Vitamin D Metabolic Ratio and Risks of Death and CKD Progression

Nisha Bansal¹, Ronit Katz¹, Lawrence Appel², Michelle Denburg³, Harold Feldman⁴, Alan S. Go⁵, Jiang He⁶, Andrew Hoofnagle⁷, Tamara Isakova⁶, Bryan Kestenbaum¹, John Kusek⁹, James Lash¹⁰, Mary Leonard¹¹, Mahboob Rahman¹², Cassianne Robinson-Cohen¹³, Myles Wolf¹⁴, Dawei Xie¹⁵, Leila Zelnick¹ and Ian H. de Boer¹; on behalf of the CRIC Study Investigators¹⁶

¹Division of Nephrology, Department of Medicine, University of Washington, Seattle, Washington, USA; ²Department of Medicine and Epidemiology, Johns Hopkins University, Baltimore, Maryland, USA; ³Division of Nephrology, Department of Pediatrics, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania, USA; ⁴University of Pennsylvania, Philadelphia, Pennsylvania, USA; ⁵Kaiser Permanente Northern California, Oakland, California, USA; ⁶Division of Nephrology, Department of Medicine, Tulane University, New Orleans, Louisiana, USA; ⁷Division of Clinical Chemistry, University of Washington, Seattle, Washington, USA; ⁸Division of Nephrology, Department of Medicine, Northwestern University, Evanston, Illinois, USA; ⁹National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, USA; ¹⁰Division of Nephrology, Department of Medicine, University of Chicago, Chicago, Illinois, USA; ¹¹Division of Nephrology, Department of Pediatrics, Stanford University, Stanford, California, USA; ¹²Division of Nephrology, Department of Medicine, Case Western Reserve University, Cleveland, Ohio, USA; ¹³Division of Nephrology, Department of Medicine, Vanderbilt University, Nashville, Tennessee, USA; ¹⁴Division of Nephrology, Department of Medicine, Duke University, Durham, North Carolina, USA; and ¹⁵Department of Biostatistics, University of Pennsylvania, Philadelphia, Pennsylvania, USA

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]2D3/25[OH]D3) 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.

Kidney Int Rep (2019) 4, 1598–1607; https://doi.org/10.1016/j.ekir.2019.08.014
KEYWORDS: kidney; mortality; vitamin D
© 2019 International Society of Nephrology. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
and its consequences, clinical decision making is limited by the lack of an effective measurement of functional 1,25(OH)2D3 deficiency; 25-hydroxyvitamin D3 (25[OH]D3) is a relatively inactive substrate form of vitamin D, circulating 1,25(OH)2D3 concentration is tightly regulated and poorly reflects tissue levels, and circulating parathyroid hormone reflects functional 1,25(OH)2D3 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)2D3 activity in animal studies because 1,25(OH)2D3 potently induces this enzyme,10 and 24,25-dihydroxyvitamin D3 (24,25[OH]2D3) is the predominant initial product of 25(OH)D3 clearance by CYP24A1. Therefore, the ratio of 24,25(OH)2D3 to 25(OH)D3 (the VDMR) is used as a measure of CYP24A1-mediated vitamin D clearance that may reflect tissue-level 1,25(OH)2D3 activity.11,12

Previous studies have shown that lower GFR and black race are associated with lower circulating concentrations of 24,25(OH)2D3, independent of 25(OH)D3.13–15 In addition, low circulating 24,25(OH)2D3 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 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)2D3, 25(OH)D3, 25(OH)D2, 1,25(OH)2D3, and 1,25(OH)2D2 on a Xevo TQ spectrometer (Waters Corp., Milford, MA) using immunoaffinity extraction and deuterated internal standards.22 The interest-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)2D3 to 25(OH)D3, (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)D2 and 25(OH)D3 and total 1,25(OH)2D as the sum 1,25(OH)2D2 and 1,25(OH)2D3.

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

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/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)2D3/25(OH)D3.

With use of inverse probability weighting to account for the case-cohort study design, 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.

