



# Does the relationship between 25-hydroxyvitamin D status and bone mass vary according to skin color in adults? Results of a Brazilian population-based study

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## Abstract

**Summary** Skin color has been indicated as an important factor in determining serum concentrations of 25-hydroxyvitamin D [25(OH)D], and consequently bone health. However, studies are controversial and scarce for mixed populations.

**Purpose/introduction** To analyze the association of 25(OH)D with bone mineral content (BMC) and bone mineral density (BMD); and to investigate the presence of interaction with skin color in Brazilian adults.

**Methods** This is a cross-sectional, population-based study conducted with adult individuals (20–59 years) of both genders. Bone health was assessed by dual energy radiological absorptometry. Vitamin D status was measured using serum 25(OH)D. Skin color and other variables in the adjusted model were collected using a questionnaire and anthropometric assessment. Associations and interactions were evaluated using linear regression models stratified according to gender.

**Results** Non-white men with vitamin D deficiency (< 20.0 ng/mL) have less bone mass than those with insufficiency and sufficiency for the femoral neck and hip sites. According to the adjusted regression analysis, the deficient status of 25(OH)D in men was associated with worse bone health for the lumbar spine sites ( $\beta = -0.1$ ;  $p = 0.006$ ), femoral neck ( $\beta = -0.08$ ;  $p = 0.006$ ), and hip ( $\beta = -0.08$ ;  $p = 0.009$ ). No statistically significant associations were observed between 25(OH)D and bone health in women. In addition, no statistical interaction was identified between skin color and vitamin D status in relation to bone health ( $p > 0.05$  for all tests) in either gender and for all bone sites evaluated.

**Conclusion** Deficient vitamin D status is associated with lower bone mass in adults with differences observed according to gender, but not according to skin color.

**Keywords** Bone mineral density · Adults, Vitamin D · Skin color · Interaction

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## Introduction

The high prevalence of vitamin D deficiency is a worldwide public health problem. This epidemiological picture may have consequences for bone health, since some studies have shown that low serum concentrations of 25-hydroxyvitamin D [25(OH)D] are associated with low bone mineral density (BMD) and consequent fractures [1, 2]. In fact, 25(OH)D acts on skeletal development and in maintaining bone health throughout life [3, 4], being one of the factors responsible for calcium absorption and consequent bone mineralization and remodeling [5].

Vitamin D can be obtained from food, but this is not the main route for obtaining it in sufficient amounts in order to meet humans' physiological needs as its intake is generally low. The highest percentage of vitamin D production occurs endogenously from exposure to UVB sunlight, which reflects the variability of its concentrations in different populations [6]. There is also a variation in its metabolism according to skin color, and white people are more exposed to sunlight with greater vitamin D production compared to Black individuals because they have less pigmentation on their skin [4, 7, 8]. Thus, skin color has been indicated as an important factor in determining 25(OH)D serum concentrations and consequently bone health [4, 5].

In this sense, some studies have pointed to a possible relationship between skin color and low bone mass [4, 9]. However, many studies have produced controversial results [5, 7, 8, 10]. It is important to note that despite having lower 25(OH)D serum concentrations [8], non-white individuals have higher mean bone mass values and have a lower risk of fractures compared to white people [4, 9]. Therefore, we hypothesized that the association between 25(OH)D status and bone mass is modified according to skin color. Although other studies have produced favorable results to this hypothesis, most investigations on this topic are conducted in Caucasian or Black populations [11, 12], with a lack of studies in mixed populations. Therefore, the aim of this study was to analyze the association of 25(OH)D with bone mineral content (BMC) and bone mineral density (BMD); and to investigate the presence of interaction with skin color in Brazilian adults.

## Methods

### Study design and population

A cross-sectional, population-based study carried out in the period of 2012–2014 with adults (20–59 years) of both genders living in the urban area of the city of Viçosa/MG, Brazil. Pregnant women, puerperal women, bedridden, and/or unable to perform measurements, as well as those who had undergone any orthopedic surgery, who used some type of

prosthesis or who had cognitive/intellectual limitations and difficulty in answering the questionnaires were excluded from the study.

A cluster sampling process was performed in two stages, with the first stage units being the census sectors [13] and the second stage units being the households [14]. The sample size calculation was performed considering the following criteria: reference population equal to 43,431 individuals; prevalence of 50% (considering the multiple outcomes assessed in the study); sampling error of 5%; and study design effect by clusters estimated to be equal to 1.55. Moreover, 10% was added to the calculated value to cover losses and control confounding factors. The final sample calculated was 651 individuals, with 701 volunteers evaluated.

This study is part of two major projects both approved by the Human Research Ethics Committee of the Federal University of Viçosa (no. 008/2012/CEPH; no. 02/2013/CEP/07-12-2013). The methodological aspects of the study are described in another article [15]. All volunteers who agreed to participate signed an informed consent form.

### Variables

The dependent variables of the study (BMC and BMD) were evaluated using a Lunar Prodigy Advance DXA System model Dual Energy X-ray Absorptiometry (DXA) (GE Healthcare), following the recommendations of the International Society for Clinical Densitometry [16]. The lumbar spine (LS-BMD: LI-L4) and the femur (femoral neck and hip) (FN-BMD and H-BMD, respectively) were mapped in addition to the total bone mineral content (BMC), being considered the absolute values of BMC (g) and BMD (g/cm<sup>2</sup>).

