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Biomarkers of Vitamin D Status and Risk of ESRD

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Supplementary Material

Table S1: Case status, racial group, and levels of mineral metabolism biomarkers according to vitamin D binding protein isoform.
Figure S1: Blood concentrations of vitamin D binding protein according to rs4588, rs7041, and isoforms.
Item S1: Calculation of free and bioavailable 25(OH)D.

Note: The supplementary material accompanying this article (doi:_____) is available at www.ajkd.org

Supplementary Material Descriptive Text for Online Delivery

Supplementary Table S1 (PDF). Case status, racial group, and levels of mineral metabolism biomarkers according to vitamin D binding protein isoform.
Supplementary Figure S1 (PDF). Blood concentrations of vitamin D binding protein according to rs4588, rs7041, and isoforms.
Supplementary Item S1 (PDF). Calculation of free and bioavailable 25(OH)D.

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Abstract

Background—Disordered mineral metabolism is characteristic of decreased kidney function. However, the prospective associations between circulating levels of vitamin D binding protein, vitamin D, and end-stage renal disease (ESRD) have not been extensively evaluated in epidemiologic studies.

Study Design—Nested case-control study

Setting & Participants—Middle-aged, black and white men and women from four US communities.

Predictors—Baseline levels of vitamin D binding protein, 25-hydroxyvitamin D (25(OH)D), and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) were measured in blood samples collected at study visit 4 (1996–1998) of the Atherosclerosis Risk in Communities (ARIC) study.

Outcome—ESRD cases (n=184) were identified through hospitalization diagnostic codes from 1996–2008 and were frequency matched to controls (n=251) on categories of estimated glomerular filtration rate, albuminuria, diabetes mellitus, sex, and race.

Measurements—Logistic regression was used to estimate the association between mineral metabolism biomarkers (vitamin D binding protein, 25(OH)D, $1,25(OH)_2D$) and incident ESRD, adjusting for age, sex, race, estimated glomerular filtration rate, albuminuria, diabetes mellitus, hypertension, education, specimen type, and serum levels of calcium, phosphate, and parathyroid hormone.

Results—Higher vitamin D binding protein levels were associated with elevated risk of incident ESRD (OR, 1.76; 95% CI, 1.22–2.54; p=0.003). Higher free and bioavailable 25(OH)D levels were associated with reduced risk of incident ESRD (ORs of 0.65 [95% CI, 0.46–0.92; p=0.02] and 0.63 [95% CI, 0.43–0.91; p=0.02] for free and bioavailable 25(OH)D, respectively). There was no association between ESRD and overall 25(OH)D (OR, 0.83; 95% CI, 0.58–1.19; p=0.3) or $1,25(OH)_2D$ (OR, 0.73; 95% CI, 0.48–1.13; p=0.2).

Limitations—Lack of direct measurement of free and bioavailable vitamin D.

Conclusions—In the general population, blood levels of vitamin D binding protein were positively associated and blood levels of free and bioavailable 25(OH)D were inversely associated with new-onset ESRD during follow-up.

Index words

biological markers; chronic renal failure; end-stage renal disease (ESRD); risk factors; vitamin D-binding protein; vitamin D; mineral metabolism biomarker; vitamin D insufficiency

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Disordered mineral metabolism is one of the earliest complications of chronic kidney disease (CKD).[1–3] Decreased glomerular filtration rate (GFR) is associated with lower 1α-hydroxylase activity, which results in decreased activation of 25-hydroxyvitamin D

(25(OH)D) to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), leading to hypocalcemia and hyperparathyroidism.[1,4–6] Adequate vitamin D levels are a therapeutic goal for kidney disease patients.[7] However, clinical recommendation to use vitamin D supplementation in CKD patients for correcting vitamin D deficiency is opinion-based and needs additional empirical evidence.[8] Detecting deficient vitamin D levels may be useful in estimating future risk of kidney disease progression.[2,9,10]

In clinical and research settings, levels of 25(OH)D are usually reported as an assessment of vitamin D status. The majority (approximately 85%–90%) of circulating 25(OH)D, like $1,25(OH)_2D$, is tightly bound to vitamin D binding protein and is thought to be biologically inactive.[11] Bioavailable 25(OH)D consists of a smaller amount (10%–15%) that is loosely bound to albumin and <1% that is circulating in a free and unbound form.[11–13] Binding affinities for 25(OH)D vary by isoforms of vitamin D binding protein; these isoforms differ by racial group, and they explain some of the variability in circulating levels of vitamin D binding protein and 25(OH)D.[14–16] Compared to previous studies of 25(OH)D, current efforts can now more completely evaluate the association of 25(OH)D with adverse outcomes using blood levels and isoforms of vitamin D binding protein to estimate free and bioavailable 25(OH)D.

