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Estimated GFR and Circulating 24,25-Dihydroxyvitamin D_3 Concentration: A Participant-Level Analysis of 5 Cohort Studies and Clinical Trials

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Supplementary Material

Table S1: Sensitivity analysis comparing association of eGFR with specific serum $24,25(OH)_2D_3$ concentration to association of eGFR with summed concentration of dihydroxyvitamin D_3 metabolites in CHS.
Figure S1: Correlation of serum concentrations of summed dihydroxyvitamin D_3 metabolites with specific $24,25(OH)_2D_3$ in CHS.
Figure S2: Correlation of serum concentrations of summed dihydroxyvitamin D_3 metabolites with specific $24,25(OH)_2D_3$ in HEMO.
Note: The supplementary material accompanying this article (doi:_____) is available at www.ajkd.org

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Abstract

Background—Decreased glomerular filtration rate (GFR) leads to reduced production of 1,25-dihydroxyvitamin D_3 from 25-hydroxyvitamin D_3 ($25(OH)D_3$). Effects of low GFR on vitamin D catabolism are less well understood. We tested associations of estimated GFR (eGFR) with the circulating concentration of 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$), the most abundant product of $25(OH)D_3$ catabolism, across populations with a wide range of GFR.

Study Design—Cross-sectional study.

Setting & Participants—9596 participants in 5 cohort studies and clinical trials: the Diabetes Control and Complications Trial (N=1193), Multi-Ethnic Study of Atherosclerosis (N=6470), Cardiovascular Health Study (N=932), Seattle Kidney Study (N=289), and Hemodialysis Study (N=712).

Predictor—eGFR.

Outcome—Circulating $24,25(OH)_2D_3$ concentration.

Measurements—GFR was estimated from serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration equation. Vitamin D metabolites were measured by mass spectrometry.

Results—Circulating $24,25(OH)_2D_3$ concentration was correlated with circulating $25(OH)D_3$ concentration (Pearson r range, 0.64–0.88). This correlation was weaker with lower eGFR.

Moreover, the increment in $24,25(OH)_2D_3$ associated with higher $25(OH)D_3$ ("slope") was lower with lower eGFR: 2.06 (95% CI, 2.01–2.10), 1.77 (95% CI, 1.74–1.81), 1.55 (95% CI, 1.48–1.62), 1.17 (95% CI, 1.05–1.29), 0.92 (95% CI, 0.74–1.10), 0.61 (95% CI, 0.22–1.00), and 0.37 (95% CI, 0.35–0.39) ng/mL $24,25(OH)_2D_3$ per 10 ng/mL $25(OH)D_3$ for eGFR ≥90, 60–89, 45–59, 30–44, 15–29, and <15 mL/min/1.73 m^2 and ESRD treated with hemodialysis, respectively. As a result, at a $25(OH)D_3$ concentration of 20 ng/mL, mean $24,25(OH)_2D_3$ concentration was 2.92 (95% CI, 2.87–2.96), 2.68 (95% CI, 2.64–2.72), 2.35 (95% CI, 2.26–2.45), 1.92 (95% CI, 1.74–2.10), 1.69 (95% CI, 1.43–1.95), 1.14 (95% CI, 0.62–1.66), and 1.04 (95% CI,1.02–1.07) ng/mL for each category, respectively. This interaction was independent of other relevant clinical characteristics. Race, diabetes, urine albumin excretion, and the circulating concentrations of parathyroid hormone and fibroblast growth factor 23 more modestly modified the association of $24,25(OH)_2D_3$ with $25(OH)D_3$.

Limitations—Lack of direct pharmacokinetic measurements of vitamin D catabolism.

Conclusions—Lower eGFR is strongly associated with reduced vitamin D catabolism as measured by circulating $24,25(OH)_2D_3$ concentration.

INDEX WORDS

decreased renal function; low estimated glomerular filtration rate; vitamin D catabolism; 1,25-dihydroxyvitamin D_3; 25-hydroxyvitamin D_3; active vitamin D; chronic kidney disease (CKD); biomarker

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Decreased glomerular filtration rate (GFR) leads to reduced production of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the active vitamin D hormone, from 25-hydroxyvitamin D_3 ($25(OH)D_3$).[1,2] Reduced $1,25(OH)_2D_3$ production is due to reduced renal mass as well as downregulation of the renal 1-α hydroxylase enzyme (CYP27B1) by fibroblast growth factor 23 (FGF-23), phosphorous excess, and metabolic acidosis.[3–5]

Less is known regarding vitamin D catabolism. Steady-state concentrations of vitamin D metabolites have to represent a balance between production and catabolism.[5] Vitamin D catabolism may therefore have important effects on vitamin D metabolite concentrations in blood and tissues. An improved understanding of vitamin D catabolism may help identify new diagnostic and therapeutic strategies to improve health in chronic kidney disease (CKD) because impaired vitamin D metabolism leads to secondary hyperparathyroidism and bone disease and may contribute to cardiovascular disease, progression to end stage renal disease, and premature death.[3–11]

To better assess vitamin D catabolism in humans, we developed a novel high-throughput assay for circulating 24,25-dihydroxyvitamin D ($24,25(OH)_2D_3$).[12] The most abundant product of vitamin D catabolism, $24,25(OH)_2D_3$, is produced from $25(OH)D_3$ by CYP24A1, the 24α-hydroxylase enzyme.[13] CYP24A1 also converts $1,25(OH)_2D_3$ to 1,24,25-trihydroxyvitamin D_3 (Figure 1). Hydroxylated products of CYP24A1 are further converted to more polar metabolites and excreted in urine or bile. In a cohort of patients referred to nephrology clinics, we demonstrated a strong, independent, direct correlation of estimated GFR (eGFR) with serum $24,25(OH)_2D_3$ concentration.[12] This observation suggests that

CKD is characterized by reduced vitamin D catabolism, in addition to reduced $1,25(OH)_2D_3$ production.

In the current study, we tested associations of eGFR with circulating $24,25(OH)_2D_3$ concentration across a wide range of eGFR using data from five cohort studies and clinical trials. Because $24,25(OH)_2D_3$ production and circulating $24,25(OH)_2D_3$ concentration are highly dependent on available substrate $25(OH)D_3$, we examined $24,25(OH)_2D_3$ in the context of $25(OH)D_3$. We hypothesized that lower eGFR is associated with smaller increments in circulating $24,25(OH)_2D_3$ concentration for a given increment in circulating $25(OH)D_3$ concentration. Such a finding would further support the theory that GFR loss leads to reduced vitamin D catabolism.

Methods

Study Populations

We measured serum or plasma $24,25(OH)_2D_3$ concentrations in five cohort studies and clinical trials: the Diabetes Control and Complications Trial (DCCT), the Multi-Ethnic Study of Atherosclerosis (MESA), the Cardiovascular Health Study (CHS), the Seattle Kidney Study (SKS), and the Hemodialysis (HEMO) Study. We included all five studies in this cross-sectional analysis.

The DCCT was a randomized clinical trial that enrolled 1,441 participants with type 1 diabetes to test the effects of intensive diabetes therapy on the development of micro- and macro-vascular complications.[14] We measured plasma vitamin D metabolite concentrations for all non-pregnant participants with available frozen samples collected at or near the end of the DCCT (N=1193).[15]

MESA is an observational cohort study of subclinical cardiovascular disease among people who were free of clinical cardiovascular disease at study entry.[16] We measured serum vitamin D metabolite concentrations for all MESA participants with available frozen samples collected at baseline (N=6470 of 6814).[17]

CHS is an observational cohort study of cardiovascular disease among adults aged 65 or older.[18] We measured serum vitamin D metabolites at the 1996–1997 CHS study visit (4–7 years after baseline) using a case-cohort design. In this study, we included the 932 participants from the randomly-selected cohort.

