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ORIGINAL ARTICLE

Serum 25 hydroxyvitamin D concentrations in individuals over 80 years old and their correlations with musculoskeletal and health parameters

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Abstract

Purpose The present study aims to evaluate the serum concentrations of 25 hydroxyvitamin D[25(OH)D] in individuals aged ≥80 years, independent, free-living in Sao Paulo, Brazil (Lat 23.5 °S), and to investigate their associations with musculoskeletal system, physical performance and health markers.

Method This cross-sectional study included 212 community dwellers aged ≥80 years and evaluated serum 25(OH)D, PTH, calcium, albumin, phosphorus, creatinine, bone markers, and bone mineral density. Physical performance was evaluated with stationary march, Flamingo, and functional reach tests, questionnaires to assess falls and fractures in the previous year, energy expenditure (MET), and Charlson index. Physical activity was evaluated with the International Physical Activity Questionnaire.

Results Vitamin D deficiency (<20 ng/mL) was observed in 56% and severe vitamin D deficiency (<10 ng/mL) in 13% of those individuals. Serum concentrations of 25(OH)D were significantly and positively associated with BMD total hip ($p = 0.001$), femoral neck ($p = 0.011$) and 33% radius ($p = 0.046$) BMDs, MET ($p = 0.03$) and functional reach test ($p = 0.037$) and negatively with age ($p = 0.021$), PTH ($p = 0.004$) and osteoporosis diagnosis ($p = 0.012$). Long-lived individuals with 25(OH)D ≥ 20 ng/mL had higher total hip and femoral neck BMDs ($p = 0.012$ and $p = 0.014$, respectively) and lower PTH ($p = 0.030$). In multiple linear regression analysis, age and osteoporosis diagnosis remained negatively associated with 25(OH)D levels ($p = 0.021$ and $p = 0.001$, respectively), while corrected calcium and cholecalciferol use remained positively associated ($p = 0.001$ and $p = 0.024$, respectively).

Conclusion We observed high vitamin D inadequacy prevalence in those Brazilian community dwellers' oldest old. Serum concentrations of 25(OH)D were positively associated with bone mass and dynamic balance, and negatively with PTH and osteoporosis diagnosis. Additionally, 25(OH)D ≥ 20 ng/mL was associated with better bone mass and lower PTH levels.

Keywords Vitamin D · Oldest old · Over 80 years · Community dwellers · Osteoporosis · Brazil

Introduction

The world's population is ageing, and chronic diseases, dependence, reduced quality of life and increased social costs will be challenges for public health systems worldwide, especially in developing countries. Between 2010 and 2050, the proportion of elderly people in developed countries is expected to grow by 56% (269 to 416 million) and in developing countries, by 224% (490 million to 1.6 billion) [1]. The age group with the highest relative growth corresponds to individuals aged 80 years or over (3.8% per year), with an estimated increase of 300% between 2015 and 2050 [2].

In this context, diseases related to the ageing process such as osteoporosis and events such as falls and fractures will grow. The estimated prevalence of osteoporosis varies according to the population studied and the methodology

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used, and was 34.9% among women aged ≥80 years in the USA [3] and 33.2% among Brazilian women aged ≥70 years [4]. As for falls, it is estimated that 1 in 3 individuals over the age of 60 years and 1 in 2 over 80 years will fall within 1 year [5].

Vitamin D, a marker related to falls and osteoporosis [6, 7], is also associated with sarcopenia [8], physical performance [9], health [10, 11], and mortality [12]. Recent studies have revealed the role of vitamin D in facilitating the crosstalk between skeletal muscle and bone by stimulating the production of factors such as osteocalcin, sclerostin, vascular endothelial growth factor (VEGF), IGF-1 and myostatin [13, 14]. In addition, vitamin D deficiency may be involved in reduced immune, antioxidant and anti-inflammatory capacity, increasing multi-organ insulin resistance, adipogenesis, lipogenesis, lipolysis and inflammation in adipose tissue, fueling not only obesity but also a new entity recognized as osteosarcopenic obesity [14]. Elderly people are at increased risk for the negative consequences of vitamin D deficiency, not only due to the lower endogenous production [15] and its greater metabolism through the interference of some drugs [16], but also due to the decrease in the renal activity of the 1-alpha-hydroxylase and in the number of vitamin D receptors, that occurs with age [15].

