dihydroxyvitamin D_3 ($24,25[OH]_2D_3$) is the predominant initial product of $25(OH)D_3$ clearance by CYP24A1. Therefore, the ratio of $24,25(OH)_2D_3$ to $25(OH)D_3$ (the VDMR) is used as a measure of CYP24A1-mediated vitamin D clearance that may reflect tissue-level $1,25(OH)_2D_3$ activity.[11,12]

Previous studies have shown that lower GFR and black race are associated with lower circulating concentrations of $24,25(OH)_2D_3$, independent of 25(OH)D_3.[11,13–15] In addition, low circulating $24,25(OH)_2D_3$ was independently associated with increased risks of secondary hyperparathyroidism and death.[16] However, these previous studies were limited by a relatively small number of participants with CKD, single measures of vitamin D metabolites, and limited numbers of important clinical outcomes. Therefore, in this study, we tested associations of baseline and time-updated VDMR with risk of progression to ESRD and death in a large, well-characterized cohort of people with CKD.

METHODS

Study Population

This study was a prospective ancillary study of the CRIC, an ongoing multicenter prospective cohort study of persons with CKD recruited from 7 clinical centers (with 13 enrolling sites).[17,18] The CRIC study initially enrolled patients with CKD who had an eGFR of 20 to 70 ml/min per 1.73 m^2 by the Modification of Diet in Renal Disease study equation. Exclusion criteria included New York Heart Association class III or IV heart failure. Institutional Review Board approval was obtained from all participating institutions, and written informed consent to participate in CRIC was obtained from all subjects.

This study utilized a case-cohort study design (Figure 1). The cohort consisted of randomly selected participants in the CRIC Mineral Metabolism Subcohort ($N = 1527$)[19–21] who attended the 1-year study visit and also had serum available for measurements ($N = 1080$). We also sampled 444 additional participants who progressed to ESRD and 413 additional participants who died between year 1 and March 31, 2013, to increase power to test associations with ESRD and death.

Exposure

We measured serum vitamin D metabolites at CRIC study years 1 and 4 to evaluate the association of baseline and time-updated measures of vitamin D metabolites with our outcomes of interest. All measurements were performed at the University of Washington using a multiplex high-performance liquid chromatography mass spectrometry assay that simultaneously measures $24,25(OH)_2D_3$, $25(OH)D_3$, $25(OH)D_2$, $1,25(OH)_2D_3$, and $1,25(OH)_2D_2$ on a Xevo TQ spectrometer (Waters Corp., Milford, MA) using immunoaffinity extraction and deuterated internal standards.[22] The interassay coefficients of variation of the 5 vitamin D metabolites ranged from 3.9% to 16.1% over several different concentrations. Our primary exposure was the ratio of serum $24,25(OH)_2D_3$ to $25(OH)D_3$, (in pg/ng) or VDMR, which was interpreted as a measure of CYP24A1-mediated 25(OH)D clearance. We calculated total serum 25(OH)D as the sum $25(OH)D_2$ and $25(OH)D_3$ and total $1,25(OH)_2D$ as the sum $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$.

Outcomes

Our primary outcomes included progression to ESRD and all-cause mortality. ESRD was identified through participant self-report, medical records review, and data from the United States Renal Data System. Deaths were identified from report from next of kin, retrieval of death certificates or obituaries, review of hospital or outpatient records, and search of Social Security death vital status and state death certificate files, if available. For the present study, follow-up was through March 31, 2013.

