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Effect of vitamin D metabolites on bone histomorphometry in healthy black and white women: An attempt to unravel the so-called vitamin D paradox in blacks

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ABSTRACT

An apparent vitamin D paradox, characterized by lower serum 25-hydroxyvitamin D (25(OH)D) levels and higher bone mineral density, is present in black population. In contrast, blacks have higher serum 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) levels. The effect of $1,25(OH)_2D$ on the skeleton is not fully understood. We examined serum 25(OH)D, $1,25(OH)_2D$ and bone histomorphometry in 50 black and white women (25 each) matched for age, menstrual status, and BMI. Histomorphometric indices related to bone structure, remodeling and mineralization were measured in cancellous bone in iliac bone biopsies. Data analyses led to the following results: 1) serum 25(OH)D was significantly lower and $1,25(OH)_2D$ was significantly higher in black than in white women, but neither blacks nor whites revealed significant correlation between these two vitamin D metabolites. 2) there was no significant difference in PTH levels between blacks and whites. 3) except for greater trabecular thickness (Tb.Th) in blacks, there were no significant differences in other histomorphometric variables between the two ethnic groups. 4) osteoid surface (OS/BS), unlabeled osteoid surface (ulOS/BS), and osteoblast surface (ObS/BS) significantly correlated with serum $1,25(OH)_2D$ levels. We conclude that lower serum 25(OH)D levels in blacks do not impair bone structure and remodeling, nor decrease bone mineralization. Higher serum $1,25(OH)_2D$ levels in blacks may help preserve bone mass by stimulating bone formation via increasing osteoblast number and function, but moderately inhibit terminal bone mineralization as shown by higher ulOS/BS.

1. Introduction

The major circulating form of vitamin D is 25-hydroxyvitamin D (25(OH)D), and is the best available index of vitamin D nutrition (Rao et al., 2020). Vitamin D deficiency is defined as a serum 25(OH)D level of <20 ng/mL, which associated with bone loss and in severe deficiency with rickets/osteomalacia (Holick, 2007; Laird et al., 2010). It has long been recognized that serum 25(OH)D levels are significantly lower in blackthan in whiteindividuals (Bell et al., 1985; Aloia et al., 2015). In the United States the prevalence of vitamin D deficiency is about 54–82 % in blacks but only 14–31 % in whites (Herrick et al., 2019; Forrest and Stuhldreher, 2011). Despite the higher prevalence of vitamin D deficiency, blacks have higher bone mineral density (BMD) and lower prevalence of osteoporosis and fragility fractures (Bell et al., 1985; Aloia et al., 2015; Cosman et al., 2000; Shieh and Aloia, 2017; Powe et al., 2013; Brown et al., 2018; Kleerekoper et al., 1994; Putman et al., 2017; Popp et al., 2017). The coexistence of lower 25(OH)D levels and better bone health in blacks has been referred to as vitamin D paradox (Brown et al., 2018; Aloia, 2008), but the underlying mechanism(s) for this paradox remains unclear.

1,25-dihydroxyvitamin D ($1,25(OH)_2D$) is the biologically active metabolite of vitamin D that stimulates intestinal calcium and phosphate absorption (Goltzman, 2018; Holick, 1996; Christakos et al., 2019), and interacts with vitamin D receptors (VDR) in osteoblasts to promote bone formation (Goltzman, 2018; Christakos et al., 2019; Reichel et al., 1989). Since $1,25(OH)_2D$ is converted from 25(OH)D in the kidney and other target tissues by the action of 1α-hydroxylase enzyme (CYP27B1) (Christakos et al., 2019), it is expected that these two vitamin D metabolites are positively correlated (Rejnmark et al., 2008; Swanson et al., 2014). However, the available data on the

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relationship between 25(OH)D and 1,25$(OH)_2$D levels are limited and inconsistent (Rejnmark et al., 2008; Swanson et al., 2014; Tsuprykov et al., 2021; Ishimura et al., 1999). There is strong evidence that blacks have lower 25(OH)D but higher 1,25$(OH)_2$D levels than their white counterparts (Bell et al., 1985; Aloia et al., 2015; Weaver et al., 2008). Thus, higher 1,25(OH)2D levels may optimize bone health (Goltzman, 2018), which can, at least partly, explain the mechanism of vitamin D paradox in blacks.