The objective of this study was to assess the relationship between vitamin D-related biomarkers with incident end-stage renal disease (ESRD) in a community-based population, the Atherosclerosis Risk in Communities (ARIC) study in collaboration with the Chronic Kidney Disease Biomarkers Consortium. We hypothesized that blood levels of vitamin D binding protein would be positively and independently associated with ESRD risk, after accounting for demographics, kidney measures, and known kidney disease risk factors. Furthermore, we hypothesized that blood levels of 25(OH)D, and, secondarily, alternative measures of vitamin D status including $1,25(OH)_2D$, free vitamin D, and bioavailable vitamin D, would be inversely and independently associated with ESRD risk. We explored the distribution of 3-*epi*-$25(OH)D_3$, a form of vitamin D that is not well characterized, according to incident ESRD case status.[17]

METHODS

Study Design

The ARIC study is a prospective cohort study of 15,792 predominantly black and white men and women, 45–64 years of age at enrollment, from four US communities: Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland. The ARIC study participants were recruited and enrolled in 1987–1989, and four follow-up study visits were conducted: 1990–1992, 1993–1995, 1996–1998, and 2011–2013. The ARIC Study is described in detail elsewhere.[18] In the present nested case-control study, ARIC study visit 4 (1996–1998) was the baseline visit. The main reason for using study visit 4 as baseline for the present analysis was that urinary albumin-creatinine ratio (UACR) was measured at this time point, which is an important indicator of kidney damage.

Study Participants

A total of 11,656 ARIC study participants (73.8% of the original ARIC cohort) completed the baseline (visit 4) examination. Study participants were excluded from the present nested case-control study if they were missing information on the factors making up the frequency matching categories (estimated GFR [eGFR], UACR, diabetes mellitus, sex, race) or developed ESRD prior to baseline. Incident ESRD cases (n=184) were defined using diagnostic codes for hospitalizations and deaths identified through active surveillance from baseline (1996–1998) through December 31, 2008. The ESRD case status was defined by: 1) International Classification of Disease (ICD) codes for hospitalizations related to kidney transplantation, dialysis, or procedural code indicating dialysis, excluding hospitalizations with concomitant ICD codes for traumatic anuria (958.5) or acute kidney injury (586, 788.9); or 2) death certificates with kidney failure-related ICD codes (584–584.9, 586, N17.0) as an underlying cause of death and history of CKD. This outcome definition is described in detail elsewhere.[19] Frequency matching was used to identify controls (n=251) based on eGFR category (<45, 45–59, 60–74, 75–89, 90–104, ≥105 mL/min/1.73 m^2), UACR category (<30, 30–300, >300 mg/g), diabetes mellitus, sex, and race. Controls were selected to match the frequency of cases within each stratum (approximately 1–2 controls per case within each stratum). These strong risk factors for ESRD (diabetes mellitus, sex, race) and indicators of kidney function (eGFR) and kidney damage (UACR) were selected as matching factors in order to evaluate the ability of novel biomarkers to predict ESRD risk beyond established factors. The study protocol was approved by the institutional review boards of all participating institutions, and written documentation of informed consent was obtained from all study participants.

