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The serum 24,25-dihydroxyvitamin D concentration, a marker of vitamin D catabolism, is reduced in chronic kidney disease

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Abstract

Chronic kidney disease is characterized, in part, as a state of decreased production of 1,25-dihydroxyvitamin D (1,25(OH)2D); however, this paradigm overlooks the role of vitamin D

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catabolism. We developed a mass spectrometric assay to quantify serum concentration of 24,25-dihydroxyvitamin D (24,25(OH)2D), the first metabolic product of 25-hydroxyvitamin D (25(OH)D) by CYP24A1, and determined its clinical correlates and associated outcomes among 278 participants with chronic kidney disease in the Seattle Kidney Study. For eGFRs of 60 or more, 45–59, 30–44, 15–29, and under 15 ml/min/1.73m^2, the mean serum 24,25(OH)2D concentrations significantly trended lower from 3.6, 3.2, 2.6, 2.6, to 1.7 ng/ml, respectively. Non-Hispanic Black race, diabetes, albuminuria, and lower serum bicarbonate were also independently and significantly associated with lower 24,25(OH)2D concentrations. The 24,25(OH)2D concentration was more strongly correlated with that of parathyroid hormone than was 25(OH)D or 1,25(OH)2D. A 24,25(OH)2D concentration below the median was associated with increased risk of mortality in unadjusted analysis, but this was attenuated with adjustment for potential confounding variables. Thus, chronic kidney disease is a state of stagnant vitamin D metabolism characterized by decreases in both 1,25(OH)2D production and vitamin D catabolism.

Introduction

Chronic kidney disease (CKD) is characterized as a state of active vitamin D deficiency. Seminal studies published in the 1970s demonstrated that the kidney produces the majority of circulating 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], the potent hormonal form of vitamin D, from 25-hydroxyvitamin D [25(OH)D].[1–5] Due in large part to 1,25(OH)$_2$D deficiency, people with CKD develop secondary hyperparathyroidism and bone disease.[6,7] Treatment of patients with CKD using 1,25(OH)$_2$D and other activated vitamin D receptor agonists is now standard of care.[8]

Compared to the focus on decreased renal production of 1,25(OH)$_2$D, relatively little attention has been paid to the potential role of altered vitamin D catabolism in CKD. Steady-state concentrations of vitamin D metabolites in blood and target tissues must necessarily represent a balance of production and catabolism. CYP24A1 is the primary enzyme responsible for the multi-step catabolism of both 25(OH)D and 1,25(OH)$_2$D. CYP24A1 is found in most tissues in the body and is rapidly induced by 1,25(OH)$_2$D.[9–11] In the kidney, CYP24A1 transcription is also induced by fibroblast growth factor-23 (FGF-23) and suppressed by parathyroid hormone (PTH).[10,12] In CKD, net effects of declining kidney function on rising FGF-23 and PTH concentrations on vitamin D catabolism are not clear.

The most abundant product of 25(OH)D catabolism by CYP24A1 is 24,25-dihydroxyvitamin D [24,25(OH)$_2$D], which has a circulating half-life of approximately 7 days and is present in nanogram/milliliter concentrations.[13] These characteristics make 24,25(OH)$_2$D amenable to clinical measurement and an attractive potential biomarker of vitamin D catabolism.

In this study, we apply a novel assay to measure circulating 24,25(OH)$_2$D concentrations along with a comprehensive panel of other circulating vitamin D metabolites and regulatory hormones in people with CKD. We then examine the clinical correlates of 24,25(OH)$_2$D and the associations of 24,25(OH)$_2$D concentration with secondary hyperparathyroidism and death.

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Results

Participant characteristics

At baseline, study participants had a mean age of 61 years; 83% were male, 68% were white, 55% had diabetes, and 95% had hypertension (Table 1). Mean estimated glomerular filtration rate (eGFR) was 46 ml/min/1.73m^2. Participants with lower 24,25(OH)$_2$D concentrations were more likely to be older and non-white, have a history of diabetes or coronary artery disease (CAD), have higher body mass index (BMI), and were less likely to take cholecalciferol supplements.

Correlates of 24,25(OH)$_2$D

Serum 24,25(OH)$_2$D concentration was positively correlated with eGFR, particularly for eGFR values less than 60 ml/min/1.73m^2 ($R^2 = 0.07$, $p < 0.0001$, Figure 1A). Similarly, 1,25(OH)$_2$D was positively correlated with eGFR ($R^2 = 0.18$, $p < 0.0001$, Figure 1B) whereas 25(OH)D concentration showed no relationship with eGFR ($R^2 = 0.01$, $p = 0.23$, Figures C). FGF-23 was strongly, negatively correlated with eGFR ($R^2 = 0.52$, $p < 0.0001$) as was PTH ($R^2 = 0.33$, $p < 0.0001$, Figure 2). Serum 24,25(OH)$_2$D concentration was correlated with serum 25(OH)D concentration but not with serum 1,25(OH)$_2$D concentration (Figure 3). FGF-23 was strongly correlated with 1,25(OH)$_2$D but was not correlated with 24,25(OH)$_2$D or 25(OH)D in unadjusted analyses (Figure 4).