Covariates

Covariates were assessed concurrently with vitamin D metabolites. Participants provided information on their sociodemographic characteristics, medical history, medication usage, and lifestyle behaviors. Race/ethnicity was categorized as non-Hispanic white, non-Hispanic black, Hispanic, or other. Comorbid diseases and medication use was ascertained by detailed participant questionnaires. Diabetes mellitus was defined as a fasting glucose >126 mg/dl, a nonfasting glucose >200 mg/dl, or use of insulin or another antidiabetic medication. Anthropometric measurements and blood pressure were assessed using standard protocols.[23] Serum creatinine concentration was measured using an enzymatic method on a Vitros 950 Chemistry Analyzer (Ortho-Clinical Diagnostics,

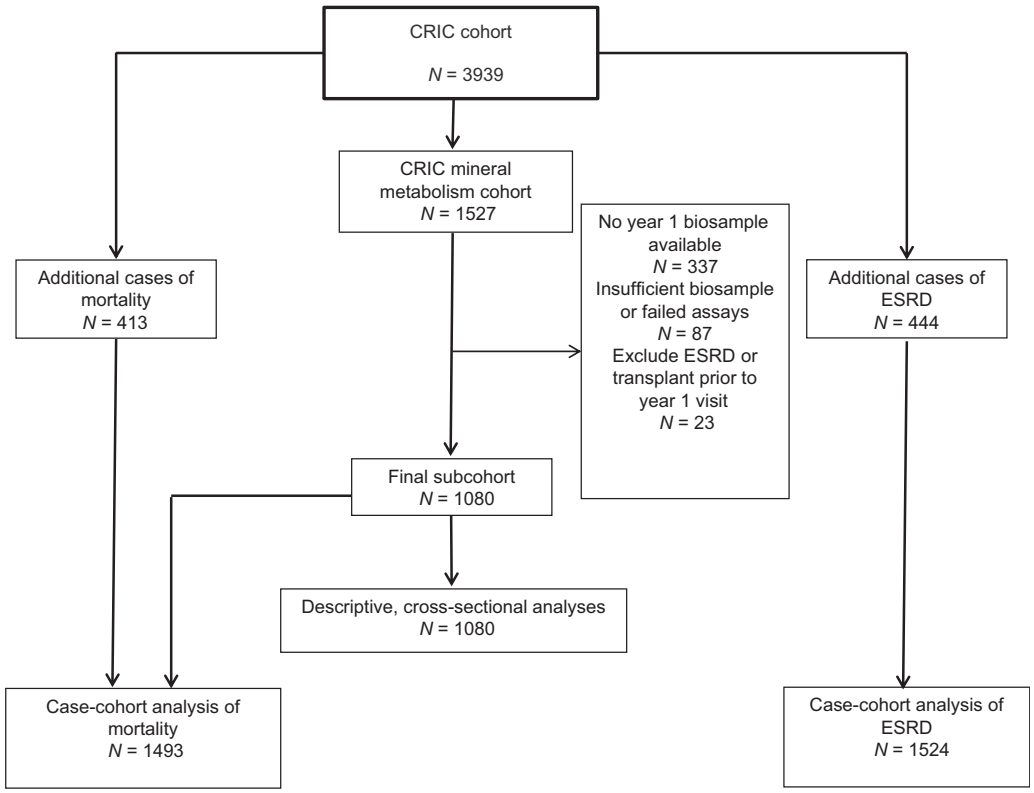

Figure 1. Cohort assembly. CRIC, Chronic Renal Insufficiency Cohort; ESRD, end-stage renal disease.

Raritan, NJ) at the CRIC Central Laboratory and standardized to isotope dilution mass spectrometry–traceable values.[24–26] Estimated GFR was calculated from serum creatinine and cystatin C using a CRIC Study equation.[26] Additional assays included serum phosphorus, 24-hour urine total protein, C-terminal fibroblast growth factor-23 (FGF-23), and total parathyroid hormone (PTH).[20,21]

Statistical Approach

Using the random subcohort, we first described characteristics of participants overall and across categories of VDMR. We then evaluated the correlations of VDMR with other vitamin D metabolites, PTH, and FGF-23 using the Spearman correlation. We generated scatterplots of VDMR versus eGFR and urine PCR. We then reported associations of participant characteristics with VDMR using multivariable linear regression, including age, sex, race, diabetes, prevalent cardiovascular disease, smoking status, body mass index, eGFR, urine PCR, PTH, FGF-23, and medication use (calciferols, vitamin D receptor activators, and cinacalcet). Models were adjusted for age (continuous), sex, race/ethnicity (4 categories), diabetes (yes/no), and eGFR (continuous). We then described the change in VDMR from year 1 to year 4 in the subcohort. We examined the association of baseline participants' characteristics with change in VDMR using linear regression, adjusting for age, sex, race/ethnicity, diabetes, and eGFR.

