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Association of 25-hydroxyvitamin D with areal and volumetric measures of bone mineral density and parathyroid hormone: impact of vitamin D-binding protein and its assays

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Abstract

Summary—A comparison of the association of different forms of 25-hydroxyvitamin D [25(OH)D] with parathyroid hormone (PTH) and with areal and volumetric bone mineral density (BMD) demonstrated that bioavailable and free 25(OH)D do not provide a better index of vitamin D status in terms of bone health compared to total 25(OH)D.

Introduction—This study aims to compare measures of vitamin D-binding protein (DBP) using a monoclonal versus polyclonal ELISA and assess correlations of total versus estimated free and bioavailable 25(OH)D with BMD and PTH concentrations.

Methods—DXA and peripheral quantitative CT (pQCT) scans were obtained in 304 adults (158 black, 146 white), ages 21–80 years. Free and bioavailable 25(OH)D were calculated from total 25(OH)D, DBP, and albumin concentrations. Multivariable linear regression with standardized beta coefficients was used to evaluate associations of bone measures and PTH with total, free, and bioavailable 25(OH)D.

Results—Measures of DBP obtained using a monoclonal versus polyclonal ELISA were not correlated ($r_s=0.02$, $p=0.76$). Free and bioavailable 25(OH)D based on the polyclonal assay were

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Conflict of interest Thomas Jemielita, Joshua Baker, Samir Sayed, Babette Zemel, Justine Shults, and Rita Herskovitz declare that they have no conflict of interest.

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lower in black versus white participants ($p<0.0001$); this race difference was not evident using the monoclonal assay. Adjusted for age, sex, calcium intake, and race, all forms of 25(OH)D were negatively associated with PTH, but the absolute coefficient was greatest for total 25(OH)D (-0.34 , $p<0.001$) versus free/bioavailable 25(OH)D ($-0.18/-0.24$ depending on DBP assay, $p=0.003$). In analyses stratified on race, none of the measures of 25(OH)D were associated with BMD across DXA and pQCT sites.

Conclusions—The monoclonal versus polyclonal ELISA yielded highly discrepant measures of DBP, particularly among black individuals, likely related to established race differences in DBP polymorphisms. Contrary to prior studies, our findings indicate that using DBP to estimate bioavailable and free 25(OH)D does not provide a better index of vitamin D status in terms of bone health.

Keywords

Bone mineral density; Parathyroid hormone; Vitamin D; Vitamin D-binding protein

Introduction

Studies of the relationship between circulating concentrations of serum 25-hydroxyvitamin D [25(OH)D], the conventional index of vitamin D status, and bone mineral density (BMD) have yielded inconsistent results. Several observational studies have demonstrated an association of higher 25(OH)D concentrations with greater BMD [1–3] and lower fracture risk [4, 5]. Randomized controlled trials have shown a beneficial effect of vitamin D supplementation on BMD and/or fracture risk [6–10]. In contrast, other observational studies and trials have been equivocal [11, 12], found no association between 25(OH)D and BMD [13, 14], showed no benefit of vitamin D supplementation on BMD and fracture risk [15, 16], or showed an increased risk of fracture following high-dose supplementation [17]. Studies have also suggested that racial differences exist in the relationship between 25(OH)D and BMD [18, 19], findings which are underscored by the fact that black individuals, compared to whites, have significantly lower fracture risk [20–24] despite consistently lower 25(OH)D concentrations [25, 26].

