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The 24,25 to 25-Hydroxyvitamin D Ratio and Fracture Risk in Older Adults: The Cardiovascular Health Study

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Abstract

25-hydroxyvitamin D [25(OH)D] may not optimally indicate vitamin D receptor activity. Higher concentrations of its catabolic product 24,25-dihydroxyvitmin D [24,25$(OH)_2$D] and a higher ratio of 24,25$(OH)_2$D to 25(OH)D (the vitamin D metabolite ratio [VMR]) may provide additional information on receptor activity. We compared the strength of associations of these markers with serum PTH concentrations, hip bone mineral density (BMD), and risk of incident hip fracture in community-living older participants in the Cardiovascular Health Study. Among 890 participants, the mean age was 78 years, 60% were women, and the mean 25(OH)D was 28 ± 11 ng/ml. In cross-sectional analysis, the strength of association of each vitamin D measure with PTH was similar; a 1% higher 25(OH)D, 24,25$(OH)_2$D, and VMR were associated with 0.32%, 0.25%, and 0.26% lower PTH, respectively ($p < 0.05$ for all). Among 358 participants with available BMD

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data, we found no associations of 25(OH)D or VMR with BMD, whereas higher 24,25$(OH)_2$D was modestly associated with greater hip BMD (1% higher 24,25$(OH)_2$D associated with 0.04% [95% CI 0.01−0.08%] higher BMD). Risk of incident hip fracture risk was evaluated using a case-cohort design. There were 289 hip fractures during a mean follow up time of 8.4 years. Both higher 24,25$(OH)_2$D and VMR were associated with lower risk of hip fracture (HR per SD higher, 0.73 [0.61, 0.87] and 0.74 [0.61, 0.88], respectively) whereas 25(OH)D was not associated with hip fracture (HR 0.93 [0.79, 1.10]). We conclude that evaluating vitamin D status by incorporating assessment of 24,25(OH)D and the VMR provides information on bone health above and beyond 25(OH)D alone.

Keywords

Vitamin D; Fracture; PTH; Bone Density; CKD-MBD; Osteodystrophy

1. INTRODUCTION

Using current definitions that rely on serum 25-hydroxyvitamin D [25(OH)D] vitamin D concentrations, vitamin D insufficiency and deficiency are common among Americans.[1] Marked skeletal abnormalities are observed in patients with severe vitamin D deficiency, and less severe vitamin D deficiency is widely hypothesized to contribute to osteoporosis, fractures and other diseases in the general population.[1–4]

Recent studies suggest that assessing vitamin D status based on concentrations of 25(OH)D alone may be suboptimal.[5–8] Studies evaluating the relationship between 25(OH)D and bone density and fractures have had mixed findings.[5–7] Additionally, it is unclear how well the serum concentration of 25(OH)D reflects the downstream effects of vitamin D receptor (VDR) signaling in response to 1,25$(OH)_2$D binding (VDR activity), as 25(OH)D may be activated, catabolized, or remain in an inactive form.[8] Furthermore, assays for 25(OH)D are affected by concentration of vitamin D Binding protein (DBP), which can vary between individuals, and may not reflect bioavailable 25(OH)D.[9] Alternative markers have been proposed.

1,25-dihydroxyvitamin D [1,25$(OH)_2$D] is the active vitamin D hormone. Intuitively, directly measuring 1,25$(OH)_2$D seems appealing. Unfortunately, direct measurement of 1,25$(OH)_2$D has several limitations. Circulating concentrations of 1,25$(OH)_2$D are approximately 1000-fold lower than that of 25(OH)D. Therefore assays require significantly more serum and extraction steps are required to account for interfering compounds.[10] Second, 1,25$(OH)_2$D has a half-life of approximately 4 hours compared to weeks for 25(OH)D.[11] Thus 1,25$(OH)_2$D values may vary significantly from measurement to measurement. For these reasons routine measurement of 1,25$(OH)_2$D is not currently recommended.

As part of vitamin D catabolism, the CYP24A1 enzyme converts 25(OH)D to 24,25-dihydroxyvitamin D [24,25$(OH)_2$D]; a process stimulated by higher 1,25$(OH)_2$D.[12] This feedback mechanism is thought to prevent tissue level vitamin D toxicity.[13] These insights provide a novel opportunity to assess adequacy of VDR activity. As a result of increased

binding and activation of the VDR in response to 1,25$(OH)_2$D, there is increased CYP24A1 enzyme expression and increased 24,25$(OH)_2$D.[12] Thus, 24,25$(OH)_2$D concentrations may reflect VDR activity. However, like 25(OH)D, 24,25$(OH)_2$D is similarly bound to DBP. Therefore, 24,25$(OH)_2$D concentrations may not reflect free 24,25$(OH)_2$D. Other investigators have proposed assessment of the 24,25$(OH)_2$D to 25(OH)D ratio; also called the vitamin D metabolite ratio (VMR); to assess VDR activity. A higher VMR is proposed to reflect greater VDR activity and may not be limited by DBP concentrations as it would affect both the numerator and the denominator of the ratio similarly and would cancel out in the VMR calculation.[14]

To our knowledge, no prior study has evaluated whether 24,25$(OH)_2$D concentrations or the VMR are more strongly associated with bone health than 25(OH)D alone. Moreover, as the CYP24A1 enzyme that regulates 24,25$(OH)_2$D generation is expressed in the kidney, whether or not the relationship of 24,25$(OH)_2$D concentrations and VMR with bone health differs in persons with chronic kidney disease (CKD) is untested. To that end, we set out to determine the associations of 24,25$(OH)_2$D and the VMR with bone health in a well characterized cohort of older individuals in the Cardiovascular Health Study (CHS). We compared the strength of these associations to that of 25(OH)D alone. *A priori*, we hypothesized that the VMR would be more strongly associated with serum parathyroid hormone (PTH), hip bone mineral density (BMD), and risk of incident hip fracture, than 25(OH)D.