We reported incident rates of our primary outcomes (death and ESRD) in the subcohort. We generated Kaplan-Meier curves to evaluate survival and ESRD-free survival among participants in the random subcohort across categories of $24,25(OH)_2D_3/25(OH)D_3$.

With use of inverse probability weighting to account for the case-cohort study design,[27] we then tested the association of VDMR with risks of death and ESRD. VDMR was modeled continuously (per 20 pg/ng decrement, approximately 1 SD) and in thirds. In secondary analyses, we modeled the association of time-updated VDMR with risk of death and ESRD. We performed nested models, adjusting for covariates ascertained concurrent with vitamin D metabolites. In model 1, we adjusted for demographics, diabetes, systolic blood pressure, number of hypertension medication classes, prevalent cardiovascular disease (which included heart failure, myocardial infarction, stroke, and peripheral artery disease), smoking status, use of renin-angiotensin-aldosterone inhibitors, use of statins, and use of calciferols and vitamin D receptor activators. Model 2 adjusted for covariates in model 1 plus eGFR and urine PCR. Finally, because vitamin D metabolism, PTH, and FGF-23 are interrelated through complex endocrine feedback loops, model 3 was a mediation model, in which we additionally adjusted for FGF-23 and PTH.

We tested for interactions by black versus non-black race because prior data have suggested that the association of vitamin D metabolites with clinical outcomes may differ by race.[28]

In a sensitivity analysis, we tested the association of $24,25(OH)_2D_3$ with risk of ESRD and death, adjusting for $25(OH)D_3$ (rather than the VDMR).

The following software was used for the analyses: IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY); Stata 13 Statistical Software (StataCorp., College Station, TX); and R: a language and environment for statistical computing (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/).

RESULTS

Study Population

Among the 1080 participants in the subcohort, mean age at the first annual CRIC study visit was 59 years, 42% were black, and 43% were female. Approximately half the study population had diabetes, and 31% had prevalent cardiovascular disease (Table 1). Only 12% were taking calciferols, and 6% were taking vitamin D

Table 1. Characteristics of a subcohort of CRIC study participants by categories of vitamin D metabolic ratio ($24,25[OH]_2D_3$:$25[OH]D_3$)