To our knowledge, there is little information regarding the relationship between 25(OH)D and 1,25$(OH)_2$D levels in normal population, particularly in blacks, and dichotomy of the effect of 1,25$(OH)_2$D on bone histomorphometry between blacks and whites. We hypothesized that the better bone health in blacks is related to increased 1,25$(OH)_2$D production despite low serum 25(OH)D levels, which is the best currently available index of vitamin D nutrition (Rao et al., 2020). To test this hypothesis, we examined serum biochemical variables, including 25(OH)D, 1,25$(OH)_2$D, calcium and parathyroid hormone (PTH), and iliac bone histomorphometry in 50 black and white women (25 each) matched for age, menstrual status, and BMI to determine the effects of vitamin D metabolites on bone histomorphometry in these two ethnic groups.

2. Materials and methods

2.1. Subject characteristics

One hundred forty-four black and white women, aged 20–73 years, were recruited between 1981 and 1993 as part of a larger study of the effect of age and menopause on bone structure and remodeling, the details of which have been reported previously (Kleerekoper et al., 1994; Han et al., 1996). All women were skeletally healthy and underwent in vivo double tetracycline labeling before a transiliac bone biopsy. Of the 144 subjects, 71 women, all recruited after 1989, in whom measurements for serum levels of calcium (Ca), Creatinine (Cr), PTH, 25(OH)D and 1,25$(OH)_2$D were available. From this sub-set, we selected 50 black and white women, 25 in each group, matched for age, menstrual status (pre- and post-menopause) and body mass index (BMI). The study was approved by the Institutional Review Board of the Henry Ford Hospital and a written informed consent was obtained from each participant.

2.2. Biochemical measurements

Serum calcium (Ca) and creatinine (Cr) was measured in the hospital laboratory by standard methods using a Hitachi-747 auto-analyzer (Hitachi, Hialeah, FL, USA). Serum Ca was adjusted for serum albumin (CCa). Serum intact parathyroid hormone (PTH) was measured by immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Serum 25(OH)D was measured by radioimmunoassay (Incstar, Stillwater, MN, USA) and serum 1,25$(OH)_2$D was measured by radioreceptor assay using kits from Nichols Institute Diagnostics. All measurements were performed within one month of bone biopsy (Rao et al., 2013).

2.3. Bone histomorphometry

Before biopsy, all patients received in vivo double tetracycline labeling with an inter-label interval of 14 days. Cylindrical transiliac bone biopsy was obtained using a trephine with an internal diameter of 7.5 mm, stained en bloc by 70 % alcohol containing 1 % basic fuchsin, and embedded, sectioned, stained, and examined as previously reported (Han et al., 1996; Rao, 1983; Han et al., 1997). All biopsy procedures were performed by a single operator (SDR). Units and symbols of bone histomorphometry were designated in accordance with the American Society for Bone and Mineral Research Histomorphometry Nomenclature Committee (Dempster et al., 2013).

The static histomorphometric indices were measured in sections stained with modified toluidine blue method, and the dynamic indices were measured in unstained sections. All the measurements were performed using a Bioquant image analysis system equipped by bright-field and fluorescent microscope.