Studies that have estimated the prevalence of hypovitaminosis D in the elderly across the world are heterogeneous. Those that evaluated individuals aged 50 years or over and considered serum 25 hydroxyvitamin D [25(OH)D] concentrations <20 ng/mL as deficient observed a prevalence ranging from 3% to 91% [17]. In Brazil, 16% of individuals aged 50 years or over [18] and 58% of individuals over 65 years old [19] living in the community in São Paulo had serum concentrations of 25(OH)D < 20 ng/mL. Studies including people aged 80 years or more, especially those living in the community, are scarce. The present study aims to evaluate the profile of a sample of individuals aged 80 years or over, independent, living in the community in Brazil, and to describe the associations of their serum concentrations of 25(OH)D with musculoskeletal system and physical performance parameters and other markers of health.

Materials and methods

Study population

This cross-sectional study was conducted in a cohort of individuals aged 80 years or over who were participating in a Division of Geriatrics program at UNIFESP. Inclusion criteria were: age ≥80 years, living in the community, with the independence to perform basic activities of daily living and ability to walk without human support. Individuals with moderate or severe dementia, clinically decompensated chronic or acute diseases, terminal illnesses, history of hospitalization in the last 6 months, those who were on dialysis or who refused to participate in the study were excluded.The study included 214 individuals who met these criteria. Of these, 212 had the serum 25(OH)D concentration measured. Methods of enrollment in the initial study are described elsewhere [20].

This research project was approved by the Research Ethics Committee at UNIFESP, and all participants gave written informed consent prior to enrollment.

Clinical evaluation

Clinical evaluation was performed at the first visit including a questionnaire about personal history, identification of diseases, description and quantification of medications, and life habits. The Charlson comorbidity index of each individual was calculated based on these data [21]. A questionnaire about the number of falls and fractures in the previous year was answered by the individuals themselves and/or their companions. A fall was defined as an unintentional change in position resulting in coming to rest on the ground or other lower level such as a chair or stair [22]. History of fractures was accepted only when the story was reliable. The level of physical activity was determined by the International Physical Activity Questionnaire (IPAQ short form) [23] from which metabolic units (MET) were calculated and quantified [24].

Anthropometry

Body mass index (BMI) was calculated using weight and height and classified according to the Lipschitz criteria, as recommended for elderly individuals [25].

Laboratory tests

Approximately 12 h after the last meal, fasting blood samples were drawn from all participants in the morning. Concentrations of 25(OH)D were quantified by chemiluminescence (LIAISON 25 OH Vitamin D Total, DiaSorin, Stillwater, MN, USA; intraassay and interassay variation coefficients 1.6% and 5.6%, respectively). PTH was measured by immunochemiluminescence (Elecsys® 2010, Roche Diagnostics, Indianapolis, IN, USA; intraassay and interassay coefficients of variation 3.0% and 3.5%, respectively) and procollagen type 1 N-propeptide (P1NP) and serum C-terminal telopeptide of type 1 collagen (CTX) by electrochemiluminescence (Elecsys® 2010; intraassay and interassay coefficients of variation 1.8% and 2.7%, respectively, for P1NP and 4.6% and 4.7% respectively, for CTX).

The concentrations of albumin, total calcium, and phosphorus were measured by an automated colorimetric method, and those of creatinine, by alkaline picrate. Calcium was corrected for albumin levels [26]. The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation [27].