SKS is an observational cohort study of patients referred to nephrology clinics associated with the University of Washington (Seattle, WA).[12] We measured baseline serum vitamin D metabolites using a case-cohort design.[12] In this study, we included the 289 participants included in the randomly-selected cohort.

The HEMO Study was a randomized clinical trial that enrolled 1846 participants with ESRD treated with maintenance hemodialysis to test the effects of dialysis dose and membrane flux on mortality.[19] In this study, we included the 712 participants for whom both $24,25(OH)_2D_3$ and its interfering analyte(s) were measured at baseline, as described below.

Measurement of $24,25(OH)_2D_3$

We measured $24,25(OH)_2D_3$ using liquid chromatography–tandem mass spectrometry (LC-MS/MS) at the University of Washington Nutrition Obesity Research Center supervised by A.N.H.. There were three variations of $24,25(OH)_2D_3$ assay used across the populations. For the DCCT, MESA, and SKS, a liquid-liquid extraction was used to prepare samples prior to LC-MS/MS.[12,15,17,20] After these analyses, it was discovered that the liquid chromatography method did not separate $24,25(OH)_2D_3$ from another analyte or analytes, present at low concentrations, which we presumed based on elution time and fragmentation pattern to be 23S,25-dihydroxyvitamin D_3 and/or 25,26-dihydroxyvitamin D_3.[21] In order to separate this interfering analyte(s) from $24,25(OH)_2D_3$, methylamine was added to the mobile phase in the liquid chromatography method. This second assay was used to analyze samples from the CHS. For the HEMO cohort, immunoaffinity enrichment was added to the newer chromatographic method to measure $1,25(OH)_2D_3$ and 1,25-dihydroxyvitaminD_2 in addition to $24,25(OH)_2D_3$, and the interfering analyte(s).[21,22] All assays measured $25(OH)D_3$ and 25-hydroxyvitamin D_2, calibrated to standards provided by the National Institute of Standards and Technology,[23] concurrently with $24,25(OH)_2D_3$.

For our primary analyses, in order to evaluate comparable $24,25(OH)_2D_3$ values across all cohorts, we summed the concentrations of $24,25(OH)_2D_3$ and the interfering analyte(s) for each participant in the CHS and the HEMO Study. In CHS, the mean concentration of the interfering dihydroxyvitamin D_3 metabolite(s) was 0.73 ± 0.35 (standard deviation) ng/mL, while the mean concentration of specific $24,25(OH)_2D_3$ was 2.77 ± 1.70 ng/mL. The correlation of the summed dihydroxyvitamin D_3 metabolites with specific $24,25(OH)_2D_3$ was 0.99 (Figure S1, available as online supplementary material). In the HEMO Study, the mean concentration of the interfering dihydroxyvitamin D_3 metabolite(s) was 0.52 ± 0.31 ng/mL, the mean concentration of specific $24,25(OH)_2D_3$ was 0.33 ± 0.23 ng/mL, and the correlation of the summed dihydroxyvitamin D_3 metabolites with specific $24,25(OH)_2D_3$ was 0.82 (Figure S2). We performed sensitivity analyses in the CHS to demonstrate that our primary associations of interest did not differ using the summed values versus the specific $24,25(OH)_2D_3$ measurements. Specifically, we compared the association of eGFR with the summed value to the association of eGFR with specific $24,25(OH)_2D_3$ concentration.

To verify that the $24,25(OH)_2D_3$ assays yielded consistent results across the populations, we regularly measured a set of 20 quality control serum samples collected from healthy donors. Frozen serum was used to reflect real-world sample testing. Twenty samples (instead of two or three) were used to evaluate for drift specific to a subset of samples with increased sensitivity. The quality control samples were collected and divided into aliquots at the University of Vermont in 2008 and stored frozen at −80°C. The set of 20 samples was measured 9 times across the study populations and included measurements of $24,25(OH)_2D_3$ using the liquid-liquid extraction method without methylamine in the chromatographic method and the method with methylamine. The average coefficient of variation (CV) observed for these 20 samples over the nine measurements (spanning 16 months) was 14.6%. Importantly, there was no significant trend detected for the mean of these 20 samples over the 16 months, and the CV of the 9 means was 7.9%, indicating that there was no drift in calibration of the assays across the populations. The measurements of $24,25(OH)_2D_3$ by

the liquid-liquid extraction method and the immunoaffinity method have previously been shown to agree well.[21]

Clinical Characteristics

In the DCCT, MESA, CHS, and SKS, serum creatinine concentrations were measured using methods traceable to isotope dilution mass spectrometry. The CKD-EPI (CKD Epidemiology Collaboration) equation was used to estimate GFR from serum creatinine and demographic variables.[24] Urine albumin excretion was quantified as the mean of two 4-hour albumin excretion rates in the DCCT[15] and as albumin-creatinine ratio from a single urine sample in MESA, CHS, and SKS. Diabetes was defined as the use of glucose-lowering medications, fasting glucose ≥126 mg/dL, or (in SKS only) random glucose ≥200 mg/dL. Hypertension was defined as the use of antihypertensive medications, systolic blood pressure ≥140 mmHg, or diastolic blood pressure ≥90 mmHg. Intact parathyroid hormone (PTH) was measured using a second-generation immunoassay on the Beckman-Coulter DxI system. Intact fibroblast growth factor 23 (FGF-23) was measured by ELISA (Kainos) for the DCCT, MESA, and SKS. Carboxy-terminal FGF-23 was measured by ELISA (Immutopics) for CHS. $1,25(OH)_2D_3$ was measured by mass spectrometry in the DCCT, SKS, and the HEMO Study.[22]

Statistical Analysis

All analyses used individual-level data from each of the five included cohorts. Bivariate relationships of $24,25(OH)_2D_3$ concentration with $25(OH)D_3$ concentration were examined using scatterplots, locally-weighted scatterplot smoothing (LOWESS), Pearson correlation, and linear regression. These relationships were examined both by cohort and by eGFR, using data pooled across cohorts. LOWESS curves were calculated using all available data but truncated below the 2.5% percentile and above the 97.5% percentile of $25(OH)D_3$ concentration for presentation. Multivariable linear regression models were used to test associations of clinical characteristics (including eGFR) with $24,25(OH)_2D_3$ concentration (the dependent variable), adjusting for potential confounding variables and cohort. We included $25(OH)D_3$ and interaction terms of each clinical characteristic with $25(OH)D_3$ to estimate how the clinical characteristics modified the relationship of $24,25(OH)_2D_3$ with $25(OH)D_3$. For each category of a covariate, its interaction with $25(OH)D_3$ was reported as a slope (increment in $24,25(OH)_2D_3$ per 10-ng/mL increment in $25(OH)D_3$) and an intercept was estimated for $25(OH)D_3$ = 20 ng/mL, a clinically relevant concentration. Mean values of other covariates were used to estimate values for the covariate of interest. The multivariable models pooled data from MESA, CHS, and SKS; the DCCT and HEMO Study were excluded because there was little or no overlap in eGFR values compared with other cohorts.

Results

Together, the DCCT, MESA, CHS, SKS, and HEMO Study covered a wide range of eGFR values (Table 1 and Figure 2). The distributions of age, sex, race/ethnicity, diabetes, hypertension, body mass index, urine albumin excretion, PTH, and FGF-23 also varied

substantially across studies. Nonetheless, mean $25(OH)D_3$ concentrations were similar, and there was substantial overlap in the distributions of circulating $25(OH)D_3$ concentration.

