Title

Vitamin D metabolites and bone mineral density: The multi-ethnic study of atherosclerosis

Authors

van Ballegooijen, Adriana J

Robinson-Cohen, Cassianne

Katz, Ronit

et al.

Publication Date

2015-09-01

DOI

10.1016/j.bone.2015.05.008

Peer reviewed

HHS Public Access

Author manuscript

Bone. Author manuscript; available in PMC 2016 September 01.

Published in final edited form as:

Bone. 2015 September ; 78: 186–193. doi:10.1016/j.bone.2015.05.008.

Vitamin D Metabolites and Bone Mineral Density: the Multi-Ethnic Study of Atherosclerosis

Adriana J. van Ballegooijen[1], **Cassianne Robinson-Cohen**[1], **Ronit Katz**[1], **Michael Criqui**[2], **Matthew Budoff**[2], **Dong Li**[3], **David Siscovick**[4], **Andy Hoofnagle**[1,5], **Steven J. Shea**[6], **Gregory Burke**[7], **Ian H. de Boer**[1], and **Bryan Kestenbaum**[1]

[1]University of Washington, Kidney Research Institute, Seattle

[2]Department of Medicine, University of California San Diego

[3]Los Angeles Biomedical Research Institute at Harbor-UCLA, Los Angeles

[4]New York Academy of Medicine, New York

[5]Department of Laboratory Medicine, University of Washington, Seattle

[6]Department of Epidemiology, Columbia University, New York

[7]Department of Public Health Sciences, Wake Forest University, Winston-Salem

Abstract

Previous studies demonstrate associations of low 25-hydroxyvitamin D (25(OH)D) concentrations with low bone mineral density (BMD) and fractures, motivating widespread use of vitamin D supplements for bone health. However, previous studies have been limited to predominantly White populations despite differences in the distribution and metabolism of 25(OH)D by race/ethnicity. We determined associations of serum 25(OH)D, 24,25-dihydroxyvitamin D ($24,25(OH_2)D_3$), and parathyroid hormone (PTH) with BMD among 1,773 adult participants in the Multi-Ethnic Study of Atherosclerosis (MESA) in a staggered cross-sectional study design. Vitamin D metabolites were measured using liquid chromatography-mass spectroscopy and PTH using a 2-site immunoassay from serum collected in 2000–2002. Volumetric trabecular lumbar BMD was measured from computed tomography scans performed in 2002–2005 expressed as g/cm^3. We used linear regression and graphical methods to compare associations of vitamin D metabolite and PTH concentrations with BMD as the outcomes measure among White (n=714), Black (n=353), Chinese (n=249), and Hispanic (n=457) participants. Serum 25(OH)D and $24,25(OH_2)D_3$ concentrations were highest among Whites and lowest among Blacks. BMD was greatest among Black participants. Higher serum 25(OH)D was only associated with higher BMD among Whites and Chinese participants (*P*-for interaction=0.054). Comparing the lowest category of 25(OH)D (<20 ng/ml) to the highest (≥30 ng/ml), the adjusted mean difference in BMD was

Corresponding author and person for reprint requests: A.J. van Ballegooijen, Kidney Research Institute, Box 35906, Seattle, WA, Phone: 206-6168574, Fax: 206-685-9399, hvb2@uw.edu.

−8.1 g/cm^3 (95% CI −14.8, −1.4) for Whites; −10.2 g/cm^3 (−20.4, 0.0) for Chinese vs. 8.8 g/cm^3 (−2.8, 20.5) for Black and −1.1 g/cm^3 (−8.3, 6.2) for Hispanic. Similar results were observed for serum 24,25(OH_2)D_3. Serum PTH was not associated with BMD. In a multi-ethnic population, associations of 25(OH)D with BMD were strongest among White and Chinese participants and null among Black and Hispanic participants. Further studies are needed to determine optimal biomarkers for bone health for multiple ethnic groups.

Keywords

Vitamin D; parathyroid hormone; quantitation of bone; general population studies; aging

1. Introduction

A body of published evidence consistently demonstrates associations of low serum 25-hydroxyvitamin D [25(OH)D] concentrations with lower bone mineral density (BMD) and fractures [1–5]. Results of these observational studies contrast to some extent with findings from meta-analyses of low-dose vitamin D supplementation trials, which demonstrate only modest or null effects on BMD and fracture prevention [6–8]. Large clinical trials of moderate-high dose vitamin D supplementation (cholecalciferol) are currently in progress.

Most observational studies of serum 25(OH)D and BMD were conducted in predominantly White populations. However, available evidence suggests differences in vitamin D metabolism by race. Blacks have lower circulating 25(OH)D concentrations, but maintain similar or higher circulating 1,25(OH_2)D concentrations compared to Whites, suggesting greater ability to maintain calcium homeostasis in states of apparent 25(OH)D deficiency [9]. Moreover, Blacks have lower urinary calcium excretion, greater skeletal calcium retention, and higher levels of parathyroid hormone (PTH) compared with Whites [10, 11]. In the Boston Area Community Health (BACH)/Bone Survey, lower serum 25(OH)D concentrations were correlated with lower BMD among White, but not Black or Hispanic men [12]. In the National Health and Nutrition Examination Survey, lower serum 25(OH)D concentrations were associated with lower BMD among White and Mexican-Americans, but not among Black individuals [13].

Understanding race/ethnic differences in relationships of vitamin D deficiency with disease may help inform the design of clinical and public health interventions to reduce ethnic disparities related to bone mineral metabolism. We used data from the Multi-Ethnic Study of Atherosclerosis (MESA), an ethnically diverse study population, to compare associations of vitamin D metabolites and PTH with BMD among White, Black, Asian, and Hispanic individuals.

