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

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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 1773 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 –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.

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

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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.

Material and methods

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 6814 men and women, between 45 and 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. MESA-AACS 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, 2202 MESA participants were invited to participate in the AACS among whom 2172 agreed to participate for computed tomographic (CT) scanning; 1968 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 1773 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.

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].

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 10 mm thick slice of trabecular bone. The stand elliptical region of interest (ROI) position was applied (6 mm region of ROI, and at least 2 mm 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.

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.

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; >20–30 ng/ml; and >30 ng/ml) and PTH categories