Densitometric evaluation

Dual Energy X-ray Absorptiometry (DXA) Hologic® (Discovery A, Waltham, MA, USA) was used to measure bone mineral density (BMD) at the lumbar spine (L1–L4), femoral neck, total hip, radius 33%, and to measure total body composition. The results were classified according to the World Health Organization (WHO) criteria [28]. The coefficients of variation were 0.8% for lumbar spine and total hip, and 1.2% for femoral neck. We calculated the participants' lean mass index (total lean mass in kilograms divided by the height squared in meters) [29], Baumgartner index [appendicular lean mass (ALM) divided by the height squared, with values above 5.45 kg/m^2 for females and 7.26 kg/m^2 for males considered normal] [30] and the ALM/BMI index (appendicular lean mass in kilograms divided by body mass index, with values equal to or above 0.512 for females and 0.789 for males considered normal) [31]. Based on these last two indices, the participants were classified as having low or adequate ALM.

Physical tests

The following physical tests were used to predict the risk of falls:

1. Flamingo balance test (assessment of static balance): the patient raises one leg forming a 90° angle with the femur, placing the hands on the waist. A stopwatch is started when the subject takes this position and is interrupted when any part of the body touches the ground or at the end of 30 s. The final score is the best of three attempts [32].
2. Functional reach (assessment of dynamic balance): a tape measure is attached to the wall, parallel to the floor, at the height of each individual's acromion, which is oriented perpendicular to the wall, with feet in parallel and arms extended at a 90° angle to the trunk. The individual is instructed to lean as far forward as possible and hold this position for 3 s without lifting the heels off the floor. The distance reached is recorded in centimeters. The best score of 3 repetitions is considered [33].
3. Stationary march - Step (assessment of aerobic capacity and lower limb strength): after prior training of a simulated gait, we count the number of times the right knee of the individual rises to at least the midpoint of the distance between the patella and the iliac crest, for a total duration of 2 min [32, 34].

Statistical analysis

Statistical analyses were performed using R, version 3.4.3 (The R Foundation for Statistical Computing, Vienna, Austria).

Twenty-five hydroxyvitamin D levels were analyzed as continuous variables and by the ranges less than 10 ng/mL (<10 ng/mL), 10 to 19.9 ng/mL (10–19.9 ng/mL) and greater than or equal to 20 ng/ml (≥20 ng/mL). Categorical variables are presented as absolute and relative frequencies, and continuous variables as measures of central tendency and dispersion: mean and standard deviation (SD) or median and interquartile range (25–75%), according to the normality of distribution assessed with the Kolmogorov–Smirnov test.

For normally distributed variables, linear associations were evaluated using Pearson's correlation and the comparison of mean values between two or more independent samples was performed using Student's *t* test and ANOVA, respectively. Tukey's multiple comparison test was used for post-hoc analysis. For non-normally distributed variables, linear associations were performed with Spearman's correlation and the mean ratings of two or more samples were compared using the Mann-Whitney or Kruskal-Wallis test alternatively. Dunn-Bonferroni test was used for post-hoc analysis. Fisher's or Pearson's exact or chi-square test were used to compare proportions between categorical variables.

Multiple linear regression models were used to evaluate the simultaneous effects of several variables on 25(OH)D using the R 4.0.2 software. To define the final model presented, the stepwise forward method with the Akaike information criterion (AIC) was used. For all tests, the level of significance was set at $p < 0.05$.

Results

Of the 212 participants (155 women and 57 men) with measured 25(OH)D serum concentrations, 3 (1.4%) had primary hyperparathyroidism (2 women and 1 man) and 9 were using calcitriol (6 women and 3 men) and were excluded from the sample. The median age of the remaining 200 participants was 85.5 years (83–90 years); 147 (73.5%) were women [median age 85 (83–90) years] and 53 (26.5%) were men [median 86 (84–89) years]. Almost 80% of the patients had a history of systemic arterial hypertension, 26.6% had diabetes mellitus, 53.6% had dyslipidemia, 24.6% referred established cardiovascular diseases (coronary insufficiency, cerebral vascular disease, peripheral

