Title

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Journal

Clinical Chemistry, 67(2)

ISSN

0009-9147

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Publication Date

2021-01-30

DOI

10.1093/clinchem/hvaa238

Peer reviewed

HHS Public Access

Author manuscript

Clin Chem. Author manuscript; available in PMC 2022 February 25.

Published in final edited form as:

Clin Chem. 2021 January 30; 67(2): 385–393. doi:10.1093/clinchem/hvaa238.

The Vitamin D Metabolite Ratio Is Independent of Vitamin D Binding Protein Concentration

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Abstract

BACKGROUND: 25-Hydroxyvitamin D [25(OH)D] may be a poor marker of vitamin D status as it reflects differences in vitamin D binding protein (VDBP) between individuals. The vitamin D metabolite ratio [VMR, ratio of $24,25(OH)_2D_3$ to $25(OH)D_3$] is a marker of vitamin D status that has been hypothesized to be independent of variability in VDBP. This hypothesis has not been directly evaluated.

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Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: A.N. Hoofnagle, *Clinical Chemistry,* AACC.

Consultant or Advisory Role: M.J. Sarnak, akebia, Bayer, Cardurian.

Stock Ownership: None declared.

Honoraria: None declared.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

METHODS: We measured 25(OH)D_3, 24,25$(OH)_2D_3$, 1,25$(OH)_2D_3$, and VDBP in 377 community-dwelling older adults that participated in the Health Aging and Body Composition Study. 24,25$(OH)_2D_3$ and 25(OH)D_3 were used to calculate the VMR. We used linear regression to assess the relationship between VDBP with the VMR, 24,25$(OH)_2D_3$, 25(OH)D_3, and 1,25$(OH)_2D_3$.

RESULTS: Participants had mean age 75±3 years, 52% were female, 40% were black, and 24% had chronic kidney disease. VDBP concentrations were associated with sex, serum albumin, and VDBP phenotype in multivariable models. In fully adjusted models, each 1% higher VDBP was associated with a 0.92%[95% CI(0.37,1.49%)], 0.76% (0.39, 1.13%), and 0.57% (0.29, 0.85%), higher 24,25$(OH)_2D_3$, 25(OH)D_3, and 1,25$(OH)_2D_3$. The VMR was independent of VDBP concentration, [0.16%(−0.11, 0.44) higher VMR per 1% higher VDBP, P=.25].

CONCLUSIONS: The VMR was independent of VDBP concentration, whereas VDBP was strongly directly associated with the individual vitamin D metabolite concentrations. Prior studies evaluating only 25(OH)D_3 may have been confounded by absence of data on VDBP status. The VMR may serve as an important biomarker of vitamin D status and clinical outcomes that can be utilized in populations with a large spectrum of VDBP concentrations.

Introduction

Vitamin D deficiency, defined as serum concentrations of 25-hydroxyvitamin D [25(OH)D] less than 20 ng/mL is common in the US (1). As of 2014, 1 in 5 Americans were taking a vitamin D supplement (2). Severe vitamin D deficiency results in osteomalacia, rickets, and fractures (3). However, recent studies have suggested that 25(OH)D may be a poor biomarker of vitamin D adequacy and bone health and may not be associated with important clinical outcomes including bone density, fracture, or death (4–6). Thus, novel biomarkers of vitamin D status and bone health are currently being investigated.

Several studies have suggested that a weakness of 25(OH)D as a biomarker of vitamin D status stems from variability in the VDBP, also known as vitamin D binding globulin) (7–9). 25(OH)D exists as either free (approximately 1%) or bound (approximately 99%, primarily to VDBP, with a small fraction being albumin bound). Both the free and albumin-bound 25(OH)D are believed to be bioavailable, whereas that bound to VDBP is not (9). Prior studies have documented substantial variability in VDBP concentrations (7–10). Moreover, common genetic variants in VDBP may lead to different binding affinity of VDBP to 25(OH)D. Therefore, bioavailability of 25(OH)D varies between individuals, depending on VDBP concentrations and phenotype.

Studies have previously demonstrated that the measurement of 24,25-dihydroxyvitamin D [24,25$(OH)_2D$] and its use to calculate the ratio of 24,25$(OH)_2D$ to 25(OH)D [the vitamin D metabolite ratio (VMR)] may serve as a surrogate marker for vitamin D sufficiency (4–6,11). 25(OH)D is catabolized to 24,25$(OH)_2D$, a process stimulated by higher 1,25$(OH)_2D$ binding to the vitamin D receptor (VDR), in order to prevent tissue level vitamin D toxicity (12). Hence, a higher 24,25$(OH)_2D$ concentration for any given concentration of 25(OH)D, leading to a higher VMR, may reflect increased VDR activity. Importantly, it has been hypothesized that the VMR is not affected by VDBP concentrations, as VDBP binds both

25(OH)D and 24,25$(OH)_2$D, thus blood concentrations of both may be affected by VDBP concentrations. However, both the numerator and denominator of the VMR calculation might cancel out VDBP and render the VMR a marker of vitamin D sufficiency that is independent of VDBP (Fig. 1). Our prior analysis supports this notion, demonstrating that a lower VMR was more strongly associated with hip fracture and allcause mortality than a lower 25(OH)D in community-dwelling older adults (4, 5). However, our prior study, and the great majority of prior clinical studies evaluating the VMR, have not measured VDBP concurrently. It is uncertain if the binding affinities of VDBP for 25(OH)D and 24,25$(OH)_2$D are similar, or may vary by vitamin D moiety or VDBP genotype. Thus, whether the VMR is truly independent of VDBP has never been directly tested.