2. SUBJECTS AND METHODS

2.1 Study Population

The CHS is a community-based longitudinal observational cohort study of older adults.[15] Briefly, enrollment began in 1989 with 5201 participants aged ≥ 65 years recruited from Medicare eligibility lists in four locations, Forsyth County, NC; Sacramento County, CA; Washington County, MD; and Allegheny County, PA. Due to low participation of African-Americans, an additional 687 predominantly African-American participants were enrolled in 1992–93. Participants underwent annual examinations until 1998–99 and a subsequent examination was performed in 2005–06. At the 1996–97 examination, 3,233 participants provided blood specimens; this visit serves as baseline for this study. Among these, we randomly selected a sub-cohort of 1000 participants, of which 890 had a complete set of data for this analysis. We investigated three distinct endpoints; each evaluated in a different manner. Sampling for each is depicted in Figure 1. All participants in CHS provided written informed consent, and the institutional review board at each center approved the study protocol.

We first evaluated serum PTH concentrations from samples drawn at the 1996–97 study visit concurrent with the vitamin D metabolite measurements, using a cross-sectional design, within the randomly selected sub-cohort. Next, we evaluated total hip BMD which was measured at 2 of the 4 clinic sites (Pennsylvania and California) at the 1994–95 study visit, approximately 2 years before the vitamin D measurements (1996–1997 visit). Among the 890 participants in the random sub-cohort, 358 provided BMD data.

Last, we evaluated longitudinal risk of incident hip fracture. For this endpoint, we set the 1996–97 visit (time of vitamin D measurements) as our baseline, and evaluated future risk of hip fracture using a case-cohort design. In addition to the 75 fractures in the randomly selected sub-cohort, we identified all CHS participants who had hip fractures after the 1996–97 visit and obtained vitamin D metabolite measurements in these 214 “cases” per standard case-cohort study design. Vitamin D metabolite measurements of the 214 “cases” were performed concurrent to the 75 fractures in the sub-cohort, as well as concurrent with all individuals in the sub-cohort, so as to avoid any lab batch effect, as described below.

2.2 Vitamin D Measurements

Participants had fasted for ≥ 8 hours at the time of blood sampling. Samples were stored at 70°C fromcollection in 1996–97 until testing in 2012. Measurements of $25(OH)D_2$, $25(OH)D_3$ and $24,25(OH)_2D_3$ were made using liquid-liquid extraction and subsequent liquid chromatography- tandem mass spectrometry.[16] Total 25(OH)D was calculated by adding $25(OH)D_2$ and $25(OH)D_3$. Coefficient of variation for 25(OH)D assays was <5.6% with an analytic range of 1–200 ng/ml.[17] Original concentrations of $24,25(OH)_2D$ were divided by two for proper calibration and traceability to NIST SRM 972a.[18] The average observed coefficient of variation was 9.9–12.7% at 2.0–5.1 ng/mL with an analytic range of 0.1–100 ng/mL. All assays measuring $25(OH)D_3$, $25(OH)D_2$, and $24,25(OH)_2D_3$ were calibrated to standards provided by the National Institute of Standards and Technology.[19] The VMR was calculated by dividing serum $24,25(OH)_2D_3$ by serum $25(OH)D_3$ and then multiplying by 100.[14] As there is no spectroscopic evidence of $24,25(OH)_2D_2$ the VMR was calculated using $24,25(OH)_2D_3$ and $25(OH)D_3$ only. Vitamin D measurements were made at the University of Washington.

2.3 Outcome Variables: PTH, BMD and Hip Fracture

Serum intact PTH levels were measured at the 1996–97 visit. The Beckman Unicell Dxl Clinical Analyzer was used with a reported inter-assay coefficient of variation of <4.5%.(20)

Dual-energy x-ray absorptiometry (DXA) was performed at the 1994–95 visit at the Pennsylvania and California sites using a Hologic QDR-2000 densitometers (Hologic, Inc).[21] Standardized positioning and use of QDR software was based on the manufacturer’s recommended protocol. The coefficient of variation for the total hip BMD was <0.75%. Interpretation of the scans was performed by investigators blinded to outcomes at the University of California San Francisco.