Blood collection was performed to evaluate the main independent variable (25(OH)D) by a qualified professional using the peripheral venous puncture technique between 7 and 10 in the morning, with the individual fasting for 12 h. Serum 25(OH)D was evaluated using chemiluminescence [17] with an ARCHITECT 25(OH)D kit using ARCHITECT/ABBOTT equipment (Abbott Architect I Instrument, IL, USA). The reference values adopted in the status classification of 25(OH)D are in accordance with the Endocrine Society Clinical Practice Guideline: sufficient (> 30.0 ng/mL), insufficient (20.1 ng/mL to 29.9 ng/mL), and deficient (< 20.0 ng/mL) [6]. In turn, the parathyroid hormone (PTH) was evaluated by ACCESS PTH Kit chemiluminescence by UNICEL DXI800 equipment and categorized in tertiles with the following reference values: P1 = 21 pg/mL, P2 = 30 pg/mL and P3 = 77 pg/mL.

The other independent variables were collected through a structured questionnaire applied at the volunteers' residence. The following variables were considered for this study: gender (men and women); age group (20–29, 30–39, 40–49, and 50–59 years); self-declared skin color according to the Brazilian

population census [13] (White, Brown, Black or Mulatto, Yellow (oriental) and indigenous, and later categorized as white and non-white). The 25 participants who declared themselves yellow or indigenous were excluded from the analysis because of their low frequency in the sample. The following additional variables were also considered: complete years of study (0–4, 5–8, 9–11, and  $\geq 12$  years), smoking (non-smokers, smokers, and ex-smokers); contraceptive use among women (yes/no); and physical activity level (PAL). This was assessed using the International Physical Activity Questionnaire (IPAQ), version-6, long format [18]. The physical activity time performed during leisure in the week prior to applying the questionnaire was considered, which was later categorized as: irregularly active (IA) ( $< 150$  min/week) and physically active (PA) ( $> 150$  min/week) [19]. The seasonality variable, which identifies the time of year when blood was collected to assess vitamin D status, was dichotomized into summer/spring and winter/autumn.

The body mass index (BMI) was calculated by the ratio of body weight to height (m) squared to classify nutritional status, and later categorized into: eutrophic ( $18.5 \text{ kg/m}^2$  to  $24.9 \text{ kg/m}^2$ ), overweight ( $25.0 \text{ kg/m}^2$  to  $29.9 \text{ kg/m}^2$ ), and obese ( $\geq 30.0 \text{ kg/m}^2$ ) [20].

## Statistical analyses

The data were double entered in the Epidata version 3.1 program. Analyses were performed on STATA version 13.1 using the Svy command after verifying the data consistency. The data were weighted according to the distribution by gender, age, and education provided by the Brazilian Institute of Geography and Statistics [13], with the weight being determined by the ratio between the proportions of individuals in the sample.

The normality of the dependent variables (BMC/BMD) was assessed using the asymmetry test considering the normal value between  $-1$  and  $+1$ . The descriptive analysis was performed by calculating relative frequency measures. The mean values of 25(OH)D and BMC/BMD according to gender and skin color were also calculated, with their respective 95% confidence intervals (95% CI) according to gender and skin color, and the mean BMC/BMD values according to 25(OH)D status, stratified by gender and skin color.

The association between 25(OH)D and BMC/BMD was evaluated using crude and adjusted linear regression modeling. The adjustment variables considered in the multiple analysis were: age (in years), education (in discrete form, in years of study), skin color, PAL (in minutes), smoking, BMI (continuous, in  $\text{kg/m}^2$ ), and seasonality, identified as potential confounders of the studied ratio according to the literature [21, 22]. In turn, the interaction between 25(OH)D and skin color was tested by inserting an interaction term in the adjusted model. All analyzes were stratified according to gender.

## Results

In the total sample, 50.29% were women, 26.15% were between 30 and 39 years old, 61.42% were non-white and 42.84% had 12 or more years of study. The population was mostly composed of eutrophic individuals regarding BMI (50.41%). For behavioral variables, most of the studied population was irregularly active (72.64%) and non-smokers (65.13%). When assessing seasonality, we found that 62.47% of the evaluations were carried out in the summer/spring period, and that the majority of the evaluated population had sufficient (42.44%) or insufficient (42.34%) vitamin D status and most individuals were categorized as Upper Tertile (37.9%) (Table 1). Information about the sample characteristics stratified by gender is described in another article [21].

We identified that white and non-white men have higher mean 25(OH)D serum concentrations when comparing men and women, with these differences being statistically significant. In addition, the mean values regarding bone mass are higher in non-white men for all evaluated bone sites. Differences were significant when comparing the mean FN-BMD and H-BMD values for white and non-white men and women. We also found that non-white men have a lower mean 25(OH)D value and higher bone mass values for all evaluated bone sites when comparing the mean values of vitamin D and bone mass between white and non-white men; however, these differences were not significant. In turn, we observed a similar situation in women (Table 2).

Figures 1 and 2 show the BMC/BMD means according to the 25(OH)D status stratified by skin color and gender. Non-white men generally have higher mean bone mass values compared to white men in the different 25(OH)D strata; however, these differences were not significant. Significant differences were identified when bone site means were compared in relation to the 25(OH)D status for non-white men in deficiency in relation to insufficiency and sufficiency for the FN-BMD site (sufficient  $1.118 \text{ g/cm}^2$ , 95%CI 1.085–1.150; insufficient  $1.105 \text{ g/cm}^2$ ; 95%CI 1.056–1.154; deficient  $1.110 \text{ g/cm}^2$ , 95%CI 0.967–1.052), and also in deficiency in relation to insufficiency and sufficiency for the H-BMD site (Sufficient:  $1.110 \text{ g/cm}^2$ , 95%CI 1.075–1.145; insufficient  $1.104 \text{ g/cm}^2$ ; 95%CI 1.065–1.143; deficient  $1.011 \text{ g/cm}^2$ , 95%CI 0.961–1.061) (Fig. 1).