To that end, we aimed to determine whether the VMR, or any vitamin D metabolites, were independent of VDBP in a cohort of community-dwelling older individuals in the Health Aging and Body Composition (ABC) Study. We had previously measured 25(OH)D, 24,25(OH)2D, and 1,25(OH)2D in this cohort. In the present analysis, we utilized additional blood samples available in the Health ABC Study to measure VDBP and serum albumin to test their inter-relationships with vitamin D metabolites. A priori, we hypothesized that higher 25(OH)D, 24,25$(OH)_2$D, and 1,25$(OH)_2$D would all be directly related to higher VDBP concentrations. In contrast, we hypothesized that the VMR would not be related to VDBP concentrations. Finally, we tested whether observed relationships were similar in men and women, blacks and whites, and in those with chronic kidney disease (CKD) vs. those without.

Methods

STUDY POPULATION

The Health ABC Study is a longitudinal cohort study designed to evaluate the relationships of age and body composition on health outcomes in healthy older adults (5). Between April 1997 and June 1998, 3075 community-dwelling adults between the age of 70 and 79 years were recruited. There were two study sites, Memphis, TN, and Pittsburgh, PA. All participants provided informed consent and the study was approved by the local institutional review boards at both study sites. The present study was also approved by the institutional review board at the University of California San Diego

Vitamin D metabolites and VDBP were measured using samples collected at the year 2 Health ABC visit, nested in a case-cohort study we had designed previously to look at the vitamin D metabolites and kidney disease and other health outcomes. We selected 239 participants at random from the overall Health ABC cohort (random subcohort), as well as 138 cases of kidney function decline (cases), defined as ≥30% decline in estimated glomerular filtration rate (eGFR) during followup. Using this cohort of 377 participants, we assessed the relationship of each vitamin D metabolite and the VMR with VDBP concentrations (Fig. 2).

VITAMIN D MEASUREMENTS

Participants had fasted for ≥8 hours at the time of blood sampling. Samples were stored at −70°C from collection in 1998–1999 until testing, as vitamin D metabolite concentrations are known to be stable at this temperature for prolonged periods (13). The exposure variable in our primary analysis was VDBP. VDBP concentration and phenotype were determined simultaneously via liquid chromatography-tandem mass spectrometry, essentially as described previously with minor assay modifications outlined in Supplemental Methods (14). Intraassay coefficient of variation ranged from 3.1% to 9.1% across a range of concentrations. This assay measures concentration of VDBP with results that are not biased by VDBP genotype, a concern that exists with other assays (15). While completing the measurement of VDBP, we noted that many samples in individual batches had markedly and implausibly high VDBP concentrations (see Supplemental Methods). All assay quality assurance parameters were satisfied (i.e., internal standard peak areas and peptide ratios) and repeated measurements of these samples confirmed the high concentrations observed initially. These observations were limited to certain samples that were labeled as experiencing a specific number of freeze-thaws before being stored in the repository and that were aliquoted differently after arriving at the University of Washington. As a result, we believe the implausibly high observed concentrations resulted from preanalytic effects on these samples and have therefore labeled all VDBP measurements from these similarly-handled samples as “untrusted,” even if a given sample had normal VDBP concentrations, since we deemed samples with these preanalytical handling characteristics as high-risk. We compared demographic and clinical characteristics among participants with samples in the “trusted” and “untrusted” boxes and determined that this effect appeared to be a random effect across all variables evaluated (Supplemental Table 1). Thus, for this analysis we used a conservative strategy and included only the results from samples in the “trusted” boxes for any analyses that involved VDBP. Based on this conservative strategy, we removed 452 samples in total.

The outcome variables were the vitamin D metabolites, including $24,25(OH)_2D_3$, $25(OH)D_3$, and $l,25(OH)_2D_3$ which were quantified using immunoaffinity enrichent and liquid chromatography-tandem mass spectrometry, as described previously elsewhere (5). The VMR served as the primary outcome for this analysis. The VMR was calculated by dividing serum $24,25(OH)_2D_3$ by serum $25(OH)D_3$ and then multiplying by 100 (4). As we found no spectrometric evidence of $24,25(OH)_2D_2$, the VMR was calculated using $24,25(OH)_2D_3$ and $25(OH)D_3$ only.

OTHER MEASUREMENTS

All participants provided a medical history and physical examination. Age, sex, race, and smoking status were determined by self-report. Height was measured using a Harpenden stadiometer (Holtain Ltd), and weight was measured using a balance beam scale. Body mass index (BMI) was calculated in kg/m^2. Baseline prevalent diabetes was defined by self-reported history, use of antidiabetic agents, fasting plasma glucose concentration ≥ 126 mg/dL, or a 2-hour oral glucose tolerance test result ≥ 200 mg/dL. Systolic and diastolic blood pressures were measured 3 times using a conventional mercury sphygmomanometer.