Hip fracture data was obtained through participant report assessed every 6 months and by linkage with Medicare files that included all hospital admissions. These participant reports were cross-referenced against Medicare claims as well as hospitalization records and discharge summaries to identify any fractures not reported by participants. ICD-9 code of 820.xx without a concomitant code for motor vehicle accident (E810–E819) or pathologic fracture (733.1x) noted on discharge summaries constituted hip fracture events. ICD-9 codes for hip fractures that occurred in this cohort included the codes 820.0–820.03, 820.08, 820.09, 820.20, 820.21, 820.22, 820.29, 820.80, and 820.90. Hip fracture data was gathered from the initiation of the study until June 2010 with a mean follow up time of 8.4 years.[21]

2.4 Other Measurements

Trained personnel performed interviews and obtained participant demographics. Participants were instructed to bring all medication to study visits, at which time the medication, dose, and frequency was recorded by the study personnel. Smoking status was determined by self-report. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. After 5 minutes rest, blood pressure was measured three times, at least 30 seconds apart. The average of the last two measurements was used to calculate blood pressure. Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or use of an anti-hypertensive agent. Diabetes status was defined by the use of insulin or hypoglycemic agent, or a fasting serum glucose of ≥126 mg/dl. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-Epidemiology formula including creatinine and cystatin C. Cystatin C concentrations were measured using a BN II nephelometer (Siemens; www.siemens.com).[22,23] Serum calcium and phosphate were measured by indirect potentiometry on a DxC Synchron analyzer (Beckman-Coulter Inc, Brea, CA) and timed-rate colorimetric reaction method, respectively.[24] Serum fibroblast growth factor 23 (FGF23) was measured using a carboxy-terminal ELISA kit (Immutopics, San Clemente, CA). Urine albumin/creatinine ratio (ACR) was performed using a single voided urine sample. Season of lab draw was defined as Winter if it occurred from January-March, Spring from April–June, Summer from July–September and Fall from October–December.

2.5 Statistical Methods

We compared baseline characteristics within the random sub-cohort across quartiles of VMR using either $\chi 2$ or ANOVA tests. We used multiple linear regression to assess the associations between 25(OH)D, 24,25$(OH)_2$D, and the VMR with PTH. To facilitate comparisons, we log transformed both the exposure (VMR, 24,25$(OH)_2$D and 25(OH)D) and outcome variable (PTH), such that beta coefficients are interpretable as the percentage change in PTH attributable to a one percent change in the predictor variable. In companion analyses, we evaluated quartiles of each vitamin D measure, setting the lowest as the reference category. 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 and FGF-23 concentrations. We then assessed race and CKD (eGFR <60 ml/min/1.73m^2 vs. higher) interactions by inclusion of multiplicative interaction terms in Model 3.

Next we evaluated the association of the vitamin D variables with BMD using multiple linear regression adjusting for identical covariates as above. We log transformed both the vitamin D and BMD variables such beta coefficients are interpretable as the percentage change in BMD attributable to a one percent change in the vitamin D variable.

Lastly, we evaluated the association of the vitamin D variables with incident hip fracture using Prentice weighted Cox models to account for the case-cohort design. Sub-cohort participants were weighted to the inverse probability of their sampling. All cases within the sub-cohort as well as outside the sub-cohort were assigned a weight of 1 at the time of failure.[25] We used the same sequence of models as above to assess the hazard ratio (HR)

of hip fracture per one standard deviation (SD) higher of the vitamin D variables. In companion models, we evaluated quartiles of vitamin D metabolites, setting the lowest as the reference category. Last, we developed spline functions using additive models, adjusted for Model 3 covariates. The extreme 2.5% of vitamin D measurements were excluded to avoid implausible extrapolations from the extremes of the data distribution in splines. Analyses were conducted in Stata SE version 14.1 (College Station, TX) for PTH and BMD outcomes and R Core Team 2016 (Vienna Austria) for hip fracture. P-values < 0.05 were considered statistically significant for all analyses including interaction terms.

3. RESULTS

3.1 Participant Characteristics

The mean age of the 890 individuals in the sub-cohort was 78 years, 60% were women, 16% were African-American, and 40% had eGFR < 60 ml/min/1.73 m^2 at baseline. The mean 25(OH)D concentration was 28 ± 11 ng/ml and 58% had levels < 30 ng/ml. The mean $24,25(OH)_2D$ concentration was 1.7 ± 1.0 ng/ml, and mean VMR was 6.84 ± 2.23 (ng/ml) / (ng/ml). Baseline characteristics across quartiles of VMR are shown in table 1. Compared to persons in the lowest VMR quartile, those with higher VMR were younger, more often male and Caucasian, less likely to have diabetes and hypertension, and they had higher eGFR. While 25(OH)D and $24,25(OH)_2D$ concentrations were highest in the Spring and Summer (Supplemental Tables 1 and 2), we found that the VMR was highest in the Fall and Winter.

3.2 Relationships of Vitamin D Measurements with PTH

25(OH)D, $24,25(OH)_2D$, and the VMR were all inversely associated with PTH and these associations were of similar strength. The associations were not materially altered across the sequence of adjusted models. In the final model, each 1% higher 25(OH)D was associated with a 0.32% lower PTH; corresponding percent changes per 1% higher $24,25(OH)_2D$ and the VMR were 0.25% and 0.26% lower PTH, respectively (Table 2). When the vitamin D variables were evaluated by quartiles, associations appeared fairly linear (Supplemental Table 3). In all cases, associations were similar irrespective of race (p-interactions all >0.630). Associations between 25(OH)D and PTH were similar irrespective of CKD status (p-interaction=0.941). Tests for interactions $24,25(OH)_2D$ and the VMR by CKD status approached statistical significance (p-interaction=0.076 and 0.063, respectively). Results stratified by CKD status suggested stronger inverse associations between VMR and $24,25(OH)_2D$ and PTH in the 357 participants with CKD (Supplemental Table 4).