In adjusted analysis, characteristics that were significantly associated with lower 24,25(OH)$_2$D concentration included black race, diabetes, lower eGFR, lower 25(OH)D concentration, and lower serum bicarbonate concentration (Table 2). All of these associations persisted after further adjustment for 1,25(OH)$_2$D, PTH, and FGF-23 concentration. Inverse associations of 24,25(OH)$_2$D concentration with urine ACR were of borderline statistical significance. Cholecalciferol use was not associated with 24,25(OH)$_2$D concentration after adjustment for 25(OH)D. After adjustment, age, sex, calcitriol use, serum phosphate, BMI, smoking status, and CAD were not significantly associated with 24,25(OH)$_2$D concentration. With adjustment, FGF-23 concentration was associated with log-transformed 24,25(OH)$_2$D concentration (41% higher 24,25(OH)2D per 2-fold increment in FGF-23, $p<0.001$) but not untransformed 24,25(OH)$_2$D concentration (Table 2).

Associations of vitamin D metabolites with PTH

24,25(OH)$_2$D was negatively correlated with PTH (Figure 4; $r = -0.44$, $p < 0.001$). This association was stronger than that of either 1,25(OH)$_2$D ($r = -0.16$, $p = 0.01$) or 25(OH)D ($r = -0.22$, $p < 0.001$). After adjustment for age, sex, race, eGFR, calcitriol use, cholecalciferol use, diabetes, serum bicarbonate, calcium, phosphate, urine ACR, 1,25(OH)$_2$D, and 25(OH)D; each 1 ng/ml lower 24,25(OH)$_2$D was associated with an estimated 13% higher geometric mean PTH (95% confidence interval (CI) 6% – 19%, $p < 0.001$), while neither 1,25(OH)$_2$D nor 25(OH)D were significantly associated with PTH. Lower 24,25(OH)$_2$D concentration was associated with hyperparathyroidism (PTH > 88 pg/dl) in a parallel adjusted model, with and odds ratio of 1.97 (95% CI 1.32 – 2.94, $p = 0.001$) for every 1 ng/ml lower 24,25(OH)$_2$D concentration.

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Association of vitamin D metabolites with mortality

Mean follow-up time for the cohort was 2.7 years (range 0 – 6.4 years). The crude incidence rate of death among the cohort was 7 per 100 person-years. In the unadjusted Cox model, 24,25$(OH)_2$D concentration below the cohort median (2.4 ng/ml) was significantly associated with increased risk of death (hazard ratio 2.04, 95% CI 1.24 – 3.36, $p = 0.005$, Supplementary Figure S2). With adjustment for age, gender, race, eGFR, urine ACR, diabetes, coronary artery disease, serum bicarbonate, and serum 25(OH)D; low 24,25$(OH)_2$D had a hazard ratio for death of 1.60 (95% CI 0.71 – 3.64 $p = 0.26$). Cox regression with 24,25$(OH)_2$D as a continuous exposure demonstrated an unadjusted hazard ratio 0.78 (95% CI 0.66 – 0.91, $p = 0.002$) per ng/ml higher 24,25$(OH)_2$D concentration and adjusted hazard ratio 0.88 (95% CI 0.66 – 1.16, $p = 0.37$) per ng/ml higher 24,25$(OH)_2$D concentration. These associations were not affected by additional adjustment for 1,25$(OH)_2$D, PTH, or FGF-23.

Discussion

Lower eGFR was strongly associated with lower circulating 24,25$(OH)_2$D concentration in a clinic-based CKD population. Black race, diabetes, lower serum bicarbonate concentration, lower serum 25(OH)D concentration, and higher urine ACR were also independently associated with lower 24,25$(OH)_2$D concentration. Serum 24,25$(OH)_2$D concentration was strongly negatively correlated with serum PTH and hyperparathyroidism, more so than either 25(OH)D or 1,25$(OH)_2$D. Low 24,25$(OH)_2$D was associated with increased risk of death in unadjusted survival analysis but lost statistical significance after adjustment for potential confounders.

CKD is widely viewed as a state of reduced 1,25$(OH)_2$D production.[1,8,14] Our finding of lower 24,25$(OH)_2$D concentrations with lower eGFR suggest that CKD is more accurately described as a state of stagnant vitamin D metabolism, with decreased vitamin D catabolism in addition to diminished 1,25$(OH)_2$D production. This conclusion is supported by a study reporting a markedly prolonged 25(OH)D half-life in anephric patients compared with normal controls, as measured using injection of radiolabeled 25(OH)D.[15] Clinically, our data suggest that 1,25$(OH)_2$D administration may not fully correct vitamin D metabolism in CKD because it addresses only the production side of vitamin D metabolism. Stagnant vitamin D metabolism in CKD parallels dysfunction seen in other metabolic and signaling pathways in kidney disease including decreased drug metabolism in the gastrointestinal tract, cytochrome dysfunction in oxidative phosphorylation, dysregulated lipid metabolism, and altered growth hormone signaling.[16–19]

The mechanism of lower serum 24,25$(OH)_2$D concentrations with lower eGFR is not known. It is possible that extra-renal CYP24A1 is an important source of circulating 24,25$(OH)_2$D that is down-regulated in CKD, perhaps due to tissue-level 1,25$(OH)_2$D deficiency.[9,11] Alternatively, the strong relationship of circulating 24,25$(OH)_2$D with eGFR may be due to decreased production of 24,25$(OH)_2$D by the kidney. PTH and FGF-23 are both elevated in CKD and regulate the renal production of 24,25$(OH)_2$D in a reciprocal manner to 1,25$(OH)_2$D production.[9,10,12] However, 24,25$(OH)_2$D and 1,25$(OH)_2$D were both strongly directly correlated with eGFR despite this reciprocal regulation. In addition,