Participants brought their medications to the study visits and study staff categorized them using the Iowa Drug Information System.

Urine albumin and urine creatinine measurements were available only at year 1, so we carried forward the year-1 urine albumin-creatinine ratio (ACR) to year 2, which was the time when vitamin D measurements were performed (5). Similarly, serum cystatin C also was measured at year 1 and carried forward. We determined the estimated GFR (eGFR) using the 2012 CKD Epidemiology Collaboration (CKD-EPI) cystatin C equation (16). Cystatin C was measured at the Health ABC core laboratory (University of Vermont, Burlington, VT) with a BNII nephelometer (Dade Behring Inc) that used a particle-enhanced immunonephelometric assay (N Latex Cystatin C). Serum calcium and phosphate were measured using direct quantitative colorimetric determination (Stanbio Laboratory). Intact parathyroid hormone (iPTH) was measured in EDTA plasma using a 2-site immunoradiometric assay kit (N-tact PTHSP; DiaSorin). Fibroblast growth factor 23 (FGF23) was measured using an intact assay (Kainos Laboratories). Serum albumin was measured using the same assay for VDBP, as mentioned above, with the addition of internal standards for 3 albumin-specific peptides: LVNEVTEFAK, TYETTLEK, and YLYEIAR, as described in more detail in Supplemental Methods.

STATISTICAL METHODS

To account for the sampling of the kidney function decline cases, subcohort participants were weighted by the inverse probability of their sampling fraction to recreate a random subcohort and avoid biasing the sample towards characteristics more common in those at risk of CKD. We compared baseline characteristics within the random subcohort across quartiles of VDBP using either chi-square or ANOVA tests. Next we evaluated for factors that were associated with VDBP concentrations. We used linear regression initially adjusting for age, sex, race, season, site, BMI, diabetes status, eGFR, urine albumin to creatinine ratio, serum phosphate, calcium, PTH, FGF23, VDBP phenotype. We used backwards modeling with a cut-off of P>.20 to develop our final model. We then used multiple linear regression to assess the associations of VDBP with the VMR, $24,25(OH)_2D_3$, $25(OH)D_3$, and $1,25(OH)_2D_3$. This served as our primary analysis. To facilitate comparisons, we log transformed both the exposure (VDBP) and outcome variables [VMR, $24,25(OH)_2D_3$, $25(OH)D_3$, and $1,25(OH)_2D_3$), such that beta coefficients could be interpreted as the percentage change in the outcome variables attributable to a 1% change in VDBP. We developed a sequence of models. Model 1 was unadjusted. Model 2 was adjusted for age, sex, race, season of blood sampling, clinic site and BMI. Model 3 was additionally adjusted for eGFR, serum calcium, phosphate, albumin, PTH and FGF-23 concentrations as well as VDBP phenotype. Last, we assessed for sex, race and CKD (eGFR <60 mL/min/1.73 m^2 vs. higher) interactions by inclusion of multiplicative interaction terms in Model 3. All analyses were conducted in Stata SE version 14.1 (College Station, TX). *P*-values <.05 were considered statistically significant for all analyses including interaction terms.

Results

The mean age of the 377 individuals in the study sample was 75 years, 52% were women, 40% were African-American, and 24% had eGFR < 60 mL/min/1.73 m^2 at

baseline. The mean±SD VDBP concentration was 259±43μg/mL. The most prevalent VDBP phenotype was homozygous group specific component 1 s (Gc1s) (25%). The mean VMR, $24,25(OH)_2D_3$, $25(OH)D_3$, and $1,25(OH)_2D_3$ concentrations were 9.2±4 (ng/mL)/(ng/mL), 2.2± 1.6 ng/mL, 22±11 ng/mL, and 42±16 pg/mL, respectively. Baseline characteristics across quartiles of VDBP are shown in Table 1. Compared to persons in the lowest VDBP quartile, those with higher VDBP were more often female and less likely to have diabetes, albuminuria, and hypertension. VDBP concentrations were highest among persons who were homozygous Gc1s and lowest among those who were homozygous Gc2. There was a trend toward higher VDBP in spring and summer, although this did not reach statistical significance (P=.06).

Next, we assessed for factors associated with VDBP concentration using multivariable models. Results from the final model are shown in Table 2. Factors associated with a higher VDBP included female sex, serum albumin and VDBP phenotype. Notably, mean VDBP concentrations were markedly lower among persons with the Gc2/Gc2 phenotype.

VDBP was strongly and directly associated with all measured vitamin D metabolites. The associations were not materially altered across the sequence of adjusted models. In the final model, each 1% higher VDBP was associated with a 0.92%, 0.76%, and 0.57% higher $24,25(OH)_2D_3$, $25(OH)D_3$, and $1,25(OH)_2D_3$ (Table 3, P≤001 for all). VDBP was not associated with the VMR (P=.25 in fully adjusted model). Quadratic regression curves depicting the unadjusted association of VDBP with each metabolite and the VMR are depicted in Fig. 3. In sensitivity analyses, we evaluated these associations using all samples, including our “untrusted” measurements (Supplemental Table 2). All 3 vitamin D metabolites remained significantly associated with VDBP (P≤.024 for all), and the VMR remained unassociated with VDBP (P=.091).