Table 1 Distribution of very old individuals by ethnicity, age group, body mass index (BMI), bone densitometry, 25(OH)D values, stages of renal function, International Physical Activity Questionnaire (IPAQ), Baumgartner, and appendicular lean mass/body mass index categories (ALM/BMI)

	n	%
Ethnicity	200	100.0
Caucasian	134	67.0
African	12	6
Asian	18	9
Hybrid (Black/White)	35	17.5
Indian	1	0.5
Age group (years)	200	100.0
80–85	100	50
86–90	63	31.5
91–95	31	15.5
≥96	6	3
BMI – classification	200	100.0
Low weight (<22 kg/m²)	25	12.5
Normal weight (22 to 26.9 kg/m²)	75	37.5
Excessive weight (≥27 kg/m²)	100	50
Bone densitometry - WHO classification	166	100.0
Normal	20	12
Osteopenia	85	51.2
Osteoporosis	61	36.8
No information	34	
25(OH)D values (ng/mL)	200	100.0
<10	26	13
10 to 19.9	86	43
20 to 29.9	59	29.5
≥30	29	14.5
eGFR stage by CKD (KDOQI)	200	100.0
1 (≥90 mL/min)	5	2.5
2 (60 to 89 mL/min)	87	43.5
3 (30 to 59 mL/min)	99	49.5
4 (15 to 29 mL/min)	9	4.5
5 (<15 mL/min)	0	0
IPAQ – Classification	155	100.0
Sedentary	19	12.3
Irregularly active A	17	11
Irregularly active B	23	14.8
Active	95	61.3
Very active	1	0.6
No information	45	
Baumgartner – Classification	162	100.0
Low appendicular lean mass	30	18.5
Adequate	132	81.5
No information	38	
ALM/BMI – Classification	162	100.0
Low appendicular lean mass	70	43.2
Adequate	92	56.8
No information	38	

WHO World Health Organization, *eGFR* estimated glomerular filtration rate, *CKD* chronic kidney disease, *KDOQI* The National Kidney Foundation - Kidney Disease Outcomes Quality Initiative

arterial disease), 3.6% had chronic obstructive pulmonary disease and 34% had a history of neoplasia. Five (2.5%) of them were taking anticonvulsants, 49 (24.5%) were on antiresorptives and none used systemic corticosteroids. Eighty (40%) were taking calcium supplements at doses ranging from 500 to 2500 mg/day and 78 (39%) were taking cholecalciferol at doses ranging from 400 to 2142 IU/day. Three (1.5%) participants were current smokers. Table 1 describes the demographic and clinical characteristics of the participants: most were Caucasians, overweight, 80 to 85 years old, active, had an adequate ALM according to the Baumgartner and ALM/BMI indices, and had osteopenia at densitometry. Information on previous falls and fractures in the last year was provided by 166 individuals, of whom 82 (49.4%) reported 179 falls (range of 0 to 10 per individual) in the previous year and 46 (27.7%) reported 2 or more falls. Eight fractures occurred after falling from a standing height in 6 individuals (1 man and 5 women), 2 of which occurred in the femur, 2 in the wrist, 1 in the lumbar vertebra, 1 in the rib, 1 in the phalanx and 1 in metacarpus.

Table 2 presents clinical and laboratory profiles of the participants. Inadequate concentrations of vitamin D [25(OH)D < 30 ng/mL] were observed in 85.5% of the subjects, and vitamin D deficiency (<20 ng/mL) was observed in 56%, whereas severe vitamin D deficiency (<10 ng/mL) was found in 13% of the population. Among those with deficiency [25(OH)D < 20 ng/mL], only 27.7% *(n = 30)* received some vitamin D supplementation, while 54% *(n = 47)* of those without deficiency were supplemented *($p = 0.0002$)*. Serum PTH levels were elevated (>65 pg/mL) in 65 (32.5%) subjects, of whom 42 (64.6%) had 25(OH)D < 20 ng/mL *($p = 0.089$)*. On the other hand, only 3 (15.7%) of those with 25(OH)D > 30 ng/mL had elevated PTH levels (>65 pg/mL) *($p = 0.006$)*. Table 2 also shows the differences between women and men: women had higher serum levels of corrected calcium and phosphorus and lower eGFR. Men had greater lumbar spine, femoral neck, total hip and 33% radius BMDs, as well as greater lean mass. Women had greater fat mass. Men performed more repetitions on the stationary march test.