Recent evidence highlights the importance of the vitamin D-binding protein (DBP) in the assessment of racial differences in vitamin D status. Both 25(OH)D and 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$] circulate bound to DBP (85–90 %) or albumin (10–15 %), with <1 % in their free forms. The binding affinity of DBP for vitamin D metabolites is >1000-fold stronger than that of albumin [7×10^8 vs. $6\times 10^5 \text{ M}^{-1}$ for 25(OH)D and 3.7×10^7 vs. $5.4\times 10^4 \text{ M}^{-1}$ for $1,25(\text{OH})_2\text{D}$], and therefore, the albumin-bound and free fractions together are considered bioavailable [27–31]. Combinations of two single-nucleotide polymorphisms, rs4588 and rs7041, produce three major polymorphic forms of DBP (Gc1F, Gc1S, and Gc2) which differ substantially in their binding affinity, circulating concentration, and racial distribution [32–34]. In a recent study, 93 % of homozygous black individuals had the high affinity Gc1F variant as compared to only 6 % of white homozygotes [34]. Pre-clinical studies have established a role for DBP in regulating the availability of 25(OH)D to certain cell types [35, 36]. For example, greater concentrations of DBP and higher affinity

forms of DBP attenuated 25(OH)D-induced monocyte antimicrobial responses, suggesting that bioavailable 25(OH)D is biologically relevant for monocytes.

A recent study in 49 healthy young adults (63 % white) showed that lumbar spine areal BMD (aBMD), measured by dual-energy x-ray absorptiometry (DXA), was positively associated with free ($r=0.413$, $p=0.003$) and bioavailable ($r=0.441$, $p=0.002$), but not total 25(OH)D ($r=0.172$, $p=0.236$) [30]. Serum DBP concentrations varied widely and were inversely associated with black race. The authors concluded that bioavailable 25(OH)D may be a better measure of vitamin D status with regards to bone health. Concern has since been raised about the validity of the monoclonal antibody enzyme-linked immunosorbent assay (ELISA) used in this study and in widespread use [37–39]. This concern centered on discrepant findings from prior studies using polyclonal DBP assays and the potential of binding affinities and glycosylation patterns of the polymorphic DBP variants leading to differential and biased performance of the monoclonal assay.

Expanding on this prior work, this study is the first to examine the comparative associations of total, free, and bio-available 25(OH)D with aBMD, measured at multiple sites by DXA, and trabecular volumetric BMD (vBMD), measured by peripheral quantitative computed tomography (pQCT), in a large cohort of adults. The racial distribution in this cohort was approximately 50 % black and 50 % white by design, facilitating the assessment of racial differences in these associations. DBP was measured by both monoclonal and polyclonal ELISA methods. The primary objective was to examine the comparative associations of total versus free and bioavailable 25(OH)D with aBMD of the lumbar spine, proximal femur, and radius, trabecular vBMD in the tibia, and parathyroid hormone (PTH) concentrations as another index of vitamin D status. The secondary objective was to compare measures of DBP and estimates of free and bioavailable 25(OH)D based on a monoclonal as compared to a polyclonal ELISA.

Methods

Study population

Adults, ages 21 to 80 years, were enrolled as healthy reference participants for bone studies at the Children's Hospital of Philadelphia (CHOP) and University of Pennsylvania (UPENN) between March of 2004 and June of 2008 as previously described [40, 41]. The study protocols were approved by the Institutional Review Boards at CHOP and UPENN, and informed consent was obtained from all participants. The study targeted equal numbers of participants in each decade of life, with 50 % black and 50 % female participants. Exclusion criteria comprised a history of chronic diseases or medications that impact skeletal health or nutrition, including diabetes, malabsorption, malignancy, and chronic kidney, liver, and thyroid disease. A total of 304 participants with complete measures of 25(OH)D, DBP, albumin, who self-identified as black ($n=158$) or white ($n=146$), and who had DXA and/or pQCT data were included here.

Anthropometrics and dietary calcium

Height was measured using a stadiometer (Holtain Ltd., Crymch, UK), and weight was measured using a digital scale (Scaltronix, White Plains, NY). Dietary information was collected using the validated National Cancer Institute Dietary History Questionnaire (DHQ) [42]. After manual review for completeness, DHQs were analyzed for daily nutrient intake using the National Cancer Institute's DietCalc software. DHQ results with extreme values for total daily energy intake were excluded (<600 or >4000 kcal for women and <800 or >5000 kcal for men), so DHQ data was available in 257 participants (85 %).