Lastly, we assessed for interactions of VDBP with sex, race, and eGFR on each metabolite and the VMR. We found no significant interactions for sex, race, or eGFR on any of the outcomes (Supplemental Table 3, P≥.52 for all).

Discussion

The VMR is purported to have advantages as a marker of vitamin D stores compared to 25(OH)D because of its hypothesized independence from VDBP concentrations, but this has not previously been tested, to our knowledge. We have demonstrated for the first time that while vitamin D metabolite concentrations are directly and strongly associated with VDBP concentrations, the VMR is indeed independent of VDBP concentrations in a diverse cohort of black and white community-living older persons. These relationships were similar irrespective of gender, race, and CKD status. Thus, the VMR appears to be a marker of a vitamin D status that is independent of the substantial interindividual variability in VDBP. Lastly, we evaluated for factors associated with VDBP concentration and demonstrated that VDBP concentrations were associated with sex, serum albumin, and VDBP phenotype.

The VMR has been evaluated as a biomarker of important clinical outcomes related to vitamin D in several settings. In our own prior studies, we demonstrated that a higher VMR was associated with lower serum PTH concentrations in cross-section, as well as

a lower risk of hip fracture and death in long-term follow-up in community-dwelling older adults (4, 5). For each endpoint, these associations were much stronger than when evaluating 25(OH)D. Bansal et al. (6) recently confirmed these findings showing a higher VMR was associated with a lower risk of death in persons with more advanced CKD. The two major hypotheses regarding why the VMR may be more strongly related to these endpoints have been that it reflects tissue level VDR activity (8) and that it is not impacted by interindividual variability in VDBP (17). Cavalier and colleagues (18) recent publication supports the former hypothesis, exploring 25(OH)D deficiency based on finding detectable $24,25(OH)_2D$ concentrations. We believe our findings presented herein are the first direct evidence to support the latter hypothesis, that the VMR is independent of VDBP concentration variability.

The finding that the VMR is indeed independent of VDBP protein concentration has important implications. First, prior studies evaluating 25(OH)D and clinical outcomes may have been biased towards the null hypothesis due to unknown and unaccounted for interindividual differences in VDBP. Indeed, this finding may explain why the VMR appeared more strongly associated with hip fracture and mortality outcomes than 25(OH)D in our prior study, and in the studies of others. Second, the $24,25(OH)_2D$ assay is now commercially available and can be measured concurrently with 25(OH)D. Measuring both metabolites could therefore allow wider use of the VMR and greater insight into relationships of tissue-level vitamin D status with clinical outcomes. Third, the finding that relationships evaluated here were similar in both genders, black and white race, and irrespective of CKD status suggest that the VMR may have utility as a marker of vitamin D sufficiency across a wide variety of settings.

Several studies have speculated that 25(OH)D deficiency is more common in African-Americans due to lower concentrations of VDBP (7, 16). VDBP knockout mice demonstrate extremely low levels of total vitamin D but do not demonstrate evidence of vitamin D deficiency unless starved of vitamin D in their diet (19). African-Americans also have lower concentrations of 25(OH)D compared to Caucasians, yet paradoxically have lower risk of hip fractures and osteoporosis, suggesting that deficiency defined by a blood level of 25(OH)D may not reflect tissue levels $1,25(OH)_2D$ concentrations or VDR activity and may instead be reflective of lower VDBP blood concentrations (20). In this analysis, we confirmed that low $25(OH)D_3$ concentrations may be attributable to low VDBP concentrations, and may not reflect true vitamin D deficiency (7). However, we did not find that VDBP concentrations differed by race. Moreover, we found that the relationships of VDBP with vitamin D metabolites and the VMR appeared similar irrespective of race. Nonetheless, our sample size was relatively modest, and we believe further work is needed, using larger, ethnically diverse cohorts, to explore the relationship of VDBP concentration and VDBP phenotype with 25(OH)D concentrations and race.

Strengths of this study include the evaluation of a diverse cohort of older men and women, as well as evaluation of black and white race, with a broad range of kidney function. We examined the relationship between the VMR and VDBP for the first time. Availability of extensive laboratory measurements, including VDBP phenotype, PTH, FGF23, and eGFR allowed us to explore other factors that may influence vitamin D metabolite concentrations.

Lastly, we were able to evaluate for factors associated with VDBP concentrations and showed a strong relationship between VDBP concentration and sex, as well as VDBP phenotype.