Within each cohort, there was a strong positive correlation of circulating $24,25(OH)_2D_3$ concentration with circulating $25(OH)D_3$ concentration (Figure 3). These relationships appeared linear when LOWESS curves were compared to estimates generated using linear regression. The correlations of $24,25(OH)_2D_3$ and $25(OH)D_3$ were strongest in DCCT and MESA (r=0.88 and r=0.84, respectively) and weaker in CHS and SKS (r=0.78 and r=0.64, respectively). Pooling data from the DCCT, MESA, CHS, and SKS, correlations of $24,25(OH)_2D_3$ and $25(OH)D_3$ were 0.87, 0.83, 0.81, 0.76, 0.76, and 0.74 for eGFR ≥90, 60–89, 45–59, 30–44, 15–29, and <15 mL/min/1.73 m^2, respectively.

The increment in $24,25(OH)_2D_3$ per unit higher $25(OH)D_3$ (slope) differed by cohort, being greatest in DCCT and MESA, followed by CHS, SKS, and the HEMO Study (Figure 4A). Pooling data across all five cohorts, the increment in $24,25(OH)_2D_3$ per increment in $25(OH)D_3$ (slope) correlated directly with eGFR (Figure 4B). As a result, unadjusted mean $24,25(OH)_2D_3$ concentration at a $25(OH)D_3$ concentration of 20 ng/mL was significantly lower with lower eGFR (Table 2).

Pooling data from MESA, CHS, and SKS in a multivariable model, lower eGFR was associated with a significantly reduced increment in $24,25(OH)_2D_3$ concentration per given increment in $25(OH)D_3$ concentration (slope) and a significantly reduced mean $24,25(OH)_2D_3$ concentration at a $25(OH)D_3$ of 20 ng/mL, adjusting for relevant potential confounding clinical characteristics (Table 3). Other clinical characteristics were also independently associated with $24,25(OH)_2D_3$ concentration, though not as strongly as eGFR. Diabetes was associated with reduced slope and reduced mean $24,25(OH)_2D_3$ concentration at a $25(OH)D_3$ of 20 ng/mL. Black or Asian race and higher urine ACR were associated with reduced slope, but no significant difference in mean $24,25(OH)_2D_3$ concentration at a $25(OH)D_3$ of 20 ng/mL. Age, sex, and body mass index were not independently associated with $24,25(OH)_2D_3$. In CHS, results were similar in terms of the comparison of specific $24,25(OH)_2D_3$ to the sum of specific $24,25(OH)_2D_3$ and its interfering analyte(s) as the outcome (Table S1).

We evaluated associations of circulating PTH and FGF-23 concentrations with circulating $24,25(OH)_2D_3$ concentration separately within MESA, CHS, and SKS because the ranges of PTH and FGF-23 varied substantially by cohort and different methods were used to assay FGF-23 across cohorts. Adjusting for potential confounders, including eGFR, higher FGF-23 was associated with a significantly higher slope and a significantly higher mean $24,25(OH)_2D_3$ concentration at a $25(OH)D_3$ of 20 ng/mL in MESA (Table 4). Similar trends were not observed in CHS and SKS. In the same models, higher PTH was associated with a significantly reduced increment in $24,25(OH)_2D_3$ per given increment in $25(OH)D_3$ (slope) in MESA, with similar trends in CHS and SKS that were not statistically significant. Higher PTH was associated with a significantly lower mean $24,25(OH)_2D_3$ concentration at a $25(OH)D_3$ of 20 ng/mL in SKS, but not MESA or CHS.

Discussion

Across five cohort studies and clinical trials with a wide range of eGFRs, the relationship of circulating $24,25(OH)_2D_3$ concentration with circulating $25(OH)D_3$ concentration was significantly modified by eGFR. Specifically, with lower eGFR, the correlation of $24,25(OH)_2D_3$ with $25(OH)D_3$ concentration was weaker, the increment in $24,25(OH)_2D_3$ per unit higher $25(OH)D_3$ (slope) was reduced, and mean $24,25(OH)_2D_3$ concentration at a given $25(OH)D_3$ concentration was lower. These observations were consistent when $24,25(OH)_2D_3$ concentration was evaluated on the study level, on the participant level, and using multivariable regression. Other clinical characteristics also modified the relationship of $24,25(OH)_2D_3$ with $25(OH)D_3$, but none as strongly as eGFR.

Our results are consistent with and extend those of smaller studies examining targeted populations. Very low or undetectable circulating concentrations of $24,25(OH)_2D_3$ were observed in hemodialysis patients.[25–30] In addition, $24,25(OH)_2D_3$ concentration was found to correlate directly with eGFR in three CKD cohorts, one of which was the SKS, included in this analysis.[12,31,32] Compared to these studies, we evaluated a much broader population, including people with type 1 diabetes and normal or high eGFR (DCCT), participants in two diverse community-based cohorts (MESA and CHS), a cohort with moderate-severe CKD not requiring renal replacement therapy (SKS), and prevalent hemodialysis patients (HEMO Study).

Accounting for available $25(OH)D_3$, $24,25(OH)_2D_3$ production is a measure of CYP24A1-mediated $25(OH)D_3$ clearance. Metabolism by CYP24A1 is thought to be the major route of $25(OH)D_3$ catabolism.[13] Therefore, assuming that circulating $24,25(OH)_2D_3$ concentration is proportional to $24,25(OH)_2D_3$ production, our results suggest that lower GFR, across its full range, is associated with reduced vitamin D catabolism. The weaker correlation of $24,25(OH)_2D_3$ with $25(OH)D_3$ further suggests that inter-individual differences in vitamin D catabolism are accentuated with lower GFR. Our results suggest that CKD is a state in which vitamin D metabolism is stagnant and vitamin D catabolism and $1,25(OH)_2D_3$ production are reduced.

A number of potential mechanisms may explain reduced CYP24A1-mediated $25(OH)D_3$ clearance in CKD. Renal tubules are a known site of CYP24A1 activity.[33] Reduced circulating $24,25(OH)_2D_3$ concentration may therefore be a sign of reduced net tubular CYP24A1 function. This could be due to decreased tubular delivery of $25(OH)D_3$ secondary to reduced glomerular filtration, impaired luminal $25(OH)D_3$ uptake by megalin and cubilin,[34] reduced CYP24A1 protein content, or impaired CYP24A1 function. Renal CYP24A1 expression is known to be stimulated by FGF-23 and inhibited by PTH.[3–5] Our observed positive and negative correlations of FGF-23 and PTH with $24,25(OH)_2D_3$ concentration suggest that these regulatory effects explain inter-individual differences in $24,25(OH)_2D_3$, to some extent. However, associations of eGFR with $24,25(OH)_2D_3$ were more consistent and much stronger than associations of PTH or FGF-23 with $24,25(OH)_2D_3$, suggesting that regulation of CYP24A1 transcription is not the predominant mechanism of reduced vitamin D catabolism in CKD.

Low circulating $24,25(OH)_2D_3$ concentration could reflect reduced non-renal CYP24A1-mediated $25(OH)D^3$ clearance, instead of or in addition to reduced renal CYP24A1-mediated $25(OH)D_3$ clearance.[29] Most cells of the body express the vitamin D receptor, and all of these are thought to express CYP24A1.[13] $1,25(OH)_2D_3$ is known to potently induce CYP24A1, probably to prevent $1,25(OH)_2D_3$ toxicity. Therefore, in CKD, low circulating $24,25(OH)_2D_3$ concentration could reflect in part systemic tissue-level $1,25(OH)_2D_3$ deficiency.