The indices related to bone structures included total bone volume per tissue volume (BV/TV [%]), trabecular thickness (Tb.Th [μm]) and trabecular number (Tb.N, [#/mm^2]). The static indices included osteoid volume as a fraction of bone volume (OV/BV [%]), eroded surface, osteoid surface and unlabeled osteoid surface as a fraction of bone surface (ES/BS [%], OS/BS [%] and ulOS/BS [%]), as well as osteoid thickness (O.Th [μm]) and wall thickness (W.Th [μm]). In addition, the fraction of bone surface covered by osteoblasts and osteoclasts was expressed as Ob.S/BS [%] and Oc.S/BS [%]. The dynamic indices were measured based on tetracycline labeling beneath the bone surface, which represents the extent of mineralizing surface (MS). MS as a fraction of bone surface and (MS/BS [%]) and osteoid surfaces (MS/OS [%]) was determined. The unlabeled osteoid surface as a fraction of bone surface (ulOS/BS [%]) was calculated as OS/BS-MS/BS. Mineral apposition rate (MAR [μm/day]) was obtained from the average distance between the two tetracycline labels divided by the interval of administration (14 days in our study). Adjusted apposition rate (Aj.Ar, μm/day) was calculated as MAR*MS/OS. Osteoid maturation time (Omt, days) and mineralization lag time (Mlt, days) were calculated as O.Th/MAR and O.Th/Aj.AR, respectively. Bone formation rate at the surface level (BFR/BS, $\mu m^3/\mu m^2$/year) was calculated as MAR*(MS/BS). Activation frequency (Ac.f, #/year), the annual probability of activation of a new remodeling site at any given locus on the bone surfaces, was derived from (BFR/BS)/W.Th. For the surface containing only a single label, a minimum value of 0.3 μm/day was assigned to MAR. If no label was present, MAR, Aj.Ar, Omt, Mlt and FP were treated as a missing value, whereas MS/BS, MS/OS, BFR/BS, and Ac.f were assigned a zero.

2.4. Statistical analysis

The data were expressed as mean ± SD and compared between black and white subjects using Student *t*-tests. Mann–Whitney test was used when a variable was not normally distributed. The categorical data, such as menopausal status and vitamin D deficiency (defined as <20 ng/mL) in blacks and whites, were compared using Fisher's exact test. Correlations between serum biochemical and bone histomorphometric variables were analysed using nonparametric Spearman rank correlation tests.

3. Results

3.1. Subject characteristics

Since the age, menstrual status and BMI were matched, there was no

Table 1
Demographic characteristics.

	Overall	Black	White	p
	n = 50	n = 25	n = 25	
Age (year)	48.3 (13.3)	48.4 (12.9)	48.2 (13.9)	0.958
Postmenopausal (#)[a]	29 (58 %)	14 (56 %)	15 (60 %)	1.000
Height (cm)	164 (6.76)	163 (6.46)	164 (7.16)	0.630
Weight (kg)	74.5 (17.9)	75.9 (14.6)	73.2 (21.0)	0.337
BMI (kb/m^2)	27.7(5.73)	28.5 (5.03)	27.0 (6.36)	0.332
25(OH)D <20 ng/mL (#)[a]	25 (50 %)	21 (84 %)	4 (16 %)	<0.001
1,25(OH)2D <18 pg/mL (#)[a]	3 (6 %)	1 (4 %)	2 (8 %)	1.000

Data expressed as mean (SD).
[a] Data expressed as number (percent).

significant difference in these variables between the 2 groups (Table 1). Half of the subjects were vitamin D deficient defined as serum 25(OH)D level < 20 ng/mL, but the prevalence was much higher in blacks than in whites (84 % vs 16 %, $p < 0.001$) (Table 1). Based on the manufacturer's reference range for serum 1,25$(OH)_2$D levels of 18–78 pg/mL (https://emedicine.medscape.com/article/2088672-overview), 1 black and 2 white women had 1,25$(OH)_2$D lower than 18 pg/mL, but none had 1,25$(OH)_2$D higher than 78 pg/mL (Table 1).

3.2. Differences in serum biochemical and bone histomorphometric indices between black and white women

The biochemical results are shown in Table 2. Serum levels of 25(OH)D were significantly lower in blacks than in whites (16.2 ± 10.1 ng/mL vs 26.4 ± 8.34 ng/mL, $p < 0.001$) (Table 2). In contrast, serum levels of 1,25$(OH)_2$D were significantly higher in blacks than in whites (41.0 ± 16.4 pg/mL vs 31.0 ± 10.7 pg/mL, $p < 0.05$) (Table 2). There were no significant differences in serum levels of CCa, Cr, or PTH between blacks and whites (Table 2).

The differences in bone histomorphometric indices between black and white women are shown in Table 3. For bone structure, Tb.Th was significantly higher in blacks than in whites ($p < 0.05$). However, there were no significant differences in static and dynamic variables between the 2 ethnic groups.

Correlation of 25(OH)D and 1,25$(OH)_2$D levels with related biochemical and bone histomorphometric indices in black and white women.