Bone outcomes

Measures of aBMD (g/cm^2) at five sites (lumbar spine, total hip, femoral neck, 1/3 radius, and ultra-distal radius) were obtained by DXA using a Hologic densitometer (Delphi Systems; Hologic, Inc., Bedford, MA). Measures of vBMD (mg/cm^3) were obtained in the left tibia by pQCT (Stratec XCT2000 12-detector unit; Orthometrix, Inc.) with a voxel size of 0.4 mm, slice thickness of 2.3 mm, and scan speed of 25 mm/s as previously described [40]. Measurements for trabecular vBMD were obtained at 3 % of tibia length proximal to the reference line at the distal endplate. The associations of pQCT outcomes with age, sex, race, and body composition have been described [40].

Vitamin D, DBP, and PTH measures

Serum 25(OH)D and 1,25(OH)₂D concentrations were measured by ¹²⁵I radioimmunoassay (RIA; DiaSorin Inc., Stillwater, MN) [43]. Intra-assay CVs were 2.2 and 7–11 %, respectively [44]. Serum DBP was measured in duplicate (CV < 10 %) using a monoclonal antibody ELISA (R&D Systems, Minneapolis, MN). The inter-assay CV was 1.6–3.6 %, and recovery was 98–103 %. Serum DBP was also measured in duplicate using a polyclonal ELISA (ALPCO Diagnostics, Salem, NH). The intra-assay variation of this ELISA was <10 %, and recovery was 99–100 %.

Intact PTH concentrations were measured by RIA with CVs of 3–10 % (Scantibodies Laboratory, Santee, CA) [45]. Serum albumin was quantified using a colorimetric method based on a bromocresol purple performed on a COBAS c501 analyzer (Roche Diagnostics, Indianapolis, IN). Total imprecision ranged from 1.0 to 1.3 %, and limit of detection was 0.2 g/dl.

Serum free and bioavailable 25(OH)D were calculated using total 25(OH)D, DBP, and albumin concentrations, according to the equations of Powe et al. [30]. Powe et al. adapted validated equations for free and bioavailable testosterone, by replacing testosterone, sex hormone-binding globulin, and albumin and their respective binding constants with those of 25(OH)D, DBP, and albumin. The equations used to calculate free and bioavailable 25(OH)D are described in detail in the Supplementary Material of that report and summarized below:

$$\text{Free 25(OH)D} = [-b + \sqrt{(b^2 - 4ac)}] / 2a,$$

where

$$\begin{aligned}
 a & K_{dbp} * K_{alb} * \text{albumin} + K_{dbp} \\
 b & (K_{dbp} * \text{DBP}) - (K_{dbp} * 25(\text{OH})\text{D}) + (K_{alb} * \text{albumin}) + 1 \\
 c & -[25(\text{OH})\text{D}] \\
 K_{dbp} & \text{affinity constant between } 25(\text{OH})\text{D} \text{ and DBP} \\
 K_{alb} & \text{affinity constant between } 25(\text{OH})\text{D} \text{ and albumin}
 \end{aligned}$$

$$\text{Bioavailable } 25(\text{OH})\text{D} = (K_{alb} * \text{albumin} + 1) * [\text{free } 25(\text{OH})\text{D}]$$

Concentrations of albumin, DBP, and 25(OH)D in these equations are in moles per liter, and free and bioavailable 25(OH)D were then converted to picograms per milliliter and nanograms per milliliter, respectively, for ease of interpretation of results.

Statistical analysis

All analyses were performed using STATA 13.0 (Stata Corporation, College Station, TX). A two-sided p value of <0.05 was considered statistically significant. Distributions of all variables were assessed for normality. All serum measures were skewed, so descriptive statistics for these continuous variables were reported as median and inter-quartile range (IQR). Group differences were assessed using Student's t , Wilcoxon rank sum, or chi-square tests as appropriate. Serum 25(OH)D concentrations were categorized as deficient (<20 ng/ml) or not, in accordance with the 2011 Institute of Medicine report [46]. Correlations were assessed by Spearman's rank correlations. The corcor program was used to test the equality of dependent correlations.