Measurement of Covariates

At visit 4 (baseline for the present study), a questionnaire was administered, blood pressure and anthropometrics were measured, and blood specimens and spot urine specimens were collected. The questionnaire was administered by trained staff to assess demographic characteristics (age, sex, race), socioeconomic status (education level), health behaviors, medical history, and medication use. Participants brought current medications to the study visit, and medications were transcribed and coded. Blood pressure measurements were taken by a certified technician using a random-zero sphygmomanometer after the participant was seated and resting for five minutes. Body mass index was calculated as weight (in kilograms) divided the the square of height (in meters) from measurements taken while participants wore light clothing without shoes. Glucose was measured by the modified hexokinase/glucose-6-phosphate dehydrogenase method. Creatinine was measured in the plasma and urine by the modified kinetic Jaffe method, and values were calibrated to the National Institute of Standards and Technology standard. Albumin was measured in urine specimens by a nephelometric method on a Dade Behring BN100 and a Beckman Image Nephelometer. Serum calcium (coefficient of variation [CV], 1.3%) and phosphorus (CV, 1.9%–2.1%) were measured by colorimetric methods and serum intact parathyroid hormone (CV, 1.4%–5.8%) was measured by a sandwich immunoassay and quantified using the Roche Cobas 6000 Chemistry Analyzer (Roche Diagnostics Corporation, Indianapolis, IN). Serum albumin was measured from specimens collected at baseline (study visit 4, 1996–1998) with bromocresol purple dye-binding assay on a Roche Cobas 6000 Chemistry

Analyzer using Roche reagents (Roche Diagnostics Corporation, Indianapolis, IN) (CV, 2.2%–2.6%).

Diabetes mellitus was defined as fasting glucose >126 mg/dL, non-fasting glucose >200 mg/dL, current medication use for diabetes mellitus, or self-report of physician-diagnosed diabetes mellitus. Hypertension status was defined as the mean of two measurements of systolic blood pressure ≥140 mm Hg, the mean of two measurements of diastolic blood pressure ≥90 mm Hg, or self-reported anti-hypertensive medication use in the past two weeks. The eGFR was calculated with serum creatinine using the CKD-EPI (CKD Epidemiology Collaboration) 2009 equation.[20]

Measurement of Vitamin D Binding Protein and Vitamin D

Blood specimens were collected at baseline (ARIC study visit 4, 1996–1998) and stored at −70°C until laboratory analysis. Vitamin D-related biomarkers (concentrations of $25(OH)D_2$, $25(OH)D_3$, $1,25(OH)_2D_2$, $1,25(OH)_2D_3$, 3-*epi*-$25(OH)D_3$, and vitamin D binding protein; isoform of vitamin D binding protein) were quantified by liquid chromatography-tandem mass spectrometry with high-sensitivity spectrometers at the Advanced Research and Diagnostic Laboratory at the University of Minnesota in 2012–2015 (AB Sciex 5500) and the Department of Laboratory Medicine at the University of Washington in 2015 (Waters Xevo TQ MS).[21] Immuno-affinity enrichment was used to measure levels of $1,25(OH)_2D$. After trypsin digestion of blood specimens, vitamin D binding protein was measured using isotopically-labeled peptides as internal standards.[22] Laboratory technicians were masked to case status and participant characteristics. Laboratory measurement of mineral metabolism biomarkers were conducted in serum specimens, except for 18% which were conducted in citrated plasma specimens. Specimen type (plasma or serum) was unrelated to levels of mineral metabolism biomarkers except for 25(OH)D, which was lower in plasma specimens. We adjusted for specimen type in regression models.

Genotyping for vitamin D binding protein single-nucleotide polymorphisms (SNPs; reference SNP identification number [rs]4588 and rs7041) was obtained from the ITMAT-Broad-CARe Chip at the Broad Institute of Massachusetts Institute of Technology and Harvard University.[23]

Calculation of Overall, Free, and Bioavailable Vitamin D

Overall $25(OH)D$ and $1,25(OH)_2D$ levels were calculated as the sum of the D_2 and D_3 isoforms. As a secondary analysis, alternative measures of vitamin D status (free and bioavailable 25(OH)D) were estimated using previously reported equations and affinity constants for albumin and vitamin D binding protein (isoform-specific; Item S1, available as online supplementary material).[12–16,24,25]

Statistical Analysis

Cases and controls were described and compared with respect to the frequency matching factors (eGFR, UACR, diabetes mellitus, sex, race), baseline characteristics, and concentrations of mineral metabolism biomarkers using descriptive statistics and χ^2 tests for

categorical variables and t tests for means. We tested for difference in log-transformed means of mineral metabolism biomarkers using linear regression and reported geometric means after race-adjustment to the overall study population. Differences in vitamin D binding protein concentrations by vitamin D binding protein SNPs (rs4588, rs7041) and vitamin D binding protein isoforms were tested using analysis of variance. In exploratory analyses, we assessed the concentrations of vitamin D markers according to isoforms of vitamin D binding protein. The cross-sectional relationship between the vitamin D-related biomarkers and eGFR was described using Spearman's rank correlation coefficients.