There is a general decline in bone mass averages as the 25(OH)D status decreases in white women. The same does not occur among non-white women, in which higher BMD values are observed among those with a deficient 25(OH)D status. However, these differences were not significant, nor the differences according to skin color (Fig. 2).

In the linear regression models for men (Table 3), it was found that the deficient status of 25(OH)D was significantly associated with worse bone health when compared to sufficiency, however with the exception of the BMC in both the crude and in the adjusted models. However, the interaction

**Table 1** Characterization of the study population. Study on Health and Food (ESA-Viçosa), 2012–2014

Variables	Proportion (%)	95%CI
Gender		
Male	49.7	45.2–54.2
Female	50.3	45.8–54.8
Age range (years)		
20–29	24.3	17.1–33.4
30–39	26.1	21.8–31
40–49	24.1	19.1–29.8
50–59	25.5	20.1–31.7
Skin color		
White	38.6	31.7–45.9
Non-white	61.4	54.1–68.3
Education (years)		
0–4	20.1	12.8–29.1
5–8	16.3	11.9–21.7
9–11	20.8	17.4–24.7
≥ 12	42.8	30.8–55.8
Contraceptive use		
Yes	18.9	15.4–23.1
No	81	76.8–84.5
BMI (kg/m <sup>2</sup> )		
Eutrophic	50.4	43.3–57.4
Overweight	33.3	28.3–38.8
Obese	16.3	12.7–20.5
Seasonality		
Summer/spring	62.4	53.1–71
Autumn/winter	37.6	28.1–46.1
PAL in leisure (min/week)		
IA	72.6	66.1–78.4
PA	27.4	21.6–33.9
Smoking		
Non-smoker	65.1	57.9–71.7
Smoker	12.9	9.1–16.6
Ex-smoker	21.9	15.8–29.5
25(OH)D (mL)		
Sufficient	42.5	35.9–49.3
Insufficient	42.3	37.1–47.7
Deficient	15.2	12–19.1
PTH		
Lower tertile	27.3	24.1–30.7
Middle tertile	34.7	30.2–39.4
Upper tertile	37.9	34.2–41.9

BMI body mass index, PAL physical activity level, IA irregularly active, PA physically active, 25(OH)D 25-Hydroxyvitamin D, PTH parathyroid hormone

**Table 2** Mean vitamin D serum concentration and bone mass values according to gender and skin color. Study on health and food (ESA-Viçosa), 2012–2014

	Men		Non-white	
	White	Non-white	Mean	95%CI
25(OH)D (ng/mL)	Mean	95%CI	Mean	95%CI
	33.8	30.2–37.5	30.5	29.1–31.9
BMC (g)	2707	2,587–2827	2,776	2633–2919
LS-BMD (g/cm <sup>2</sup> )	1.174	1.137–1.211	1.226	1.197–1.255
FN-BMD (g/cm <sup>2</sup> )	1.074	1.046–1.102*	1.109	1.083–1.136 <sup>+</sup>
H-BMD (g/cm <sup>2</sup> )	1.073	1.051–1.095*	1.107	1.083–1.130 <sup>+</sup>
	Women		Non-white	
	White	Non-white	Mean	95%CI
25(OH)D (ng/mL)	Mean	95%CI	Mean	95%CI
	27.8	26.2–29.5	28.5	26.5–30.5
BMC (g)	2571	2486–2657	2,626	2547–2705
LS-BMD (g/cm <sup>2</sup> )	1.115	1.085–1.144	1.170	1.142–1.197
FN-BMD (g/cm <sup>2</sup> )	0.965	0.939–0.992*	1.004	0.982–1.026 <sup>+</sup>
H-BMD (g/cm <sup>2</sup> )	0.975	0.952–0.998*	1.013	0.986–1.039 <sup>+</sup>

CI confidence interval, 25(OH)D 25-Hydroxyvitamin D, BMC bone mineral content, LS-BMD lumbar spine bone mineral density, FN-BMD femoral neck bone mineral density, H-BMD hip bone mineral density

\*Statistically significant difference,  $p < 0.05$ , for comparison between white men and women