The correlations between biochemical markers in the entire cohort and subgroups of blacks and whites are shown in Table 4. Serum 25(OH)D levels were not significantly correlated with 1,25$(OH)_2$D, CCa or Cr in either group but inversely correlated with serum PTH levels ($r = -0.507$, $p < 0.05$) only in white women. Additionally, 1,25$(OH)_2$D did not correlated with CCa, Cr or PTH in either group.

In the entire cohort, 1,25$(OH)_2$D had significantly positive correlation with several bone static variables including Oc.S/BS, OV/BV, OS/BS, ulOS/BS, W.Th and ObS/BS, (all $p < 0.05$). The correlation trends for these variables were identical between blacks and whites (Table 5). It is worth noting that 1,25$(OH)_2$D was significantly positively correlated with OS/BS, ulOS/BS in both black and white women ($p < 0.05$) (Table 5). There was no significant correlation of 1,25$(OH)_2$D with other histomorphometric variables. However, 25(OH)D levels did not correlate to any bone histomorphometric variable in either black or white women.

4. Discussion

There is evidence that serum 25(OH)D levels are affected by age, menstrual status and BMI (Perez-Lopez et al., 2011; Adami et al., 2009; Holick, 2006; Rajakumar et al., 2011). Compared to white women, we found that serum 25(OH)D levels were significantly lower but 1,25$(OH)_2$D levels significantly higher in black women matched for age, menstrual status and BMI, suggesting that the differences in 25(OH)D and 1,25$(OH)_2$D levels might be independent of these factors. Decreased serum 25(OH)D levels in blacks results from attenuated cutaneous production of vitamin D because of dark skin pigmentation (Holick, 2004; Chen et al., 2007). In addition, there were no significant differences in serum Ca and PTH levels between the 2 ethnic groups. Our results agree with other studies that reduced serum 25(OH)D levels do not alter serum Ca and PTH levels in blacks (Bell et al., 1985; Cosman et al., 2000; Meier et al., 1991), most likely due to the result of calcium economy (Aloia, 2008; Cosman et al., 1997). Blacks are more efficient than whites in absorbing dietary calcium, preserving calcium in bones, and reabsorbing calcium in the kidney (Aloia, 2008; Cosman et al., 1997), and thus maintain adequate calcium hemostasis despite lower 25

Table 2
Differences in serum biochemical data between black and white women.

	Overall	Black	White	p
	n = 50	n = 25	n = 25	
25(OH)D (ng/mL)	21.3 (10.5)	16.2 (10.1)	26.4 (8.34)	<0.001
1,25$(OH)_2$D (pg/mL)	36.0 (14.6)	41.0 (16.4)	31.0 (10.7)	0.014
Corrected Calcium (mg/dL)	9.44 (0.469)	9.43 (0.496)	9.44 (0.449)	0.953
Creatinine (mg/dL)	1.07 (0.184)	1.10 (0.181)	1.04 (0.185)	0.195
PTH (pg/mL)	34.5 (12.0)	34.9 (13.5)	34.0 (10.5)	0.801

Data expressed as mean (SD).

Table 3
Differences in bone static and dynamic indices between black and white women.