Characteristic	Total	$24,25(OH)_2D_3/25(OH)D_3$ (pg/ng)		
		T1	T2	T3
$24,25(OH)_2D_3$:$25(OH)D_3$ range (pg/ng)		0.00–26.47	26.48–43.16	43.17–136.97
N	1080	348	365	367
24,25(OH)2D3, ng/ml, mean (SD)	0.78 (0.69)	0.26 (0.20)	0.65 (0.37)	1.41 (0.75)
25(OH)D3, ng/ml, mean (SD)	18.6 (10.4)	13.5 (8.1)	18.6 (9.9)	23.5 (10.6)
Demographics				
Age, yr, mean (SD)	59 (11)	58 (11)	61 (10)	58 (10)
Female, *N* (%)	464 (43)	151 (43)	152 (42)	161 (44)
Race/ethnicity, *N* (%)				
Non-Hispanic white	463 (43)	99 (28)	148 (41)	216 (59)
Non-Hispanic black	457 (42)	197 (57)	152 (42)	108 (29)
Hispanic	124 (12)	44 (13)	51 (14)	29 (8)
Other	36 (3)	8 (2)	14 (4)	14 (4)
Medical history, *N* (%)				
Diabetes	534 (49)	203 (58)	205 (56)	126 (34)
Current smoker	113 (11)	53 (15)	30 (8)	30 (8)
Prevalent cardiovascular disease	362 (34)	123 (35)	142 (39)	97 (26)
Prevalent heart failure	98 (9)	33 (10)	37 (10)	28 (8)
Prevalent myocardial infarction	242 (22)	85 (24)	85 (23)	72 (20)
Prevalent stroke	108 (10)	38 (11)	43 (12)	27 (7)
Prevalent peripheral arterial disease	69 (6)	21 (6)	35 (10)	13 (4)
Hypertension	960 (89)	325 (93)	339 (93)	296 (81)
Medications, *N* (%)				
Calciferols	132 (12)	28 (8)	37 (10)	67 (18)
Vitamin D receptor agonists	69 (6)	36 (10)	29 (8)	4 (1)
Cinacalcet	2 (0.2)	2 (0.6)	0 (0)	0 (0)
Phosphate binders				
Calcium-based	68 (6)	35 (10)	10 (3)	23 (6)
Non–calcium-based	2 (0.2)	1 (0.3)	0 (0)	1 (0.3)
Physical examination, mean (SD)				
Body mass index, kg/m^2	32.3 (8.1)	34.0 (9.4)	32.7 (7.7)	30.2 (6.5)
Systolic blood pressure, mm Hg	126 (21)	129 (22)	129 (22)	121 (19)
Diastolic blood pressure, mm Hg	70 (14)	71 (13)	69 (14)	70 (13)
Laboratory data				
eGFR CKD-EPI, ml/min per 1.73 m^2, mean (SD)	43 (16)	35 (14)	41 (14)	52 (14)
Proteinuria, median [IQR] g/24 hr	0.13 [0.05–0.72]	0.28 [0.08–1.11]	0.13 [0.05–0.66]	0.08 [0.04–0.34]
Calcium, mg/dl, mean (SD)	9.3 (0.5)	9.2 (0.6)	9.3 (0.5)	9.4 (0.4)
Phosphate, mg/dl, mean (SD)	3.69 (0.64)	3.75 (0.69)	3.75 (0.66)	3.57 (0.55)
FGF-23, pg/ml, median [IQR]	128 [85–217]	166 [107–308]	137 [86–217]	101 [69–153]
Total 25(OH)D, ng/ml, mean (SD)	20.6 (10.7)	15.0 (8.7)	20.8 (10.3)	25.7 (10.2)
Total 1,25(OH)D, pg/ml, mean (SD)	32.0 (14.5)	32.4 (15.0)	31.1 (13.8)	32.4 (14.6)
Intact PTH, pg/ml, median [IQR]	62 [41–99]	93 [64–161]	65 [45–96]	44 [29–62]

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRIC, Chronic Renal Insufficiency Cohort; eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; IQR, interquartile range; PTH, parathyroid hormone.

receptor agonists. Participants in the lowest tertile of VDMR were more likely to be black, be current smokers, take vitamin D receptor agonists, and have a lower eGFR, higher urine PCR, higher FGF-23, lower total 25(OH)D, and lower urinary calcium excretion (Table 1).

Correlates of Serum VDMR Ratio

The VDMR ratio was most strongly correlated with eGFR (correlation coefficient 0.49), PTH (–0.53), and FGF-23 (–0.36) and weaker with urine PCR (correlation coefficient –0.28; Table 2 and Supplementary Figure S1). In models adjusted for age, sex, race/ethnicity, diabetes, and eGFR, the following characteristics were significantly associated with lower VDMR: younger age, black race, diabetes, tobacco use, higher body mass index, lower eGFR, and higher PTH (Supplementary Table S1).