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previous animal experimental models have demonstrated conflicting results regarding renal CYP24A1 expression in CKD, and one human biopsy study showed no association of CKD with CYP24A1 expression.[20–22] In the Col4a3$^{-/-}$ mouse, circulating 24,25$(OH)_2$D concentrations decline with loss of GFR despite increased renal CYP24A1 expression, suggesting that renal expression and total enzymatic activity diverge in CKD (L.D. Quarles, personal communication). Together, these observations suggest that PTH and FGF-23 are not the main drivers of vitamin D catabolism in CKD. Instead, we believe that an overall decrease in renal mass, less delivery of 25(OH)D to proximal tubular cells, or lower net metabolic capacity of the proximal tubular cells is the major determinant of renal 24,25$(OH)_2$D production and serum 24,25$(OH)_2$D concentration.

Small, early studies observed lower 24,25$(OH)_2$D concentrations in both pre-dialysis CKD and ESRD.[23–27] These studies, however, used a competitive binding assay which have the potential for cross-reactivity with 25(OH)D and 1,25$(OH)_2$D. Substantial cross-reactivity has been demonstrated with a 1,25$(OH)_2$D competitive binding assay compared to mass-spectrometry.[28] Moreover, our study was larger and more diverse than prior studies of 24,25$(OH)_2$D and included a comprehensive panel of other circulating vitamin D metabolites and regulatory hormones, allowing assessment of clinical determinants of 24,25$(OH)_2$D for the first time. We found that 25(OH)D, race, serum bicarbonate, urine ACR, and diabetes were strong predictors of 24,25$(OH)_2$D concentration, after adjustment for eGFR. The association with 25(OH)D is probably a simple relationship between CYP24A1 substrate and product. The association with race, however, is more complex, since this association is present after adjustment for 25(OH)D concentration.[29,30] We speculate that the associations of 24,25$(OH)_2$D concentration with diabetes, serum bicarbonate, and ACR are due to proximal tubular dysfunction that is not fully captured by measurement of eGFR. The proximal tubule, which is impaired by diabetes, is the site of vitamin D metabolism as well as bicarbonate reabsorption, ammonia production for acid excretion, and protein reabsorption.[31–33]

Low 24,25$(OH)_2$D may identify risk of CKD complications. Low serum 24,25$(OH)_2$D concentration was strongly associated with hyperparathyroidism, perhaps because 24,25$(OH)_2$D concentration reflects the extent to which vitamin D metabolism is deranged in CKD. Our results also provide preliminary evidence that low serum 24,25$(OH)_2$D concentrations may be related to an elevated risk of death. Although this association was not statistically significant after adjustment, participants with 24,25$(OH)_2$D below 2.4 ng/ml had an estimated 60% greater mortality rate than those with levels above this threshold. The wide confidence interval (95% CI −29% to 264%) indicates that our data are consistent with an increased mortality risk of substantial magnitude. Further study is necessary to investigate this potential link.

There was no difference in 25(OH)D concentration across eGFR in our study. Other studies have variously shown stable or declining 25(OH)D concentration with lower eGFR.[34–37] Our data suggest that 25(OH)D concentrations may not decrease with falling eGFR due to counterbalanced decrements in both 25(OH)D production and 25(OH)D catabolism.

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This study has several strengths. First, it includes a well-characterized, racially diverse population with a spectrum of kidney function in whom a comprehensive panel of circulating vitamin D metabolites and regulatory are rigorously quantified. To our knowledge, it is the largest study of 24,25$(OH)_2D$ concentration performed to date. Second, we applied a novel and accurate mass-spectrometry assay for 24,25$(OH)_2D$. Third, we concurrently evaluated complementary measurements of mineral metabolism. Our study also has some limitations. Power for detecting associations with mortality was marginal, and associations of 24,25$(OH)_2D$ with clinical outcomes require further study. Our study population is derived from only two Nephrology clinic sites and may not be generalizable to the CKD population as a whole. Because CYP24A1 is a multicatalytic enzyme that not only produces but also degrades 24,25$(OH)_2D$, lower 24,25$(OH)_2D$ concentrations in CKD could conceivably result from increased catabolism of 24,25$(OH)_2D$ by CYP24A1 rather than decreased 24,25$(OH)_2D$ production. We expect, however, that all catalytic functions of CYP24A1 are similarly altered in CKD. Therefore, circulating 24,25$(OH)_2D$ likely reflects overall CYP24A1 activity. Finally, analyses of interrelated circulating biomarkers were cross-sectional, so causality cannot be determined.

In conclusion, CKD is a state of stagnant vitamin D metabolism characterized by decreased vitamin D catabolism in addition to decreased 1,25$(OH)_2D$ production. Decreased 25(OH)D catabolism, as measured by lower circulating 24,25$(OH)_2D$ concentration, is associated with increased risks of secondary hyperparathyroidism and possibly with death. Further studies are needed to test associations of 24,25$(OH)_2D$ with clinical outcomes and to determine whether 24,25$(OH)_2D$ is a modifiable therapeutic target in CKD.

Methods

Study Population

The Seattle Kidney Study (SKS) is an ongoing Nephrology clinic-based cohort study of people with CKD. Beginning in June of 2004, participants were recruited from outpatient Nephrology clinics at Harborview Medical Center and the Veterans Affairs Puget Sound Health Care Center (VAPSHC) in Seattle, Washington. Inclusion criteria are age 18 years and either an estimated glomerular filtration rate (eGFR) 90ml/min/1.73m^2 or a urinary protein to creatinine ratio 30 mg/g. Exclusion criteria are dialysis, previous kidney transplant, inability to provide informed consent, and expectation of dialysis initiation within 3 months. This study was approved by the University of Washington Institutional Review Board.