Multivariable linear regression analysis was used to compare the association of the total, free, and bioavailable 25(OH)D measures with PTH, adjusted for age, sex, race, and calcium intake. Multivariable linear regression analysis was used to evaluate the association of 25(OH)D measures with trabecular vBMD in the tibia and with aBMD in the lumbar spine, femoral neck, total hip, 1/3 radius, and ultra-distal radius. Skewed bone outcome variables (lumbar spine aBMD) were natural log transformed to achieve normality. Base models were first established for each bone outcome, examining independent associations with age, sex, race, and BMI as well as a two-way interaction between age and sex. Once a base model was established for each outcome, models were compared, with each model including a different form of 25(OH)D (total, free, or bioavailable) as an independent variable. Models were also stratified by race. Model assumptions were assessed by graphical checks, particularly checking the normality of residuals. To facilitate comparison of the individual vitamin D variables with their different units of measurement and their respective associations with bone outcomes, standardized regression coefficients were presented in each model rather than conventional beta coefficients. The value of a standardized regression coefficient can be interpreted as follows: one standard deviation greater value of the variable is associated with that magnitude of difference in standard deviation(s) of the outcome.

Results

Participant characteristics

Participant characteristics are summarized by sex and race in Table 1. By design, participants were evenly distributed across race and sex, and age distribution did not differ by race. BMI was significantly higher among black versus white females, but there was no racial difference in males. Black participants of both sexes had significantly lower total 25(OH)D concentrations. There were no sex differences in total 25(OH)D concentrations. The prevalence of vitamin D deficiency [25(OH)D<20 ng/ml] was 63 % among black males versus 21 % among white males ($p<0.001$), and 57 % compared to 13 % ($p<0.001$) among black and white females, respectively. Black participants had significantly higher PTH concentrations, consistent with prior publications [47]. The 13 values of PTH concentration 70 pg/ml were observed only among black participants (10 women and 3 men). Among black ($p=0.01$) but not white ($p=0.25$) participants, women had higher PTH concentrations than men, potentially related to their lower calcium intake. No racial difference was found in 1, 25(OH)₂D concentrations, but women had higher concentrations than men among both black ($p=0.02$) and white ($p<0.001$) participants. Black participants of both sexes had significantly lower average dietary calcium intake.

Measures of DBP concentrations differed markedly between the monoclonal and polyclonal assays, with the greatest discrepancies between assay results observed among black participants. Results from the two assays were not correlated overall ($r_s=0.02$, $p=0.76$) and poorly correlated when stratified by race (black, $r_s=0.19$, $p=0.02$; white, $r_s=0.22$, $p=0.01$). Using the monoclonal assay, black participants of both sexes had DBP concentrations that were more than 50 % lower than white participants. In contrast, using the polyclonal assay, DBP concentrations were significantly higher in black females compared to white females, with no race difference among males. Not surprisingly, given the discrepant results of the monoclonal versus polyclonal DBP assay, the estimation of free and bioavailable 25(OH)D concentrations differed considerably depending on which DBP assay was used (Fig. 2). Using the monoclonal assay results, no statistically significant race difference was found in free or bioavailable 25(OH)D concentrations except for slightly greater free 25(OH)D in black males compared to white males (Table 1). In stark contrast, based on DBP results from the polyclonal assay and in keeping with their lower total 25(OH)D concentrations, black participants of both sexes had significantly lower free and bioavailable 25(OH)D compared to white participants ($p<0.001$).