Change in Serum VDMR Ratio

Overall, the median (interquartile range) absolute change in VDMR from year 1 to year 4 was a median [interquartile range] decline of 1.11 [–10.30, 7.23] pg/ng (Supplementary Figure S2). The unadjusted absolute change in VDMR from year 1 to year 4 was greatest in participants who were older; male; Hispanic; diabetic; had cardiovascular disease; were smokers; were taking calciferol; were not taking vitamin D receptor agonists, cinacalcet, or phosphate binders; and had higher body mass index and lower eGFR. In adjusted models, higher baseline urine PCR and higher baseline PTH were significantly associated with a greater decline in VDMR (Supplementary Table S2). Initiation of a calciferol supplement between years 1 and 4 was associated with an increase in VDMR (Supplementary Table S3).

End-Stage Renal Disease

A total of 708 ESRD events occurred over a mean (SD) follow-up period of 4.9 (2.9) years. The unadjusted incidence rate of ESRD was highest among participants in the lowest tertile of VDMR (Figure 2a and Table 3). In models adjusted for demographics, comorbidity, and pertinent medication use, participants in the lowest tertile of VDMR had a greater risk of ESRD compared with those in the highest tertile. However, with additional adjustment for eGFR and urine PCR, the association between VDMR and risk of ESRD was attenuated and no longer statistically significant (Table 3). There was no significant heterogeneity by race.

In unadjusted models with time-updated $24,25(OH)_2D_3/25(OH)D_3$, the association of VDMR with risk of ESRD was even stronger than that observed with baseline VDMR (HR, 2.26; 95% CI, 2.01–2.54 per every 20 pg/ng decrement). Similarly to that seen with the baseline VDMR models, the association of time-updated VDMR with risk of ESRD was attenuated with adjustment for eGFR and urine PCR and no longer statistically significant (Supplementary Table S4).

In a sensitivity analysis, we examined the association of $24,25(OH)_2D_3$ with risk of ESRD, also adjusting for $25(OH)D_3$. The results of this analysis were similar to results of the primary analysis (Supplementary Table S5).

Mortality

There were 650 deaths over a mean (SD) follow-up period of 6.5 (2.5) years. When VDMR was modeled continuously, a significant association was found between VDMR and risk of mortality in model 1, which adjusted for potential confounders (HR, 1.18; 95% CI, 1.02–1.36 per every 20 pg/ng decrement in $24,25[OH]_2D_3/25[OH]D_3$). This association was attenuated but remained statistically significant after adjustment for eGFR and urine PCR (HR, 1.17; 95% CI, 1.01–1.36 per every 20 pg/ng decrement in $24,25[OH]_2D_3/25[OH]D_3$). Little change occurred in the risk estimate with further adjustment for possible mediators PTH and FGF-23 (Table 3). There was no significant heterogeneity by race.

In models with time-updated $24,25(OH)_2D_3/25(OH)D_3$, the association of VDMR with risk of death was similar to that observed for baseline VDMR (Supplementary Table S4).

In a sensitivity analysis, we examined the association of $24,25(OH)_2D_3$ with risk of death, also adjusting

Table 2. Correlation matrix of vitamin D metabolites and other mineral metabolism and kidney function measures in subcohort

Measure of vitamin D metabolite or kidney function	$24,25(OH)_2D_3$	$25(OH)D_3$	$24,25(OH)_2D_3/25(OH)D_3$	eGFR	PCR	FGF-23	PTH
$24,25(OH)_2D_3$	1.00	0.87[a]	0.83[a]	0.35[a]	–0.30[a]	–0.28[a]	–0.52[a]
$25(OH)D_3$		1.00	0.46[a]	0.11[a]	–0.24[a]	–0.17[a]	–0.36[a]
$24,25(OH)_2D_3/25(OH)D_3$			1.00	0.49[a]	–0.27[a]	–0.31[a]	–0.53[a]
eGFR				1.00	–0.39	–0.52[a]	–0.53[a]
PCR					1.00	–0.33[a]	0.36[a]
FGF-23						1.00	0.36[a]
PTH							1.00

eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone.
[a]Correlation significant at the 0.01 level.