2. Material and Methods

2.1 Design and sample

The methods of the Multi-Ethnic Study of Atherosclerosis have been described previously [14]. Briefly, MESA participants were recruited between July 2000 and August 2002 from 6 field centers across the United States. The study population consisted of 6,814 men and

women, between 45–84 years of age who were free of clinical cardiovascular disease at baseline and who identified themselves as White (38%), Asian (12%), Black (28%) or Hispanic (22%). This report describes a random subsample of MESA participants who participated in the MESA Abdominal Aortic Calcium Study (MESA-AACS) during follow-up visit 2 and 3. One third of the MESA cohort was invited between August 2002 and November 2003 and the other two-third participated between March 2004 and September 2005. MESAAACS participants were recruited from 5 MESA centers: Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County, California; New York, New York; and St. Paul, Minnesota.

In total, 2,202 MESA participants were invited to participate in the AACS among whom 2,172 agreed to participate for computed tomography (CT) scanning; 1,968 satisfied eligibility criteria of no recent prior diagnostic abdominal CT and completed scanning. Subsequently, we excluded 59 participants because of vertebral pathology complicating BMD measurement. We further excluded 106 participants with inadequate sample volume for vitamin D measurements, one participant with serum 25(OH)D >100 ng/mL (to convert to nmol/L, multiply by 2.496) suggestive of high-dose vitamin D supplementation, 2 participants with serum PTH > 200 pg/mL, and 27 participants who were using oral corticosteroids, resulting in a final sample of 1,773 participants. Institutional review board approval was obtained from each participating institution and was in agreement with the Declaration of Helsinki. All participants provided written informed consent.

2.2 Mineral metabolism markers

Serum vitamin D metabolites and PTH concentrations were measured in baseline fasting samples that were collected during 2000–2002, an average of 2.6 years (interquartile range: 1.5–3.3) prior to the BMD measurements. The University of Washington Clinical Nutrition Research Unit performed mineral metabolism measurements. Samples were stored at −80°C and thawed before analysis in 2011. Liquid chromatography-mass spectroscopy on a Quattro Micro mass spectrometer (Water, Milford, Connecticut) was used to measure total 25(OH)D ($25(OH)D_2$ + $25(OH)D_3$) and $24,25(OH)_2D_3$. Inter-assay coefficients of variation (COV) were calculated using repeat measurements of quality control specimens from each sample plate: 8.5% at 24.8 ng/mL for $25(OH)D_3$, 11.8% at 7.0 ng/mL for $25(OH)D_2$, and 14.7% at 2.7 ng/mL for $24,25(OH)_2D_3$. Calibration of serum 25(OH)D concentrations was verified using SRM 972 from the National Institutes of Standards and Technology [15] with accuracy of 91–95% for $25(OH)D_3$ and 100–116% for $25(OH)D_2$. The inter-assay coefficient of variation for total $25(OH)D_3$ was 4.4% at 10.4 ng/mL. Serum $24,25(OH_2)D_3$ is the predominant product of CYP24A1-mediated 25(OH)D catabolism, which occurs throughout the body [16]. The CYP24A1 enzyme is potently induced by 1,25-dihydroxyvitamin D, such that increased $24,25(OH)_2D_3$ concentration may indicate higher tissue-level 1,25(OH)D activity. Serum intact PTH concentrations were measured using an automated 2-sited immunoassay (Beckman-Coulter, Inc., Brea, California) inter-assay CV 3.4–6.1% [17].

2.3 Bone density measurement

MESA study personnel performed CT scans of the abdomen [18] using an electron-beam CT scanner (Chicago, and Los Angeles; Imatron C-150, General Electric Medical Systems) [19] or with a multi-detector CT system (New York, Forsyth County, and St. Paul field centers; Siemens Inc., GE Medical Systems). Participants were scanned along with phantoms of known physical calcium concentration to convert CT values directly to equivalent volumetric BMD in mg/cm^3 [20]. Scans were read centrally at the MESA Reading Center (Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Los Angeles, California). Each vertebra was analyzed with the Image Analysis NVivo workstation (QCT, Columbia, Kentucky) to determine BMD in a virtual 10mm thick slice of trabecular bone. The stand elliptical region of interest (ROI) position was applied (6mm region of ROI, and at least 2mm away from the spinal cortical edge). A trained reader, blinded to the results of arterial calcium scoring, examined each region of interest and changed its placement to exclude vertebral abnormalities, including bone islands and diffuse density variations, or excluded a vertebra entirely if any of the following abnormalities were noted: fractures, metastatic lesions, osteophytes, or benign focal lesions within the vertebra. In the current analyses, we used bone volumetric trabecular BMD of the three consecutive thoracic vertebrae within the T7–T10 range vertebra [18]. In a random sample of 25 scan re-reads on three occasions, there was 100% agreement between blinded scan readers and no evidence of systematic differences between reads or a time effect in the data. Pearson's correlation for pairwise rereads was >0.98.

2.4 Measurement of covariates

Study personnel collected detailed data regarding demographics, comorbidities, and medication use including use of calcium/vitamin D supplements at the baseline exam [14]. Participants completed questionnaires to determine race/ethnicity, smoking status, physical activity, and attained education. Level of education was defined as some high school or less, some college/technical school certificate, and completed college or more. Leisure-time physical activity was estimated as the total amount of intentional exercise performed in a usual week and measured in metabolic equivalent task–minutes. Estimated GFR was calculated based on the combination of serum creatinine and Cystatin C concentrations using the CKD-EPI equation [21].

Both serum and urine calcium and phosphate were measured using the timed-rate colorimetry reaction on a Beckman-Coulter DxC automated analyzer. A random urine sample was collected and samples were acidified prior to measurement to reduce calcium-phosphorus precipitation.