This study has important limitations. First, while our data demonstrate that the VMR is independent of VDBP concentrations, we cannot exclude a weak association that did not reach statistical significance in this study. The results should be interpreted within the confines of the 95% confidence intervals. Second, we had to exclude a large number (55%) of samples due to preanalytical sample-handling issues, which reduced our overall sample size. Nonetheless, these sample-handling issues appear to have been randomly distributed across the participants and our overall findings were consistent, even when including these "untrusted" data. Third, a hypothesis imbedded in the independence of the VMR from VDBP is that the binding affinities of 25 D and 24,25$(OH)_2$D with VDBP are similar. While the associations tested here support that hypothesis, binding affinities were not directly measured. Modest differences in binding affinities may exist and remain consistent with our findings. Additionally, a larger sample size would be helpful to further explore the different VDBP phenotypes across different subgroups such as race, gender, and seasons of blood measurements. Finally, the study samples were collected from well-functioning community-living elders. Few had advanced CKD, and all were of black or white race. While relationships appeared similar across these subgroups, future studies should confirm these findings in other settings.

In summary, we have demonstrated that the VMR is independent of VDBP concentrations, while 25$(OH)D_3$, 24,25$_2(OH)D_3$, and 1,25$_2(OH)D_3$ concentrations are highly dependent on circulating VDBP concentrations. The VMR has previously been more strongly associated with important clinical outcomes than 25(OH)D in several large prospective cohort studies, which may reflect unmeasured confounding due to differences in VDBP concentrations. Overall, these findings support wider use of the VMR as a method to overcome limitations in 25(OH)D as a marker of vitamin D sufficiency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

The authors acknowledge the services of the Health ABC Study, contributing research centers, and all study participants.

C. Ginsberg, financial support, statistical analysis, administrative support; A. N. Hoofnagle, administrative support; R. Katz, statistical analysis; S. B. Kritchevsky, provision of study material or patients; J. H. Ix, financial support, administrative support.

Research Funding: This study was supported by grants from the, National Institute of Diabetes, Digestive, and Kidney Diseases K23DK118197 and Loan Repayment Program (C. Ginsberg), R01DK101720 and K24 DK110427 (J.H. Ix), the National Institute on Aging (NIA) 5R01AG027002 (M.J. Sarnak and M. G. Shlipak), 0R01AG033087 (S.B. Kritchevsky), and University of Washington Nutrition and Obesity Research Center P30DK035816 (A.N. Hoofnagle). J.H. Ix was additionally supported by an Established Investigator Award from the American Heart Association (14EIA18560026) This research was additionally supported by National Institute on Aging (NIA) Contracts N01-AG-6-2101; N01-AG-6-2103; N01-AG-6-2106; NIA grant R01-AG028050, and NINR grant R01-

NR012459. This research was funded in part by the Intramural Research Program of the NIH, National Institute on Aging. A.N. Hoofnagle, funding from Waters, Inc. to institution.

Expert Testimony: A.N. Hoofnagle, Kilpatrick Townsend & Stockton LLP.

Patents: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Nonstandard Abbreviations:

25(OH)D	25-hydroxyvitamin D
VDBP	vitamin D binding protein
VMR	vitamin D metabolite ratio
24,25(OH)2D	24,25-dihydroxyvitamin D
1,25(OH)2D	1,25-dihydroxyvitamin D
VDR	vitamin D receptor
ABC	Aging and Body Composition
BMI	body mass index
CKD	chronic kidney disease
eGFR	estimated glomerular filtration rate
ACR	albumin-creatinine ratio
iPTH	intact parathyroid hormone
FGF23	fibroblast growth factor23

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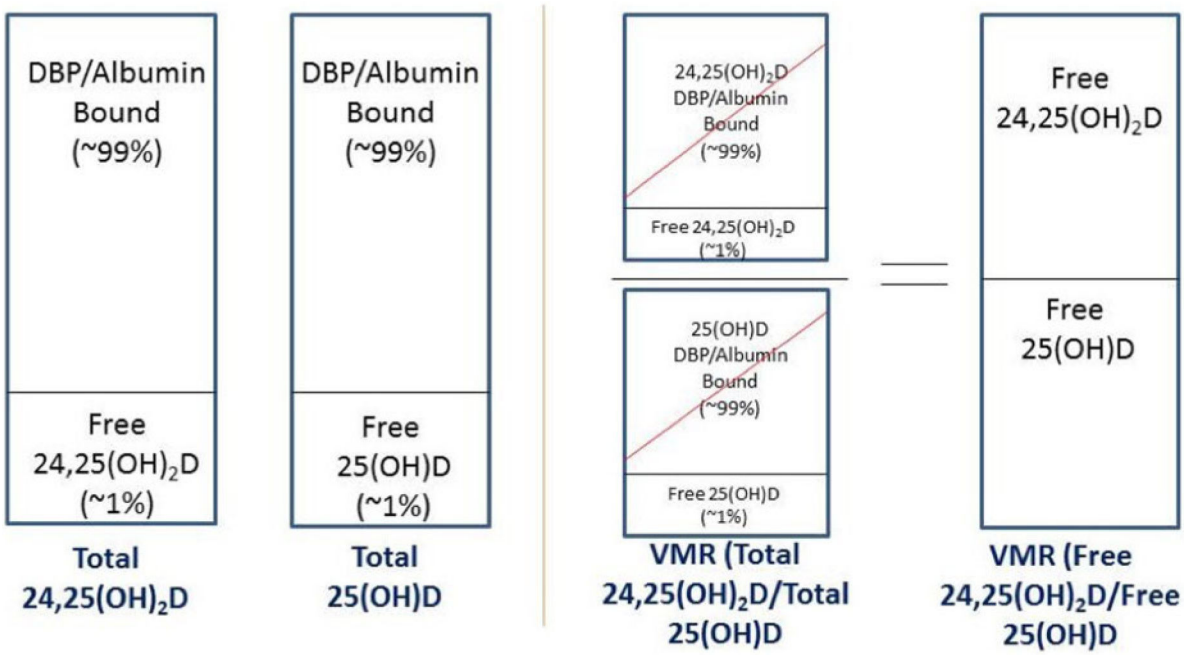