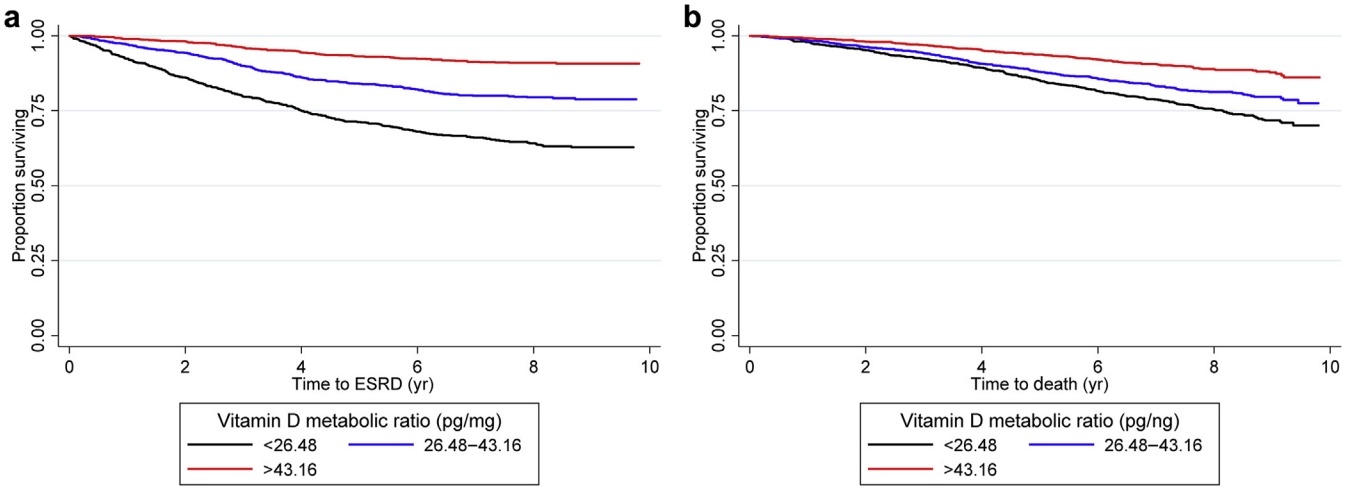

Figure 2. Kaplan-Meier curves for (a) end-stage renal disease (ESRD)–free survival and (b) overall survival across categories of vitamin D metabolic ratio ($24,25[OH]_2D_3/25[OH]D_3$) in the subcohort.

for $25(OH)D_3$. The results of this analysis were similar to results of the primary analysis (Supplementary Table S5).

DISCUSSION

In this longitudinal study of participants with prevalent CKD, we found that lower baseline serum $24,25(OH)_2D_3/25(OH)D_3$, reflecting reduced CYP24A1-mediated vitamin D clearance, was modestly but significantly associated with all-cause mortality. Lower eGFR and higher urine PCR were strongly associated with lower VDMR in cross-sectional analyses, but VDMR was not associated with risk of ESRD after adjustment for eGFR and urine PCR. These data suggest that reduced vitamin D clearance, as measured by serum $24,25(OH)_2D_3/25(OH)D_3$, is a consequence but not a cause of progressive CKD. Furthermore, these data suggest that reduced vitamin D clearance (or related abnormalities in vitamin D metabolism) may be a mortality risk factor in persons with CKD.

The strong cross-sectional correlation of eGFR with serum $24,25(OH)_2D_3/25(OH)D_3$, which has been previously observed,[11,13–15,29] probably largely reflects reduced renal production of serum $24,25(OH)2D_3$ in CKD. Vitamin D metabolites bound to vitamin D binding globulin are filtered and reabsorbed into proximal tubular cells via megalin and cubilin,[30] and flux through this pathway likely decreases with reduced glomerular filtration. In a study of anephric pigs that were given cholecalciferol, the rise in circulating $24,25(OH)_2D_3$ concentration was delayed and concentrations were lower than in control pigs.[31] In addition, a study of humans found a 22% lower metabolic clearance rate of 1,25(OH)D, which is also cleared by CYP24A1, in persons who had CKD compared with normal control subjects.[32] In this study, we also noted a significant inverse correlation