Correlations between PTH and total, free, and bioavailable 25(OH)D

Figure 1 summarizes the Spearman correlation matrix for PTH with each form of 25(OH)D. Among black participants, total, free, and bioavailable 25(OH)D were all significantly and inversely associated with PTH. Correlations were greatest for total 25(OH)D ($r_s=-0.30$) and free and bioavailable 25(OH)D estimated from the polyclonal DBP assay ($r_s=-0.32$ and -0.34 , respectively), as compared to those for free and bio-available 25(OH)D estimated from the monoclonal DBP assay ($r_s=-0.22$ and -0.23 , respectively), though this difference in correlations was not statistically significant. Among white participants, only total 25(OH)D was significantly correlated with PTH. There was no association between PTH

and 1, 25(OH)₂D concentrations. In multivariable linear regression analysis adjusted for age, sex, calcium intake, and race (Table 2), all forms of 25(OH)D were negatively associated with PTH, but the absolute standardized beta coefficient was greatest for total 25(OH)D (−0.34, $p<0.001$) as compared to free/bioavailable 25(OH)D, and the standardized beta coefficients were greater for estimates of free and bioavailable 25(OH)D based on the polyclonal (−0.24, $p<0.001$) as compared to the monoclonal DBP assay (−0.18/−0.19, $p=0.003$).

Associations between BMD and total, free, and bioavailable 25(OH)D

Table 3 summarizes the multivariable regression models relating DXA aBMD at several sites and trabecular vBMD to the multiple forms of 25(OH)D. Among black participants, only 5 of the 25 DXA models summarized in the table achieved statistical significance, but no consistent pattern of association with a form of 25(OH)D emerged. Within the group of white participants, none of the forms of 25(OH)D was associated with aBMD at any DXA site. Among black participants, total and bioavailable 25(OH)D (based on the monoclonal DBP assay) were independently and positively associated with trabecular vBMD. Among white participants, there was no association between any form of 25(OH)D and trabecular vBMD.

Discussion

This is the first and largest study to investigate the comparative associations of total, free, and bioavailable 25(OH)D with both aBMD, measured at multiple sites by DXA, and trabecular vBMD, measured by pQCT. The primary objective was to determine if estimates of free and bioavailable 25(OH)D provide a better index of vitamin D status in terms of bone health, as suggested by the prior report that lumbar spine aBMD was positively associated with free and bioavailable, but not total 25(OH)D in 49 healthy young adults [30]. Our results did corroborate this finding in terms of bioavailable 25(OH)D at the lumbar spine; however, this pattern was only present in black participants and only based on the monoclonal DBP assay. Furthermore, this finding was not observed at any other DXA site, nor was it supported by pQCT measures of vBMD. Indeed, among black participants, findings were highly inconsistent across aBMD sites, and total and bioavailable 25(OH)D were similarly associated with trabecular vBMD. Among white participants, no association was found between any form of 25(OH)D and any measure of aBMD or trabecular vBMD.

Similarly, there was no evidence that free and bioavailable 25(OH)D were more strongly associated with PTH compared to total 25(OH)D. In fact, the magnitude of the correlation of total 25(OH)D with PTH was greater than the correlations of free or bioavailable 25(OH)D. This is in contrast to the prior finding of Bhan et al. [27] that only bioavailable 25(OH)D was significantly associated with PTH in 94 hemodialysis patients. However, as expected, the dialysis patients had considerably higher and more variable PTH and lower albumin levels than our healthy control population. Furthermore, consistent with the findings of this study, in our prior study of 148 children with chronic kidney disease, PTH was inversely and comparably associated with total, free, and bioavailable 25(OH)D (Spearman's rho of −0.48, −0.38, and −0.41, respectively) [48].