In addition to informing pathophysiology, better understanding of the effects of low $24,25(OH)_2D_3$ concentration may eventually help guide vitamin D–related interventions in CKD. Current clinical care focuses on measuring 25-hydroxyvitamin D and PTH concentrations to direct the initiation and titration of vitamin D supplements and vitamin D receptor agonists. However, these biomarkers may not adequately capture tissue $1,25(OH)_2D_3$ function: $25(OH)D_3$ is relatively inactive and requires regulated conversion to $1,25(OH)_2D_3$ for full hormonal activity, while PTH reflects functional $1,25(OH)_2D_3$ deficiency at only one of many relevant biological sites and is influenced by factors other than $1,25(OH)_2D_3$, such as calcium. Low $24,25(OH)_2D_3$ relative to $25(OH)D_3$ may better reflect impaired vitamin D metabolic function and therefore provide a stronger indication for treatment. Ultimately, to determine whether $24,25(OH)_2D_3$ is a clinically useful biomarker, additional studies are needed to determine whether low $24,25(OH)_2D_3$ is associated with clinical outcomes, is modifiable, and identifies patients who respond favorably to vitamin D–related interventions.

Furthermore, $25(OH)D_3$ is also metabolized by pathways that do not produce $24,25(OH)_2D_3$. For example, metabolism of $25(OH)D_3$ by cytochrome P450 3A4 (CYP3A4) in the liver and intestine produces 4β,25-dihydroxyvitamin D_3.[35] Neither the effects of CKD on alternate catabolic pathways nor the net effects of CKD on overall vitamin D catabolism are known. One pharmacokinetic study documented a nearly 50% reduction in the clearance of radiolabeled $25(OH)D_3$ in hemodialysis patients, compared with healthy control participants.[25] This is consistent with our findings utilizing circulating $24,25(OH)_2D_3$ and strongly suggests that net vitamin D catabolism is reduced in hemodialysis patients. Two similar pharmacokinetic studies in CKD yielded conflicting results.[36,37] Additional pharmacokinetic studies and studies assessing multiple catabolic pathways across a wide range of GFR would be useful to better define this pathophysiology.

Limitations of this study include the lack of direct pharmacokinetic measurements of vitamin D catabolism; the cross-sectional design, which is subject to confounding and precludes assessment of changes in $24,25(OH)_2D_3$ within individuals over time; the focus on a single biomarker of vitamin D catabolism; and the inclusion of an interfering analyte(s) in the measurement of $24,25(OH)_2D_3$. Regarding the interfering analyte(s), its concentration was low compared with specific $24,25(OH)_2D_3$ in CHS, the summed value utilized in primary analyses correlated strongly with specific $24,25(OH)_2D_3$ (r=0.99 in CHS), and sensitivity analyses in the CHS verified that associations of eGFR with specific $24,25(OH)_2D_3$ were similar to those of the summed value used in primary analyses.

This study also has important strengths, including the use of five diverse cohorts that together cover the full range of GFR and enhance external validity, the large number of novel $24,25(OH)_2D_3$ measurements utilized to evaluate vitamin D catabolism on an unprecedented scale, and the ability to assess the impact of important regulatory hormones. As such, it represents an important initial step in understanding vitamin D catabolism in CKD.

In conclusion, lower eGFR is strongly associated with reduced vitamin D catabolism as measured by circulating $24,25(OH)_2D_3$ concentration. Further studies are needed to more fully understand vitamin D catabolism and its changes in CKD, to determine whether assessment of vitamin D catabolism augments clinical care, and to develop new therapeutic approaches to treat impaired vitamin D metabolism in CKD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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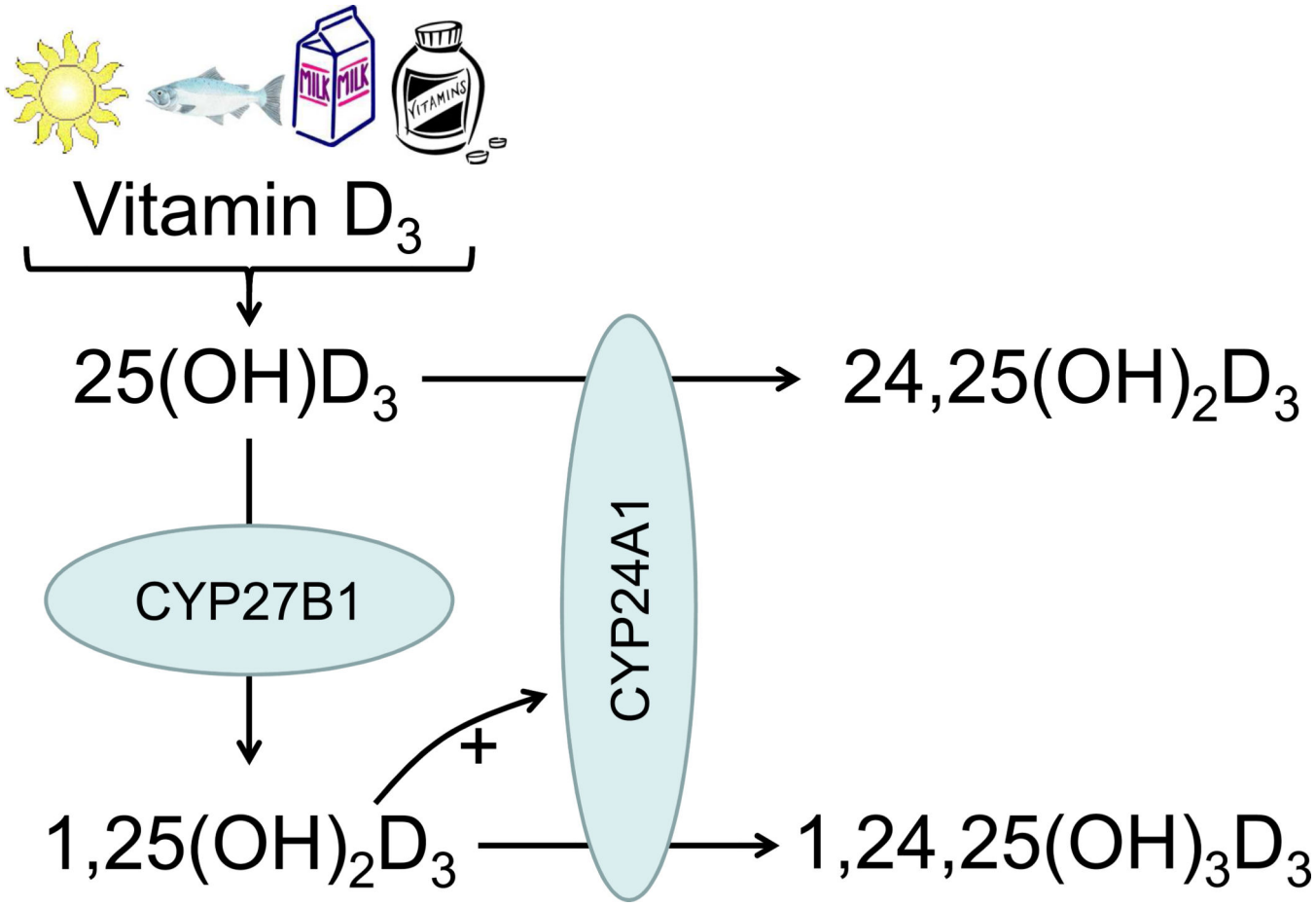

Figure 1. Vitamin D_3 metabolism

Vitamin D_3 synthesized in the skin or consumed by mouth is metabolized to 25-hydroxyvitamin D_3 ($25(OH)D_3$), which can then be metabolized to 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$, the active vitamin D hormone) by CYP27B1. Vitamin D_3 catabolism is accomplished predominantly by CYP24A1, which metabolizes $25(OH)D_3$ to 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) and $1,25(OH)_2D_3$ to 1,24,25-trihydroxyvitamin D_3 ($1,24,25(OH)_3D_3$). CYP24A1 is induced by $1,25(OH)_2D_3$.