Table 3. Associations of vitamin D metabolic ratio ($24,25[OH]_2D_3/25[OH]D_3$) with risk of ESRD and death in case cohort

$24,25(OH)_2D_3/25(OH)D_3$ ratio	No. of events	Incidence rate (%/yr)[a]	HR (95% CI) model 1	HR (95% CI) model 2	HR (95% CI) model 3 (mediation)
ESRD					
Tertile 1	371	7.00	3.20 (2.41–4.27)	0.88 (0.63–1.23)	0.79 (0.55–1.16)
Tertile 2	225	4.42	1.90 (1.43–2.52)	0.89 (0.64–1.26)	0.75 (0.51–1.09)
Tertile 3	112	1.32	Ref	Ref	Ref
Per 20 pg/ng (1 SD) decrement			1.80 (1.56–2.08)	0.94 (0.81–1.10)	0.86 (0.72–1.02)
Death					
Tertile 1	278	4.80	1.10 (0.80–1.51)	1.09 (0.79–1.50)	1.06 (0.72–1.56)
Tertile 2	227	3.26	1.19 (0.89–1.58)	1.15 (0.86–1.54)	1.10 (0.78–1.55)
Tertile 3	145	1.49	Ref	Ref	Ref
Per 20 pg/ng (1 SD) decrement			1.18 (1.02–1.36)	1.17 (1.01–1.36)	1.18 (0.99–1.41)

CI, confidence interval; ESRD, end-stage renal disease; HR, hazard ratio; Ref, reference.
[a]Incidence rate is based on the subcohort only.
Model 1: Adjusted for age, sex, race, diabetes, systolic blood pressure, number of antihypertensive medication classes, prevalent cardiovascular disease, smoking status, renin-angiotensin-aldosterone inhibitors, statin use, calciferol use, and vitamin D receptor activators.
Model 2: Model 1 + estimated glomerular filtration rate and urine protein-to-creatinine ratio.
Model 3: Model 2 + parathyroid hormone and fibroblast growth factor-23.

of urine PCR with serum $24,25(OH)_2D_3$. It is possible that proteinuria impairs recovery or metabolism of filtered vitamin D metabolites. Instead of or in addition to CKD causing low serum VDMR through reduced renal production, lower serum $24,25(OH)_2D_3$ may reflect decreased vitamin D clearance in non-kidney tissues as a result of systemic 1,25(OH)2D deficiency. Either way, it is likely that low serum VDMR is an overall marker of impaired CKD-related vitamin D metabolism.

Contrary to our hypothesis, we did not find evidence that impaired CKD-related vitamin D metabolism, manifest as low serum $24,25(OH)_2D_3/25(OH)D_3$, was independently associated with progression to ESRD. Animal studies have demonstrated that impaired 1,25(OH)2D signaling promotes kidney injury.[33–35] Epidemiologic studies have observed that low circulating concentrations of 25(OH)D and $1,25(OH)_2D$ are associated with increased risks of albuminuria and CKD progression,[36–39] and $1,25(OH)_2D$ analogues reduced proteinuria in clinical trials.[34,40,41] However, our findings suggest that, whereas CKD is strongly associated with low $24,25(OH)_2D_3/25(OH)D_3$, low VDMR is not a risk factor for CKD progression, independent of baseline eGFR and urine PCR. It is plausible that other confounders such as variability of vitamin D metabolites and use of other therapies (e.g., renin-angiotensin-aldosterone inhibitors) may have influenced our findings.

We found that lower VDMR was significantly associated with greater risk of all-cause mortality. From experimental work, pleiotropic actions of impaired vitamin D are well recognized.[42–47] These actions include broad effects on cell differentiation and proliferation, immune cell function, and the renin-angiotensin system. Epidemiologic studies in persons with CKD and non-CKD populations suggest that low circulating concentrations of 25(OH)D and $1,25(OH)_2D$ are associated with increased risks of heart failure, atherosclerotic cardiovascular disease events, and death.[15,16,37,38,48–55] Thus our study provides further evidence that impaired CKD-related vitamin D metabolism may have adverse clinical consequences.