Multivariable logistic regression was used to estimate the association between mineral metabolism biomarkers and risk of ESRD, accounting for matching factors. The continuous form of eGFR and UACR were used in regression models to account for residual confounding after matching on categories of eGFR and UACR. Regression models were additionally adjusted for age and hypertension status due to their known, strong associations with ESRD, for education level as an indicator of socioeconomic status, and for other markers of mineral metabolism (serum levels of calcium, phosphate, and parathyroid hormone). We also adjusted for specimen type (plasma or serum). Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated per one interquartile range (IQR) higher level of biomarkers and, for overall 25(OH)D concentrations, according to clinical categories (assessing two cut-points: 20 and 30 ng/mL). Due to the non-normal distribution, log-transformed 25(OH)D was used in regression models. Stata version 13.1 statistical software was used for analysis (StataCorp LP, College Station, TX, USA).

RESULTS

Baseline Characteristics

In the overall study population, 57% of participants had diabetes mellitus, 48% were women, and 44% were black. Cases and controls were similar with respect to demographic characteristics, level of education, and kidney disease risk factors, with the exception of eGFR (16.3% of cases and 2.8% of controls had eGFR <30 mL/min/1.73 m^2; p<0.001) and UACR (36.4% of cases and 17.1% of controls had UACR >300 mg/g; p<0.001) (Table 1).

Levels of Mineral Metabolism Biomarkers

Blood levels of vitamin D binding protein were higher among cases than controls and 25(OH)D and $1,25(OH)_2D$ were lower among cases than controls after race adjustment (Table 2). Overall, levels of the mineral metabolism biomarkers were correlated with each other and baseline eGFR ($p \leq 0.01$ for all; Table 3). In contrast, $1,25(OH)_2D$ was not correlated with overall, free, and bioavailable 25(OH)D. Free and bioavailable 25(OH)D were not correlated with eGFR.

For the SNP rs4588, vitamin D binding protein levels were highest for the CC genotype and lowest for the AA genotype (p<0.001; Figure S1A). For rs7041, vitamin D binding protein levels were highest for the GG genotype and lowest for the TT genotype (p<0.001; Figure S1B). Blood levels of vitamin D binding protein varied by isoform of vitamin D binding protein (p<0.001; Figure S1C). In addition, blood levels of free and bioavailable 25(OH)D

and 1,25(OH)$_2$D differed according to vitamin D binding protein isoforms (p <0.009 for all; Table S1).

Association Between Mineral Metabolism Biomarkers and ESRD

After adjusting for eGFR, UACR, age, sex, race, diabetes mellitus, hypertension, specimen type, education, and serum levels of calcium, phosphate, and parathyroid hormone, one IQR higher in vitamin D binding protein was associated with higher odds of incident ESRD (OR, 1.76; 95% CI, 1.22–2.54; p = 0.003; Table 4). In the fully adjusted model, higher levels of 25(OH)D were not associated with ESRD when expressed continuously (OR per 1 IQR higher, 0.83; 95% CI, 0.58–1.19; p = 0.3) or according to clinical categories (ORs of 1.11 [95% CI, 0.59–2.07; p=0.7] and 0.95 [95% CI, 0.57–1.56; p=0.8) for ≤30 versus >30 ng/mL and ≤20 versus >20 ng/mL, respectively).

Higher levels of estimated free and bioavailable 25(OH)D were significantly associated with lower odds of ESRD (ORs of 0.65 [95% CI, 0.46–0.92; p=0.02] and 0.63 [95% CI, 0.43–0.91; p=0.02] for free and bioavailable 25(OH)D, respectively). Levels of 1,25(OH)$_2$D were not associated with ESRD risk.

When vitamin D binding protein, 25(OH)D, and 1,25(OH)$_2$D were included in the same model, the results were similar to the results for the biomarkers modelled separately. Vitamin D binding protein was more strongly associated with ESRD than the other biomarkers (for the fully adjusted model, ORs were 2.08 [95% CI, 1.40–3.08; p<0.001], 0.71 [95% CI, 0.44–1.12; p=0.1], and 0.64 [95% CI, 0.39–1.04; p=0.1] for vitamin D binding protein, 25(OH)D, and 1,25(OH)$_2$D, respectively).