Variable	Overall	Black	White	p
	n = 50	n = 25	n = 25	
Structural indices				
BV/TV (%)	23.6 (6.95)	24.7 (6.42)	22.5 (7.43)	0.274
Tb.Th (μm)	142 (27.6)	152 (30.8)	132 (19.4)	0.026
Tb.N (#/mm)	1.66 (0.383)	1.63 (0.319)	1.68 (0.443)	0.649
Static indices				
ES/BS (%)	7.21 (3.50)	6.94 (3.76)	7.48 (3.28)	0.558
Oc.S/BS (%)	1.21 (0.905)	1.33 (1.01)	1.09 (0.792)	0.423
OV/BV (%)	2.38 (1.50)	2.26 (1.63)	2.50 (1.37)	0.410
OS/BS (%)	20.0 (13.2)	19.7 (15.1)	20.4 (11.3)	0.541
ulOS/BS (%)	14.1 (9.26)	12.9 (8.50)	15.3 (9.98)	0.357
O.Th (μm)	8.23 (1.62)	8.32 (1.87)	8.14 (1.35)	0.892
W.Th (μm)	34.4 (3.85)	35.0 (4.12)	33.8 (3.55)	0.269
Ob.S/BS (%)	6.92 (5.36)	6.23 (6.69)	7.21 (3.70)	0.154
Dynamic indices				
MS/BS (%)	5.94 (6.10)	6.83 (7.93)	5.05 (3.39)	0.503
MS/OS (%)	27.9 (17.3)	31.1 (14.9)	24.7 (19.2)	0.194
MAR (μm/day)	0.547 (0.153)	0.543 (0.120)	0.551 (0.182)	0.734
Aj.AR (μm/day)	0.172 (0.108)	0.182 (0.082)	0.163 (0.128)	0.156
Omt (days)	17.7 (14.0)	16.5 (6.46)	18.8 (18.7)	0.674
MLT (days)	74.6 (63.3)	59.4 (40.1)	89.2 (77.6)	0.271
BFRs ($\mu m^3/\mu m^2$/year)	11.0 (8.09)	11.3 (7.95)	10.7 (8.37)	0.705
Ac.f (#/year)	0.319 (0.228)	0.326 (0.229)	0.312 (0.230)	0.764

Data expressed as mean (SD).

Table 4
Correlations among biochemical indices.

	1,25(OH)D	Corrected Ca	Creatinine	PTH
Overall				
25(OH)D (ng/mL)	−0.224	0.051	0.027	−0.198
1,25$(OH)_2$D (pg/mL)		−0.223	0.372	0.200
Corrected calcium (mg/dL)			−0.138	−0.063
Creatinine (mg/dL)				0.143
Black				
25(OH)D (ng/mL)	0.112	0.181	0.387	−0.078
1,25$(OH)_2$D (pg/mL)		−0.179	0.380	0.212
Corrected calcium (mg/dL)			−0.200	−0.078
Creatinine (mg/dL)				0.059
White				
25(OH)D (ng/mL)	−0.255	0.024	−0.172	−0.507*
1,25$(OH)_2$D (pg/mL)		−0.326	0.240	0.235
Corrected calcium (mg/dL)			−0.172	−0.026
Creatinine (mg/dL)				0.194

Data expressed as correlation coefficient r value.
* $p < 0.05$.

Table 5
Correlations of 25(OH)D and 1,25$(OH)_2$D levels with bone histomorphometric indices.

Variables	25(OH)D			1,25$(OH)_2$D		
	Overall	Black	White	Overall	Black	White
	n = 50	n = 25	n = 25	n = 50	n = 25	n = 25
Structural indices						
BV/TV (%)	−0.172	−0.403	−0.002	0.142	0.103	0.121
Tb.Th (μm)	−0.228	−0.008	−0.347	0.185	−0.024	0.188
Tb.N (#/mm)	−0.008	−0.307	0.168	−0.017	0.043	0.031
Static indices						
ES/BS (%)	0.149	0.221	0.290	0.104	0.177	0.085
Oc.S/BS (%)	−0.029	0.038	0.200	0.311*	0.302	0.325
OV/BV (%)	0.151	0.331	0.039	0.321*	0.362	0.456*
OS/BS (%)	0.099	0.334	−0.165	0.380**	0.446*	0.496*
ulOS/BS (%)	0.107	0.300	−0.177	0.393**	0.476*	0.476*
O.Th (μm)	0.060	0.164	0.211	−0.034	0.073	−0.151
W.Th (μm)	−0.194	−0.181	−0.073	0.331*	0.231	0.387
Ob.S/BS (%)	0.187	0.351	−0.057	0.319*	0.445*	0.410*
Dynamic indices						
MS/BS (%)	−0.011	0.369	−0.131	0.250	0.224	0.263
MS/OS (%)	−0.101	0.029	0.234	0.246	0.170	0.231
MAR (μm/day)	0.040	−0.125	0.132	−0.272	−0.401	−0.063
Aj.AR (μm/day)	−0.092	0.061	0.138	−0.175	−0.396	−0.163
Omt (days)	0.027	0.319	−0.026	0.090	0.220	−0.131
MLT (days)	0.067	−0.046	−0.149	0.195	0.357	0.223
BFRs ($\mu m^3/\mu m^2$/year)	−0.005	0.280	−0.008	0.155	0.100	0.230
Ac.f (#/year)	−0.012	0.297	−0.032	0.125	0.106	0.153

Data expressed as correlation coefficient r value.
* $p < 0.05$.
** $p < 0.01$.