3.3 Relationships of Vitamin D Measurements and Hip Bone Mineral Density

Among those sampled in the random sub-cohort, there were 358 participants who had had BMD measurements previously at the 1994–95 study visit; approximately 2 years before blood samples were taken for vitamin D measurements. Demographics of this subset were similar to the remainder of the sub-cohort (Supplemental Table 5). There was no significant association of either 25(OH)D or the VMR with BMD across the sequence of models (Table 3). In contrast, we observed that higher $24,25(OH)_2D$ was associated with higher BMD, but the magnitude of the association was modest. In unadjusted models, each 1% higher $24,25(OH)_2D$ was associated with a 0.06% higher BMD. This association was modestly

attenuated across the sequence of models such that 1% higher $24,25(OH)_2D$ was associated with a 0.04% higher BMD in the final model. We found no evidence for effect modification by race (all p-interactions > 0.12) or CKD status (all p-interactions > 0.06) with any of the vitamin D measures.

3.4 Relationships of Vitamin D Measurements and Hip Fracture Risk

In the case-cohort analysis for hip fracture, we excluded 36 individuals from the sub-cohort and an additional 10 hip fracture cases due to missing covariate data. Among the remaining 1116 participants, there were 902 participants in the sub-cohort, and 289 hip fracture cases (75 cases arose from within the random subcohort and 214 occurred in the remaining CHS population). The incidence rate of hip fracture was 9.1 events per 1000 person-years within the random sub-cohort. We observed no association of 25(OH)D with risk of hip fracture; a finding that was consistent across the sequence of models (Table 4). In contrast, each SD higher $24,25(OH)_2D$ was associated with 24% lower risk of hip fracture in unadjusted models. This strength of association remained similar across the sequence of models such that each SD higher $24,25(OH)_2D$ was associated with 27% lower risk of hip fracture in the fully adjusted model. Results were similar with the VMR, where each SD higher VMR was associated with 26% lower risk of hip fracture in both unadjusted and fully adjusted models. Spline functions depicting the nature of the relationships across the range of each marker are shown in figure 2; associations were fairly linear across the range of available values. We observed no evidence of effect modification by race (p interactions all > 0.22) or CKD status (p interactions all >0.31) for the association of any of the vitamin D metabolites with risk of hip fracture.

4. DISCUSSION

This study represents the first, to our knowledge, to investigate the relationship of $24,25(OH)_2D$ and the VMR with bone health in older, community-living adults. We demonstrate that while lower serum 25(OH)D was associated with higher serum PTH, we observed no association of 25(OH)D with either hip BMD or longitudinal risk of hip fracture. $24,25(OH)_2D$ and the VMR were similarly associated with PTH, but in contrast to 25(OH)D, lower $24,25(OH)_2D$ and VMR were both independently associated with hip fracture risk. We hypothesized that VMR would provide the most comprehensive data on VDR activity, and thus the strongest associations with PTH, BMD, and hip fracture risk. We found that $24,25(OH)_2D$ in isolation had similar associations with PTH and fracture risk, and was associated with BMD while VMR was not.

Several prior studies have evaluated the cross-sectional relationship of the VMR with PTH, including a prior study from the CHS population. [14,26,27] This prior study evaluated the relationship of quartiles of $24,25(OH)_2$ D and the VMR with PTH and showed a trend of lower VMR with higher PTH across quartiles (p=0.08). Our findings are similar, but by designing the analyses as per SD change in each vitamin D variable, we were able to compare strengths of association across vitamin D markers. We demonstrate that all 3 markers had similar strengths of association with PTH. Results evaluating the relationship of the VMR with PTH are generally similar in most, but not all, prior studies. In the Multi-

Ethnic Study of Atherosclerosis (MESA), there was an inverse association of the VMR with PTH concentrations.[26] Berg et al. evaluated a cohort of 376 community-living adults (mean age 48 years) and also found an inverse relationship between VMR and PTH.(14) In contrast, Wagner et al. evaluated 80 participants receiving oral vitamin D supplementation and reported no association between the VMR or $24,25(OH)_2D$ with PTH concentrations. Mean age in this study was 28 years, thus substantially younger than participants in the CHS, MESA, or the study by Berg et al. Thus, with regards to VMR and PTH, we confirm findings in MESA and by Berg and colleagues. Similarly, we confirm findings by Binkley et al. in which they used a vitamin D composite score that sums total 25(OH)D, $25(OH)D_3$ and 24,25(OH)D (instead of a ratio), and found it was inversely associated with PTH.[28] Beyond this confirmation, we meaningfully extend the findings by comparing strengths of association relative to other markers of vitamin D. As the relative strengths of association of VMR and 25(OH)D with PTH were of similar strength, we lack evidence to support one measure over the other from the perspective of the PTH end-point.

Numerous studies in a variety of settings have evaluated the relationship of 25(OH)D with BMD. Results have been conflicting.[7,8,29,30] We found no association between 25(OH)D and BMD in our study, consistent with several prior studies in older adults.[7,30] For the first time, we evaluated the associations of $24,25(OH)_2D$ and the VMR with BMD. We found a direct association of $24,25(OH)_2D$ with BMD. While significant, the association was quite modest in strength. We observed no association of VMR with BMD. Our sample size for this endpoint was modest (N=358), thus these results should be evaluated within the context of the 95% confidence intervals. While it is possible that an association of VMR with BMD may exist, our findings suggest that any such association would be modest in strength, at best.