The primary limitations of this study are the lack of data on DBP polymorphisms and the cross-sectional design. Compared to the Gc1F allele, the relative affinities of the Gc1S and Gc2 alleles for 25(OH)D are 0.536 and 0.321, respectively [32]. Therefore, the six allelic combinations differ considerably in their affinity for 25(OH)D and 1,25(OH)₂D. Circulating concentrations of 25(OH)D and racial distribution also differ by allelic combination [33]. In a recent population-based cohort study of healthy adults, Powe et al. [34] showed that 54 % of black participants were homozygous for the Gc1F allele. In contrast, only 2.6 % of white participants were homozygous for the Gc1F allele. The equations used to estimate free and bioavailable 25(OH)D in our study used the affinity constants of the Gc1F allele, as did the equations in the 2011 study by Powe et al. [30] reporting that DXA spine aBMD was correlated with free and bioavailable 25(OH)D, but not total 25(OH)D. This results in a systematic underestimation of the bioavailable and free forms of 25(OH)D for all five allelic combinations other than the Gc1F homozygote and particularly for those combinations with no Gc1F allele, which predominate in non-black populations. Indeed, 63 % of the 49 participants in the 2011 study by Powe et al. [30] were white. Given that young white adults have lower spine aBMD compared with young black adults (as confirmed in our study here), this under-estimation of free and bioavailable 25(OH)D among white participants may have contributed to the observed positive associations with aBMD. Furthermore, even with available genotypic data and the ability to apply genotype-specific affinity constants to homozygous individuals, the relative circulating concentrations of different allelic forms of DBP in heterozygotes is unknown. The stratified analyses performed in this study begin to address confounding by race. DXA aBMD at the lumbar spine, femoral neck, and hip were significantly lower in white versus black participants (all $p < 0.002$). Thus, under-estimation of free and bioavailable 25(OH)D in the white participants could potentially have driven an association between these measures and BMD in the overall cohort, making the stratified analyses critical. Last, the cross-sectional nature of the study limits our ability to infer causality from the associations observed.

The direct comparison of findings based on a monoclonal versus polyclonal assay for DBP highlights an issue of profound importance, namely the lack of a gold standard measure of DBP and concern about differential performance of the monoclonal assay by genotype [37–39]. The primary monoclonal antibody of the R&D ELISA binds to a single peptide fragment of DBP and may bind differently to Gc1F and Gc1S variants, yielding underestimated concentrations in black individuals [37, 39]. Consistent with this concern, DBP results between the two assays were most discrepant among black participants in our study, with concentrations >50 % lower than among white participants based on the monoclonal assay. In keeping with prior studies which did not find racial differences in circulating DBP using polyclonal assays [37, 49–51], the only difference we observed using the polyclonal ELISA was actually higher DBP concentrations in black versus white females and no racial difference among males. While there may be cross-reactivity of polyclonal antibodies with other proteins [52, 53] which likely explains the systematically higher DBP results with the polyclonal assay, this is non-differential by race. The markedly discrepant DBP measures had a considerable impact on interpretation of racial differences in free and bioavailable 25(OH)D. Using the monoclonal assay would lead to the conclusion that despite lower total 25(OH)D concentrations, black individuals do not have lower free and

bioavailable 25(OH)D, as put forth in the Powe et al. study [34]. As shown in Fig. 2, when not stratified by sex, black participants actually had higher free and bioavailable 25(OH)D estimated using the monoclonal assay. However, using the polyclonal assay, black participants had significantly (approximately 50 %) lower free and bioavailable 25(OH)D. The impact in multivariable models comparing associations of total, free, and bioavailable 25(OH)D with measures of BMD and PTH was mitigated by the fact that much of the variability in these outcomes was explained by other factors in the model and the associations were driven primarily by the total 25(OH)D.

In summary, this study does not support the prior suggestion that using total 25(OH)D, DBP, and albumin to calculate bioavailable or free 25(OH)D provides a better index of vitamin D status in terms of bone health. Furthermore, our findings substantiate the concern about differential performance of the monoclonal ELISA according to genotype and, therefore, race. The polymorphism of DBP challenges its accurate measurement, and until the methodologic issues of specificity and differential performance by genotype are addressed, cautious interpretation of results is warranted. Whether direct measurement of free or bioavailable 25(OH)D will provide better indices of vitamin D-related bone health remains to be seen.