When considering the whole group ($n = 200$), the PTH levels were the variable that showed the best correlation with 25(OH)D ($r = -0.255$; $p < 0.0001$), and the correlation remained significant after adjustment for renal function ($p < 0.005$). In Fig. 1, the mathematical equation $PTH = 19.79\ln[25(OH)D] + 116.7$ reached a $R^2 = 8.6\%$. A more pronounced increase in PTH is noted with 25(OH)D levels below 20 ng/mL.

To assess the relationship between 25(OH)D and bone mass, we excluded individuals who were taking antiresorptive drugs, leaving 148 individuals. The significant correlations found between 25(OH)D and the various parameters in this sample are described in Table 3. The 25(OH)D correlated with total hip (highest correlation: $r = 0.294$), femoral neck, 33% radius and total body BMDs. The correlation with PTH remained but showed a slight

Table 2 Clinical, laboratory and densitometric characteristics of the very old individuals participating in the study and description of characteristics between women and men

Variable	Reference values	Total Mean ± SD or Median [25–75%] (*n*)	Female (*n*)	Male (*n*)	P^{abc}value
Age	Years	85.5 [83.0–90.0] (200)	85 (147)	86 (53)	NS[a]
Weight	Kg	63.8 [55.5–69.7] (200)	60.4 (147)	69.5 (53)	<0.000[a]
Height	M	1.54 [1.48–1.60] (200)	1.51 (147)	1.63 (53)	<0.000[a]
BMI	22.0–26.9 kg/m^2	27.0 ± 4.3 (200)	27.0 (147)	26.9 (53)	NS[b]
25(OH)D	>30 ng/mL	18.1 [12.8–26.3] (200)	17.1 (147)	18.1 (53)	NS[a]
25(OH)D with supplementation	>30 ng/mL	23.3 ± 11.3 (78)	22.7 (70)	28.6 (8)	NS[a]
25(OH)D without supplementation	>30 ng/mL	16.9 [11.8–22.3] (122)	15.1(77)	17.4 (45)	NS[a]
PTH	15 to 65 pg/mL	52.2 [36.8–70.7] (200)	53.9 (147)	48.5 (53)	NS[a]
CTX	F: < 0.650 ng/mL; M: < 0.850 ng/mL	0.258 [0.154–0.391] (200)	0.268 (147)	0.232 (53)	NS[a]
P1NP	F (premenopausal): 13.8 to 60.9 ng/mL; M: 13.9 to 85.5 ng/mL	35.9 [23.1–52.3] (200)	37.4 (147)	33.7 (53)	NS[a]
Corrected calcium	8.8 to 10.6 mg/dL	9.2 [8.9–9.4] (198)	9.2 (145)	9.02 (53)	<0.001[a]
Albumin	3.4 to 4.8 g/dL	4.2 [4.0–4.5] (200)	4.2 (147)	4.2 (53)	NS[a]
Phosphorus	F: 2.3 to 4.3 mg/dL; M: 2.4 to 4.6 mg/dL	3.3 [3.0–3.6] (196)	3.4 (144)	3.0 (52)	<0.000[b]
Creatinine	F: 0.60 to 1.00 mg/dL; M: 0.80 to 1.20 mg/dL	0.94 [0.78–1.09] (200)	0.86 (147)	0.94 (53)	<0.000[a]
eGFRCKD	>90 mL/min	58.1 ± 15.3 (200)	56.7 (147)	62.1 (53)	0.041[b]
lumbar spine BMD	g/cm^2	0.897 [0.802–1.059] (164)	0.882 (117)	1.043 (47)	<0.000[a]
femoral neck BMD	g/cm^2	0.651 [0.579–0.745] (166)	0.624 (119)	0.725 (47)	<0.000[a]
total hip BMD	g/cm^2	0.758 [0.673–0.838] (166)	0.721 (119)	0.841 (47)	<0.000[a]
33% radius BMD	g/cm^2	0.585 ± 0.120 (97)	0.559 (75)	0.746 (22)	<0.000[a]
total body BMD	g/cm^2	0.999 ± 0.1256 (162)	0.962 (119)	1.102 (43)	<0.000[a]
BMC	G	1,780 [1,532–2,092] (162)	1,643 (119)	2,231 (43)	<0.000[a]
Lean mass	Kg	38.7 [34.6–44.2] (162)	36.4 (119)	47.5 (43)	<0.000[a]
Lean mass index	F > 13.8 kg/m^2 M > 16.8 kg/m^2	16.8 ± 2.1 (162)	16.3 (119)	18.1 (43)	<0.000[a]
ALM	F > 15.02; M > 19.75 kg	15.87 [13.52–18.60] (162)	14.47 (119)	19.39 (43)	<0.000[a]
Baumgartner	F > 5.45; M > 7.26 kg/m^2	6.83 ± 1.05 (162)	6.57 (119)	7.52 (43)	<0.000[a]
ALM/BMI	F ≥ 0.512; M ≥ 0.789	0.584 [0.512–0.683] (162)	0.545 (119)	0.755 (43)	<0.000[a]
Fat	Kg	22.4 ± 6.9 (162)	23,293.3 (119)	19,864.1	0.003[b]
%fat	%	34.6 ± 6.9 (162)	37.1 (119)	27.6 (43)	<0.000[a]
Flamingo	S	3.7 [1.9–9.4] (166)	3.6 (119)	3.4 (47)	NS[a]
Functional reach	Cm	23.8 ± 6.9 (165)	24 (118)	25 (47)	NS[a]
Stationary march	Rep/2 min.	49 [40–61] (165)	46 (118)	55 (47)	0.002[a]
Falls in the previous year	NA	0 [0–2] (166)	0 (119)	1 (47)	NS[a]