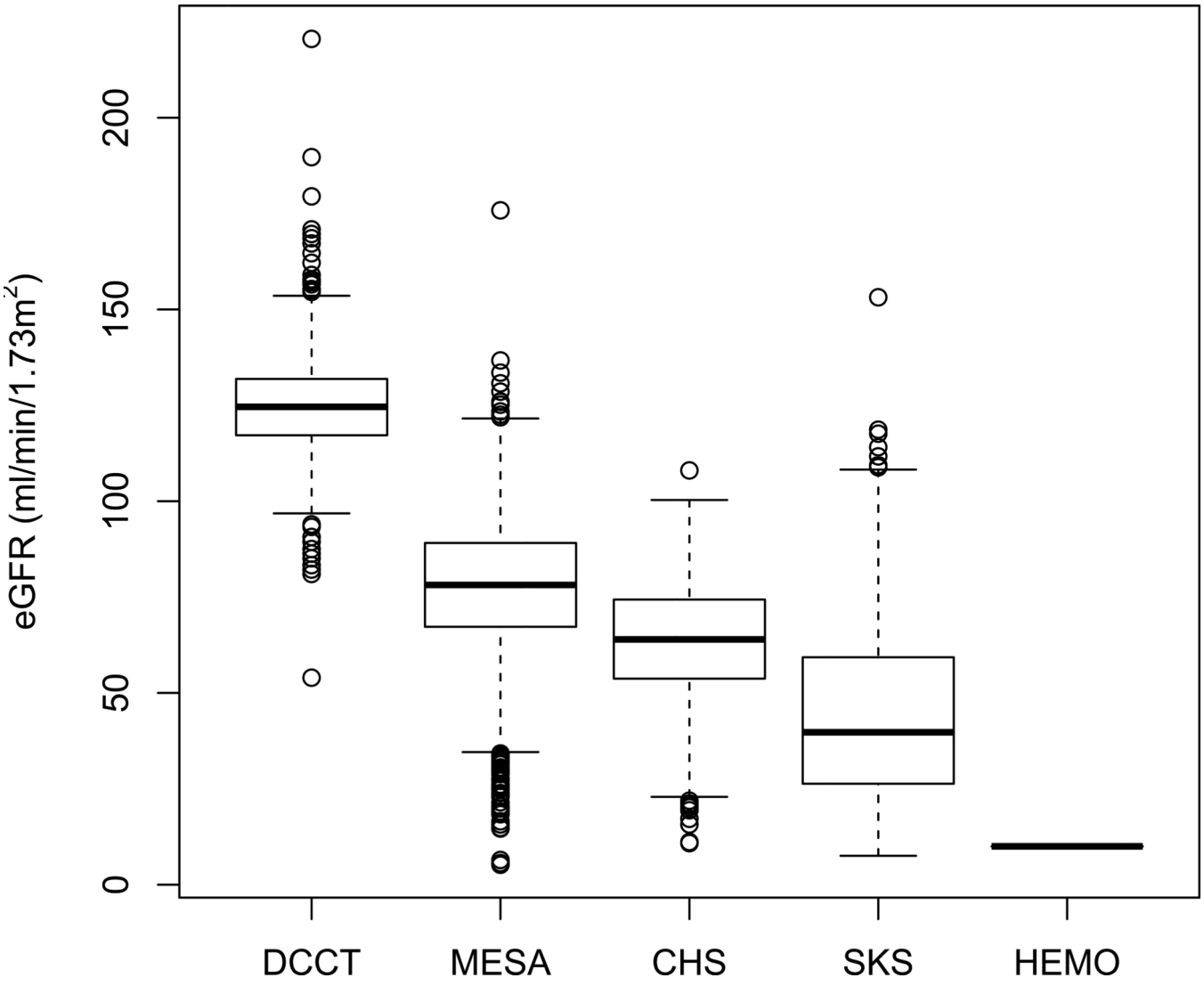

Figure 2. Distribution of estimated GFR among 9596 participants in the Diabetes Control and Complications Trial (DCCT), Multi-Ethnic Study of Atherosclerosis (MESA), Cardiovascular Health Study (CHS), Seattle Kidney Study (SKS), and Hemodialysis (HEMO) Study

Box plots demonstrate the 5^{th}, 25^{th}, 50^{th}, 75^{th}, and 95^{th} percentiles, with dots representing estimated GFR outside the 5^{th} – 95^{th} percentiles. All HEMO Study participants have end-stage renal disease treated with hemodialysis, represented here as an estimated GFR of 10 mL/min/1.73 m^2.