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Fig. 1.

a Correlations of parathyroid hormone (PTH) with total 25(OH)D. **b** Correlations of PTH with free 25(OH)D. **c** Correlations of PTH with bioavailable 25(OH)D

Fig. 2.
Estimation of free and bioavailable 25(OH)D concentrations

Table 1

Participant characteristics according to sex and race

	Male		Female		<i>p</i> value ^a
	Black	White	Black	White	
<i>N</i>	71 (48.3 %)	76 (51.7 %)	87 (55.4 %)	70 (44.6 %)	
Age, years	47.7 (37.2, 61.2)	49.4 (27.9, 65.4)	48.5 (38.2, 62.1)	45.0 (35.0, 56.7)	0.10
BMI, kg/m ²	26.1 (22.7, 29.3)	24.9 (22.9, 28.0)	28.1 (24.8, 33.0)	23.6 (20.6, 27.2)	<0.0001
Total 25D, ng/ml	16.0 (10.3, 23.6)	28.4 (21.2, 35.9)	18.6 (13.0, 25.0)	30.6 (23.6, 37.2)	<0.0001
Vitamin D-binding protein, mg/dl					
Polyclonal	41.6 (35.4, 47.1)	38.4 (33.0, 44.2)	48.1 (40.1, 56.0)	40.9 (37.5, 48.9)	0.002
Monoclonal	7.5 (5.9, 9.8)	22.2 (17.4, 28.6)	9.1 (6.4, 16.9)	21.5 (16.9, 27.1)	<0.0001
Albumin	4.1 (4.0, 4.3)	4.3 (4.0, 4.5)	4.0 (3.8, 4.2)	4.0 (3.9, 4.3)	0.12
Free 25(OH)D, pg/ml					
Polyclonal	3.1 (2.0, 4.4)	6.1 (4.0, 7.4)	2.9 (2.1, 4.0)	5.3 (4.4, 6.7)	<0.0001
Monoclonal	11.8 (7.7, 19.1)	10.0 (6.7, 13.0)	12.4 (6.8, 16.7)	9.7 (8.1, 12.9)	0.16
Bioavailable 25(OH)D, ng/ml					
Polyclonal	1.2 (0.7, 1.7)	2.2 (1.5, 2.8)	1.0 (0.7, 1.5)	2.0 (1.6, 2.5)	<0.0001
Monoclonal	4.4 (2.7, 7.1)	3.7 (2.6, 5.0)	4.3 (2.6, 5.9)	3.5 (2.9, 4.8)	0.18
1,25D, pg/ml	37.0 (31.2, 43.9)	36.4 (28.7, 45.9)	41.6 (33.1, 51.1)	45.7 (35.2, 53.1)	0.22
Parathyroid hormone, pg/ml	31.0 (24.0, 46.0)	25.0 (19.4, 34.0)	37.0 (29.0, 52.0)	28.0 (22.0, 35.2)	<0.0001
Dietary calcium intake, mg/day ^b	709 (447, 890)	857 (526, 1132)	530 (389, 837)	768 (551, 1014)	0.0003

Results are presented as median (inter-quartile range)

^aRefers to a rank-sum test between blacks and whites^b*N*=355

Table 2

Multivariable regression analysis of the association between parathyroid hormone (PTH) and total, free, and bioavailable 25(OH)D concentrations

Model R ²	0.25	0.21	0.19	0.21	0.19
Age	0.27 (<0.001)	0.26 (<0.001)	0.24 (<0.001)	0.25 (<0.001)	0.24 (<0.001)
Female	0.14 (0.01)	0.10 (0.08)	0.12 (0.03)	0.09 (0.11)	0.12 (0.04)
Black	0.14 (0.02)	0.20 (0.002)	0.34 (<0.001)	0.20 (0.002)	0.34 (<0.001)
Dietary calcium intake, mg/day	-0.02 (0.77)	-0.03 (0.56)	-0.02 (0.73)	-0.03 (0.60)	-0.02 (0.78)
Vitamin D measure	Total 25(OH)D	Free 25(OH)D (Polyclonal)	Free 25(OH)D (Monoclonal)	Bioavailable 25(OH)D (Polyclonal)	Bioavailable 25(OH)D (Monoclonal)
	-0.34 (<0.001)	-0.24 (<0.001)	-0.18 (0.003)	-0.24 (<0.001)	-0.19 (0.002)