Table 2 (continued)

Variable	Reference values	Total Mean ± SD or Median [25–75%] (*n*)	Female (*n*)	Male (*n*)	P^{abc}value
Fractures in the previous year	NA	0 [0–0] (166)	5 (119)	1 (47)	NS[c]
Number of medications	NA	6 [4–8] (194)	6 (144)	5 (50)	NS[a]
Number of comorbidities	NA	4 [3–6] (199)	4(146)	4(53)	NS[a]
Charlson Index	NA	2 [1–3] (197)	2 (144)	2 (53)	NS[a]
MET	NA	819.0 [297.0–1368.0] (156)	840 (109)	765 (47)	NS[a]

BMI body mass index, *eGFR CKD* estimated glomerular filtration rate by the CKD-EPI formula, *PTH* parathyroid hormone, *CTX* C-terminal telopeptide of type 1 collagen, *P1NP* procollagen type 1 N-propeptide, *ALM* appendicular lean mass, *MET* energy expenditure, *NA* Not applicable, *NS* no significant difference between the group

[a]Wilcox test

[b]*t* test

[c]Fisher´s Exact

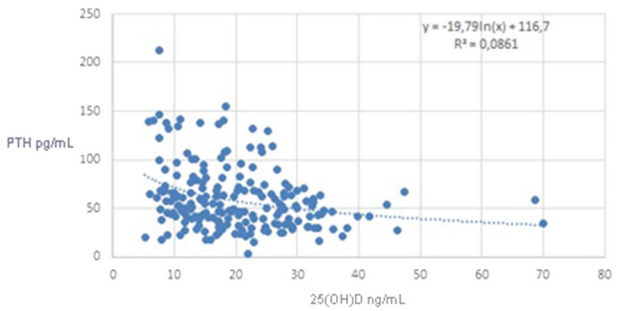

Fig. 1 Relationship between serum PTH and 25(OH)D values ($n = 200$), after excluding those with primary hyperparathyroidism ($n = 3$) or taking calcitriol ($n = 9$)