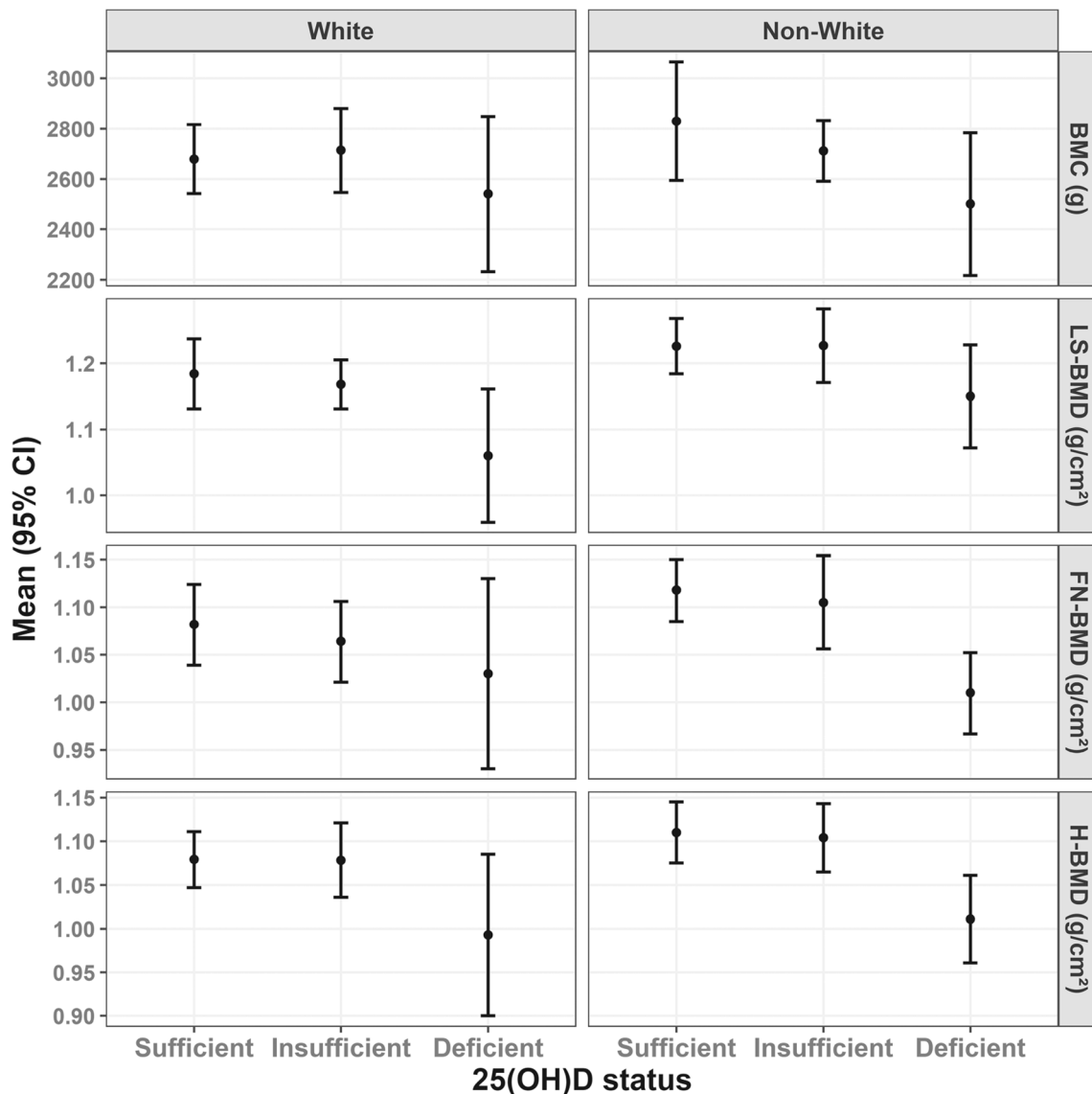
<sup>+</sup> Statistically significant difference,  $p < 0.05$ , for comparison between non-white men and women

between 25(OH)D serum concentrations and skin color was not significant in the evaluated bone sites.

For women (Table 4), 25(OH)D deficiency compared to sufficiency was not significantly associated with bone health in any of the evaluated sites in either the crude or adjusted models. There was also no significant interaction between 25(OH)D serum concentrations and skin color.

## Discussion

The results of this study generally indicate that the 25(OH)D deficiency status was significantly associated with worse bone health when compared to sufficiency in men, but not in women, with no interaction in the skin color in either gender. In view of these findings, it is important to note that this article has the fact that it is a study with a mixed population and mostly composed of non-whites (61.42%) as a strength, and there was stratification by gender; thus, these data fill an important gap considering that there is a scarcity of studies which consider skin color and gender when assessing the relationship between 25(OH)D status and bone health. In addition, it is a population-based study, which enables extrapolating the results to the studied population and establishing analogies for other populations which have similar characteristics.



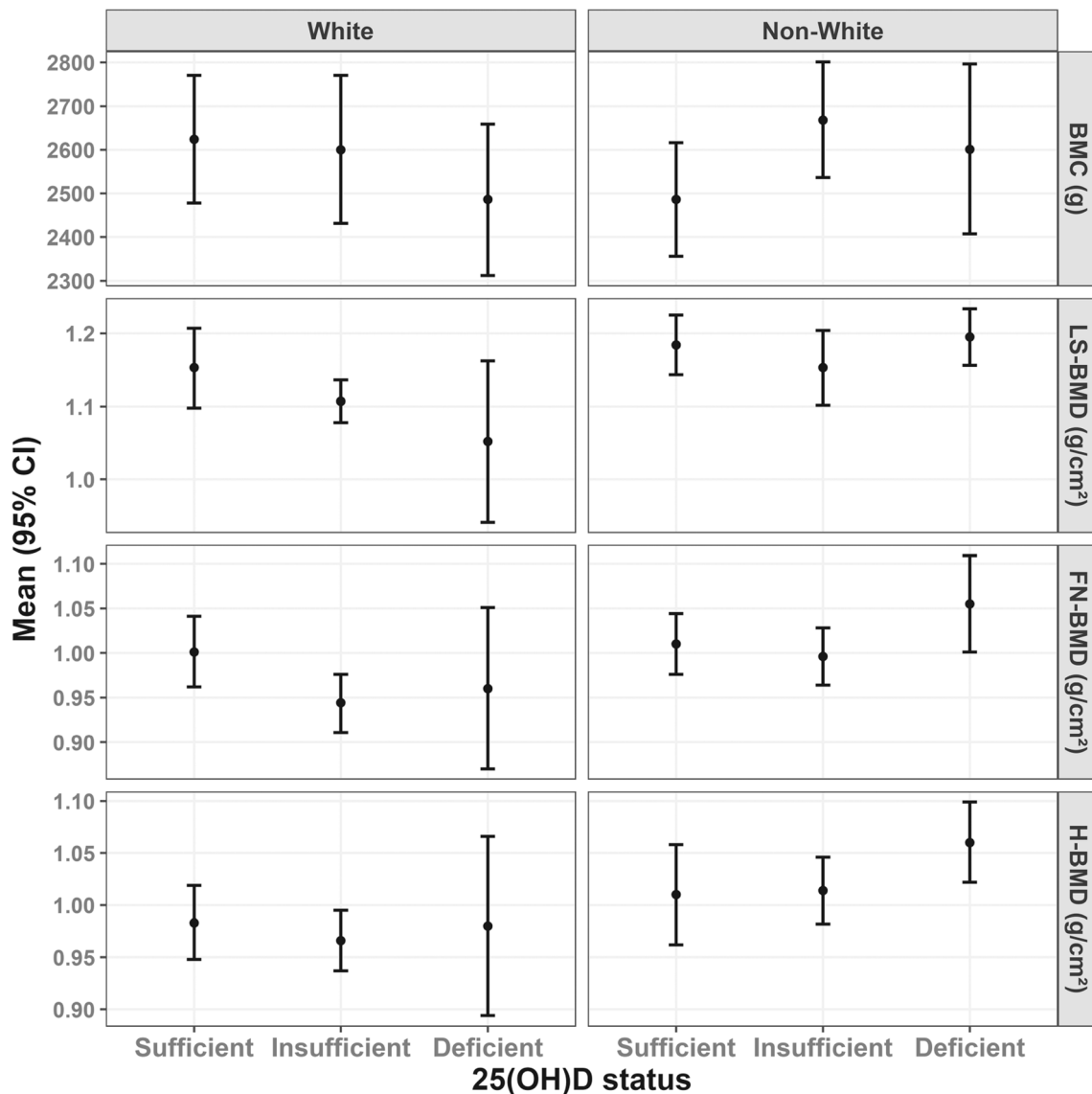
**Fig. 1** Mean values of bone mineral content and density according to 25-Hydroxyvitamin D status for white and non-white men. Study on Health and Food (ESA-Viçosa), 2012–2014. *BMC* bone mineral content, *LS-*