Figure 1. Cohort assembly. CRIC, Chronic Renal Insufficiency Cohort; ESRD, end-stage renal disease.
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)2D3 with risk of ESRD and death, adjusting for 25(OH)D3 (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)2D3:25(OH)D3)

| Characteristic | Total | T1 | T2 | T3 |
|---------------|-------|----|----|----|
| 24,25(OH)2D3:25(OH)D3 (pg/ng) | 0.00–26.47 | 28.48–43.16 | 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) | 52 (15) | 30 (8) | 30 (8) |
| Prevalent cardiovascular disease | 362 (34) | 122 (35) | 142 (39) | 97 (26) |
| Prevalent heart failure | 96 (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/m² | 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 m², mean (SD) | 43 (16) | 36 (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 [88–217] | 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) decline 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)2D3/25(OH)D3, 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)2D3 with risk of ESRD, also adjusting for 25(OH)D3. 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)2D3/25(OH)D3). 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)2D3/25(OH)D3). 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)2D3/25(OH)D3, 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)2D3 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)2D3 | 25(OH)D3 | 24,25(OH)2D3/25(OH)D3 | eGFR | PCR | FGF-23 | PTH |
|-------------------------------------------------|-------------|----------|------------------------|-----|-----|--------|-----|
| 24,25(OH)2D3                                   | 1.00        | 0.87a    | 0.83a                  | 0.35a| −0.30a| −0.28a | −0.52a|
| 25(OH)D3                                        | 1.00        | 0.46a    | 0.11a                  | −0.24a| −0.17a| −0.36a | −0.52a|
| 24,25(OH)2D3/25(OH)D3                           | 1.00        | 0.49a    | −0.27a                 | −0.31a| −0.53a|        |      |
| eGFR                                           | 1.00        | −0.39    | −0.52a                 | −0.53a|        |        |      |
| PCR                                            | 1.00        | −0.33a   | 0.36a                  |      |      |        |      |
| FGF-23                                         | 1.00        | 0.36a    |                        |      |      |        |      |
| PTH                                            | 1.00        |          |                        |      |      |        |      |

*Correlation significant at the 0.01 level.

eGFR, estimated glomerular filtration rate; FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone.
for 25(OH)D₃. 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)₂D₃/25(OH)D₃, 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)₂D₃/25(OH)D₃, 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)₂D₃/25(OH)D₃, which has been previously observed, likely largely reflects reduced renal production of serum 24,25(OH)₂D₃ in CKD. Vitamin D metabolites bound to vitamin D binding globulin are filtered and reabsorbed into proximal tubular cells via megalin and cubilin, 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)₂D₃ concentration was delayed and concentrations were lower than in control pigs. 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. In this study, we also noted a significant inverse correlation

![Figure 2](image_url). 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)₂D₃/25(OH)D₃) in the subcohort.

| 24,25(OH)₂D₃/25(OH)D₃ ratio | No. of events | Incidence rate (%/yr) | 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.

*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)2D3. 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)2D3 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)2D3/25(OH)D3, was independently associated with progression to ESRD. Animal studies have demonstrated that impaired 1,25(OH)2D signaling promotes kidney injury. 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, and 1,25(OH)2D analogues reduced proteinuria in clinical trials. However, our findings suggest that, whereas CKD is strongly associated with low 24,25(OH)2D3/25(OH)D3, 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. 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. 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 (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. 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. 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. 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. 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)2D3/25(OH)D3, 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**

CRIC Study Investigators include Lawrence J. Appel, Harold I. Feldman, Alan S. Go, Jiang He, John W.
Kusek, James P. Lash, Panduranga S. Rao, Mahboob Rahman, and Raymond R. Townsend.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