At the time of this study, 531 SKS participants had completed a baseline study visit including collection of serum specimens. We excluded 15 participants taking ergocalciferol (vitamin D_2) supplements because our assay detected 24,25$(OH)_2D_3$ but not 24,25$(OH)_2D_2$. Vitamin D_2 has an additional methyl group at the 24-carbon, which may alter hydroxylation at that site. Due to cost constraints, we randomly selected a cohort of 278 SKS participants (approximately 50%) for this study. To test associations of 24,25$(OH)_2D$ with mortality using a case-cohort design, we also studied the remaining 34 of the 531 participants who died during longitudinal follow-up (Supplementary Figure S1). Two participants taking

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cinacalcet were included in our analyses. Exclusion of these two participants did not substantially alter any of the results.

Measurement of 24,25(OH)$_2$D, 25(OH)D, and 1,25(OH)$_2$D

24,25(OH)$_2$D and 25(OH)D were measured simultaneously with the same extraction and analysis procedures. The full method of extraction and analysis for 25(OH)D has been described previously.[38] The only difference in the combined assay was the use of 50% *n*-heptane:50% methyl-tert-butyl-ether instead of *n*-heptane alone. Briefly, 200 µl of thawed serum specimens, previously frozen at −80°C, were alkalinized with sodium hydroxide, covered, vortexed, and incubated at room temperature for 15 minutes. 25(OH)D$_2$-d$_3$, 25(OH)D$_3$-d$_6$ (Medical Isotopes Inc., Pelham, NH) and 24,25(OH)$_2$D$_3$-d$_6$ (Toronto Research Chemicals Inc., North York, Ontario) were added as internal standards, and specimens were vortexed for an additional 15 seconds. Extraction solvent (1 ml of 50% *n*-heptane:50% methyl-tert-butyl-ether) was added, and each aliquot was vortexed for 5 minutes followed by centrifugation. The aliquots were then placed in a dry-ice acetone bath for 50 minutes to freeze the lower aqueous layer. The organic layer was removed, dried, and reconstituted in 100 µL 0.5 mg/mL PTAD in acetonitrile. Ten microliters of dissolved extracts were injected and developed using a Acquity UPLC (Waters Corp., Milford, MA). Compounds were then analyzed using isotope dilution tandem mass spectrometry in ESI positive mode on a Xevo TQ (Waters Corp., Milford, MA). Dwell time was 42 milliseconds. The calibration for the measurement of 25(OH)D was verified using SRM 972 from NIST with accuracy of 91–95% for 25(OH)D$_3$ and 100–116% for 25(OH)D$_2$. The method has lower limits of quantification (20% coefficient of variation (CV)) of 0.5 ng/mL, 1.0 ng/mL, and 5.0 ng/mL for 24,25(OH)$_2$D$_3$, 25(OH)D$_3$, and 25(OH)D$_2$, respectively. Interassay imprecision was 8.58% at 1.5 ng/mL for 24,25(OH)$_2$D$_3$, 4.40% at 10.4 ng/mL for 25(OH)D$_3$, and 4.35% at 9.7 ng/mL for 25(OH)D$_2$. Among a subset of 22 subjects with repeat 24,25(OH)$_2$D measurements a mean of 1.9 years (SD ± 0.9) after the baseline examination, 24,25(OH)$_2$D intraclass correlation coefficient, a measure of intra-individual variation, was 0.75 (95% CI 0.56 – 0.94).

Serum concentrations of 1,25(OH)$_2$D$_2$ and 1,25(OH)$_2$D$_3$ were measured by immunoaffinity extraction and HPLC-mass spectrometry (Xevo TQ, Waters Corp., Milford, MA), which has been previously described.[28] The method has lower limits of quantification (20% CV) of 1.25 pg/mL for 1,25(OH)$_2$D3 and 0.64 pg/mL for 1,25(OH)$_2$D$_2$. Published interassay and intraassay imprecision for this method, for both 1,25(OH)$_2$D$_3$ and 1,25(OH)$_2$D$_2$, are < 14%. We report total 1,25(OH)$_2$D as the sum of 1,25(OH)$_2$D$_2$ and 1,25(OH)$_2$D$_3$.

Measurement of Covariates

Race, ethnicity, and smoking status were ascertained by questionnaire. Diabetes was defined as fasting blood glucose greater than 126 mg/dL, non-fasting blood glucose greater than 200 mg/dL, hemoglobin A1c 6.5%, or use of glucose lowering medications.[39] Blood pressure was measured 3 times 5 minutes apart on an automated sphygmomanometer, and the last two measurements were averaged for analysis. Hypertension was defined as systolic blood pressure 140 mmHg, diastolic blood pressure 90 mmHg, or use of antihypertensive medications.[40] CAD was defined as history of myocardial infarction, cardiac arrest,

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coronary artery bypass graft, or percutaneous coronary intervention. Medications were ascertained from the computerized pharmacy database for participants from the VAPSHC or by direct transcription of medication bottle labels for participants at HMC.