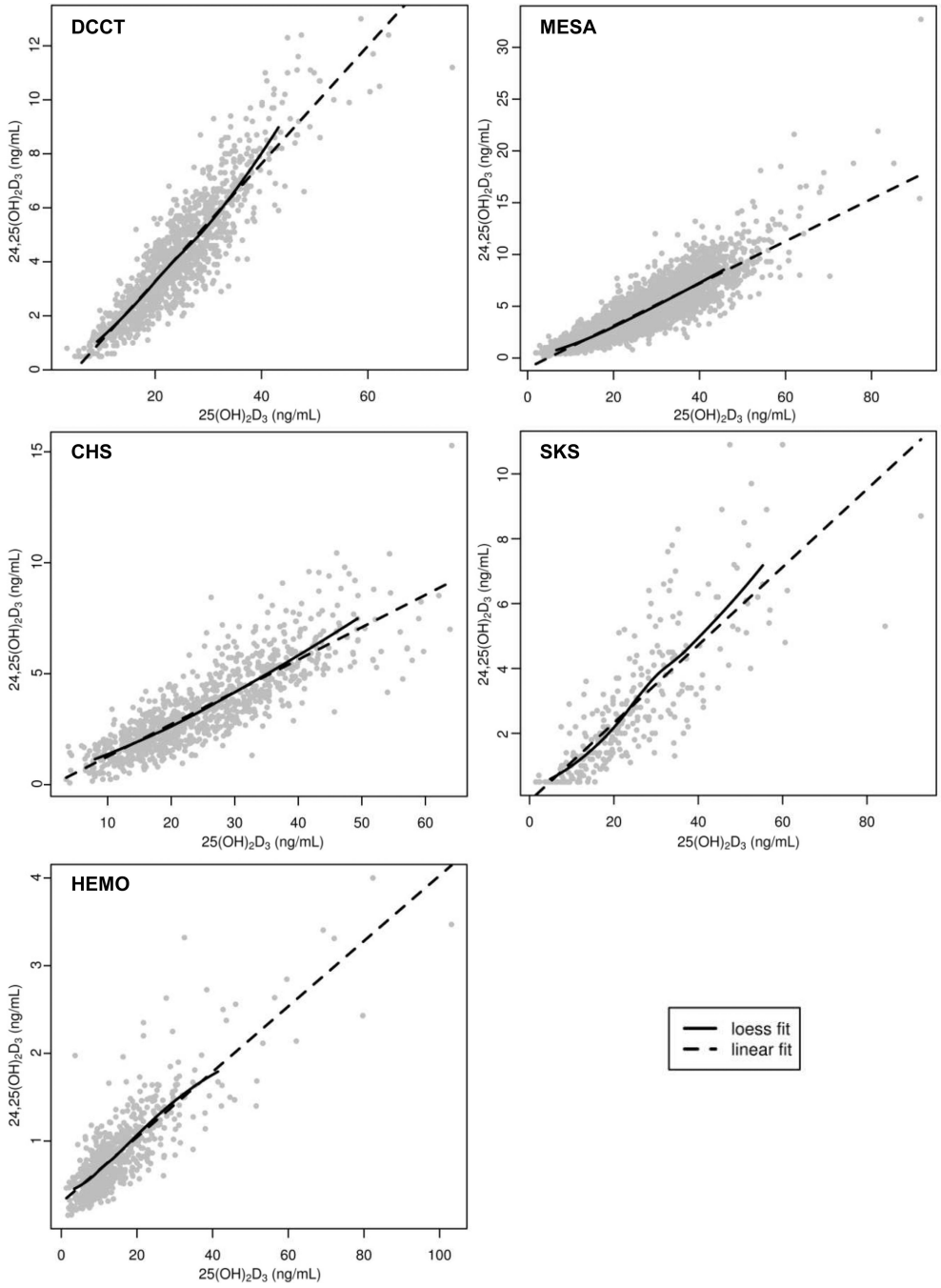

Figure 3. Relationships of 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) with 25-hydroxyvitamin D_3 ($25(OH)D_3$) in five cohort studies and clinical trials

The scatter plots display individual values for each participant. Solid lines represent mean $24,25(OH)_2D_3$ concentrations estimated using locally-weighted scatterplot smoothing (LOWESS). Broken lines represent linear fit generated using unadjusted linear regression. LOWESS curves were calculated using all available data but truncated below the 2.5% percentile and above the 97.5% percentile of $25(OH)D_3$ concentration for presentation. Note that the ranges of the×and y axes vary in each panel. DCCT = Diabetes Control and

Complications Trial; MESA = Multi-Ethnic Study of Atherosclerosis; CHS = Cardiovascular Health Study; SKS = Seattle Kidney Study; HEMO = Hemodialysis Study.

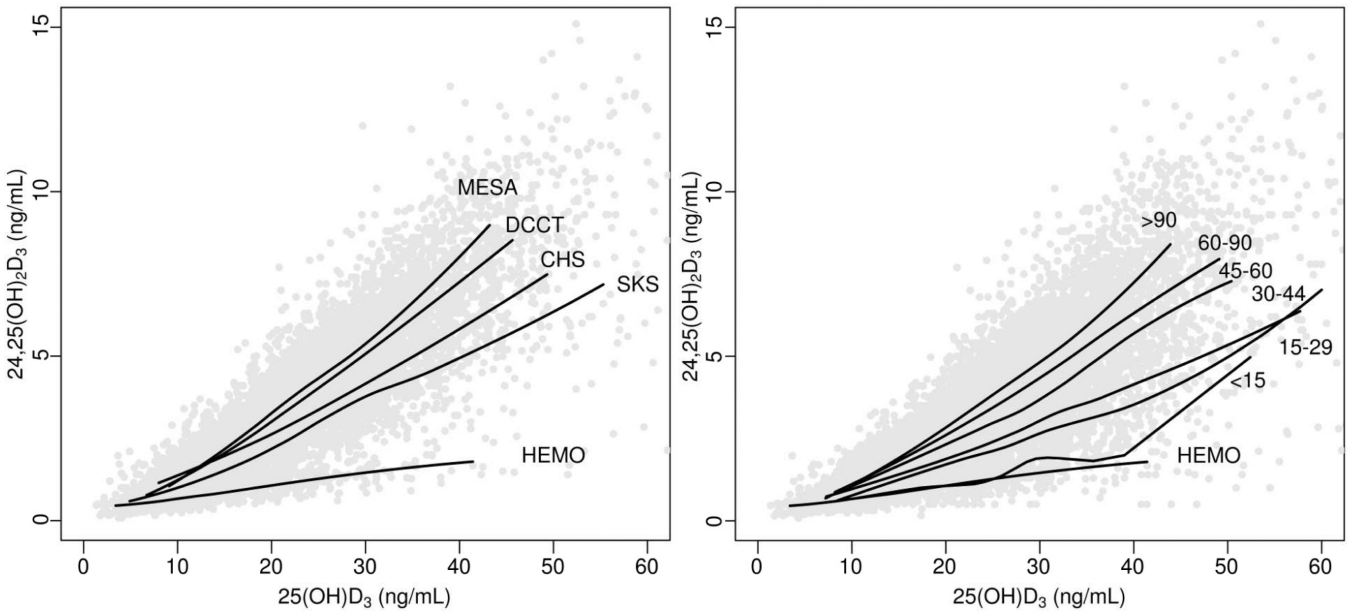

Figure 4. Relationships of 24,25-dihydroxyvitamin D_3 ($24,25(OH)_2D_3$) with 25-hydroxyvitamin D_3 ($25(OH)D_3$) among 9596 participants in the Diabetes Control and Complications Trial (DCCT), Multi-Ethnic Study of Atherosclerosis (MESA), Cardiovascular Health Study (CHS), Seattle Kidney Study (SKS), and Hemodialysis (HEMO) Study

The scatter plots display individual values for each participant. Solid lines represent mean $24,25(OH)_2D_3$ concentrations estimated using locally-weighted scatterplot smoothing (LOWESS) among subgroups defined by cohort (Panel A) or category of estimated GFR (Panel B). LOWESS curves were calculated using all available data but truncated below the 2.5% percentile and above the 97.5% percentile of $25(OH)D_3$ concentration for presentation. The X axis was truncated at 60 ng/mL. Estimated GFR is in mL/min/1.73 m^2.

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Table 1

Participant characteristics by cohort.

	DCCT	MESA	CHS	SKS	HEMO
No. analyzed	1193	6470	932	289	712
Age (y)	26.9 (7)	62.1 (10.3)	78.1 (4.8)	60.7 (13)	56.7 (14)
Female sex	564 (47)	3446 (53)	372 (40)	49 (17)	402 (56)
Race/ethnicity					
White	1151 (96.5)	2509 (38.8)	778 (83.5)	197 (68.2)	235 (33.2)
Black	24 (2.0)	1762 (27.2)	148 (15.9)	56 (19.4)	449 (63.4)
Hispanic	13 (1.1)	1412 (21.8)	2 (0.2)	12 (4.2)	21 (3.0)
Asian	4 (0.3)	787 (12.2)	3 (0.3)	8 (2.8)	7 (1.0)
Other	1 (0.1)	0 (0)	1 (0.1)	16 (5.5)	0 (0)
Diabetes	1193 (100)	803 (12)	131 (14)	161 (56)	320 (45)
Hypertension	104 (9)	4266 (66)	744 (80)	274 (95)	676 (95)
BMI category ()					
<25 kg/m^2	560 (46.9)	1865 (28.8)	323 (35.2)	48 (16.6)	356 (50.3)
25-<30 kg/m^2	487 (40.8)	2552 (39.4)	400 (43.6)	89 (30.8)	338 (47.7)
≥30 kg/m^2	146 (12.2)	2053 (31.7)	194 (21.2)	152 (52.6)	14 (2.0)
Mean eGFR (mL/min/1.73 m^2)	125 (12.5)	78.2 (16.3)	63.2 (16.5)	45.6 (25.8)	-
eGFR category					
≥ 90 mL/min/1.73 m^2	1185 (99.3)	1518 (23.5)	47 (5)	22 (7.6)	0
60–89 mL/min/1.73 m^2	7 (0.6)	4125 (63.8)	499 (53.5)	46 (15.9)	0
45–59 mL/min/1.73 m^2	1 (0.1)	699 (10.8)	265 (28.4)	52 (18)	0
30–44 mL/min/1.73 m^2	0	96 (1.5)	94 (10.1)	80 (27.7)	0
15–29 mL/min/1.73 m^2	0	21 (0.3)	24 (2.6)	71 (24.6)	0
< 15* mL/min/1.73 m^2	0	5 (0.1)	3 (0.3)	18 (6.2)	0
Hemodialysis	0	0	0	0	712 (100)
UAE (mg/d or mg/g**)	10.0 [7.1–16.2]	5.3 [3.3–11.0]	9.8 [5.4–23.0]	143.3 [20.6–704.3]	-
UAE category (mg/d or mg/g**)					