*BMD* lumbar spine bone mineral density, *FN-BMD* femoral neck bone mineral density, *H-BMD* hip bone mineral density, *25(OH)D* 25-Hydroxyvitamin D

Two criteria for determining satisfactory 25(OH)D serum concentrations are identified in the literature: one established by the Endocrine Society Clinical Practice Guideline [6], which proposes that serum concentrations for vitamin D insufficiency be below 30 ng/mL [6, 23, 24], and which was adopted in the present study; and another established by the Institute of Medicine (IOM), in which a lower threshold of 20 ng/mL is considered [25]. The Brazilian Society of Endocrinology and Metabolism has also recently adopted this last threshold as a reference value for the diagnosis of vitamin D deficiency in the Brazilian population, under the argument that the 30 ng/mL threshold was not suitable for a tropical country like Brazil and therefore provides sun exposure for

most of the year and regions of the country [26]. However, the 20 ng/mL threshold was established based on a cohort study in a predominantly white population [6], which suggests that extrapolation to other ethnic-racial or miscegenate groups should be performed with caution.

It is noteworthy that the use of the cut-off points for vitamin D status proposed by the Endocrine Society Clinical Practice Guideline enables greater sensitivity in analyzes related to bone health, as it presents a range of insufficiency classification. In this sense, significant differences were identified for non-white men in the 25(OH)D deficiency status in relation to insufficiency and sufficiency for FN-BMD and H-BMD. In addition, only worse bone health in men with vitamin D



**Fig. 2** Mean bone mineral content and density values according to 25-Hydroxyvitamin D status for white and non-white women. Study on Health and Food (ESA-Viçosa), 2012–2014

deficiency was observed when compared to sufficiency in the regression models in this study; however, our results suggest that it may be necessary to review the cut-off points for women in a miscegenated population, requiring further research on the topic. Although there is still no consensus regarding the 25(OH)D cut-off points, it is noteworthy that the literature has reported that this is an important biological parameter for assessing bone health [27].

We identified in this study that non-white individuals generally had a tendency to have higher bone mass averages than white individuals. The fact that non-whites have lower 25(OH)D serum concentrations and greater bone mass has also been confirmed in other studies [4, 9, 28, 29]. In a study conducted with healthy white and Black individuals aged 24–36

years of age of both genders, a positive association was found between 25(OH)D and H-BMD, LS-BMD, and BMC, while BMD showed higher values for Black men than for white men [7]. It has also been observed that American Blacks aged between 28 and 48 years, supply the parathormone (PTH) and absorb calcium from minimum concentrations of 20 ng/mL of 25(OH)D, maintaining themselves with adequate BMD values even in this range [9]. This result corroborates another study carried out with African immigrants in the USA, in which PTH was suppressed from the same 25(OH)D concentration and the evaluated population showed adequate bone health [30]. Therefore, such a 25(OH)D status threshold appears to be adequate for this population. However, it is not clear whether there is a need for vitamin D supplementation for Black individuals

**Table 3** Coefficients and *p*-value of linear regression models for the association between 25(OH)D status and bone mass in the total population and according to skin color in men. Study on Health and Food (ESA - Viçosa), 2012–2014