Finally, we evaluated the associations of the vitamin D measures with longitudinal risk of hip fracture. We found no association between serum 25(OH)D and hip fracture risk, consistent with prior studies in CHS.[31] In contrast, we found that each SD higher VMR was associated with a 26% lower risk of hip fracture during follow-up, and individuals in the highest VMR quartile had nearly half the risk of hip fracture compared to the lowest quartile. Thus, the VMR provided more information on fracture risk than 25-hydroxyvitmain D alone. We also found that lower $24,25(OH)_2D$ was associated with risk of hip fracture; an association that was similar in magnitude of that of the VMR. This suggests that the value of the VMR may be driven primarily by information garnered through measurement of $24,25(OH)_2D$.

The etiology for the association between VMR and $24,25(OH)_2D$ with bone outcomes are not completely understood. We hypothesized that higher VMR and $24,25(OH)_2D$ reflect greater VDR activity driving catabolism. This hypothesis was driven in part by experimental evidence in dogs. When treated with cholecalciferol, the animals expressed higher concentrations of renal 24-hydroxylase as well as higher concentrations of $24,25(OH)_2D$.[12] $24,25(OH)_2D$ is typically thought to be an inert catabolic product of vitamin D. However, some studies suggest that $24,25(OH)_2D$ may be bioactive itself. Ornoy et al. demonstrated improvements in bone health in rachitic chicks after treatment with $24,25(OH)_2D$.[32] Additionally, Curtis et al. found that $24,25(OH)_2D$ drives osteoblastic

differentiation of mesenchymal stem cells.[33] Thus, while VMR and $24,25(OH)_2D$ may reflect VDR activity under the influence of $1,25(OH)D_2$, it is possible that $24,25(OH)_2D$ and the VMR may mark additional biological effects.

Strengths of our study include evaluation of a study sample of older men and women from multiple centers across the US. We examined the relationships between $24,25(OH)_2D$ and the VMR with BMD and hip fracture for the first time. Availability of PTH, BMD, and hip fracture data allowed us the opportunity to comprehensively examine relationships of these markers with bone health. The availability of men and women, whites and blacks, persons with and without CKD, and detailed measurements of covariates including serum calcium, phosphate, and FGF23 are additional strengths.

The study also has important limitations. BMD measurements were made 2 years prior to vitamin D measurements. Prior studies demonstrate that BMD changes slowly, thus we believe this design feature is unlikely to have biased our results.[34] BMD was only available from two clinical centers and the available sample size was modest. Nonetheless, the null findings and relatively tight confidence intervals suggest that any missed association is likely to be modest in strength, at most. We lack data on bisphosphonates or vitamin D supplements use. As such, we do not have data regarding the effect of treatment with ergocalciferol, cholecalciferol or calcitriol on $24,25(OH)_2D$ or the VMR. All vitamin D metabolites were made in one point in time. Future studies are required to determine whether longitudinal changes in these measures provide information on hip fracture risk above and beyond measurements at one point in time. Additionally this study is observational, hence causality cannot be determined. These important questions require future study.

5. CONCLUSION

In summary, we demonstrate that lower $24,25(OH)_2D$ concentrations and lower VMR are associated with increased hip fracture risk community-living older men and women. In contrast, 25(OH)D was not associated with hip fracture risk. If confirmed, measurement of $24,25(OH)_2D$, and potentially calculation of the VMR may provide additional insights to the sufficiency of VDR activity and bone health above and beyond 25(OH)D measurements alone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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principal CHS investigators and institutions can be found at CHS-NHLBI.org. This material is also the result of their work.

Abbreviations

25(OH)D	25 hydroxyvitamin D
24,25(OH)$_2$D	24,25-dihydroxyvitmin D
1,25(OH)$_2$D	1,25-dihydroxyvitamin D, ratio of 24,25(OH)$_2$D to 25(OH)D
VMR	the vitamin D metabolite ratio
VDR	Vitamin D Receptor
DBP	Vitamin D Binding Protein
CHS	Cardiovascular Health Study
eGFR	Estimated Glomerular Filtration Rate
FGF23	Fibroblast Growth Factor 23
ACR	Albumin/Creatinine Ratio

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HIGHLIGHTS

- Serum 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D and the vitamin D Metabolite ratio (VMR) were similarly inversely associated with serum PTH.
- Higher 24,25-dihydroxyvitamin D, but not 25-hydroxyvitamin D or the VMR, was associated with increased bone density.
- The VMR was strongly associated with hip fracture risk while 25-hydroxyvitamin D has no association with hip fracture risk.