2.5 Statistical analyses

Serum 25(OH)D concentrations were converted to their season-adjusted means using the cosinor model previously established in MESA [22]. We tabulated the distribution of mineral metabolism markers by race/ethnicity using histograms and determined correlations among the markers using Pearson's correlation and locally weighted regression plots. We evaluated vitamin D metabolites and PTH using previously published categories [17, 23] – 25(OH)D categories (<20 ng/ml; >20 to 30 ng/ml; and >30 ng/ml) and PTH categories that

combined tertiles with a threshold value of 65 pg/ml (<33 pg/ml; >33 to 44.2 pg/ml; >44.2 to 65 pg/ml; >65 ng/ml).

To evaluate functional associations of mineral metabolism markers with BMD we constructed cubic smoothing splines with evenly spaced knots, among the inner 95% of concentrations, stratified by race/ethnicity groups and adjusted for age and sex by race/ethnicity group [24].

We used linear regression with robust 95% confidence intervals to estimate associations of each mineral metabolism marker with BMD after adjustment for literature defined general osteoporosis risk factors: age (years; continuous), sex, body mass index (BMI; kg/m^2; continuous), MESA study site, exam (visit 2/visit 3) smoking (never, former, current), education (categories), physical activity (MET/min/wk; continuous), estrogen use (yes/no) and estimated GFR ($ml/min/1.73m^2$; continuous). We included separate terms for each race/ethnicity group and each race/ethnicity*exposure interaction term in the linear regression model to calculate race/ethnicity-specific associations via a linear combination of regression coefficients for main effect and cross-product terms. We tested for interactions by comparing the likelihoods of the nested models with and without the specific race/ethnicity*exposure terms using the chi-square test. As a sensitivity analysis, we excluded participants that used bisphosphonates. All analyses were conducted with R software version 3.1.1 (R Foundation Statistical Computing).

3 Results

3.1 Study sample

Among the 1,773 person MESA study sample the mean age was 62±10 years; 886 (50%) were female, and approximately 13% were current smokers. Black and Hispanic participants had the highest mean BMI; Asian participants had the lowest physical activity levels (Table 1). Among women, 798 (90%) were post-menopausal and 278 (31%) were using estrogen.

3.2 Description of vitamin D metabolites by race/ethnicity

Serum 25(OH)D, $24,25(OH_2)D_3$ and PTH concentrations were roughly normally distributed within each race/ethnicity. Mean serum 25(OH)D and $24,25(OH_2)D_3$ concentrations were highest among White participants, intermediate among Asian and Hispanic participants, and lowest among Black participants (Table 2). In contrast, Black participants had the highest serum PTH concentrations, the highest BMD scores, and the lowest urinary calcium excretion. Serum calcium and phosphate concentrations were similar across race/ethnicity groups.

Lower serum 25(OH)D concentrations were associated with higher serum PTH concentrations within each race/ethnicity group (Figure 1). Pearson's correlation coefficients of 25(OH)D with PTH were: −0.25, −0.26, −0.25, and −0.29 among White, Black, Asian, and Hispanic participants, respectively (all *P*-values <0.001). The correlation of serum 25(OH)D with $24,25(OH_2)D_3$ concentrations was r=0.80; this correlation was similar across race/ethnicity.

3.3 Associations of 25(OH)D concentrations with bone mineral density

Among White and Asian participants, lower serum 25(OH)D concentrations tended to track with lower BMD values (Figure 2). In contrast, BMD values appeared, graphically, to be generally similar across the distribution of serum 25(OH)D concentrations among Black and Hispanic participants. Similar graphical distinctions were observed for associations of serum 24,25(OH_2)D_3 with BMD by race/ethnicity (supplemental figures 1 & 2). Unadjusted results can be found in the supplemental (Table S1).

After adjustment for osteoporosis risk factors, lower serum 25(OH)D categories were associated with significantly lower BMD among White participants (Table 3). Similar trends and borderline statistical significance was observed among Asian participants. In contrast, serum 25(OH)D was not associated with BMD among Black or Hispanic participants. These contrasts in associations of 25(OH)D concentrations with BMD were different by race/ethnicity (*P*-for-interaction=0.054). Repeating analyses using race-specific quartiles for serum 25(OH)D concentrations yielded similar results (supplemental table 2; *P*-for-interaction=0.045). Similar findings were also obtained when serum 25(OH)D was analyzed as a continuous linear variable by race/ethnicity (*P*-for-interaction=0.044). For each 10 ng/ml lower serum 25(OH)D, adjusted BMD was −3.1 g/cm^2 lower (95% CI −5.4, −0.9) among White participants, −2.7 g/cm^2 lower (−7.0, 1.5) among Asian participants, 3.0 g/cm^2 higher (1.3, 7.4) among Black participants, and −0.2 g/cm^2 lower (−3.3, 3.0) among Hispanic participants.

3.4 Associations of 24,25(OH_2)D_3 and PTH concentrations with bone mineral density

We observed similar trends for associations of serum 24,25(OH_2)D_3 concentrations with BMD (Table 2). Among the full study cohort, adjusted BMD values were, on average, 5.5 g/cm^2 lower among participants who had serum PTH concentrations >65 pg/mL (*P*-for trend=0.07). However, PTH was not associated with BMD in any of the four race/ethnicity groups, the magnitude of associations were similar by race/ethnicity (*P*-for-interaction=0.690).

In a sensitivity analyses, excluding participants using bisphosphonates (n=62) did not change the associations between, 25(OH)D, 24,25(OH_2)D_3 and PTH with BMD. Sex-specific analyses by race/ethnicity for 25(OH)D, 24,25(OH_2)D_3, and PTH and BMD can be found in the supplement (Table S3), *P*-for-interaction=0.117.

