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Original Investigation

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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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 GFRs.

Study Design: Cross-sectional study.

Setting & Participants: 9,596 participants in 5 cohort studies and clinical trials: the Diabetes Control and Complications Trial (N = 1,193), Multi-Ethnic Study of Atherosclerosis (N = 6,470), 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 eGFRs. Moreover, the increment in 24,25$(OH)_2D_3$ concentration associated with higher 25(OH)D_3 concentration (slope) was lower with lower eGFRs: 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 of 24,25$(OH)_2D_3$ per 10 ng/mL of 25(OH)D_3 for eGFRs $\geq$ 90, 60-89, 45-59, 30-44, 15-29, and $<$15 mL/min/1.73 m^2 and end-stage renal disease treated with hemodialysis, respectively. As a result, at a 25(OH)D_3 concentration of 20 ng/mL, mean 24,25$(OH)_2D_3$ concentrations were 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 circulating parathyroid hormone and fibroblast growth factor 23 concentrations 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 associated strongly with reduced vitamin D catabolism, as measured by circulating 24,25$(OH)_2D_3$ concentration.

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INDEX WORDS: Decreased renal function; low estimated glomerular filtration rate; vitamin D catabolism; 1,25-dihydroxyvitamin D3; 25-hydroxyvitamin D3; active vitamin D; chronic kidney disease (CKD); biomarker.

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), phosphorus excess, and metabolic acidosis.[3-5]

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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 therefore may 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_3 (24,25[OH]$_2$$D_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 (Fig 1). Hydroxylated products of CYP24A1 are converted further to more polar metabolites and excreted in urine or bile. In a cohort of patients referred to nephrology clinics, we showed 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 present study, we tested associations of eGFRs with circulating 24,25$(OH)_2D_3$ concentrations across a wide range of eGFRs using data from 5 cohort studies and clinical trials. Because 24,25$(OH)_2D_3$ production and circulating 24,25$(OH)_2D_3$ concentrations 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$(OH)_2D_3$ concentrations for a given increment in circulating 25$(OH)D_3$ concentrations. Such a finding would further support the theory that GFR loss leads to reduced vitamin D catabolism.

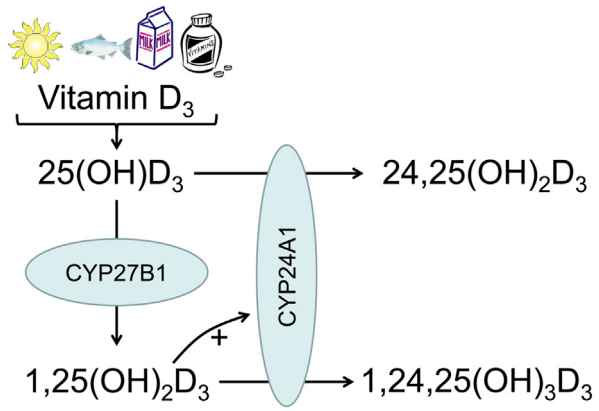

Figure 1. Vitamin D_3 metabolism. Vitamin D_3 synthesized in the skin or consumed by mouth is metabolized to 25-hydroxyvitamin D_3 (25[OH]D_3), which then can be metabolized to 1,25-dihydroxyvitamin D_3 (1,25[OH]$_2$$D_3$, the active vitamin D hormone) by CYP27B1. Vitamin D_3 catabolism is accomplished predominantly by CYP24A1, which metabolizes 25$(OH)D_3$ to 24,25-dihydroxyvitamin D_3 (24,25[OH]$_2$$D_3$) and 1,25$(OH)_2D_3$ to 1,24,25-trihydroxyvitamin D_3 (1,24,25[OH]$_3$$D_3$). CYP24A1 is induced by 1,25$(OH)_2D_3$.

METHODS

Study Populations

We measured serum or plasma 24,25$(OH)_2D_3$ in 5 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 5 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 macrovascular complications.[14] We measured plasma vitamin D metabolites for all nonpregnant participants with available frozen samples collected at or near the end of the DCCT (N = 1,193).[15]

MESA is an observational cohort study of subclinical cardiovascular disease in people who were free of clinical cardiovascular disease at study entry.[16] We measured serum vitamin D metabolites for all MESA participants with available frozen samples collected at baseline (n = 6,470 of 6,814).[17]

CHS is an observational cohort study of cardiovascular disease in adults 65 years 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 1,846 participants with end-stage renal disease 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 next.

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 3 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 Study cohort, immunoaffinity enrichment was added to the newer chromatographic method to measure 1,25$(OH)_2D_3$ and 1,25-dihydroxyvitamin D_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