decrease ($r = -0.236$). However, PTH maintained the positive correlation with CTX and P1NP in the group not using antiresorptives ($r = 0.498$, $p < 0.0001$ and $r = 0.431$, $p < 0.0001$, respectively). We also obtained a negative correlation with age ($p = 0.021$) and a positive one with corrected calcium ($p = 0.040$), energy expenditure (MET) ($p = 0.03$) and functional reach test ($p = 0.037$). 25(OH)D did not correlate with the bone remodeling markers (CTX: $p = 0.486$ and P1NP: $p = 0.358$). We neither observed a correlation between serum 25(OH)D concentrations and the number of falls or fractures in the previous year ($p = 0.822$ and $p = 0.964$, respectively), sex ($p = 0.708$), ethnicity ($p = 0.688$), BMI ($p = 0.351$), diabetes diagnosis ($p = 0.487$), smoking ($p = 0.818$), nor with fat ($p = 0.386$) or fat percentage ($p = 0.763$) or appendicular lean mass ($p = 0.092$) and their indices - Baumgartner ($p = 0.059$) and ALM/BMI ($p = 0.164$).

Table 3 Statistically significant ($p < 0.05$) correlations* observed between 25(OH)D concentrations and clinical and laboratory variables without antiresorptives ($n = 148$)

	R	*p*
Positive correlations		
Corrected calcium	0.168	0.040
BMC	0.218	0.016
Total body BMD	0.209	0.021
33% radius BMD	0.237	0.046
Femoral neck BMD	0.227	0.011
Total hip BMD	0.294	0.001
Functional reach	0.186	0.037
MET	0.195	0.03
Negative correlations		
Age	−0.188	0.021
PTH	−0.236	0.004

PTH parathyroid hormone, *CTX* C-terminal telopeptide of type 1 collagen, *P1NP* procollagen type 1 N-propeptide

*Spearman's test

A multiple linear regression model was performed with the subgroup of 148 individuals who were not on anti-resorptive drugs. All the variables with a statistical significance of $p < 0.15$ in the univariate analysis were included: densitometric diagnosis, PTH classification (>65 or ≤65 pg/mL), IPAQ classification, age, corrected calcium, PTH levels, albumin levels, MET, Baumgartner index, ALM, BMC, total body BMD, lumbar spine BMD, femoral neck BMD, total hip BMD, functional reach, cholecalciferol use, furosemide use, number of comorbidities and calcium

Table 4 Results of the final linear regression model adjusted for 25(OH)D

Coefficient	Estimated	Standard error	*t* value	*p*-value
(Intercept)	5.60	16.27	0.34	0.731
corrected calcium	4.89	1.43	3.43	0.001
Densitometric diagnosis - osteoporosis	−4.72	1.40	−3.37	0.001
Use of cholecalciferol - yes	3.31	1.44	2.29	0.024
Age	−0.33	0.14	−2.33	0.021
R^2 adjusted = 0.189				

supplement dosage. The final adjusted model showed that, for each additional unit of corrected calcium, serum concentrations of 25(OH)D increased by an average of 4.9 ng/mL ($p = 0.001$). The 25(OH)D concentrations in those with a diagnosis of osteoporosis were on average 4.7 ng/mL lower than in those with DXA scans showing normality or osteopenia ($p = 0.001$). Those who used cholecalciferol had, on average, 3.3 ng/mL higher 25(OH)D values than those who did not use it ($p = 0.024$), and for each year added in age, the serum concentrations of 25(OH)D decreased by 0.33 ng/mL ($p = 0.021$) (Table 4). The BMD and BMC of all sites were included in the regression model but did not remain because they presented collinearity with densitometric diagnosis, which showed a better association with 25(OH)D in the final model.