4 Discussion

In this ethnically diverse community-based population, we observed significant associations of lower serum 25(OH)D concentrations with lower BMD among White and Asian participants. However, associations of serum 25(OH)D with BMD were null among Black and Hispanic participants, with paradoxically higher BMD values observed for lower serum 25(OH)D concentrations among Blacks. Associations of serum 24,25(OH_2)D_3 concentrations with BMD followed a generally similar pattern as those for 25(OH)D. No significant associations of PTH with BMD were observed. When considered in the context

of other 25(OH)D studies, these results add to accumulating evidence that low serum 25(OH)D concentrations may serve as a disease marker in White, but not Black populations.

Despite considerably lower circulating 25(OH)D levels, Black individuals have substantially higher BMD compared with any other racial/ethnic group [12, 13, 25]. This was confirmed in our study. The null association between 25(OH)D and BMD in Blacks was suggested along a broader spectrum of 25(OH)D concentrations. In Black men residing in the Caribbean with mean serum 25(OH)D levels of 35 ng/ml no association was observed between 25(OH)D and BMD [25]. Consistent with these data, a trial of vitamin D_3 supplementation or placebo for 3 years had no impact on bone turnover markers among Black women [26], despite a significant increase in 25(OH)D concentrations in the treatment group.

New insight into the biological difference in mineral and bone metabolism came from studies that examined the contribution of genetic ancestry. This technique takes advantage of the fact that individuals within heterogeneous populations are admixed with different genetic ancestry. In the Women's Health Initiative, women with a higher percentage of African admixture had higher BMD suggesting an overall protective benefit of higher African ancestry on bone quality in postmenopausal women [27]. A similar study among children indicated that a greater percentage of European admixture was inversely related to BMD, whereas a greater percentage of African admixture was positively related to BMD [28]. Interestingly, in that study no statistically significant association of self-reported race or ethnicity with BMD was observed.

There are several potential explanations for race-specific differences in the associations of serum 25(OH)D concentration with BMD. First, it is possible that Black individuals more efficiently utilize vitamin D substrate to maintain similar of higher tissue concentrations of $1,25(OH_2)D$, for a given concentration of 25(OH)D [29, 30]. Concentrations of $1,25(OH_2)D$, the biologically potent form of vitamin D, are approximately 1,000-fold smaller than those of 25(OH)D, providing for tight regulation of vitamin D activity [31]. Race-specific differences in the activities of primary vitamin D metabolizing enzymes e.g. CYP27B1 and CYP24A1 could partly explain differences in vitamin D regulation. Second, differences in vitamin D binding protein (VDBP) polymorphisms and concentrations may contribute to racial differences in total 25(OH)D concentrations [32]. Bioavailable 25(OH)D, the portion that is free or loosely bound to albumin, may better reflect 25(OH)D that is available to target organs. The total amount of 25(OH)D may therefore not accurately represent bioavailable 25(OH)D in Blacks [32]. Third, it is possible that serum 25(OH)D concentrations more strongly reflect other adverse metabolic processes, such as adipose tissue activity, among White individuals [33]. Altogether, testing and treating specific 25(OH)D targets should be viewed with caution, primarily among non-White participants.

Strengths and limitations

We compared associations of serum 25(OH)D concentration with BMD in a community-based population composed of four race/ethnicities, a population that is more diverse than most previously examined. We focused on associations with BMD, an established marker of osteoporosis and a proven risk factor for osteoporotic fractures in both men and women [34].

Further, we used novel mass spectroscopy methods to measure vitamin D metabolites with high precision. In addition, we converted 25(OH)D concentrations to their season adjusted means using a previously developed cosinor model.

The relatively small sample sizes within each race/ethnicity group leaves residual uncertainty as to the true magnitude of associations of 25(OH)D concentrations with BMD among the racial/ethnic groups studied. Additional studies are needed to improve precision of race-specific estimates of associations for 25(OH)D. Differences in associations of serum 25(OH)D concentrations with BMD and other health outcomes may not reflect differences in the response to vitamin D treatment. Results from ongoing large clinical trials of vitamin D supplementation may help clarify race/ethnicity specific differences in treatment response. Although BMD is an established surrogate marker of fracture, study findings would be strengthened by the evaluation of clinical fractures. This study was limited by only a small number of fracture outcomes (n=14); however, future studies could evaluate this important outcome. Lastly, serum 25(OH)D measurements were performed 1.5–3.3 years prior to BMD measurements, which could increase measurement error in BMD and dilute the hypothesized associations.

Conclusion

In conclusion, our results suggest different relationships between serum 25(OH)D concentrations and BMD by race/ethnicity. Higher 25(OH)D concentrations were associated with higher BMD among White individuals, but not among Black or Hispanic individuals. Further research to confirm and elucidate the basis of these findings could enhance our understanding of ethnic differences in bone density and subsequently fracture risk. Our results suggest that optimal serum 25(OH)D concentrations for bone health may differ by race and ethnicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors wish to thank the investigators, staff, and participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

Funding

This research was supported by grant R01-HL-096875 and Niels Stensen Fellowship 2014. MESA is supported by contracts N01-HC-95159–N01-HC-95166, N01-HC-95169, R01-HL-071739 and R01-HL-072403 from the NHLBI.

Conflict of interest

Dr. Kesten aum reports receiving consulting fees from eryx Biopharmaceuticals Inc. Dr. de Boer reports receiving research grant funding from Abbvie.