Fig. 1.
Hypothesized vitamin D metabolite protein binding and the VMR.

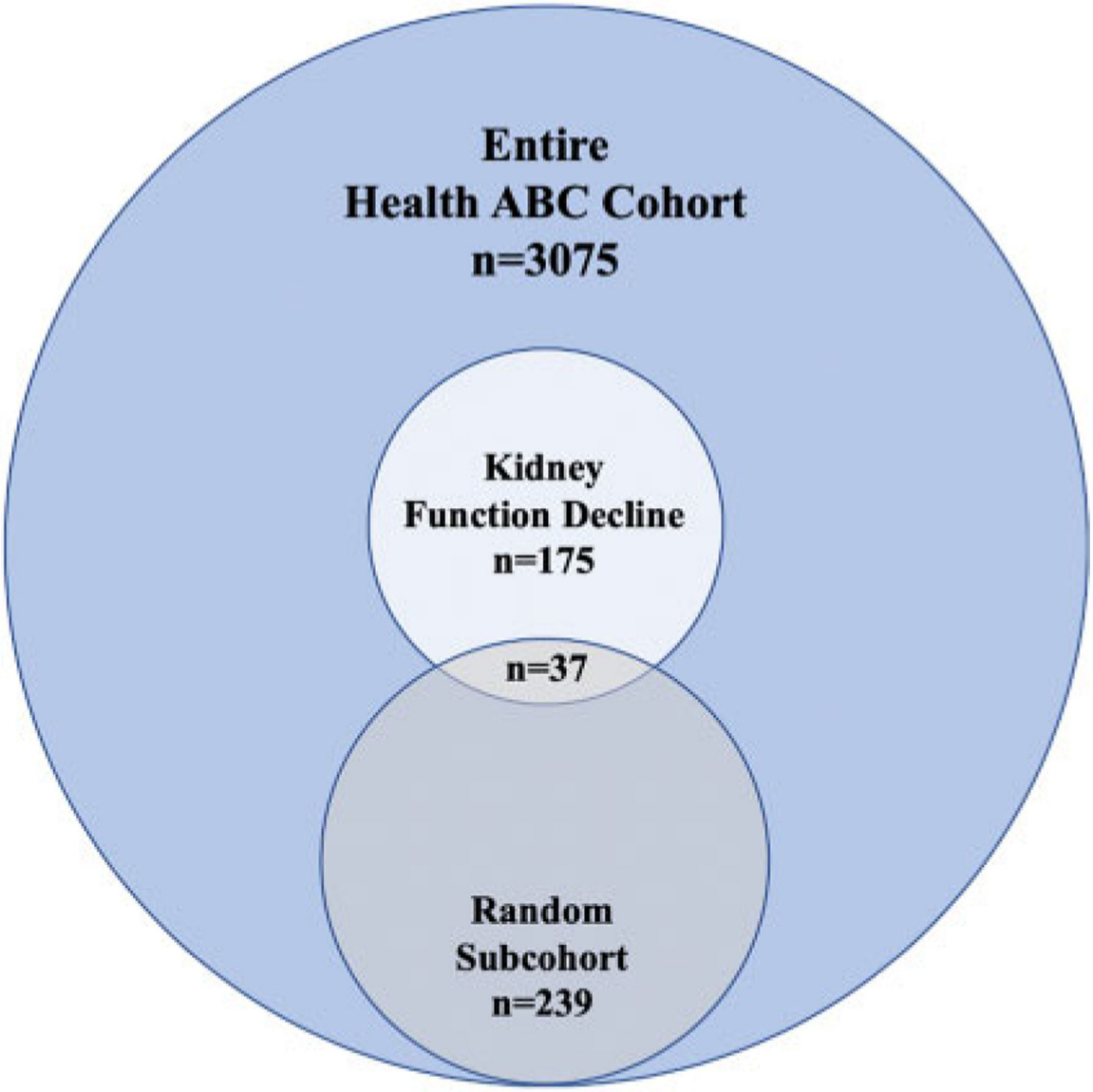

Fig. 2.
Health ABC Study sampling.