This work was supported by R01DK099199 (IHdB). Funding for the CRIC Study was obtained under a cooperative agreement from National Institute of Diabetes and Digestive and Kidney Diseases (U01DK060990, U01DK060984, U01DK061022, U01DK061021, U01DK061028, U01DK060980, U01DK060963, and U01DK060992). In addition, this work was supported in part by the Perelman School of Medicine at the University of Pennsylvania Clinical and Translational Science Award National Institutes of Health/National Center for Advancing Translational Sciences (NIH/NCATS) UL1TR000003, Johns Hopkins University UL1TR-000424, University of Maryland GCRC M01 RR-16500, Clinical and Translational Science Collaborative of Cleveland, UL1TR000439 from the NCATS component of the NIH and NIH roadmap for Medical Research, Michigan Institute for Clinical and Health Research UL1TR000433, University of Illinois at Chicago CTSA UL1RR029879, Tulane COBRE for Clinical and Translational Research in Cardiometabolic Diseases P20 GM109036, and Kaiser Permanente NIH/National Center for Research Resources UCSF-CTSI UL1RR-024131.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Cross-sectional associations of clinical characteristics with vitamin D metabolic ratio (24,25[OH]2D3/25[OH]D3).

Table S2. Association of baseline clinical characteristics with change in vitamin D metabolic ratio (24,25[OH]2D3/25[OH]D3).

Table S3. Association of change in baseline clinical characteristics with change in vitamin D metabolic ratio (24,25[OH]2D3/25[OH]D3).

Table S4. Association of time-updated vitamin D metabolic ratio (24,25[OH]2D3/25[OH]D3) with risk of end-stage renal disease and death.

Table S5. Associations of 24,25(OH)2D3 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]2D3/25[OH]D3) from year 1 to year 4.

REFERENCES

1. Kodicek E, Lawson DE, Wilson PW. Biological activity of a polar metabolite of vitamin D. Nature. 1970;228:763–764.

2. Myrtle JF, Haussler MR, Norman AW. Evidence for the biologically active form of cholecalciferol in the intestine. J Biol Chem. 1970;245:1190–1196.

3. Blunt JW, Tanaka Y, DeLuca HF. Biological activity of 25-hydroxycholecalciferol, a metabolite of vitamin D3. Proc Natl Acad Sci U S A. 1968;61:1503–1506.

4. Fraser DR, Kodicek E. Unique biosynthesis by kidney of a biological active vitamin D metabolite. Nature. 1970;228:764–766.

5. Gray R, Boyle I, DeLuca HF. Vitamin D metabolism: the role of kidney tissue. Science. 1971;172:1232–1234.

6. Palmer SC, McGregor DO, Macaskill P, et al. Meta-analysis: vitamin D compounds in chronic kidney disease. Ann Intern Med. 2007;147:840–853.

7. Silver J, Naveh-Many T, Mayer H, et al. Regulation by vitamin D metabolites of parathyroid hormone gene transcription in vivo in the rat. J Clin Invest. 1986;78:1296–1301.

8. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266–281.

9. 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.

10. Knutson JC, DeLuca HF. 25-Hydroxyvitamin D3-24-hydroxylase. Subcellular location and properties. Biochemistry. 1974;13:1543–1548.

11. Bosworth CR, Levin G, Robinson-Cohen C, et al. The serum 24,25-dihydroxyvitamin D concentration, a marker of vitamin D catabolism, is reduced in chronic kidney disease. Kidney Int. 2012;82:693–700.

12. Binkley N, Lappe J, Singh RJ, et al. Can vitamin D metabolite measurements facilitate a “treat-to-target” paradigm to guide vitamin D supplementation? Osteoporos Int. 2015;26:1655–1660.

13. de Boer IH, Sachs MC, Cleary PA, et al. Circulating vitamin D metabolites and kidney disease in type 1 diabetes. J Clin Endocrinol Metab. 2012;97:4780–4788.

14. Sachs MC, Shoben A, Levin GP, 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.

15. Kestenbaum B, Katz R, de Boer I, et al. Vitamin D, parathyroid hormone, and cardiovascular events among older adults. J Am Coll Cardiol. 2011;58:1433–1441.

16. de Boer IH, Levin G, Robinson-Cohen C, et al. Serum 25-hydroxyvitamin D concentration and risk for major clinical disease events in a community-based population of older adults: a cohort study. Ann Intern Med. 2012;156:627–634.