Baseline laboratory measurements were obtained from serum and urine that were stored at −80°C. General chemistries were measured on a Beckman-Coulter DxC autoanalyzer. Urine albumin was measured by a timed endpoint method, and urine creatinine was measured with the modified rate Jaffe method. Serum creatinine was measured by the modified rate Jaffe method with an assay traceable to isotope dilution mass spectrometry. The CKD-Epi formula was used to calculate eGFR.[41] Serum PTH was measured with the Beckman-Coulter DxI automated immunoassay. Hyperparathyroidism was defined as intact PTH concentration above 88 pg/ml (upper limit of normal for assay). Intact FGF-23 was measured via ELISA (Kainos Laboratories Inc., Tokyo, Japan).

Ascertainment of death

Deaths were identified during twice yearly surveillance calls and through linkage with the Social Security Death Index. Living participants were censored at the date of their last SKS contact. As of December 31, 2010, 57 of 531 SKS participants had either withdrawn or were lost to follow-up.

Statistical analysis

Cross-sectional analyses included the 278 participants in the randomly-selected cohort. We tabulated participant characteristics by 24,25$(OH)_2$D concentration, categorized as high or low using the cohort population median value (2.4 ng/ml). We examined interrelationships of 24,25$(OH)_2$D with 25(OH)D, 1,25$(OH)_2$D, PTH, and eGFR using Pearson correlation coefficients, scatter plots, and fractional polynomials (second degree). We used multivariable linear regression to assess independent determinants of 24,25$(OH)_2$D. Primary analyses evaluated 24,25$(OH)_2$D concentration without transformation; results examining log-transformed 24,25$(OH)_2$D concentration were similar except where noted. Age, sex, race, cholecalciferol use, calcitriol use, 25(OH)D concentration, eGFR (categories), log-transformed urine albumin-creatinine ratio [log(ACR)], and body mass index (BMI) were forced into the model. Diabetes, CAD, smoking, serum albumin, serum phosphate, and serum bicarbonate were tested for inclusion in the final model and retained if they were significantly associated with 24,25$(OH)_2$D ($p < 0.05$). Parallel linear regression models were used to assess determinants of PTH. We tested associations of 24,25$(OH)_2$D with death using Cox proportional hazards regression. We used the Barlow method to account for the case-cohort study design[42] and stratified by clinic site. We computed Kaplan Meier survival estimates by weighting individual observations according to the inverse of their estimated sampling probability.[43]

Analyses were performed using Stata version 11.1 (College Station, TX) and R 2.12.1 (http://www.r-project.org). All p-values are two-sided, and values below 0.05 were considered significant.

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Supplementary Material

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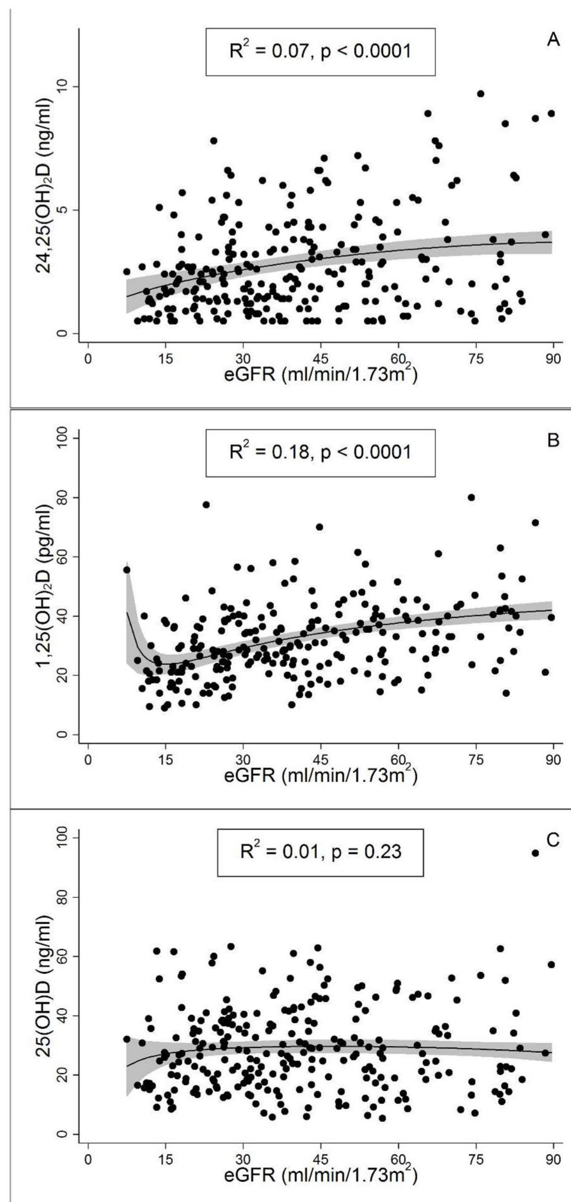

Figure 1.

Correlations of circulating vitamin D metabolites with estimated glomerular filtration rate (eGFR). (A) 24,25(OH)$_2$D versus eGFR. (B) 1,25(OH)$_2$D versus eGFR. (C) 25(OH)$_2$D versus eGFR. Values estimated using second degree fractional polynomial (solid line with gray shading indicating 95% confidence interval of estimate) are superimposed on standard scatter plots. Plots are truncated at eGFR < 90, but all points were used for line fit.