Serum VDMR ultimately could serve as a clinically useful biomarker. Compared with circulating concentrations of 25(OH)D (which is relatively inactive), $1,25(OH)_2D_3$ (which is tightly regulated), and PTH (which is variable and influenced by many factors), serum VDMR may reflect a more useful aspect of CKD-related impaired vitamin D metabolism or tissue-level 1,25(OH)2D deficiency. To this point, one recent study of older adults found that lower VDMR was associated with higher risk of hip fracture; however, no association was seen with 25(OH)D.[56]

Ideally, clinically useful biomarkers should be modifiable and identify which patients derive clinical benefit from available therapeutic interventions, such as vitamin D supplementation. We and other investigators have shown that cholecalciferol, ergocalciferol, 1,25(OH)2D3, or paricalcitol each increase the circulating VDMR ratio.[57–60] The increase in serum VDMR ratio observed in this study among CRIC participants who initiated cholecalciferol between study year 1 and 4 is consistent with this literature. However, no available biomarker has been shown to identify patient subsets who are likely to derive clinical benefits from vitamin D–related interventions; this subject requires further study.

There are well-known differences by race in vitamin D metabolites. Blacks have lower levels of 25(OH)D because the melanin-rich skin reduces absorption of ultraviolet B light needed for vitamin D synthesis.[61] We and other investigators have reported that blacks had significantly lower VDMR compared with whites, suggesting that reduced vitamin D clearance may help compensate for reduced vitamin D production.[28,62] In the present study, black participants had lower VDMR than did whites, but we did not note significant interactions by race of VDMR with study outcomes.

Our study had several strengths. We studied a large, well-characterized cohort of patients who had CKD with longitudinal follow-up. Vitamin D metabolites were measured longitudinally using an established and precise mass spectrometry assay. We were able to consider a broad range of potential confounders in our analysis. We recognize some limitations as well. We cannot be certain that VDMR accurately assesses vitamin D clearance. Although we could adjust for use of vitamin D supplementation, doses and duration of therapies were not known. Finally, this study was observational, and we cannot determine whether the association of VDMR with mortality is causal in nature.

In conclusion, our data suggest that impaired CYP24A1-mediated vitamin D clearance, measured as low serum $24,25(OH)_2D_3/25(OH)D_3$, is a consequence of CKD but not a risk factor for progression of established CKD. Moreover, lower VDMR was significantly associated with all-cause mortality, providing further evidence that impaired vitamin D metabolism may be an important pathway through which CKD increases cardiovascular risk. Further studies are needed to determine whether circulating VDMR may be a clinically actionable measure of impaired vitamin D metabolism in CKD.

APPENDIX

CRIC Study Investigators

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DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Cross-sectional associations of clinical characteristics with vitamin D metabolic ratio ($24,25[OH]_2D_3/25[OH]D_3$).

Table S2. Association of baseline clinical characteristics with change in vitamin D metabolic ratio ($24,25[OH]_2D_3/25[OH]D_3$).

Table S3. Association of change in baseline clinical characteristics with change in vitamin D metabolic ratio ($24,25[OH]_2D_3/25[OH]D_3$).

Table S4. Association of time-updated vitamin D metabolic ratio ($24,25[OH]_2D_3/25[OH]D_3$) with risk of end-stage renal disease and death.

Table S5. Associations of $24,25(OH)_2D_3$ with risk of end-stage renal disease and death in the case cohort.

Figure S1. Scatterplots of 24,25(OH)2D3/24(OH)D3 with (A) estimated glomerular filtration rate and (B) urine polymerase chain reaction.

Figure S2. Histogram of change in vitamin D metabolic ratio ($24,25[OH]_2D_3/25[OH]D_3$) from year 1 to year 4.

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