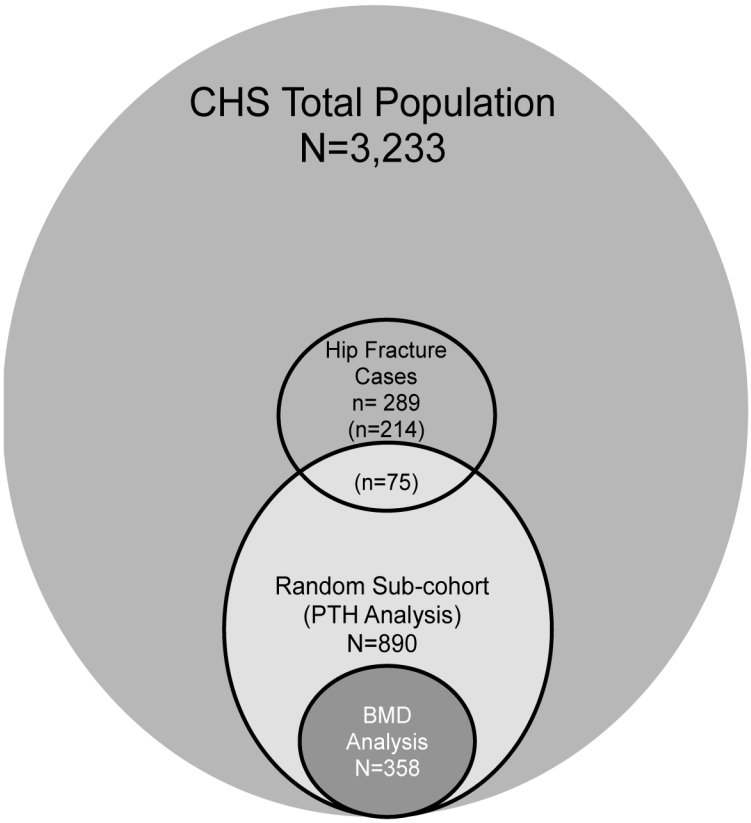
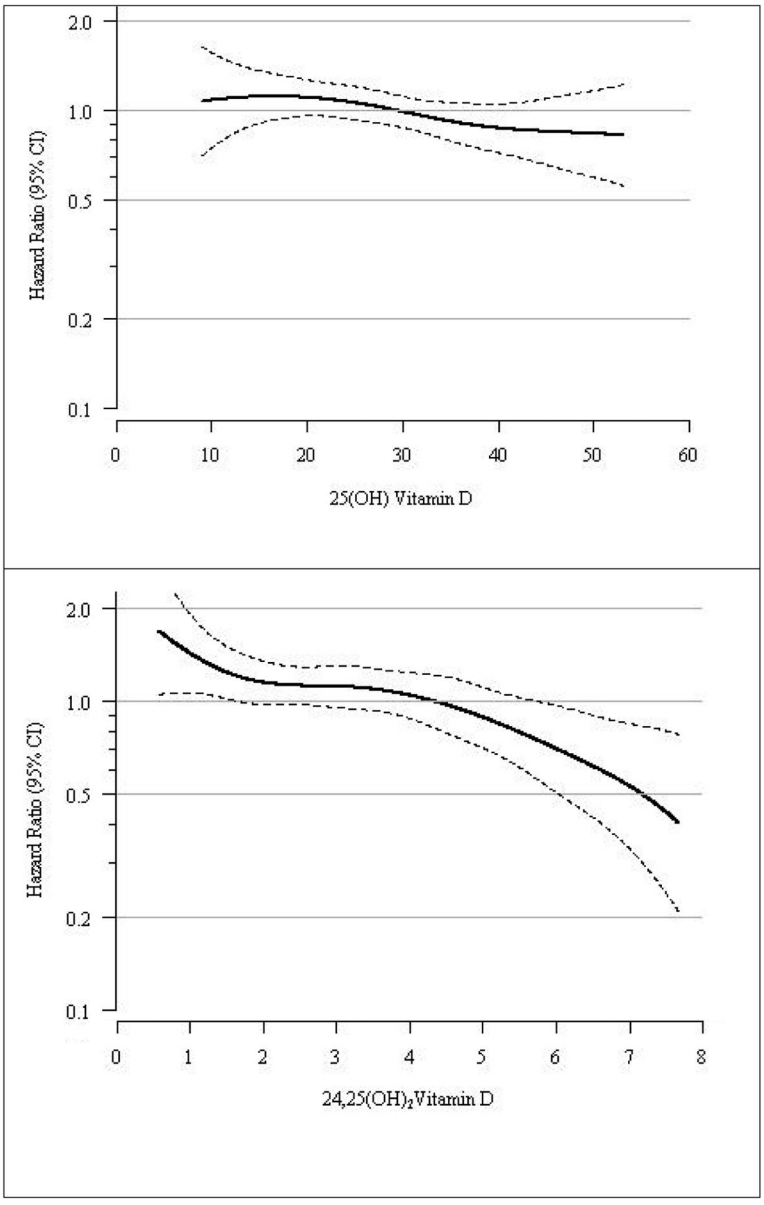


Figure 1.

Study Sampling Relative to the Study Endpoints

Study Populations for each endpoint in CHS. There were 3,233 total participants at the 1996–97 visit in CHS. A random sub-cohort of 1,000 persons (890 with complete data set) were evaluated for PTH analysis. 358 participants underwent DXA and were included for the BMD analysis. All fractures in CHS were included in the analysis, 75 fractures occurred in the random sub-cohort and 214 fractures occurred in the remaining CHS population.