DISCUSSION

In this community-based study population, blood levels of vitamin D binding protein were positively associated with incident ESRD during follow-up even after complete multivariable adjustment including other mineral metabolism biomarkers. In addition, higher levels of estimated free and bioavailable 25(OH)D, which were calculated by incorporating concentrations of binding proteins for 25(OH)D (vitamin D binding protein and albumin) and vitamin D binding protein isoforms as a proxy for binding affinity, were associated with lower ESRD risk.

We did not find a significant association between 25(OH)D levels and ESRD risk in the fully adjusted model. The existing literature on this topic is inconsistent. For example, vitamin D insufficiency (<30 ng/mL) was not independently associated with ESRD in the Chronic Renal Insufficiency Cohort (CRIC) Study.[2] In contrast, among 13,328 NHANES (National Health and Nutrition Examination Survey) participants, those with lower 25(OH)D levels (<15 ng/mL) had higher ESRD risk (adjusted incidence rate ratio, 2.64; 95% CI, 1.00–7.05).[10] In a small CKD clinic study of 168 patients, 25(OH)D levels ≥15 ng/mL were significantly associated with lower risk of initiating dialysis.[9] Inconsistencies in the vitamin D literature may be due to differences in study populations, ESRD ascertainment, or assays or due to the failure to measure the physiologically-relevant component of vitamin D.

Vitamin D binding protein binds tightly to the majority of 25(OH)D in the circulation and may be important to measure to accurately assess 25(OH)D status.[14,16] When levels of vitamin D binding protein are elevated, more 25(OH)D is bound to vitamin D binding protein, and there is less 25(OH)D in the circulation able to interact with other receptors.[26,27] Free and bioavailable 25(OH)D represent the physiologically-active portion of vitamin D levels, accounting for blood levels of vitamin D binding protein and albumin and binding affinities of albumin and vitamin D binding protein to 25(OH)D using isoform-specific information.[13–16,24] No studies have previously related free and bioavailable 25(OH)D to kidney disease risk. Our findings extend recent studies documenting that bioavailable vitamin D levels are related to other markers of mineral metabolism and that the vitamin D binding protein isoforms explain racial differences in overall 25(OH)D levels.[13,16] Blood levels of free and bioavailable 25(OH)D may better characterize vitamin D status than overall levels of 25(OH)D.

We did not find that $1,25(OH)_2D$ levels were related to ESRD risk in the present study. This is consistent with the recommendation not to measure this active form of vitamin D for the evaluation of vitamin D status since it is typically normal or elevated in individuals with vitamin D deficiency.[28] Levels of $1,25(OH)_2D$ are tightly regulated by parathyroid hormone in the kidney, and low levels are detected only in late stages of kidney disease.[29] Since extrarenal tissues express 1α-hydroxylase and vitamin D receptors, $1,25(OH)_2D$ levels may represent other disease states.[30]

This study adds to a broader body of literature on mineral metabolism biomarkers and kidney disease risk. In particular, fibroblast growth factor 23 (FGF-23), a bone-derived hormone, has many endocrine functions in the kidney, such as urinary phosphorus excretion and suppression of parathyroid hormone synthesis.[31,32] Studies have also shown a bidirectional relationship between FGF-23 and vitamin D: FGF-23 inhibits vitamin D production by regulating key enzymes and vitamin D supplementation influences blood FGF-23 levels.[33–35] The associations between mineral metabolism markers (FGF-23, vitamin D-related biomarkers) and ESRD from our present and previous studies remained significant after adjusting for calcium, phosphate, and parathyroid hormone; thus, there may be additional pathways by which markers of mineral metabolism confer risk.[36] Future research should focus on identifying these pathways. Further, the use of multiple biomarkers of mineral metabolism may improve risk prediction and evaluation of pharmacologic and dietary interventions to reduce kidney disease risk.[37,38]