17. Feldman HI, Appel LJ, Chertow GM, et al. The Chronic Renal Insufficiency Cohort (CRIC) Study: design and methods. J Am Soc Nephrol. 2003;14:S148–S153.

18. Lash JP, Go AS, Appel LJ, et al. Chronic Renal Insufficiency Cohort (CRIC) Study: baseline characteristics and associations with kidney function. Clin J Am Soc Nephrol. 2009;4:1302–1311.

19. Ky B, Shults J, Keane MG, et al. FGF23 modifies the relationship between vitamin D and cardiac remodeling. Circ Heart Fail. 2013;6:817–824.

20. Scialla JJ, Xie H, Rahman M, et al. Fibroblast growth factor-23 and cardiovascular events in CKD. J Am Soc Nephrol. 2014;25:349–360.
21. Isakova T, Cai X, Lee J, et al. Longitudinal FGF23 trajectories and mortality in patients with CKD. *J Am Soc Nephrol*. 2018;29:579–589.

22. de Boer IH, Sachs MC, Chonchol M, 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.

23. National Center for Health Statistics. National health and nutrition examination survey. Available at: https://www.cdc.gov/nchs/nhanes/index.htm. Accessed September 9, 2019.

24. Joffe M, Hsu CY, Feldman HI, et al. Variability of creatinine measurements in clinical laboratories: results from the CRIC study. *Am J Nephrol*. 2010;31:426–434.

25. Levey AS, Coresh J, Greene T, et al. 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.

26. Anderson AH, Yang W, Hsu CY, et al. Estimating GFR among adults: a comparison across equations. *J Am Soc Nephrol*. 2012;60:250–261.

27. Prentice R. A case-cohort design for epidemiologic cohort studies and disease prevention trials. *Biometrika*. 1986;73:1–11.

28. Robinson-Cohen C, Hoofnagle AN, Ix JH, et al. Racial differences in the association of serum 25-hydroxyvitamin D concentration with coronary heart disease events. *JAMA*. 2013;310:179–188.

29. Eknoyan G, Beck GJ, Cheung AK, et al. Effect of dialysis dose and membrane flux in maintenance hemodialysis. *N Engl J Med*. 2002;347:2010–2019.

30. Bosworth C, de Boer IH. Impaired vitamin D metabolism in CKD. *Semin Nephrol*. 2013;33:158–168.

31. Horst RL, Littledike ET, Gray RW, et al. Impaired 24,25-dihydroxyvitamin D production in anephric human and pig. *J Clin Invest*. 1981;67:274–280.

32. Hsu CH, Patel S, Buchsbaum BL. Calcitriol metabolism in patients with chronic renal failure. *Am J Kidney Dis*. 1991;17:185–190.

33. Goncalves JG, de Braganca AC, Canale D, et al. Vitamin D deficiency aggravates chronic kidney disease progression after ischemic acute kidney injury. *PLoS One*. 2014;9:e107228.

34. de Borst MH, Vervloet MG, ter Wee PM, et al. Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease. *J Am Soc Nephrol*. 2011;22:1603–1609.

35. Doorenbos CR, van den Born J, Navis G, et al. Possible renoprotection by vitamin D in chronic renal disease: beyond mineral metabolism. *Nat Rev Nephrol*. 2009;5:691–700.

36. de Boer IH, Katz R, Chonchol M, et al. Serum 25-hydroxyvitamin D and change in estimated glomerular filtration rate. *Clin J Am Soc Nephrol*. 2011;6:2141–2149.

37. Ravani P, Malberti F, Tripepi G, et al. Vitamin D levels and patient outcome in chronic kidney disease. *Kidney Int*. 2009;75:88–95.

38. Kendrick J, Cheung AK, Kaufman JS, et al. 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.

39. Rehholz CM, Grams ME, Lutsey PL, et al. Biomarkers of vitamin D status and risk of ESRD. *Am J Kidney Dis*. 2016;67:235–242.

40. Fishbane S, Chittineni H, Packman M, et al. Oral paricalcitol in the treatment of patients with CKD and proteinuria: a randomized trial. *Am J Kidney Dis*. 2009;54:647–652.