	DCCT	MESA	CHS	SKS	HEMO
<30	1073 (89.9)	5829 (90.5)	676 (80)	82 (28.6)	-
30–299	105 (8.8)	525 (8.1)	137 (16.2)	90 (31.4)	-
≥300	15 (1.3)	89 (1.4)	32 (3.8)	115 (40.1)	-
24,25(OH)***2***D***3***(ng/mL)	4.1 (2.1)	3.7 (2.4)	3.5 (1.9)	2.8 (2.1)	0.8 (0.5)
25(OH)D***3***(ng/mL)	23.9 (8.9)	22.7 (10.6)	25.3 (11.1)	24 (14.1)	14.6 (10.5)
Total 25(OH)D (ng/mL)	25.4 (8.7)	25.3 (10.9)	28.1 (11.3)	29.3 (15.3)	18.6 (13.0)
Total 1,25(OH)***2***D (pg/mL)	41.3 (11.8)	-	-	33.6 (14.9)	10.9 (11.9)
Calcium (mg/dL)	-	9.6 (0.4)	9.8 (0.6)	9.0 (0.8)	9.3 (0.9)
Phosphorus (mg/dL)	-	3.7 (0.5)	3.8 (0.6)	3.8 (0.7)	5.8 (1.9)
Parathyroid hormone (pg/mL)	28.5 [21.6–37.2]	40.6 [31.2–53.3]	43.1 [33.0–57.0]	72.8 [43.3–130.2]	190 [83–426]
FGF-23 (pg/mL or RU/mL[***])	30.8 [24.9–35.9]	37.7 [30.5–46.4]	69.7 [53.3–96.7]	59.8 [42.6–97.9]	3118 [726–129278]

Note: Unless otherwise indicated, values for categorical variables are given as number (percentage); values for continuous variables are given as mean ± standard deviation or median [interquartile range].

Limited data are missing for BMI (CHS), eGFR (MESA), and UAE (MESA, CHS, SKS). Conversion factors for units: calcium in mg/dL to mmol/L, ×0.2495; phosphorus in mg/dL to mmol/L, ×0.3229;

[*] Not treated with dialysis.

[**] UAE is based on albumin excretion rate (in mg/d) for DCCT, otherwise it reflects albumin-creatining ratio (mg per g of creatinine).

[***] FGF-23 is in RU/mL for CHS, otherwise pg/mL.

Abbreviations and definitions: DCCT = Diabetes Control and Complications Trial; MESA = Multi-Ethnic Study of Atherosclerosis; CHS = Cardiovascular Health Study; SKS = Seattle Kidney Study; HEMO = Hemodialysis Study; BMI, body mass index; eGFR, estimated glomerular filtration rate; UAE, urinary albumin excretion; FGF, fibroblast growth factor; total 1,25(OH)***2***D, 1,25-dihydroxyvitamin D_2 and 1,25-dihydroxyvitamin D_3; 25(OH)D_3, 25-hydroxyvitamin D_3; 24,25(OH)$_2D_3$, 24,25-dihydroxyvitamin D_3; total 25(OH)D, 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3

Table 2

Relationship of $24,25(OH)_2D_3$ with $25(OH)D_3$ in all 5 studies.

	Mean $24,25(OH)_2D_3$ at $25(OH)D_3$ = 20 ng/mL (ng/mL)	**Increment in $24,25(OH)_2D_3$ per increment in $25(OH)D_3$ (slope, ng/mL per 10 ng/mL)**
Cohort (Model A)		
DCCT	2.97 (2.90 to 3.03)	2.16 (2.10 to 2.23)
MESA	2.68 (2.65 to 2.72)	1.84 (1.81 to 1.87)
CHS	2.40 (2.30 to 2.49)	1.33 (1.26 to 1.40)
SKS	1.98 (1.77 to 2.20)	0.88 (0.76 to 1.00)
HEMO	1.04 (1.02 to 1.07)	0.37 (0.35 to 0.39)
eGFR category (Model B)		
≥ 90 mL/min/1.73 m^2	2.92 (2.87 to 2.96)	2.06 (2.01 to 2.1)
60–89 mL/min/1.73 m^2	2.68 (2.64 to 2.72)	1.77 (1.74 to 1.81)
45–59 mL/min/1.73 m^2	2.35 (2.26 to 2.45)	1.55 (1.48 to 1.62)
30–44 mL/min/1.73 m^2	1.92 (1.74 to 2.10)	1.17 (1.05 to 1.29)
15–29 mL/min/1.73 m^2	1.69 (1.43 to 1.95)	0.92 (0.74 to 1.10)
< 15* mL/min/1.73 m^2	1.14 (0.62 to 1.66)	0.61 (0.22 to 1.00)
Hemodialysis	1.04 (1.02 to 1.07)	0.37 (0.35 to 0.39)

Note: N=9596. Two parallel models are presented evaluating this relationship: (A) by cohort, or (B) by eGFR, pooling cohorts. The regression model evaluates $24,25(OH)_2D_3$ as dependent variable and $25(OH)D_3$, cohort or eGFR, and the interaction of $25(OH)D_3$ with cohort or eGFR as independent variables. No additional covariates are included. intercepts (mean $24,25(OH)_2D_3$ concentration at $25(OH)D_3$ = 20 ng/mL) and Slopes (increment in $24,25(OH)_2D_3$ per 10-ng/mL increment in $25(OH)D_3$) are derived from linear regression.. 95% confidence intervals are given in parentheses.

Abbreviations: eGFR, estimated glomerular filtration rate; DCCT = Diabetes Control and Complications Trial; MESA = Multi-Ethnic Study of Atherosclerosis; CHS = Cardiovascular Health Study; SKS = Seattle Kidney Study; HEMO = Hemodialysis Study. $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3

*Patients treated with hemodialysis are not included in this category.

Table 3

Adjusted associations of eGFR and other clinical characteristics with serum $24,25(OH)_2D_3$ concentration in MESA, CHS, and SKS.