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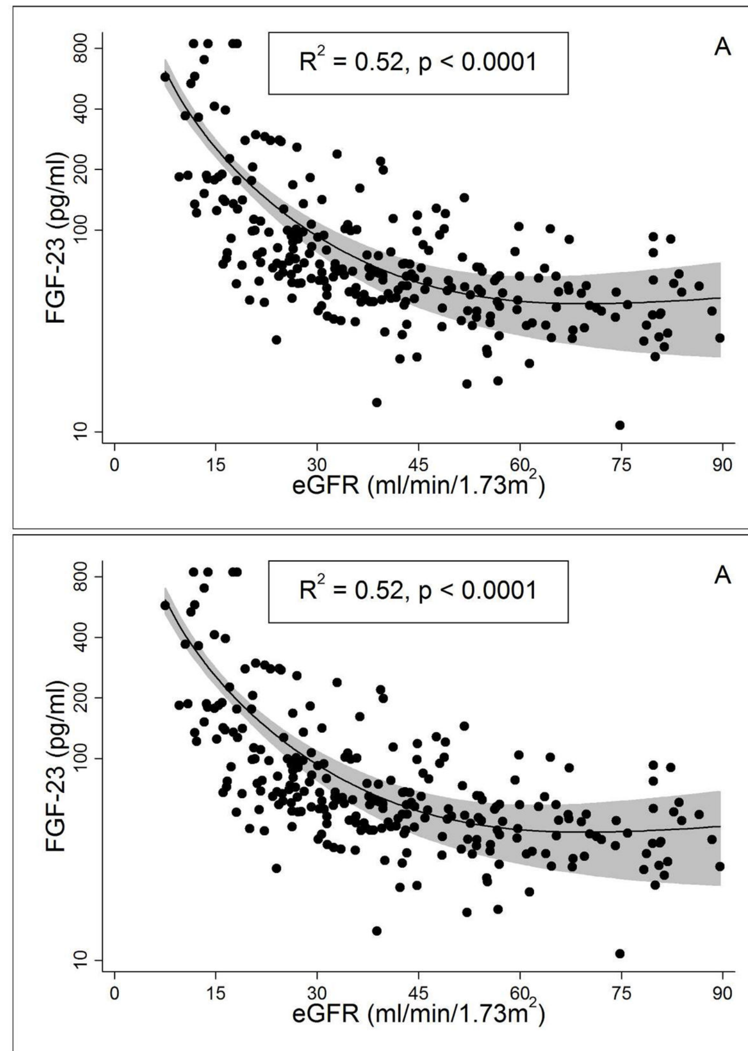

Figure 2.

Correlations of eGFR with (A) FGF-23 and (B) PTH. Values estimated using second degree fractional polynomial models (solid line with gray shading indicating 95% confidence interval of estimate) are superimposed on standard scatter plots.

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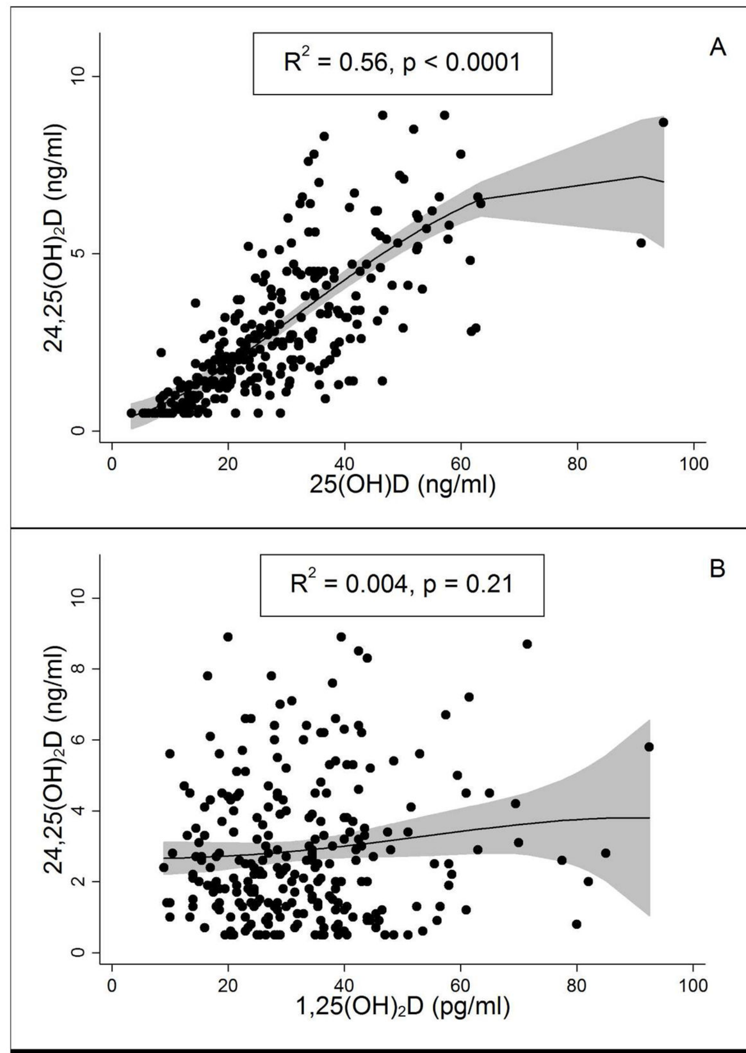

Figure 3.

Correlations of 24,25OH2D concentration with (A) 25(OH)D and (B) 1,25(OH)$_2$D. Values estimated using second degree fractional polynomial models (solid line with gray shading indicating 95% confidence interval of estimate) are superimposed on standard scatter plots.