References

1. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev. 2001; 22:477–501. [PubMed: 11493580]
2. Kuchuk NO, Pluijm SM, van Schoor NM, Looman CW, Smit JH, Lips P. Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. J Clin Endocrinol Metab. 2009; 94:1244–1250. [PubMed: 19158198]
3. Hwang S, Choi HS, Kim KM, Rhee Y, Lim SK. Associations between serum 25-hydroxyvitamin D and bone mineral density and proximal femur geometry in Koreans: the Korean National Health and Nutrition Examination Survey (KNHANES) 2008–2009. Osteoporos Int. 2014; 26:163–171. [PubMed: 25262060]
4. Melhus H, Snellman G, Gedeborg R, Byberg L, Berglund L, Mallmin H, et al. Plasma 25-hydroxyvitamin D levels and fracture risk in a community-based cohort of elderly men in Sweden. J Clin Endocrinol Metab. 2010; 95:2637–2645. [PubMed: 20332246]
5. Steingrimsdottir L, Halldorsson TI, Siggeirsdottir K, Cotch MF, Einarsdottir BO, Eiriksdottir G, et al. Hip fractures and bone mineral density in the elderly--importance of serum 25-hydroxyvitamin D. PLoS One. 2014; 9:e91122. [PubMed: 24621578]
6. Reid IR. Effects of vitamin D supplements on bone density. J Endocrinol Invest. 2014
7. Chung M, Lee J, Terasawa T, Lau J, Trikalinos TA. Vitamin D with or without calcium supplementation for prevention of cancer and fractures: an updated meta-analysis for the U.S. Preventive Services Task Force. Ann Intern Med. 2011; 155:827–838. [PubMed: 22184690]
8. Bischoff-Ferrari HA, Willett WC, Orav EJ, Lips P, Meunier PJ, Lyons RA, et al. A pooled analysis of vitamin D dose requirements for fracture prevention. N Engl J Med. 2012; 367:40–49. [PubMed: 22762317]
9. Cosman F, Shen V, Morgan D, Gordon S, Parisien M, Nieves J, et al. Biochemical responses of bone metabolism to 1,25-dihydroxyvitamin D administration in black and white women. Osteoporos Int. 2000; 11:271–277. [PubMed: 10824245]
10. Braun M, Palacios C, Wigertz K, Jackman LA, Bryant RJ, McCabe LD, et al. Racial differences in skeletal calcium retention in adolescent girls with varied controlled calcium intakes. Am J Clin Nutr. 2007; 85:1657–1663. [PubMed: 17556706]
11. Kleerekoper M, Nelson DA, Peterson EL, Flynn MJ, Pawluszka AS, Jacobsen G, et al. Reference data for bone mass, calciotropic hormones, and biochemical markers of bone remodeling in older (55–75) postmenopausal white and black women. J Bone Miner Res. 1994; 9:1267–1276. [PubMed: 7976509]
12. Hannan MT, Litman HJ, Araujo AB, McLennan CE, McLean RR, McKinlay JB, et al. Serum 25-hydroxyvitamin D and bone mineral density in a racially and ethnically diverse group of men. J Clin Endocrinol Metab. 2008; 93:40–46. [PubMed: 17986641]
13. Gutierrez OM, Farwell WR, Kermah D, Taylor EN. Racial differences in the relationship between vitamin D, bone mineral density, and parathyroid hormone in the National Health and Nutrition Examination Survey. Osteoporos Int. 2011; 22:1745–1753. [PubMed: 20848081]
14. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, et al. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol. 2002; 156:871–881. [PubMed: 12397006]
15. Phinney KW. Development of a standard reference material for vitamin D in serum. Am J Clin Nutr. 2008; 88:511S–512S. [PubMed: 18689392]
16. de Boer IH, Sachs MC, Chonchol M, Himmelfarb J, Hoofnagle AN, Ix JH, et al. Estimated GFR and circulating 24,25-dihydroxyvitamin D3 concentration: a participant-level analysis of 5 cohort studies and clinical trials. Am J Kidney Dis. 2014; 64:187–197. [PubMed: 24703961]
17. Bosworth C, Sachs MC, Duprez D, Hoofnagle AN, Ix JH, Jacobs DR Jr, et al. Parathyroid hormone and arterial dysfunction in the multi-ethnic study of atherosclerosis. Clin Endocrinol (Oxf). 2013; 79:429–436. [PubMed: 23402353]

18. Hyder JA, Allison MA, Wong N, Papa A, Lang TF, Sirlin C, et al. Association of coronary artery and aortic calcium with lumbar bone density: the MESA Abdominal Aortic Calcium Study. Am J Epidemiol. 2009; 169:186–194. [PubMed: 19064643]
19. Breen JF, Sheedy PF, Schwartz RS, Stanson AW, Kaufmann RB, Moll PP, et al. Coronary artery calcification detected with ultrafast CT as an indication of coronary artery disease. Radiology. 1992; 185:435–439. [PubMed: 1410350]
20. Cann CE. Quantitative CT for determination of bone mineral density: a review. Radiology. 1988; 166:509–522. [PubMed: 3275985]
21. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012; 367:20–29. [PubMed: 22762315]
22. Sachs MC, Shoben A, Levin GP, Robinson-Cohen C, Hoofnagle AN, Swords-Jenny N, et al. Estimating mean annual 25-hydroxyvitamin D concentrations from single measurements: the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr. 2013; 97:1243–1251. [PubMed: 23615830]
23. Robinson-Cohen C, Hoofnagle AN, Ix JH, Sachs MC, Tracy RP, Siscovick DS, et al. Racial differences in the association of serum 25-hydroxyvitamin D concentration with coronary heart disease events. JAMA. 2013; 310:179–188. [PubMed: 23839752]
24. Eilers P, Marx D. Flexible smoothing with B-splines and penalties. Stat Sci. 1996; 11:89–121.
25. Barbour KE, Zmuda JM, Horwitz MJ, Strotmeyer ES, Boudreau R, Evans RW, et al. The association of serum 25-hydroxyvitamin D with indicators of bone quality in men of Caucasian and African ancestry. Osteoporos Int. 2011; 22:2475–2485. [PubMed: 21104232]
26. Aloia JF, Talwar SA, Pollack S, Feuerman M, Yeh JK. Optimal vitamin D status and serum parathyroid hormone concentrations in African American women. Am J Clin Nutr. 2006; 84:602–609. [PubMed: 16960175]
27. Chen Z, Qi L, Beck TJ, Robbins J, Wu G, Lewis CE, et al. Stronger bone correlates with African admixture in African-American women. J Bone Miner Res. 2011; 26:2307–2316. [PubMed: 21590740]
28. Casazza K, Thomas O, Dulin-Keita A, Fernandez JR. Adiposity and genetic admixture, but not race/ethnicity, influence bone mineral content in peripubertal children. J Bone Miner Metab. 2010; 28:424–432. [PubMed: 20087611]
29. Bell NH, Yergey AL, Vieira NE, Oexmann MJ, Shary JR. Demonstration of a difference in urinary calcium, not calcium absorption, in black and white adolescents. J Bone Miner Res. 1993; 8:1111–1115. [PubMed: 8237481]
30. Cosman F, Morgan DC, Nieves JW, Shen V, Luckey MM, Dempster DW, et al. Resistance to bone resorbing effects of PTH in black women. J Bone Miner Res. 1997; 12:958–966. [PubMed: 9169356]
31. Lips P. Relative value of 25(OH)D and 1,25(OH)2D measurements. J Bone Miner Res. 2007; 22:1668–1671. [PubMed: 17645404]
32. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med. 2013; 369:1991–2000. [PubMed: 24256378]
33. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000; 72:690–693. [PubMed: 10966885]
34. Johnell O, Kanis JA, Oden A, Johansson H, De Laet C, Delmas P, et al. Predictive value of BMD for hip and other fractures. J Bone Miner Res. 2005; 20:1185–1194. [PubMed: 15940371]