Independent variable	Mean $24,25(OH)_2D_3$ at $25(OH)D_3$ = 20 ng/mL (ng/mL)		Increment in $24,25(OH)_2D_3$ per increment in $25(OH)D_3$ (slope, ng/mL per 10 ng/mL)	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
Age Category		0.02		<0.001
<55 y	2.94 (2.83 to 3.06)		2.12 (2.03 to 2.21)	
55–64 y	2.91 (2.78 to 3.04)		2.16 (2.05 to 2.26)	
65–74 y	2.83 (2.70 to 2.97)		2.16 (2.05 to 2.28)	
≥75 y	2.78 (2.63 to 2.93)		1.94 (1.82 to 2.06)	
Sex		<0.001		0.5
Male	2.94 (2.83 to 3.06)		2.12 (2.03 to 2.21)	
Female	2.82 (2.71 to 2.94)		2.14 (2.05 to 2.23)	
Race/ethnicity		0.07		<0.001
White	2.94 (2.83 to 3.06)		2.12 (2.03 to 2.21)	
Black	2.99 (2.87 to 3.11)		2.03 (1.92 to 2.13)	
Hispanic	3.07 (2.94 to 3.19)		2.15 (2.05 to 2.26)	
Asian	2.92 (2.78 to 3.05)		1.85 (1.72 to 1.97)	
Other	2.62 (1.91 to 3.32)		2.76 (2.28 to 3.24)	
Diabetes		<0.001		<0.001
No	2.94 (2.83 to 3.06)		2.12 (2.03 to 2.21)	
Yes	2.74 (2.59 to 2.89)		1.88 (1.76 to 2.01)	
BMI category		0.3		<0.001
<25 kg/m^2	2.94 (2.83 to 3.06)		2.12 (2.03 to 2.21)	
25-<30 kg/m^2	2.98 (2.87 to 3.10)		2.06 (1.97 to 2.15)	
≥30 kg/m^2	2.93 (2.81 to 3.05)		1.96 (1.87 to 2.06)	
eGFR category		<0.001		<0.001
>90 mL/min/1.73 m^2	2.94 (2.83 to 3.06)		2.12 (2.03 to 2.21)	
60–89 mL/min/1.73 m^2	2.81 (2.70 to 2.93)		1.92 (1.84 to 1.99)	
45–59 mL/min/1.73 m^2	2.58 (2.42 to 2.73)		1.74 (1.63 to 1.85)	
30–44 mL/min/1.73 m^2	2.29 (2.07 to 2.52)		1.25 (1.11 to 1.39)	
15–29 mL/min/1.73 m^2	2.07 (1.76 to 2.38)		0.94 (0.74 to 1.15)	
<15 mL/min/1.73 m^2	1.45 (0.86 to 2.04)		1.10 (0.68 to 1.52)	
Urine ACR *		0.9		0.001
<30 mg/g	2.94 (2.83 to 3.06)		2.12 (2.03 to 2.21)	
30–299 mg/g	2.92 (2.76 to 3.07)		1.98 (1.86 to 2.11)	
≥300 mg/g	2.96 (2.72 to 3.19)		1.90 (1.72 to 2.08)	

Note: N=7691. A single combined regression model evaluates 24,25(OH)$_2$D$_3$ as dependent variable and 25(OH)D$_3$, clinical characteristics (including eGFR), and interactions of 25(OH)D$_3$ with each clinical characteristic as independent variables. All variables included in the model are shown. intercepts (mean 24,25(OH)$_2$D$_3$ concentration at 25(OH)D$_3$ = 20 ng/mL) and Slopes (increment in 24,25(OH)$_2$D$_3$ per 10-ng/mL increment in 25(OH)D$_3$) are derived from linear regression. 95% confidence intervals are listed in parentheses.

Reported are the results of a single multivariable regression model, with circulating 24,25(OH)$_2$D$_3$ concentration as dependent variable. In addition to circulating 25(OH)D$_3$ concentration, the model includes age, sex, race/ethnicity, diabetes, body mass index, estimated GFR, urine ACR, and interactions of each of these variables with circulating 25(OH)D$_3$ concentration as independent variables. Estimates are reported at mean values of the other independent variables. P-values test the null hypothesis that the slope (or intercept) does not differ by level of the independent variable (p-value for interaction).

ACR, albumin-creatinine ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; 24,25(OH)$_2$D$_3$, 24,25-dihydroxyvitamin D$_3$; 25(OH)D$_3$, 25-hydroxyvitamin D$_3$

Table 4

Adjusted associations of circulating PTH and FGF-23 concentrations with serum $24,25(OH)_2D_3$ concentration, evaluated in 3 individual cohorts.

Independent variable	Mean $24,25(OH)_2D_3$ at $25(OH)D_3$ = 20 ng/mL (ng/mL)		Increment in $24,25(OH)_2D_3$ per increment in $25(OH)D_3$ (slope, ng/mL per 10 ng/mL)	
	Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
MESA				
PTH ()		0.2		<0.001
≤ 31.2	3.21 (2.96 to 3.46)		2.54 (2.35 to 2.73)	
31.3–40.6	3.22 (2.97 to 3.47)		2.34 (2.14 to 2.54)	
40.7–53.3	3.23 (2.98 to 3.48)		2.18 (1.98 to 2.38)	
≥ 53.4	3.14 (2.88 to 3.40)		2.21 (2.00 to 2.41)	
FGF-23		<0.001		<0.001
≤ 30.5	3.16 (2.92 to 3.41)		2.41 (2.21 to 2.6)	
30.6–37.7	3.26 (3.01 to 3.51)		2.5 (2.30 to 2.69)	
37.8–46.4	3.31 (3.05 to 3.56)		2.65 (2.45 to 2.85)	
≥ 46.5	3.39 (3.14 to 3.64)		2.51 (2.31 to 2.70)	
CHS				
PTH		0.6		0.08
≤ 33.2	2.78 (0.94 to 4.62)		1.89 (0.50 to 3.28)	
33.3–42.6	2.91 (1.05 to 4.77)		1.64 (0.24 to 3.04)	
42.7–56.8	2.84 (0.96 to 4.73)		1.71 (0.30 to 3.12)	
≥ 56.9	2.73 (0.84 to 4.62)		1.61 (0.18 to 3.04)	
FGF-23		0.7		0.6
≤ 53.0	2.59 (0.74 to 4.44)		2.12 (0.73 to 3.51)	
53.1–69.0	2.71 (0.88 to 4.54)		2.02 (0.65 to 3.39)	
69.1–93.8	2.66 (0.82 to 4.49)		2.12 (0.72 to 3.51)	
≥ 93.9	2.78 (0.92 to 4.63)		2.15 (0.74 to 3.56)	
SKS				
PTH		0.05		0.4
≤ 43.4	3.15 (1.60 to 4.7)		1.3 (0.38 to 2.21)	
43.5–72.8	3.02 (1.40 to 4.63)		1.12 (0.13 to 2.10)	
72.9–130.0	2.60 (0.96 to 4.23)		1.25 (0.25 to 2.24)	
≥ 130.1	2.33 (0.7 to 3.95)		0.95 (-0.08 to 1.98)	
FGF-23		0.5		0.8
≤ 42.6	3.00 (1.41 to 4.59)		1.55 (0.59 to 2.51)	
42.7–59.8	3.42 (1.76 to 5.09)		1.40 (0.45 to 2.36)	
59.9–97.9	3.48 (1.78 to 5.18)		1.42 (0.37 to 2.46)	
≥ 98.0	3.47 (1.70 to 5.24)		1.35 (0.26 to 2.44)	

Note: PTH and FGF-23 expressed in pg/mL, except that FGF-23 expressed in RU/mL for CHS. Reported are the results of three multivariable regression models (one for each cohort), with circulating $24,25(OH)_2D_3$ concentration as dependent variable. In addition to circulating $25(OH)D_3$ concentration, each model includes PTH, FGF-23, age, sex, race/ethnicity, diabetes, body mass index, estimated glomerular filtration rate, urine albumin-creatinine ratio, and interactions of each of these variables with circulating $25(OH)D_3$ concentration as independent variables. Estimates are reported at mean values of the other independent variables. P-values test the null hypothesis that the slope or intercept does not differ by level of PTH or FGF-23.

Abbreviations: MESA = Multi-Ethnic Study of Atherosclerosis; CHS = Cardiovascular Health Study; SKS = Seattle Kidney Study. FGF, fibroblast growth factor; PTH, parathyroid hormone; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3