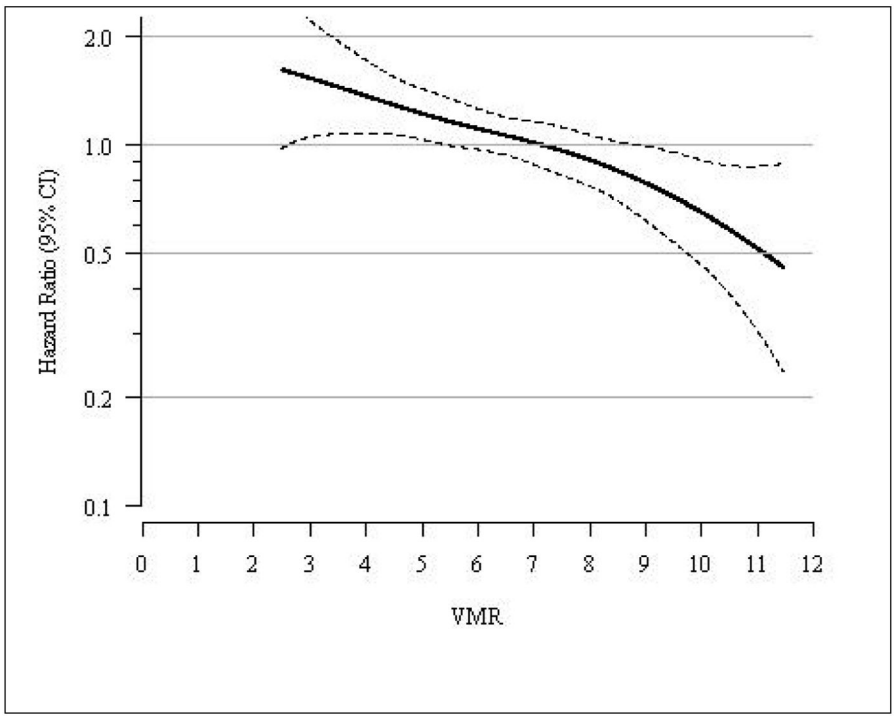


Figure 2.

Spline Function Depicting the Relationship of Vitamin D Measurements with Risk of Hip

Figure 2: Spline function of HR of hip fracture by vitamin D metabolite. Solid lines represent the spline function. Dashed lines represent 95% CI.

Table 1

Baseline Characteristics by Vitamin D Metabolite Ratio (VMR)

	VMR Quartiles				
	Quartile 1 (n=223)	**Quartile 2 (n=222)**	**Quartile 3 (n=223)**	**Quartile 4 (n=222)**	**P Value**
Range	1.05–5.39	5.39–6.70	6.70–8.08	8.08–22.63	
Age (years) ± SD	78.8 (5.1)	77.9 (4.4)	77.6 (4.4)	77.7 (4.6)	0.030
Male, n (%)	64 (28.7)	95 (42.8)	91 (40.8)	104 (46.9)	0.001
Black, n (%)	55 (24.7)	27 (12.2)	30 (13.5)	30 (13.5)	0.001
Clinic site, n(%)					0.236
North Carolina	60 (26.9)	57 (25.7)	45 (20.2)	47 (21.2)	
California	65 (29.2)	55 (24.8)	52 (23.3)	74 (33.3)	
Maryland	39 (17.5)	50 (22.5)	59 (26.5)	45 (20.3)	
Pennsylvania	59 (26.5)	60 (27.0)	67 (30.0)	56 (25.2)	
Season of blood measurement, n (%)					<0.001
Winter	37 (16.6)	54 (24.3)	67 (30.0)	80 (36.0)	
Spring	95 (42.6)	27 (12.2)	27 (12.1)	13 (5.9)	
Summer	64 (28.7)	75 (33.8)	66 (29.6)	39 (17.6)	
Fall	27 (12.1)	66 (29.7)	63 (28.3)	90 (40.5)	
BMI (kg/m^2) ± SD	27.3 (5.0)	26.9 (4.9)	26.3 (4.7)	26.8 (4.2)	0.156
Smoking status, n (%)					0.619
Never	120 (55.3)	110 (50.0)	102 (46.2)	104 (48.6)	
Former	82 (37.8)	96 (43.6)	103 (46.6)	93 (43.5)	
Current	15 (6.9)	14 (6.4)	16 (7.2)	17 (7.9)	
Diabetes, n (%)	44 (19.7)	33 (14.9)	25 (11.2)	24 (10.8)	0.024
Systolic BP (mm Hg) ± SD	138.4 (20.4)	137.4 (21.7)	135.8 (22.9)	134.9 (18.4)	0.293
Diastolic BP (mm Hg) ± SD	69.7 (12.1)	69.4 (10.4)	69.2 (11.3)	69.9 (10.7)	0.931
Use of anti-hypertensive medications, n (%)	123 (55.2)	103 (46.4)	103 (46.2)	89 (40.1)	0.016
CKD, n (%)	111 (49.8)	110 (49.6)	80 (35.9)	56 (25.8)	<0.001
eGFR ($ml/min/1.73m^2$) ± SD	58.4 (17.5)	60.2 (16.6)	66.2 (15.7)	71.2 (14.9)	<0.001
Albumin/Creatinine, median [IQR]	9.3 [5.2–23.6]	7.9 [4.6–17.9]	8.8 [4.8–22.1]	8.00 [4.7–16.5]	0.291
Calcium (mg/dl) ± SD	9.8 (0.5)	9.8 (0.6)	9.8 (0.6)	9.8 (0.6)	0.936

	VMR Quartiles				
	Quartile 1 (n=223)	**Quartile 2 (n=222)**	**Quartile 3 (n=223)**	**Quartile 4 (n=222)**	**P Value**
Phosphate (mg/dl) ± SD	3.8 (0.5)	3.8 (0.6)	3.7 (0.5)	3.8 (0.6)	0.414
Parathyroid hormone (pg/ml), median [IQR]	48.2 [36.7–66.8]	42.3 [32.7–56.2]	40.4 [32.6–56.3]	40.7 [31.3–53.1]	0.001
FGF23 (RU/ml), median [IQR]	73.1 [54.6–107.8]	71.7 [52.8–107.8]	67.0 [52.4–93.4]	68.2 [52.9–87.0]	0.156
Bone mineral density (g/cm^2) ± SD *	0.81 (0.18)	0.85 (0.20)	0.85 (0.18)	0.82 (0.19)	0.256