Bone sites	BMC (g)		LS-BMD (g/cm <sup>2</sup> )		FN-BMD (g/cm <sup>2</sup> )		H-BMD (g/cm <sup>2</sup> )	
<b>Crude Linear Model</b>								
25(OH)D	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>
Insufficient	-47.74	0.496	-0.02	0.942	-0.01	0.624	-0.01	0.951
Deficient	-240.41	0.083	-0.10	0.004	-0.08	0.006	-0.09	0.004
<b>Adjusted Linear Model</b>								
25(OH)D	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>
Insufficient	-62.42	0.384	-0.02	0.321	-0.02	0.208	-0.01	0.601
Deficient	-263.05	0.090	-0.10	0.006	-0.08	0.006	-0.08	0.009
<b>Interaction</b>								
25(OH)D * skin color	<i>p</i>		<i>p</i>		<i>p</i>		<i>p</i>	
	0.182		0.986		0.091		0.252	

*BMC* Bone Mineral Content; *LS-BMD* Lumbar Spine Bone Mineral Density; *FN-BMD* Femoral Neck Bone Mineral Density; *H-BMD* Hip Bone Mineral Density; *25(OH)D* 25-Hydroxyvitamin D

$\beta$  linear regression coefficient value

Adjustment variables: age, education, physical activity level, body mass index (continuous); seasonality, skin color and smoking (categorical)

with lower concentrations than this value given the lack of evidence regarding low BMD [9, 31–34].

Still in line with these findings, the present study also enabled us to observe that non-white men with sufficient levels of 25(OH)D had higher mean bone mass compared to white men, despite being non-statistically significant differences. In turn, higher mean bone mass values were generally found in the insufficient and deficient 25(OH)D status in non-white women in

relation to white women. These observations reinforce that non-white individuals possibly have higher mean bone mass values regardless of the lower 25(OH)D serum concentration, corroborating the findings of other studies [4, 7, 9, 38, 35]. This difference in the relationship between 25(OH)D and bone mass according to ethnicity can be explained by the greater skin pigmentation of non-white individuals which inhibits the production of cholecalciferol, which is the precursor to 25(OH)D synthesis [4].

**Table 4** Coefficients and *p*-value of linear regression models for the association between 25(OH)D status and bone mass in the total population and according to skin color in women. Study on Health and Food (ESA- Viçosa), 2012–2014

Bone sites	BMC (g)		LS-BMD (g/cm <sup>2</sup> )		FN-BMD (g/cm <sup>2</sup> )		H-BMD (g/cm <sup>2</sup> )	
<b>Crude Linear Model</b>								
25(OH)D	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>
Insufficient	114.60	0.062	-0.04	0.110	-0.03	0.044	-0.07	0.720
Deficient	31.60	0.685	-0.03	0.332	0.01	0.603	0.03	0.250
<b>Adjusted Linear Model</b>								
25(OH)D	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>	$\beta$	<i>p</i>
Insufficient	52.62	0.370	-0.04	0.850	-0.01	0.441	-0.01	0.559
Deficient	-43.08	0.617	-0.02	0.904	0.03	0.246	0.01	0.460
<b>Interaction</b>								
25(OH)D * skin color	<i>p</i>		<i>p</i>		<i>p</i>		<i>p</i>	
	0.360		0.587		0.541		0.814	

*BMC* Bone Mineral Content; *LS-BMD* Lumbar Spine Bone Mineral Density; *FN-BMD* Femoral Neck Bone Mineral Density; *H-BMD* Hip Bone Mineral Density; *25(OH)D* 25-Hydroxyvitamin D

$\beta$  linear regression coefficient value

Adjustment variables: age group, education, physical activity level, body mass index (continuous); seasonality, contraceptive use, skin color and smoking (categorical)

Thus, non-white individuals produce less 25(OH)D from sun exposure [9].

In addition, it is observed that non-white individuals have higher dietary calcium absorption, greater renal calcium absorption [4], and lower bone resorption markers [28]. Thus, despite having lower 25(OH)D concentrations, this would be sufficient to activate bone metabolism, causing non-white adults to have greater bone mass regardless of 25(OH)D serum concentrations [4, 7, 8]. Another hypothesis for these findings is linked to BMI, and an inverse association has already been observed between BMI and Black individuals with vitamin D binding protein (DBP); and this in turn has been inversely correlated with BMD, suggesting that DBP is an inhibitor of the 25(OH)D actions in humans [10]. Bearing in mind that non-white individuals seem to have a higher BMI than white individuals [4, 7], these findings support the fact that non-white individuals have greater bone mass than white individuals, despite having lower 25(OH)D serum concentrations.

However, our results did not indicate an association between 25(OH)D and BMC/BMD in women. Other studies carried out with a mixed [35] or non-white [5] female population also found no association between 25(OH)D and bone health. One study found no association between 25(OH)D and BMD assessed by DXA in 67 Somali women who lived in Switzerland who were similar in age to our sample (18 to 56 years old) [5]. However, the prevalence of hypovitaminosis D found by these authors was high, with 73% presenting serum 25(OH)D levels below 10 ng/mL. In turn, another study that evaluated the relationship between 25(OH)D and bone health in a mixed population of 485 volunteers aged between 32 and 96 years old of both genders, observed that white women had higher 25(OH)D serum concentrations and lower bone quality values compared to non-white women as assessed by the broadband ultrasound attenuation method (BUA–Broadband Ultrasound Attenuation). However, corroborating our findings, a significant relationship was only observed between 25(OH)D serum concentrations and bone health in men, but not in women [35].

The cross-sectional character of this study stands out among its limitations, which does not enable ensuring the temporality of the associations found. Another possible limitation is that the volunteers who declared as Black ( $n = 70$ ) were grouped with Brown individuals ( $n = 325$ ) in the non-white group due to their low frequency in the sample, which may have influenced the results found. An additional limitation refers to the fact that we do not have data regarding the menstrual history of the evaluated women. However, it is important to note that several studies have carried out this division between white and non-white individuals [10, 29, 35], thus enabling a comparison between the results and greater statistical power for the analyses. Regarding seasonality,

the city where the study was conducted has a tropical altitude climate with two well-defined seasons. We consider that this limitation has been circumvented, since the seasonality variable was included as an adjustment in the regression model.