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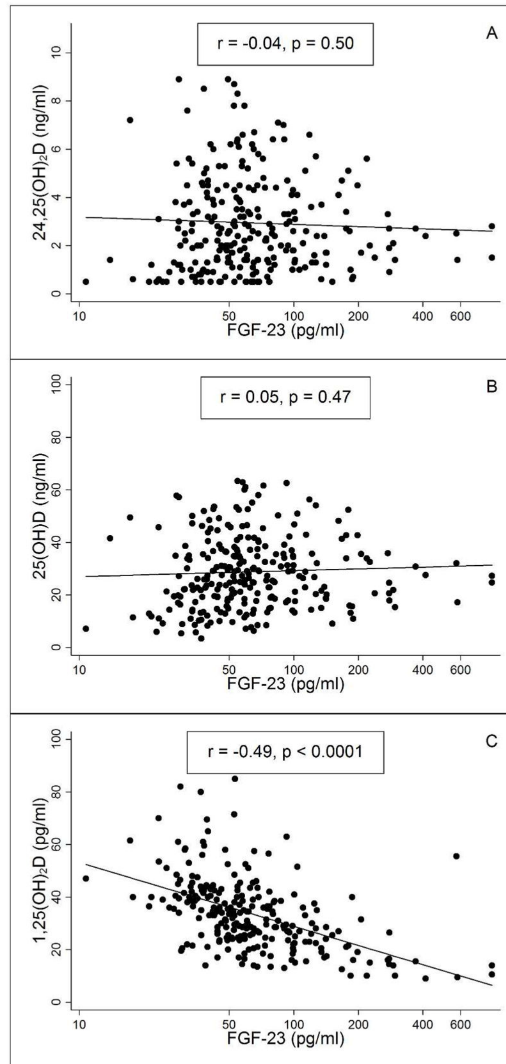

Figure 4.

Correlations of FGF-23 with (A) 24,25$(OH)_2$D, (B) 25(OH)D, and (C) 1,25$(OH)_2$D. Line fit with linear regression. P-values are for the correlation coefficients.

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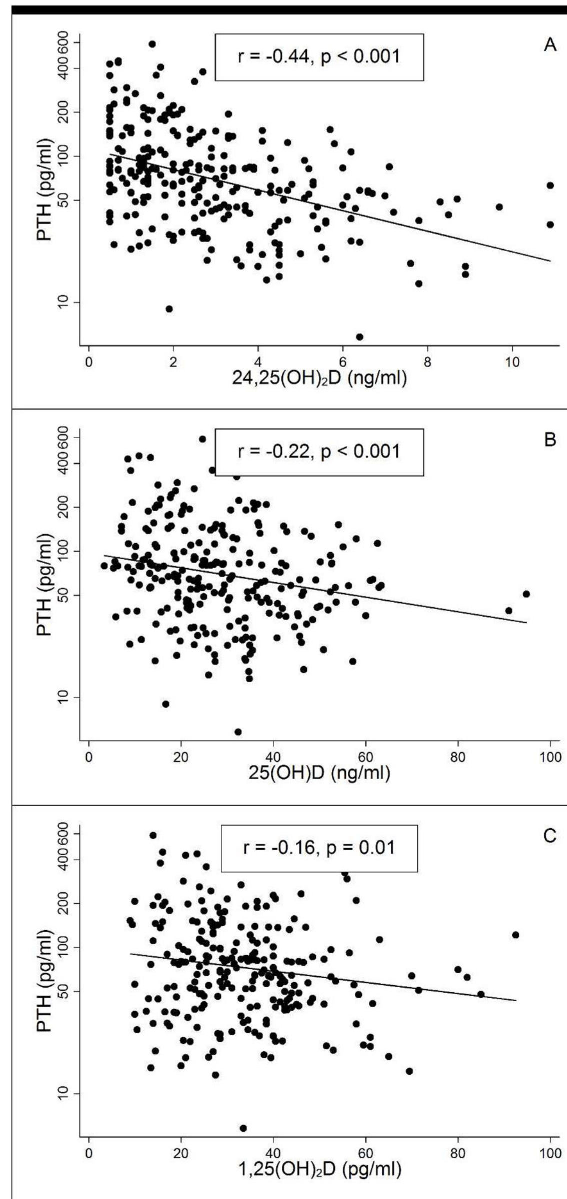

Figure 5.

Correlations of parathyroid hormone with (A) 24,25$(OH)_2$D, (B) 25(OH)D, and (C) 1,25$(OH)_2$D. Line fit with linear regression. P-values are for the correlation coefficients.

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Table 1

Characteristics of 278 Seattle Kidney Study participants by serum 24,25-dihydroxyvitamin D concentration.