Highlights

- Results of the Multi-Ethnic Study of Atherosclerosis suggest different relationships between serum 25(OH)D concentrations and bone mineral density by race/ethnicity.
- Higher 25(OH)D concentrations were associated with higher bone mineral density among White individuals, but not among Black or Hispanic individuals.
- Optimal serum 25(OH)D concentrations for bone health may differ by race and ethnicity.

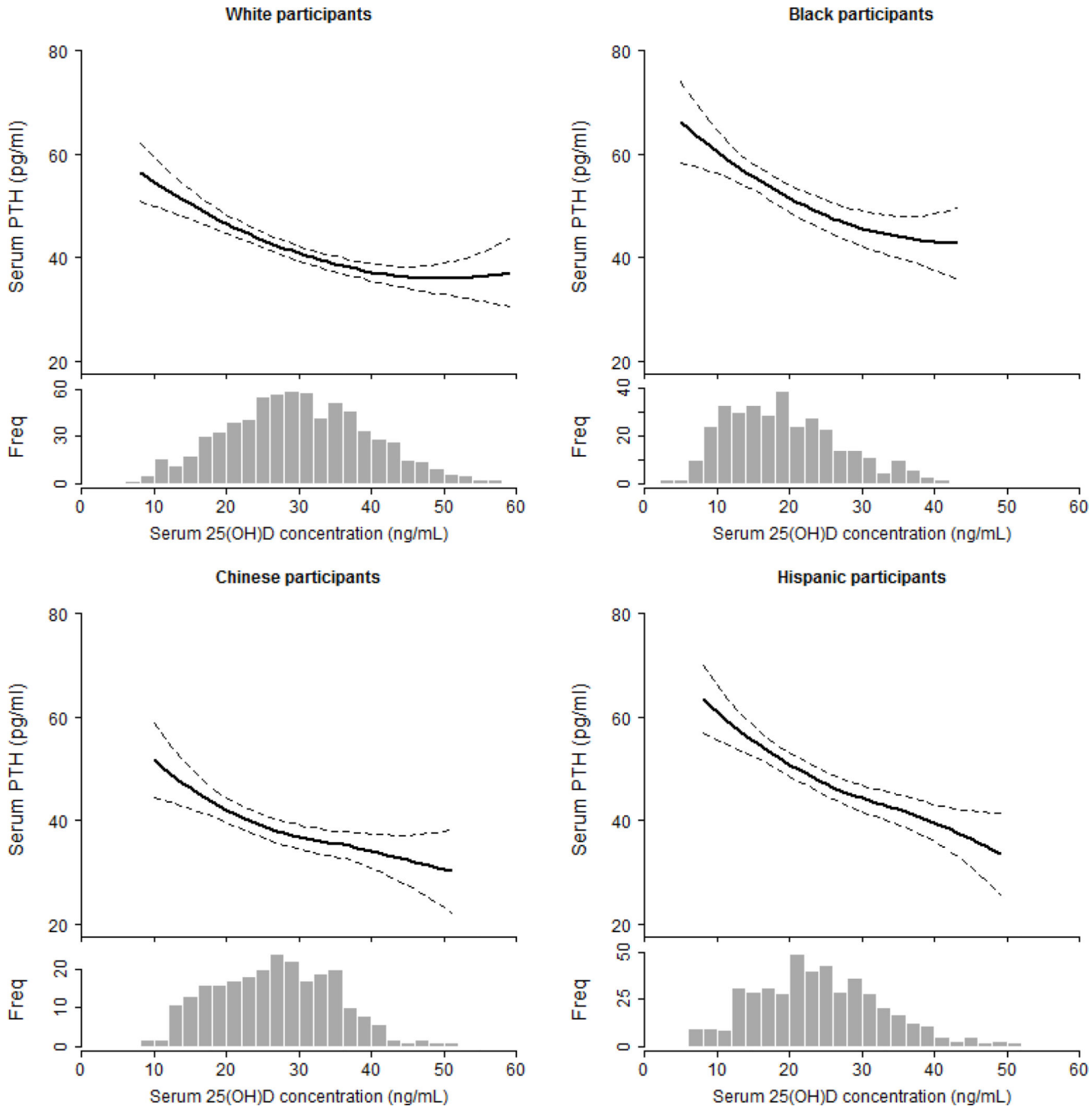

Figure 1.
Loess of serum 25(OH)D by parathyroid hormone stratified by race/ethnicity