There are some limitations of this study that should be considered when interpreting these findings. We were unable to find exact matches for ESRD cases and controls based on kidney function measures. To account for these differences, we adjusted for the continuous forms of eGFR and UACR in multivariable regression models. The blood specimens were stored for approximately 15 years before laboratory measurement of vitamin D binding protein, 25(OH)D, and $1,25(OH)_2D$. Previous research has documented the stability of blood levels of vitamin D with a long duration of freezer storage.[39] The ESRD cases were identified based on diagnostic codes for hospitalizations and deaths. Use of hospitalization data has been shown to be a valid method for defining ESRD and is more representative of the overall burden of kidney disease by incorporating treated and untreated kidney disease

including deaths due to kidney disease.[40,41] We previously reported that, compared to medical record review, our composite definition including ICD-9/10 codes for hospitalizations has a sensitivity of 88% and specificity of 97%.[41] Assessment of free and bioavailable 25(OH)D was based on calculations rather than direct measurement. Future studies that directly measure free and bioavailable 25(OH)D may provide a better assessment of vitamin D status.

The present study has several strengths. This case-control study was nested within a well-characterized prospective cohort. As such, it was possible to match on several important factors due to the large sample size of the overall ARIC study. Strong confounders were selected as matching factors, resulting in an efficient study design, which mitigated confounding and maximized statistical power to detect the association between mineral metabolism biomarkers and ESRD. The findings in our study were robust to adjustment for demographics, kidney function, established risk factors, and other mineral metabolism markers (serum calcium, phosphate, and parathyroid hormone). Multiple methods to evaluate vitamin D status, as well as the isoform of vitamin D binding protein, were assessed in the present study, providing a comprehensive analysis of these important indicators of decreased kidney function.

In conclusion, the present study demonstrates that vitamin D binding protein is positively associated with ESRD risk in a community-based population, independent of demographics, baseline kidney function, established kidney disease risk factors, and other mineral metabolism markers (calcium, phosphate, parathyroid hormone). Furthermore, estimated free and bioavailable 25(OH)D were inversely associated with ESRD risk. Directly measuring the free and bioavailable fractions of overall 25(OH)D concentrations may be worthwhile for clinical and research purposes. Further research is warranted to determine whether these markers can be intervened upon to reduce ESRD risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline demographic, clinical characteristics, and biochemical measurements of study participants by incident ESRD case status

	Incident ESRD Case Status		
	Case (n=184)	**Control (n=251)**	**P-value[b]**
Female sex[c]	87 (47.3)	47.8% (120)	0.9
Black race[c]	45.7% (84)	41.8% (105)	0.5
Diabetes mellitus[c]	60.3% (111)	54.0% (136)	0.2
eGFR[c]			
≥60 mL/min/1.73 m^2	46.7% (86)	63.1% (159)	
30–59 mL/min/1.73 m^2	37.0% (68)	34.1% (86)	<0.001
<30 mL/min/1.73 m^2	16.3% (30)	2.8% (7)	
UACR[c]			
<30 mg/g	37.0% (68)	52.8% (133)	
30–300 mg/g	26.6% (49)	30.2% (76)	<0.001
>300 mg/g	36.4% (67)	17.1% (43)	
Age, y	64.5 (5.6)	64.7 (5.8)	0.7
Hypertension status	75.3% (137)	68.6% (170)	0.1
Body mass index, kg/m^2	30.5 ±6.2	30.2 ±6.0	0.7
Education level			
<High school	37.7% (69)	28.9% (72)	
High school	37.2% (68)	39.0% (97)	0.1
>High school	25.1% (46)	32.1% (80)	
Serum albumin, g/dL	3.6 ±0.4	3.7 ±0.3	0.005
Serum calcium, mg/dL	9.2 ±0.5	9.2 ±0.4	0.5
Serum phosphorus, mg/dL	3.5 ±0.8	3.4 ±0.5	0.08
Serum PTH, pg/mL	77.3 ±106.4	44.2 ±27.0	<0.001

eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; PTH, parathyroid hormone; UACR, urine albumin-creatinine ratio

Note: Values for categorical variables are given as number (percentage); for continuous variables, as mean ± standard deviation.

[b] P-value from χ^2 test for categorical variables and *t* test for continuous variables

[c] Sex, race, diabetes mellitus, eGFR category, and UACR category were matching factors and therefore would not be expected to differ by case status. Due to the differences in eGFR and UACR categories by case status, the continuous form of eGFR and UACR were used in multivariable regression models to account for potential residual confounding.