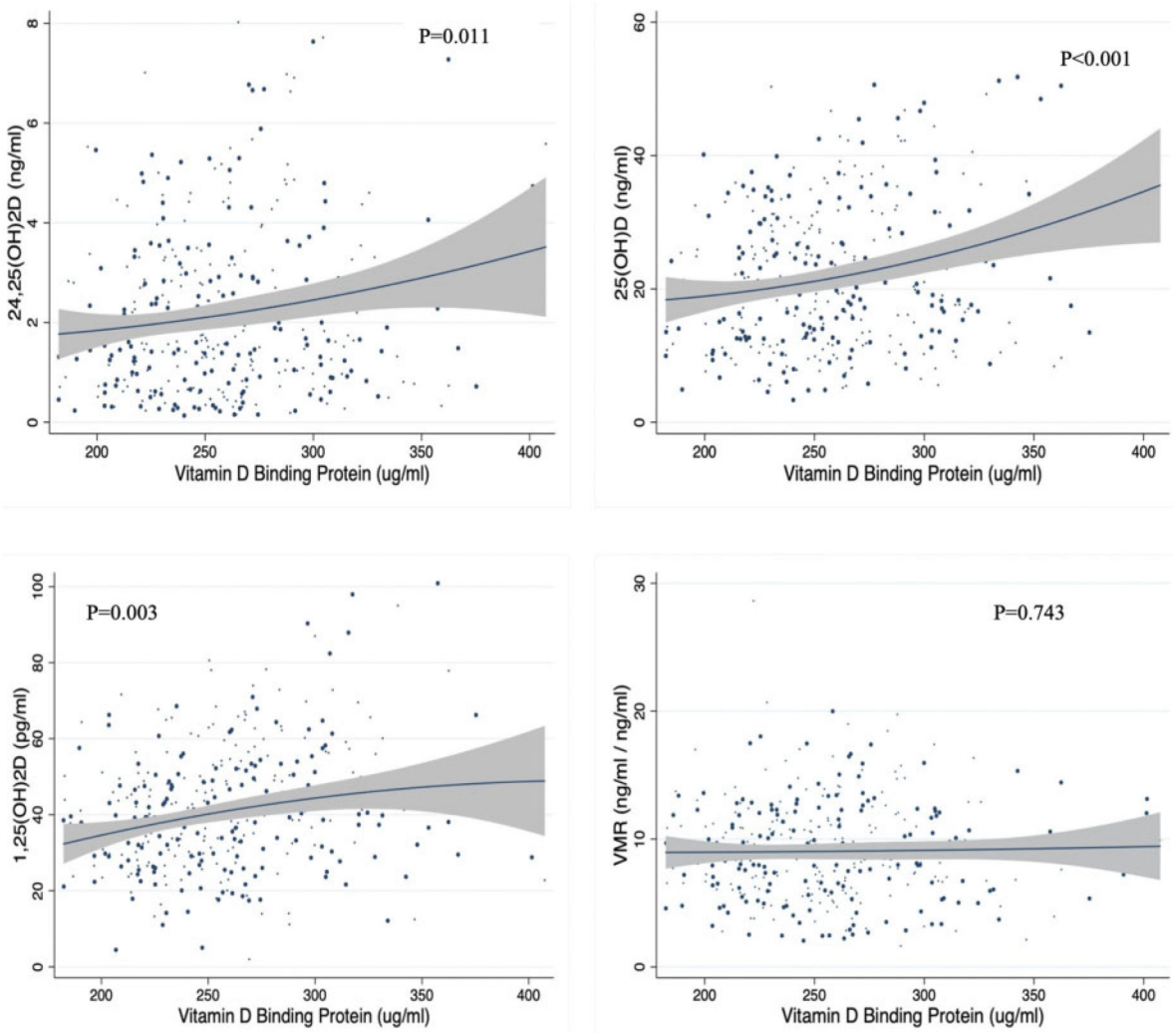

Fig. 3.

Unadjusted quadratic regression curves of vitamin D metabolites and the VMR vs. vitamin D binding protein concentration (shaded areas around the regression lines represent the 95% confidence interval of the estimates).

Table 1.

Baseline characteristics by vitamin D binding protein quartile.[a]