* N=358

Table 2

Cross-sectional Relationship of Vitamin D Measures with PTH (All Measured in 1996–97)*

	25(OH) Vitamin D		$24,25(OH)_2$ Vitamin D		VMR	
	β coef (95% CI)	P Value	β coef (95% CI)	P Value	β coef (95% CI)	P Value
Model 1**	−0.363 (−0.430 , −0.297)	<.001	−0.282 (−0.329 , −0.234)	<.001	−0.303 (−0.391, − 0.214)	<0.001
Model 2	−0.316 (−0.389 , −0.243)	<0.01	−0.289 (−0.342, −0.236)	<.001	−0.361 (−0.455, −0.266)	<0.001
Model 3	−0.324 (−0.394, −0.255)	<.001	−0.252 (−0.303, −0.200)	<.001	−0.257 (−0.352, −0.161)	<0.001

*Data reported is for natural logarithm of vitamin D metabolites and natural logarithm of PTH

**Model 1 is an unadjusted model. Model 2 is adjusted for age, sex, race, season of measurements, site of measurement and BMI, Model 3 is adjusted for the same variables as model 2 as well as eGFR, serum calcium, phosphate and FGF-23.

Table 3

Cross-sectional Relationship of Vitamin D Measures (1996–97) with BMD (1994–95)*

	25-hydroxyvitamin D		24,25-dihydroxyvitamin D		VMR	
	β coef (95% CI)	**P Value**	**β coef (95% CI)**	**P Value**	**β coef (95% CI)**	**P Value**
Model 1**	0.038 (−0.003, 0.093)	0.162	0.058 (0.019, 0.098)	0.004	0.013(−0.057, 0.084)	0.705
Model 2	0.022 (−0.023, 0.067)	0.337	0.040 (0.007, 0.074)	0.018	0.017 (−0.041, 0.075)	0.562
Model 3	0.022 (−0.023, 0.067)	0.344	0.043 (0.009, 0.078)	0.013	0.026 (−0.037, 0.088)	0.422

*Data reported is for natural logarithm of vitamin D metabolites and natural logarithm of BMD

**Model 1 is an unadjusted model. Model 2 is adjusted for age, sex, race, season of measurements, site of measurement and BMI, Model 3 is adjusted for the same variables as model 2 as well as eGFR, serum calcium, phosphate and FGF-23

Table 4

Prospective Association of Vitamin D Measures (1996–97) with Incident Hip Fracture (Case-Cohort)

25-hydroxyvitamin D, HR (95% CI), ng/ml						
	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	**Per SD=11.26 Higher**	**P-value(continuous analysis)**
	HR (95% CI)	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	**HR (95% CI)**	
N Fracture / N at Risk	66/271	77/276	83/294	63/275	289/1116	
Incident Rate (per 1000 person years)	10.91	12.69	7.10	6.15	9.14	
Model 1*	Ref	1.11 (0.76, 1.65)	1.10 (0.75, 1.60)	0.84 (0.56, 1.24)	1.00 (0.87, 1.13)	p=0.968
Model 2	Ref	1.01 (0.65, 1.55)	0.96 (0.62, 1.47)	0.70 (0.44, 1.12)	0.94 (0.81, 1.11)	p=0.514
Model 3	Ref	0.99 (0.65, 1.54)	0.91 (0.59, 1.41)	0.66 (0.42, 1.06)	0.93 (0.79, 1.10)	p=0.374
24,25-dihydroxyvitamin D, HR (95% CI), ng/ml						
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Per SD=0.96 Higher	
N Fracture / N at Risk	79/289	76/274	85/287	49/266	289/1116	
Incident Rate (per 1000 person years)	12.22	12.32	9.17	3.60	9.14	
Model 1	Ref	0.90 (0.62, 1.31)	0.95 (0.66, 1.36)	0.50 (0.34, 0.75)	0.76 (0.67, 0.89)	p<0.001
Model 2	Ref	0.91 (0.61, 1.37)	0.83 (0.54, 1.26)	0.48 (0.30, 0.77)	0.72 (0.60, 0.85)	p<0.001
Model 3	Ref	0.87 (0.58, 1.31)	0.82 (0.54, 1.25)	0.48 (0.30, 0.78)	0.73 (0.61, 0.87)	p<0.001
VMR, HR (95% CI), (ng/ml) / (ng/ml)						
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Per SD=2.23 Higher	
N Fracture / N at Risk	80/291	84/290	64/274	51/261	289/1116	
Incident Rate (per 1000 person years)	14.77	8.21	6.68	7.48	9.14	
Model 1	Ref	0.83 (0.58, 1.19)	0.61 (0.42, 0.89)	0.49 (0.33, 0.72)	0.74 (0.63, 0.85)	p<0.001
Model 2	Ref	0.82 (0.55, 1.23)	0.61 (0.40, 0.93)	0.48 (0.30, 0.76)	0.70 (0.58, 0.84)	p<0.001
Model 3	Ref	0.82 (0.55, 1.22)	0.64 (0.41, 0.99)	0.54 (0.34, 0.87)	0.74 (0.61, 0.88)	p=0.001

Model 1 is an unadjusted model. Model 2 is adjusted for age, sex, race, season of measurements, site of measurement and BMI, Model 3 is adjusted for the same variables as model 2 as well as eGFR, serum calcium, phosphate and FGF-23