41. Keyzer CA, van Breda GF, Vervloet MG, et al. Effects of vitamin D receptor activation and dietary sodium restriction on residual albuminuria in CKD: the ViRTUE-CKD trial. *J Am Soc Nephrol*. 2017;28:1296–1305.

42. Dusso AS, Tokumoto M. Defective renal maintenance of the vitamin D endocrine system impairs vitamin D renoprotection: a downward spiral in kidney disease. *Kidney Int*. 2011;79:715–729.

43. Andress DL. Vitamin D in chronic kidney disease: a systemic role for selective vitamin D receptor activation. *Kidney Int*. 2006;69:33–43.

44. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *Am J Physiol Renal Physiol*. 2005;289:F8–F28.

45. Weishaar RE, Simpson RU. Vitamin D3 and cardiovascular function in rats. *J Clin Invest*. 1987;79:1706–1712.

46. Xiang W, Kong J, Chen S, et al. Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *Am J Physiol Endocrinol Metab*. 2005;288:E125–E132.

47. Zhou C, Lu F, Cao K, et al. Calcium-independent and 1,25(OH)2D3-dependent regulation of the renin-angiotensin system in 1alpha-hydroxylase knockout mice. *Kidney Int*. 2008;74:170–179.

48. Wolf M, Shah A, Gutierrez O, et al. Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int*. 2007;72:1004–1013.

49. de Boer IH, Kestenbaum B, Shoben AB, et al. 25-hydroxyvitamin D levels inversely associate with risk for developing coronary artery calcification. *J Am Soc Nephrol*. 2009;20:1805–1812.

50. Melamed ML, Michos ED, Post W, et al. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med*. 2008;168:1629–1637.

51. Navaneethan SD, Schold JD, Arrigain S, et al. Low 25-hydroxyvitamin D levels and mortality in non-dialysis-dependent CKD. *Am J Kidney Dis*. 2011;58:536–543.

52. Wang TJ, Pencina MJ, Booth SL, et al. Vitamin D deficiency and risk of cardiovascular disease. *Circulation*. 2008;117:503–511.

53. Giovannucci E, Liu Y, Hollis BW, et al. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch Intern Med*. 2008;168:1174–1180.

54. Pilz S, Marz W, Wollner B, et al. Association of vitamin D deficiency with heart failure and sudden cardiac death in a large cross-sectional study of patients referred for coronary angiography. *J Clin Endocrinol Metab*. 2008;93:3927–3935.

55. Kendrick J, Targher G, Smits G, et al. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. *Atherosclerosis*. 2009;205:255–260.
56. Ginsberg C, Katz R, de Boer IH, 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.

57. Batacchi Z, Robinson-Cohen C, Hoofnagle AN, et al. Effects of vitamin D2 supplementation on vitamin D3 metabolism in health and CKD. Clin J Am Soc Nephrol. 2017;12:1498–1506.

58. Zelnick LR, de Boer IH, Kestenbaum BR, et al. Comparative effects of cholecalciferol and calcitriol on circulating markers of CKD mineral bone disorder: a randomized clinical trial. Clin J Am Soc Nephrol. 2018;13:927–928.

59. Stubbs JR, Zhang S, Friedman PA, et al. Decreased conversion of 25-hydroxyvitamin D3 to 24,25-dihydroxyvitamin D3 following cholecalciferol therapy in patients with CKD. Clin J Am Soc Nephrol. 2014;9:1965–1973.

60. de Boer IH, Sachs M, Hoofnagle AN, et al. Paricalcitol does not improve glucose metabolism in patients with stage 3-4 chronic kidney disease. Kidney Int. 2013;83:323–330.

61. Armas LA, Dowell S, Akhter M, et al. Ultraviolet-B radiation increases serum 25-hydroxyvitamin D levels: the effect of UVB dose and skin color. J Am Acad Dermatol. 2007;57:588–593.

62. Berg AH, Powe CE, Evans MK, et al. 24,25-Dihydroxyvitamin d3 and vitamin D status of community-dwelling black and white Americans. Clin Chem. 2015;61:877–884.