Table 2

Vitamin D-related biomarkers by incident ESRD case status

Marker	Incident ESRD Case Status		P-value[b]
	Cases (n=184)	Controls (n=251)	
Vitamin D binding protein, μg/mL	260 (233, 288)	250 (225, 277)	0.02
Detectable 3-*epi*-25(OH)D_3	30 (16.3)	53 (21.1)	0.2
25(OH)D, ng/mL	18.6 (13.5, 25.7)	20.3 (15.0, 27.3)	0.04
Deficient 25(OH)D[c]	155 (84.2)	204 (81.0)	0.4
Free 25(OH)D, pg/mL	6.79 (4.91, 9.44)	8.08 (5.66, 11.21)	0.003
Bioavailable 25(OH)D, ng/mL	2.21 (1.63, 3.15)	2.69 (1.83, 3.79)	0.001
$1,25(OH)_2D$, pg/mL	34.0 (26.0, 45.6)	39.0 (30.4, 50.4)	0.001

Note: Values for categorical variables are given as number (percentage); for continuous variables, as geometric mean (25^{th}, 75^{th} percentile). Total cases and total controls were race-adjusted to the overall population. Conversion factors for units: 25(OH)D, free 25(OH)D, and bioavailable 25(OH)D in ng/mL to nmol/L, ×2.496; $1,25(OH)_2D$ in pg/mL to pmol/L, ×2.6.

ESRD, end-stage renal disease; 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D

[b] P-value from χ^2 test or linear regression testing the difference in proportions or means

[c] Deficient 25(OH)D: ≤30 ng/mL

Table 3

Cross-sectional relationship between vitamin D-related biomarkers and kidney function

	VDBP	25(OH)D	Free 25(OH)D	Bioavailable 25(OH)D	$1,25(OH)_2D$	eGFR
Vitamin D binding protein	1					
25(OH)D	r=0.14; p=0.01	1				
Free 25(OH)D	r= −0.18; p=0.002	r=0.58; p<0.001	1			
Bioavailable 25(OH)D	r= −0.18; p=0.002	r=0.60; p<0.001	r=0.98; p<0.001	1		
$1,25(OH)_2D$	r=0.20; p<0.001	r= −0.08; p=0.1	r=0.01; p=0.9	r=0.03; p=0.6	1	
eGFR	r= −0.16; p=0.002	r= −0.18; p<0.001	r=0.001; p=0.9	r=0.03; p=0.6	r=0.44; p<0.001	1

eGFR, estimated glomerular filtration rate; 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D

Am J Kidney Dis. Author manuscript; available in PMC 2017 February 01.

Table 4

Adjusted ORs for incident ESRD per 1 IQR higher concentration of mineral metabolism biomarkers

Biomarker and Model	OR (95% CI)	P-value
Vitamin D binding protein		
Model 1	1.48 (1.08, 2.02)	0.02
Model 2	1.41 (1.02, 1.95)	0.04
Model 3	1.76 (1.22, 2.54)	0.003
25(OH)D		
Model 1	0.70 (0.51, 0.97)	0.03
Model 2	0.73 (0.53, 1.00)	0.05
Model 3	0.83 (0.58, 1.19)	0.3
Free 25(OH)D		
Model 1	0.64 (0.47, 0.89)	0.007
Model 2	0.64 (0.46, 0.89)	0.009
Model 3	0.65 (0.46, 0.92)	0.02
Bioavailable 25(OH)D		
Model 1	0.63 (0.44, 0.88)	0.007
Model 2	0.63 (0.44, 0.90)	0.01
Model 3	0.63 (0.43, 0.91)	0.02
$1,25(OH)_2D$		
Model 1	0.87 (0.61, 1.26)	0.5
Model 2	0.82 (0.56, 1.20)	0.3
Model 3	0.73 (0.48, 1.13)	0.2

CI, confidence interval; OR, odds ratio

Note: Interquartile ranges of vitamin D binding protein, log-transformed 25(OH)D,, free 25(OH)D, and bioavailable 25(OH)D are 54.24, 0.25, 4.91, and 1.74.respectively. Model 1: sex, race, diabetes mellitus, estimated glomerular filtration rate, log(urine albumin-creatinine ratio), specimen type; Model 2: variables in model 1 plus age, hypertension status, education; Model 3: variables in model 2 plus calcium, phosphorus, parathyroid hormone.