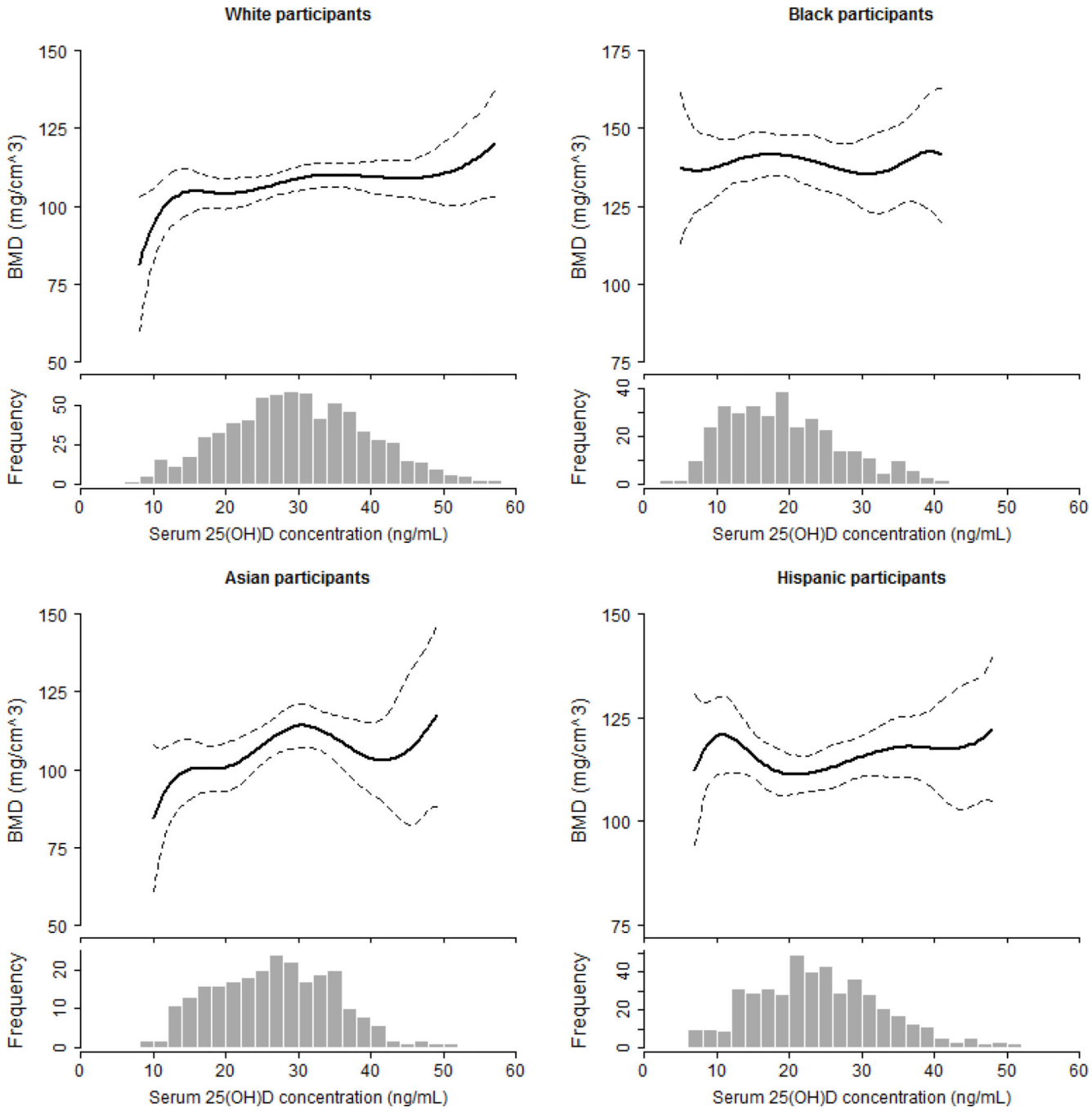

Figure 2.
Cubic splines of serum 25(OH)D and bone mineral density stratified by race/ethnicity

Table 1

Characteristics of 1,773 MESA participants by race/ethnicity

	MESA participants			
	White	**Asian**	**Black**	**Hispanic**
Demographics				
N	714	249	353	457
Age	62.7±9.7	62.3± 10.2	62.2±9.7	61±9.9
Female	336 (47.1)	115 (46.2)	206 (56.7)	229 (50.1)
Education				
Some High School	27 (3.8)	57 (22.9)	39 (11.0)	202 (44.2)
Completed High School	299 (41.9)	72 (28.9)	166 (47.0)	189 (41.4)
Completed College or more	388 (54.4)	119 (47.8)	148 (41.9)	66 (14.4)
BMI, kg/m^2	27.6±4.7	24.1± 3.1	29.2±5	29.5±5
Smoking				
Never	326 (45.7)	180 (72.3)	162 (45.9)	229 (50.1)
Former	302 (42.3)	55 (22.1)	129 (36.5)	166 (36.3)
Current	86 (12.0)	13 (5.2)	62 (17.6)	62 (13.6)
Physical Activity, MET/min/wk	4331(2385–7326)	2775 (1470–5483)	4275 (2194–7976)	4620 (2122–8865)
Systolic blood pressure, mm Hg	125±20.4	124±20.7	133±21.4	128± 23.1
Diastolic blood pressure, mm Hg	71±10.2	72±9.5	75±10.2	72.1±9.9
eGFR, $ml/min/1.73m^2$*	87.8±16.9	94.4±20	88.7±18	92.2±17.5
C-reactive protein, mg/l	3.4±5	1.7±3.2	4.0±5.2	4±5.1
Menopause**	312 (93.0)	106 (92.2)	174 (84.5)	206 (90.0)
Estrogen use**	164 (47.3)	18 (15.6)	49 (23.8)	47 (20.0)
Bisphosphonate use	24 (3.4)	15 (6.0)	8 (2.3)	15 (3.3)
Calcium supplements	338 (47.3)	130 (52.2)	224 (63.5)	305 (66.7)
Calcium intake, mg/d	758±500	587±505	630±448	827±611

Values are mean±SD or frequency and (%)

BMI: body mass index, eGFR: estimated glomerular filtration rate, LDL: low-density lipoprotein. Physical activity: expressed as median and interquartile range.