Data presented are standardized regression coefficients with *p* value in parentheses. PTH natural log-transformed

Table 3

Multivariable regression analysis of the association of DXA areal bone mineral density (aBMD) and trabecular volumetric BMD (vBMD) with total, free, and bioavailable 25(OH)D concentrations

Site	Measure of 25(OH)D	Black	White
		Standardized regression coefficient (<i>p</i> value)	
Lumbar spine aBMD ^a	Total 25(OH)D	0.09 (0.25)	−0.04 (0.62)
	Free 25(OH)D (polyclonal)	0.13 (0.10)	0.02 (0.78)
	Free 25(OH)D (monoclonal)	0.15 (0.06)	0.02 (0.73)
	Bioavailable 25(OH)D (polyclonal)	0.15 (0.06)	0.02 (0.78)
	Bioavailable 25(OH)D (monoclonal)	0.17 (0.03)	0.02 (0.76)
Femoral neck aBMD	Total 25(OH)D	0.12 (0.09)	0.02 (0.75)
	Free 25(OH)D (polyclonal)	0.15 (0.03)	0.01 (0.88)
	Free 25(OH)D (monoclonal)	0.10 (0.18)	0.08 (0.20)
	Bioavailable 25(OH)D (polyclonal)	0.17 (0.02)	0.01 (0.84)
	Bioavailable 25(OH)D (monoclonal)	0.12 (0.10)	0.08 (0.20)
Total hip aBMD	Total 25(OH)D	0.11 (0.14)	0.005 (0.94)
	Free 25(OH)D (polyclonal)	0.12 (0.10)	−0.004 (0.95)
	Free 25(OH)D (monoclonal)	0.08 (0.30)	0.05 (0.50)
	Bioavailable 25(OH)D (polyclonal)	0.14 (0.06)	0.0005 (0.99)
	Bioavailable 25(OH)D (monoclonal)	0.09 (0.20)	0.05 (0.49)
Ultra-distal radius aBMD	Total 25(OH)D	0.14 (0.04)	−0.05 (0.49)
	Free 25(OH)D (polyclonal)	0.07 (0.29)	0.01 (0.93)
	Free 25(OH)D (monoclonal)	0.12 (0.07)	−0.01 (0.93)
	Bioavailable 25(OH)D (polyclonal)	0.08 (0.23)	−0.002 (0.98)
	Bioavailable 25(OH)D (monoclonal)	0.13 (0.047)	−0.01 (0.91)
1/3 radius aBMD	Total 25(OH)D	0.10 (0.12)	0.004 (0.96)
	Free 25(OH)D (polyclonal)	0.06 (0.40)	−0.002 (0.98)
	Free 25(OH)D (monoclonal)	0.05 (0.47)	−0.02 (0.72)
	Bioavailable 25(OH)D (polyclonal)	0.06 (0.40)	0.0002 (1.00)
	Bioavailable 25(OH)D (monoclonal)	0.05 (0.42)	−0.02 (0.75)
Trabecular vBMD	Total 25(OH)D	0.17 (0.04)	0.03 (0.65)
	Free 25(OH)D (polyclonal)	0.13 (0.11)	0.03 (0.67)
	Free 25(OH)D (monoclonal)	0.16 (0.05)	0.11 (0.12)
	Bioavailable 25(OH)D (polyclonal)	0.15 (0.07)	0.03 (0.65)
	Bioavailable 25(OH)D (monoclonal)	0.17 (0.04)	0.11 (0.11)

All models were adjusted for BMI, age, and sex, and the lumbar spine and 1/3 radius models also included an age-by-sex interaction term. Bolded values refer to point estimates with *p* < 0.05

^aLog transformed to achieve normality