	Quartile 1 (n = 104)	Quartile 2 (n = 85)	Quartile 3 (n = 82)	Quartile 4 (n = 105)
Range (μg/mL)	182–230	230–254	254–277	277–408
Age (years) (SD)	75.0 (3.0)	74.9(2.9)	74.4 (2.8)	74.2 (2.8)
Male, n (%)	64 (62)	45 (53)	41 (50)	30 (29)
Black, n (%)	43 (41)	38 (49)	31 (38)	37 (35)
Clinic site, n (%)				
Memphis	55 (55)	37 (44)	43 (52)	57 (54)
Pittsburgh	47 (45)	48 (56)	39 (48)	48 (46)
Season of blood measurement, n (%)				
Winter	36 (35)	19 (22)	19 (23)	22 (21)
Spring	26 (25)	20 (24)	26 (32)	39 (37)
Summer	17 (16)	26 (31)	18 (22)	25 (24)
Fall	25 (24)	20 (24)	19 (23)	19 (18)
BMI (kg/m^2) (SD)	27.9 (4.8)	27.1 (3.9)	26.7 (4.3)	27.1 (5.2)
Smoking status, n (%)				
Never	41 (39.3)	35 (41)	36 (44)	49 (47)
Former	57 (55)	42 (49)	41 (50)	45 (43)
Current	6 (6)	8 (79)	5 (6)	11 (10)
Diabetes, n (%)	52 (50)	40 (47)	33 (40)	30 (29)
Systolic BP (mm Hg) (SD)	134 (22)	138 (23)	131 (19)	137 (21)
Diastolic BP (mm Hg) (SD)	71 (11)	71 (12)	69 (11)	72 (11)
On anti-HTN medications, n (%)	70 (67)	58 (69)	43 (52)	51 (61)
CKD, n (%)	24 (23)	19 (22)	19 (23)	24 (29)
eGFR (mL/min/1.73 m^2) (SD)	74 (19)	70 (17)	72 (20)	72 (21)
Additional kidney cases[a], n(%)	43 (43)	24 (29)	26 (32)	45 (44)
Albumin/creatinine, median [IQR]	13 [5–38]	11 [5–33]	7 [4–22]	8 [4–20]
Calcium (mg/dL) (SD)	8.8 (0.4)	8.9 (0.5)	8.9 (0.5)	9.0 (0.5)
Phosphate (mg/dL) (SD)	3.5 (0.5)	3.5 (0.5)	3.6 (0.5)	3.7 (0.5)
PTH (pg/mL), median [IQR]	36 [27–46]	35 [24–52]	31 [24–46]	34 [22–46]

	Quartile 1 (n = 104)	Quartile 2 (n = 85)	Quartile 3 (n = 82)	Quartile 4 (n = 105)
FGF23 (pg/mL), median [IQR]	44 [33, 59]	44 [32–60]	47 [34–59]	44 [34–62]
Phenotype, n (%)				
Gc1f/Gc1f	21 (20)	16 (19)	20 (24)	15 (14)
Gc1f/Gc1s	16 (15)	13 (15)	11 (13)	21 (20)
Gc1s/Gc1s	15 (14)	18 (21)	24 (29)	36 (34)
Gc2/Gc1f	16 (15)	11 (13)	10 (12)	12 (11)
Gc2/Gc1s	23 (22)	21 (25)	17 (21)	19 (18)
Gc2/Gc2	13 (13)	6 (7)	0 (0)	2 (2)
Albumin (g/dl) (SD)	4.2 (0.4)	4.2 (0.4)	4.4 (0.5)	4.4 (0.4)

[a]Quartile ranges defined by random subcohort ranges. Additional cases of kidney function decline were added in after quartile definitions were set from the random subcohort; thus, number of participants is not equivalent in all quartiles.

Table 2.

Factors associated with vitamin D binding protein concentration[a].

	% Higher VDBP (95% CI)	*P* value
Gender (ref = Male)	8 (4, 12)	<0.001
Season (ref = Winter)		0.058
Spring	7 (1, 13)	
Summer	8 (1, 14)	
Fall	3 (−3, 10)	
Diabetes	−3 (−7, 0.6)	0.101
Calcium (per 1 mg/dL higher)	3(−1, 7)	0.143
Albumin(per 1 g/dL higher)	11 (5, 16)	<0.001
DBP Phenotype (ref = Gc1f)		<0.001
Gc1f/Gc1s	3 (−4, 10)	
Gc1s/Gc1s	5 (−1, 12)	
Gc2/Gc1f	0.3 (−5, 7)	
Gc2/Gc1s	−0.4 (−6, 6)	
Gc2/Gc2	−11 (−17, −6)	

[a]Initial model adjusted for age, sex race, season, site, BMI, diabetes status, eGFR, urine albumin to creatinine ratio, phosphate, calcium, PTH, FGF23, VDBP phenotype, and albumin. Backwards modeling, *P*-value cutoff of 0.2, used to produce final model.

Table 3.

Association of VDBP with vitamin D metabolites and the vitamin D metabolite ratio.[a]

	24,25 D		25D		1,25D		VMR	
	β coef (95% CI)	***P* value**	**β coef (95% CI)**	***P* value**	**β coef (95% CI)**	***P* value**	**β coef (95% CI)**	***P* value**
Model 1[b]	0.81 (0.23, 1.39)	0.011	0.76 (0.39, 1.13)	<0.001	0.51 (0.18, 0.84)	0.003	0.05 (−0.26, 0.36)	0.743
Model 2[c]	085 (0.32, 1.38)	0.002	0.77 (0.44, 1.11)	<0.001	0.41 (0.20, 0.81)	0.002	0.07 (−0.29, 0.36)	0.510
Model 3[d]	0.92 (0.37, 1.49)	0.001	0.76 (0.39, 1.13)	<0.001	0.57 (0.29, 0.85)	<0.001	0.16 (−0.11, 0.44)	0.247

[a]Data reported is for natural logarithm of the VMR, vitamin D metabolites, and natural logarithm of DBP.

[b]Model 1 is unadjusted.

[c]Model 2 is adjusted for race, sex, age, season, site and BMI.

[d]Model 3 is additionally adjusted for above as well as eGFR, phosphate, calcium, PTH, FGF23, VDBP phenotype, and albumin.