In conclusion, the results of this study indicate an inverse association between vitamin D deficiency and bone mass in men, but not in women. There was no statistically significant interaction between 25(OH)D and skin color in relation to bone health in adults. Thus, when considering the clinical investigation of the effects of 25(OH)D concentrations on bone health status, our results suggest that there appears to be no significant influence of skin color, but there is regarding gender. Therefore, it is suggested that the relationship between vitamin D and bone health be explored in more studies with mixed and gender-stratified populations.

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## Declarations

**Conflict of interest** Kátia Josiany Segheto, Leidjaira Lopes Juvanhol, Danielle Cristina Guimarães da Silva, Cristiane Junqueira de Carvalho, Fernanda Hansen, Mariana Papini Gabiatti, Adriana Maria Kakehasi, Giana Zarbato Longo declare that they have no conflict of interest.

## References

1. Hwang S, Choi HS, Kim KM et al (2014) 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* 26:163–171. <https://doi.org/10.1007/s0198-014-2877-0>
2. Steingrimsdottir L, Halldorsson TI, Siggeirsdottir K, Cotch MF, Einarsdottir BO, Eiriksdottir G, Sigurdsson S, Launer LJ, Harris TB, Gudnason V, Sigurdsson G (2014) Hip fractures and bone mineral density in the elderly—importance of serum 25-hydroxyvitamin D. *PLoS One* 9:e91122. <https://doi.org/10.1371/journal.pone.0091122>
3. Moayyeri A, Hammond CJ, Hart DJ, Spector TD (2012) Effects of age on genetic influence on bone loss over 17 years in women: The Healthy Ageing Twin Study (HATS). *J Bone Miner Res* 27:2170–2178. <https://doi.org/10.1002/jbmr.1659>
4. Gutiérrez OM, Farwell WR, Kermah D, Taylor EN (2011) Racial differences in the relationship between vitamin D, bone mineral density, and parathyroid hormone in the National Health and Nutrition Examination Survey. *Osteoporos Int* 22:1745–1753. <https://doi.org/10.1007/s00198-010-1383-2>
5. Demeke T, El-Gawad GA, Osmanovic A et al (2015) Lower bone mineral density in Somali women living in Sweden compared with African-Americans. *Arch Osteoporos* 10:208. <https://doi.org/10.1007/s11657-015-0208-5>



6. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM, Endocrine Society (2011) Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 96:1911–1930. <https://doi.org/10.1210/jc.2011-0385>
7. Fujiyoshi A, Polgreen LE, Hurley DL, Gross MD, Sidney S, Jacobs DR Jr (2013) A cross-sectional association between bone mineral density and parathyroid hormone and other biomarkers in community-dwelling young adults: the CARDIA study. *J Clin Endocrinol Metab*. 98:4038–4046. <https://doi.org/10.1210/jc.2013-2198>
8. Cosman F, Beur SJ, LeBorr MS et al (2014) Clinician's Guide to Prevention and Treatment for Osteoporosis. *Osteoporosis Int* 25: 2359–2381. <https://doi.org/10.1007/s00198-014-2794-2>
9. Thoreson CK, Chung ST, Ricks M, Reynolds JC, Remaley AT, Periwal V, Li Y, Sumner AE (2015) Biochemical and clinical deficiency is uncommon in African immigrants despite a high prevalence of low vitamin D: the Africans in America study. *Osteoporosis Int* 26:2607–2615. <https://doi.org/10.1007/s00198-015-3169-z>
10. Powe CE, Ricciardi C, Berg AH, Erdenesanaa D, Collierone G, Ankers E, Wenger J, Karumanchi SA, Thadhani R, Bhan I (2011) Vitamin D-binding protein modifies the vitamin D-bone mineral density relationship. *J Bone Miner Res* 26:1609–1616. <https://doi.org/10.1002/jbmr.387>
11. Joo N-S, Dawson-Hughes B, Yeum K-J (2013) 25-Hydroxyvitamin D, calcium intake, and bone mineral content in adolescents and young adults: analysis of the fourth and fifth Korea National Health and Nutrition Examination Survey (KNHANES IV-2, 3, 2008-2009 and V-1, 2010). *J Clin Endocrinol Metab* 98:3627–3636. <https://doi.org/10.1210/jc.2013-2013-1480>
12. Lim JS, Kim KM, Rhee Y, Lim SK (2012) Gender-dependent skeletal effects of vitamin D deficiency in a younger generation. *J Clin Endocrinol Metab* 97:1995–2004. <https://doi.org/10.1210/jc.2011-3098>
13. IBGE (2010) Censo Demográfico de 2010. <https://ww2.ibge.gov.br/home/estatistica/populacao/censo2010/default.shtm>. Acessado 01 março 2011
14. Silva NN (1998) Amostragem probabilística: um curso introdutório. EDUSP, São Paulo
15. Segheto W, Silva DCG, Coelho FA et al (2015) Body adiposity index and associated factors in adults: method and logistics of a population-based study. *Nutr Hosp* 32:101–109. <https://doi.org/10.3305/nh.2015.32.1.8391>
16. Lewiecki EM, Binkley N, Morgan SL, Shuhart CR, Camargos BM, Carey JJ, Gordon CM, Jankowski LG, Lee JK, Leslie WD (2016) Best practices for dual-energy X-ray absorptiometry measurement and reporting: International Society for Clinical Densitometry Guidance. *J Clin Densitom* 19:127–140. <https://doi.org/10.1016/j.jocd.2016.03.003>
17. Alves M, Bastos M, Leitão F, Marques G, Ribeiro G, Carrilho F (2013) Vitamin D - Importance of laboratory evaluation. *Rev Port Endocrinol Diabetes Metab* 8:32–39. <https://doi.org/10.1016/j.rpedm.2012.12.001>
18. Pardini R, Matsudo S, Araujo T et al (2001) Validation of the International Physical Activity Questionnaire (IPAQ version 6): pilot study in Brazilian young adults. *R bras Ci e Mov* 9:45–51. <https://doi.org/10.18511/rbcm.v9i3.393>
19. Haskell WL, Lee I, Pate RR et al (2007) Physical activity and public health: updated recommendation for adults from the American College of Sports Medicine and the American Heart Association. *Med Sci Sports Exerc* 39:1423–1434. <https://doi.org/10.1249/mss.0b013e3180616b27>
20. WHO (1998) Obesity: preventing and managing the global epidemic. [https://www.who.int/nutrition/publications/obesity/WHO\\_TRS\\_894/en/](https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/). Accessed March 01, 2011.
21. Segheto KJ, Juvanhol LL, Carvalho CJ et al (2020) Factors associated with bone mineral content in adults: A population-based study. *Einstein* 18:1–7. <https://doi.org/10.31744/einstein.journal/2020AO4694>
22. Silva ACV, Rosa MI, Fernandes B et al (2015) Factors associated with osteopenia and osteoporosis in women undergoing bone mineral density test. *Rev Bras Reumatol* 55:223–228. <https://doi.org/10.1016/j.rbr.2014.08.012>
23. Heaney RP, Holick MF (2011) Why the IOM recommendations for vitamin D are deficient. *J Bone Miner Res* 26:455–457. <https://doi.org/10.1002/jbmr.328>
24. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM (2012) Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. *J Clin Endocrinol Metab*. 97:1153–1158. <https://doi.org/10.1210/jc.2011-2601>
25. Ross AC, Manson JE, Abrams SA et al (2011) The (2011) report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 96:53–58. <https://doi.org/10.1210/jc.2010-2704>
26. Ferreira CES, Maeda SS, Batista MC, Lazaretti-Castro M, Vasconcellos LS, Madeira M, Soares LM, Borba VZC, Moreira CA (2017) Consensus-reference ranges of vitamin D [25(OH)D] from the Brazilian medical societies. Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC/ML) and Brazilian Society of Endocrinology and Metabolism (SBEM). *J Bras Patol Med Lab* 53:377–381. <https://doi.org/10.5935/1676-2444.20170060>
27. Shivane VK, Sarathi V, Lila AR, Bandgar T, Joshi SR, Menon PS, Shah NS (2012) Peak Bone Mineral Density and Its Determinants in an Asian Indian Population. *J Clin Densitom* 15:152–158. <https://doi.org/10.1016/j.jocd.2011.12.007>
28. Hannan MT, Litman HJ, Araujo AB, McLennan CE, McLean RR, McKinlay JB, Chen TC, Holick MF (2008) Serum 25-hydroxyvitamin D and bone mineral density in a racially and ethnically diverse group of men. *J Clin Endocrinol Metab*. 93:40–46. <https://doi.org/10.1210/jc.2007-1217>
29. Barbour KE, Zmuda JM, Horwitz MJ et al (2011) The association of serum 25-hydroxyvitamin D with indicators of bone quality in men of Caucasian and African ancestry. *Osteoporosis Int* 22:2475–2485. <https://doi.org/10.1007/s00198-010-1484-1>
30. Lips P (2007) Vitamin D status and nutrition in Europe and Asia. *J Steroid Biochem Mol Biol* 103:620–625. <https://doi.org/10.1016/j.jsbmb.2006.12.076>
31. Maeda SS, Borba VZC, Carmargo MB et al (2014) Recommendations of the Brazilian Society of Endocrinology and Metabolism (SBEM) for the diagnosis and treatment of hypovitaminosis D. *Arq Bras Endocrinol Metab* 58:411–433. <https://doi.org/10.1590/0004-2730000003388>
32. De Paula FJA, Rosen CJ (2012) Vitamin D safety and requirements. *Arch Biochem Biophys* 523:64–72. <https://doi.org/10.1016/j.abb.2011.12.002>
33. Sempos CT, Durazo-Arvizu RA, Dawson-Hughes B, Yetley EA, Looker AC, Schleicher RL, Cao G, Burt V, Kramer H, Bailey RL, Dwyer JT, Zhang X, Gahche J, Coates PM, Picciano MF (2013) Is there a reverse J-shaped association between 25-hydroxyvitamin D and all-cause mortality? Results from the U.S. nationally representative NHANES. *J Clin Endocrinol Metab* 98:3001–3009. <https://doi.org/10.1210/jc.2013-1333>
34. Amrein K, Quraishi SA, Litonjua AA, Gibbons FK, Pieber TR, Camargo CA Jr, Giovannucci E, Christopher KB (2014) Evidence for a U-shaped relationship between prehospital vitamin

- D status and mortality: a cohort study. *J Clin Endocrinol Metab* 99: 1461–1469. <https://doi.org/10.1210/jc.2013-3481>
35. Sakamoto R, Thorpe D, Knutsen R, Beeson L, Knutsen S (2018) Ethnic variations in serum 25(OH)D levels and bone ultrasound attenuation measurements in blacks and whites. *J Racial Ethn Health Disparities* 5:439–448. <https://doi.org/10.1007/s40615-017-0387-4>

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