* Based on CKD-EPI equation 2012 including creatinine and cystatin C

** among women

To convert 25(OH)D in ng/mL to nmol/L multiply by 2.496

Table 2

Mineral metabolism markers 1,773 MESA participants by ethnicity/race

Variable	MESA participants				P-value*
	White	Asian	Black	Hispanic	
N	714	249	353	457	
Total 25-hydroxyvitamin D, ng/ml	30.1±10.0	26.8±8.7	20.1±9.1	24.7±11.9	<0.001
24,25-dihydroxyvitamin D_3, ng/ml	4.5±2.4	3.5±1.7	2.6±1.8	3.6±2.9	<0.001
Parathyroid hormone, pg/ml	41.4±16.2	38.7±14.7	53.1±25.3	48.7±24.6	<0.001
Phosphate, mg/dl	3.7±0.5	3.7±0.5	3.6±0.5	3.7±0.5	0.080
Calcium, mg/dl	9.7±0.4	9.5±0.3	9.7±0.4	9.7±0.4	<0.001
U calcium, mg/dl	8.4±6.1	11.0±7.5	8.1±6.2	10.0±6.8	<0.001
U calcium/creatinine, mg/g	0.10±0.06	0.12±0.09	0.08±0.07	0.10±0.07	<0.001
Bone mineral density (mg/cm^3)	108±35.7	107±36.6	139±45.5	115±35.5	<0.001

U: urinary

*P-values derived using ANOVA test

Table 3

Associations of vitamin D metabolites and bone mineral density among 1,773 MESA participants stratified by race/ethnicity

	Bone mineral density (mg/cm^3)									
	All participants		White		Asian		Black		Hispanic	
	Unadjusted mean±SD	Beta (95% CI)		Beta (95% CI)		Beta (95% CI)		Beta (95% CI)		Beta (95% CI)
25(OH)D (ng/ml)										
<20	123±43	−3.4 (−7.7, 0.9)	105±37	−8.1 (−14.8, −1.4)	103±33	−10.2 (−20.4, 0.0)	143±45	8.8 (−2.8, 20.5)	116±32	−1.1 (−8.3, 6.2)
20–30	115±39	−4.0 (−7.8, −0.3)	108±34	−6.1 (−11.1, −1.0)	110±32	0.1 (−8.8, 9.1)	136±47	4.9 (−8.2, 17.9)	114±37	−4.5 (−11.3, 2.3)
≥30	110±38	*0.0 (Ref)*	108±38	*0.0 (Ref)*	104±41	*0.0 (Ref)*	126±48	*0.0 (Ref)*	116±37	*0.0 (Ref)*
P-for trend		0.102		0.006		0.069		0.126		0.849
						P-for interaction=0.054				
24,25(OH$_2$)D$_3$ (ng/ml)										
<2.0	114±38	−6.8 (−14.1, 0.6)	102±34	−6.0 (−14.4, 2.3)	99±36	−12.6 (−26.2, 1.0)	143±46	6.4 (−9.3, 22.1)	113±34	−3.7 (−12.6, 5.1)
2.0–3.3	119±43	−3.8 (−10.5, 2.9)	104±36	−3.3 (−10.7, 4.1)	110±38	−4.8 (−18.2, 8.6)	139±48	2.2 (−13.6, 17.9)	113±35	−6.2 (−14.1, 1.7)
3.3–5.0	116±41	−1.6 (−7.5, 4.3)	109±35	−1.3 (−7.3, 4.7)	107±34	−1.8 (−14.3, 10.7)	134±42	−1.3 (−17.3, 14.7)	114±37	−4.4 (−12.6, 3.7)
≥5.0	114±38	*0.0 (Ref)*	110±36	*0.0 (Ref)*	110±40	*0.0 (Ref)*	130±41	*0.0 (Ref)*	119±37	*0.0 (Ref)*
P-for trend		0.058		0.047		0.023		0.208		0.232
						P-for interaction=0.397				
PTH (pg/ml)										
<33	116±40	0.0 (Ref)	112±34	*0.0 (Ref)*	95.7±39	*0.0 (Ref)*	143±56	*0.0 (Ref)*	122±38	*0.0 (Ref)*
33–44.2	115±40	−3.1 (−7.1, 97)	107±37	−4.2 (−10.0,1.1.5)	109±35	−1.1 (−10.2, 8.0)	140±44	−6.3 (21.6, 9.0)	115±36	0.5 (−7.5, 8.5)
44.2–65	116±39	−2.7 (−6.8, 1.5)	106±34	−3.2 (−9.2, 2.7)	108±35	−4.6 (−13.9, 4.7)	141±41	−0.1 (−14.6, 14.8)	111±33	−3.2 (−10.8, 4.3)
≥65	114±42	−5.6 (−11.0, 0.1)	96±38	−9.5 (−19.1, 0.1)	102±38	−3.3 (−22.2, 15.5)	132±47	−8.7 (−24.3, 6.9)	113±34	0.7 (−7.9, 9.4)
P-for trend		0.064		0.071		0.373		0.571		0.776
						P-for interaction=0.690				

BMD: bone mineral density; CI: confidence interval

Betas represent adjusted differences in bone mineral density in mg/cm^3 compared to reference groups.
Betas are adjusted for age, sex, BMI, site, exam, race/ethnicity, education, smoking, physical activity, estrogen use and eGFR