	Full Cohort (N = 278)	**24,25(OH)$_2$D 2.4 ng/ml (N = 142)**	**24,25(OH)$_2$D < 2.4 ng/ml (N = 136)**	**p-value**
Demographic Data				
Age (years)	60.7 ± 13.0	60.0 ± 13.3	61.5 ± 12.7	0.38
Male Sex	231 (83%)	118 (83%)	113 (83%)	0.99
Race				0.001
Non-Hispanic white	190 (68%)	111 (78%)	79 (58%)	
Non-Hispanic black	53 (19%)	21 (15%)	32 (24%)	
Other	35 (13%)	10 (7%)	25 (18%)	
Study Site				0.96
VAPSHC	159 (57%)	81 (57%)	78 (57%)	
HMC	119 (43%)	61 (43%)	58 (43%)	
Medical History				
Current Smoking	48 (18%)	29 (21%)	19 (14%)	0.14
Diabetes	153 (55%)	66 (46%)	87 (64%)	0.003
Hypertension	264 (95%)	132 (93%)	132 (97%)	0.12
Coronary Artery Disease	92 (33%)	38 (27%)	54 (40%)	0.02
Medications				
Phosphate Binder	32 (12%)	14 (10%)	18 (13%)	0.38
Cholecalciferol	46 (17%)	32 (23%)	14 (10%)	0.006
Calcitriol	22 (8%)	8 (6%)	14 (10%)	0.15
Any Anti-hypertensive	257 (92%)	127 (89%)	130 (96%)	0.05
Erythropoietin	12 (4%)	7 (5%)	5 (4%)	0.61
Physical Examination				
Systolic Blood Pressure (mmHg)	133 ± 21	130 ± 21	136 ± 22	0.01
Body Mass Index (kg/m^2)	31.5 ± 7.5	29.8 ±6.1	33.4 ± 8.3	0.0002
Laboratory Measurements				
Creatinine (mg/dL)	2.0 ± 1.0	1.8 ± .95	2.2 ± 1.1	0.0009
eGFR (mL/min/1.73m^2)	45.9 ± 26.1	50.4 ± 27.2	41.3 ± 24.2	0.003
eGFR Groups				0.04
> 60 mL/min/1.73m^2	67 (24%)	41 (29%)	26 (19%)	
45–59 mL/min/1.73m^2	46 (17%)	28 (20%)	18 (13%)	
30–44 mL/min/1.73m^2	76 (27%)	32 (23%)	44 (32%)	
15–29 mL/min/1.73m^2	72 (26%)	36 (25%)	36 (26%)	
< 15 mL/min/1.73m^2	17 (6%)	5 (4%)	12 (9%)	
Urine ACR (mg/g)	132 (19, 696)	111 (15, 445)	213 (27, 1233)	0.05
25(OH)D (ng/ml)	28.6 ± 14.5	37.5 ± 13.3	19.3 ± 8.8	< 0.0001
24,25(OH)$_2$D (ng/ml)	2.9 ± 2.1	4.40 ± 1.85	1.3 ± .6	< 0.0001
1,25(OH)$_2$D (pg/ml)	33.1 ± 14.3	34.4 ± 15.5	31.7 ± 12.8	0.19
PTH (pg/ml)	71.9 (43.4, 126.9)	54.3 (35.8, 83.0)	92.0 (63.7, 180.7)	< 0.0001

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	Full Cohort (N = 278)	**24,25(OH)$_2$D 2.4 ng/ml (N = 142)**	**24,25(OH)$_2$D < 2.4 ng/ml (N = 136)**	**p-value**
FGF-23 (pg/ml)	59.4 (42.4, 97.9)	58.7 (42.7, 94.6)	60.5(40.3, 100.5)	0.86

*All values mean ± standard deviation or n (%); except urine ACR, PTH, and FGF-23 which are median (interquartile range). P-values are two-sided using Wilcoxon rank-sum for continuous variables and chi-squared for proportions. VAPSHC, Veterans Affairs Puget Sound Health Care Center; HMC, Harborview Medical Center; ACE, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate; ACR, albumin to creatinine ratio; PTH, parathyroid hormone; FGF-23, fibroblast growth factor-23.

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Table 2

Correlates of 24,25$(OH)_2D$ as determined using multiple linear regression.

	Model 1		Model 2		Model 3	
Variables	**Coefficient**	**p-value**	**Coefficient**	**p-value**	**Coefficient**	**p-value**
Age (per 10 years)	−0.08	0.159	−0.05	0.442	−0.02	0.696
Sex	−0.05	0.835	−0.19	0.382	−0.26	0.182
Race						
-Non-Hispanic white	Reference		Reference		Reference	
-Non-Hispanic black	−0.82	<0.001	−0.95	<0.001	−0.57	0.003
-Other	−0.20	0.307	−0.28	0.162	−0.26	0.18
eGFR (ml/min/1.73m2)						
>60	Reference		Reference		Reference	
45–59	−0.30	0.276	−0.30	0.251	−0.36	0.183
30–44	−1.11	<0.001	−1.11	<0.001	−0.99	<0.001
15–29	−1.17	<0.001	−1.15	<0.001	−1.21	<0.001
<15	−1.65	<0.001	−1.56	<0.001	−1.61	0.001
25(OH)D	0.10	<0.001	0.10	<0.001	0.10	<0.001
Calcitriol use			−0.06	0.842	0.08	0.804
Cholecalciferol use			−0.21	0.323	0.01	0.955
Serum bicarbonate (mg/dL)			0.05	0.011	0.05	0.019
Diabetes			−0.46	0.007	−0.63	<0.001
Urine ACR (mg/g, per twofold higher ACR)			0.06	0.042	0.05	0.057
Body Mass Index (kg/m2)			−0.01	0.286	−0.004	0.708
1,25(OH)2D (pg/ml)					−0.02	0.001
PTH (pg/ml, per two-fold higher PTH)					−0.33	<0.001
FGF-23 (pg/ml, per two-fold higher FGF-23)					0.11	0.329

eGFR, estimated glomerular filtration rate; ACR, albumin to creatinine ratio. All covariates adjusted for the other covariates included in each model.