(OH)D levels (Heaney, 2002; Gutierrez et al., 2011). Higher levels of serum 1,25$(OH)_2$D in blacks would stimulate intestinal calcium absorption (Christakos et al., 2019; Christakos et al., 2020). It is well-known that 25(OH)D is the substrate for synthesis of 1,25$(OH)_2$D in the kidney via 1a-hydroxylase (CYP27B1) activity. Therefore, there is likely to be a significantly positive correlation between 25(OH)D and 1,25$(OH)_2$D levels in blood circulation (Rejnmark et al., 2008; Swanson et al., 2014). However, this relationship was not significant in either blacks or whites in our study. In the black cohort, only 1 of 21 (~5 %) subjects with 25(OH)D deficiency had subnormal level of 1,25$(OH)_2$D (<18 pg/mL). The possible mechanism is that healthy black individuals with 25(OH)D deficiency usually have adequate levels of 1,25$(OH)_2$D, which may be caused by increased activity of 1-α-hydroxylase (CYP27B1) or reduced 1,25$(OH)_2$D catabolism (Robinson-Cohen et al., 2013). Increased CYP27B1 activity in blacks could be driven by increased parathyroid hormone or by racial differences in CYP27B1 affinity for 25 (OH)D (Robinson-Cohen et al., 2013).

Despite significantly lower serum 25(OH)D levels, blacks generally have higher bone mineral density (BMD) and lower risk of fragility fractures than whites (Aloia et al., 2015; Shieh and Aloia, 2017; Powe et al., 2013; Brown et al., 2018). This contradiction is commonly referred to as "vitamin D paradox". However, BMD only represents bone mass rather than other components, such as bone microarchitecture, remodeling and mineralization, all of which are very important to bone health. Bone histomorphometry is able to assess bone structure, resorption, formation, remodeling and mineralization in the same specimen (Han et al., 1996; Han et al., 1997; Dempster et al., 2013; Parfitt et al., 1997). Our previous studies demonstrated that many bone histomorphometric variables relative to bone structure, mineralization and remodeling were remarkably different between blacks and whites with different ages and menstrual status (Han et al., 1996; Han et al., 1997; Parfitt et al., 1997). In order to reduce the effects of these confounding factors, we designed a nested-case control study to adjust for age, menstrual status and BMI. The results showed that, except for greater Tb.Th in blacks, there was no significant difference in any other histomorphometric variables between the 2 ethnic groups. The values of bone histomorphometry are well within the normal range in both black and white women (Qiu et al., 2020). In addition, 25(OH)D levels did not correlate with any bone histomorphometric variables in either black or white women. The reported findings on the effect of serum 25(OH)D levels on bone are inconsistent. High-resolution peripheral quantitative computed tomography (HR-pQCT) and finite element measurements at the radius and tibia demonstrated that serum 25(OH)D levels was moderately associated with poor or deteriorated bone microarchitecture (Boyd et al., 2015; Garrahan et al., 2022) but no significant effect on bone strength (failure load) (Burt et al., 2019). Also, there are inconsistent data on the relationship between serum 25(OH)D level and BMD measured by dual-energy x-ray absorptiometry (DXA). Interestingly, more recent studies reported insignificant correlation between serum 25 (OH)D level and DXA measured BMD (Alkhenizan et al., 2017; Allison et al., 2018). Furthermore, the clinical trial data suggest that vitamin D neither improves bone health nor reduce the risk osteoporotic fractures (Reid et al., 2014; Bolland et al., 2018; Torjesen, 2018; LeBoff et al., 2022). Based on these findings, absence of correlation between 25(OH)D levels and bone histomorphometry is not surprsing. Our data, although collected several decades ago, also support the current perspectives that 25(OH)D is not essential for protecting bone health (Torjesen, 2018; LeBoff et al., 2022). Accordingly, there is little justification to use vitamin D supplements to maintain or improve musculoskeletal health (Bolland et al., 2018; LeBoff et al., 2022).

It has been reported that 1,25$(OH)_2$D increases bone volume by stimulating osteoblastic bone formation but decreases bone mineralization by raising local and systemic inhibitors of osseous mineralization (Goltzman, 2018; Wronski et al., 1986). However, most of these observations came from in vitro and animal experiments. Our study showed that Ob.S/BS and W.Th increase with increasing serum 1,25$(OH)_2$D levels, suggesting that circulating 1,25$(OH)_2$D may facilitate bone formation by increasing osteoblast number and function. Cosman et al. (2000) reported that administration of 1,25$(OH)_2$D caused a more robust increase in two markers of bone formation (osteocalcin and carboxyterminal propeptide of type 1 procollagen) in black women than in white women, indicating that the sensitivity of osteoblasts to circulating 1,25$(OH)_2$D is greater in black women. Although 1,25$(OH)_2$D is primarily synthesized in the kidney (Zofkova, 2018), there is a number of bone cells, especially osteoblasts and osteocytes, can produce it as well (Turner et al., 2014; Lanske et al., 2014). The renal 1,25$(OH)_2$D affects the skeleton via endocrine pathway (Anderson, 2017), but the locally produced 1,25$(OH)_2$D in bone cells affect the skeleton via autocrine and intracrine pathways (Anderson, 2017; Labrie, 1991).

Vitamin D metabolites have been considered to play an important role in maintenance of bone mineralization. In bone histomorphometry, O.Th and MLT are specific indices used for the assessment of bone mineralization and diagnosis of osteomalacia (Bhan et al., 2018). Our results demonstrated that there was no significant difference in O.Th and MLT between black and white women and no subject fell into the category of osteomalacia (O.Th > 12.5 μg and MLT > 100 days, data not shown). In addition, O.Th and MLT were not correlated with serum 25 (OH)D and 1,25$(OH)_2$D levels. These findings indicate that neither 25 (OH)D nor 1,25$(OH)_2$D levels in blacks or whites affect general bone mineralization. A less frequently reported osteoid-related variable, ulOS/BS ([OS-MS]/BS), was included in our study. This variable

represents the fraction of bone surface covered with osteoid which is lack of tetracycline labeling. The change in ulOS/BS may be attributed to one or more interruptions of mineralization during the life span of the osteoid seam (Parfitt et al., 1997). Lack of label occurs preferentially at locations where the osteoid is thin and distant from the cement line, and covered by flat cells that have probably stopped making bone matrix (Parfitt et al., 1997). Thus ulOS/BS reflects the status of bone mineralization at the terminal stage of bone remodeling, because bone formation is extremely low or even ceased in this period (Parfitt et al., 1997). The positive correlation between $1,25(OH)_2D$ levels and ulOS/BS indicates that $1,25(OH)_2D$ may attenuate terminal mineralization of bone.

There are some limitations in our study. First, this is a cross-sectional and retrospective study with relatively small sample size. Retrospective nature of the study may generate less valid conclusion usually due to missing data. Second, we did not have data on routine or daily intake of vitamin D supplementation. Third, our study did not include healthy male subjects for examining gender differences in vitamin D paradox in black male population.

5. Conclusion

Although black women have significantly lower serum 25(OH)D levels and higher prevalence of vitamin D deficiency, their bone mass, remodeling and mineralization are well-maintained. However, higher serum $1,25(OH)_2D$ levels may facilitate bone formation by increasing osteoblast number and function, and moderately inhibit bone mineralization at the terminal stage of bone remodeling. These results indicate that vitamin D deficiency, as currently defined by serum 25(OH)D, does not compromise bone health, particularly in blacks.

CRediT authorship contribution statement

Shijing Qiu: Conceptualization, Investigation, Data curation, Writing-Original draft preparation, Writing-Reviewing and editing.

George Devine: Data curation, Formal analysis, Writing-Original draft preparation.

Sudhaker D Rao: Conceptualization, Supervision, Project administration, Funding acquisition, Writing-Original draft preparation, Writing-Reviewing and editing.

Declaration of competing interest

All authors state no conflicts of interest.

Data availability

Data will be made available on request.

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