The subgroup with 148 elderly was divided according to the 25(OH)D serum concentrations into 3 ranges (<10, between 10 to 19.9, and ≥20 ng/mL). Only 10 individuals had 25(OH)D ≥ 30 ng/mL, and they were included in the group with levels ≥20 ng/mL. We observed that individuals who had serum 25(OH)D concentrations ≥20 ng/mL had a lower proportion of osteoporosis diagnosis ($p = 0.009$; Fig. 2) and higher BMD of the total hip and femoral neck ($p = 0.012$ and $p = 0.014$, respectively; Fig. 2) than individuals with concentrations <10 ng/mL. For the lumbar spine, there was a trend towards higher BMD among individuals in the highest range of 25(OH)D levels than in the lowest ($p = 0.083$; Fig. 2). We also observed that the individuals in the ≥20 ng/mL range had lower serum PTH concentrations than those in the <10 ng/mL range ($p = 0.030$; Fig. 3) and a tendency to have a higher Baumgartner index and higher energy expenditure ($p = 0.094$ and $p = 0.056$, respectively; Fig. 3). Individuals in the <10 ng/mL range, in turn, were older than individuals in the other ranges (Fig. 3). The number of falls or fractures in the previous year was not different among the 3 ranges of 25(OH)D, but levels of 25(OH)D < 10 ng/mL tended to be more frequent in those who reported 2 or more falls ($p = 0.068$).

Discussion

This study evaluated serum 25(OH)D concentrations in a singular sample, characterized by individuals aged 80 years or more, living independently in the community and with chronic conditions under control, in São Paulo, Brazil (Latitude 23.5 °S). In this community-dwelling over-80 individuals, we observed a high percentage of vitamin D inadequacy. Serum levels of 25(OH)D < 20 ng/mL were observed in 56% of the oldest old, in line with the prevalence of 58.0% and 55.8% obtained by Lopes et al. and Saraiva et al., respectively, in individuals aged 65 years or over residing in the community in São Paulo [19, 35]. However, Saraiva et al. described a mean concentration of PTH higher than ours (82.5 pg/mL versus 61.4 pg/mL) and observed a prevalence of secondary hyperparathyroidism of 54% in these elderly people living in the community [35], higher than that we found (32.5%). Vitamin D supplementation was less prevalent in the study by Saraiva et al., collected 15 years earlier (9.5% versus 39.0% in our study), which may explain this difference. And it may reflect a greater awareness of the harmful effects of vitamin D deficiency conquered today and of the importance of supplementation in the elderly. These findings are relevant, as higher levels of PTH are associated with increased mortality in the elderly [36] and also may contribute to studies on the determination of the ideal 25(OH)D concentrations in this population.

We observed a 36.8% prevalence of osteoporosis, in agreement with the approximately 30% observed in the compilation of Brazilian data on postmenopausal women by Pinheiro et al. [37]; the 33.2% prevalence found in women aged ≥ 70 years in the city of São Paulo by Camargo et al. [4] and the 34.9% among women aged ≥ 80 years by Wright et al. [3]. The positive correlation found between BMD and 25(OH)D concentrations is in line with other studies [38–40], with the highest correlation observed in total hip BMD ($r = 0.294$). We could not find an association between low concentrations of 25(OH)D and fractures, as other authors reported in larger groups [41, 42], possibly because of the small number of fractures in our sample that probably compromised this analysis. Fractures were self-reported and vertebral fractures were not actively investigated, which probably underestimated the prevalence of fractures [43]. However, BMD itself can be considered a surrogate marker to assess fracture risk [44]. Moreover, the highly significant positive correlation found between PTH levels and the bone markers may be considered as another indicator of bone fragility associated with the poor status of Vitamin D observed in this population. Nonetheless, the effects of vitamin D supplementation on fracture events remain uncertain, with inconsistent interventional study results, some finding evidence of benefit, some reporting no

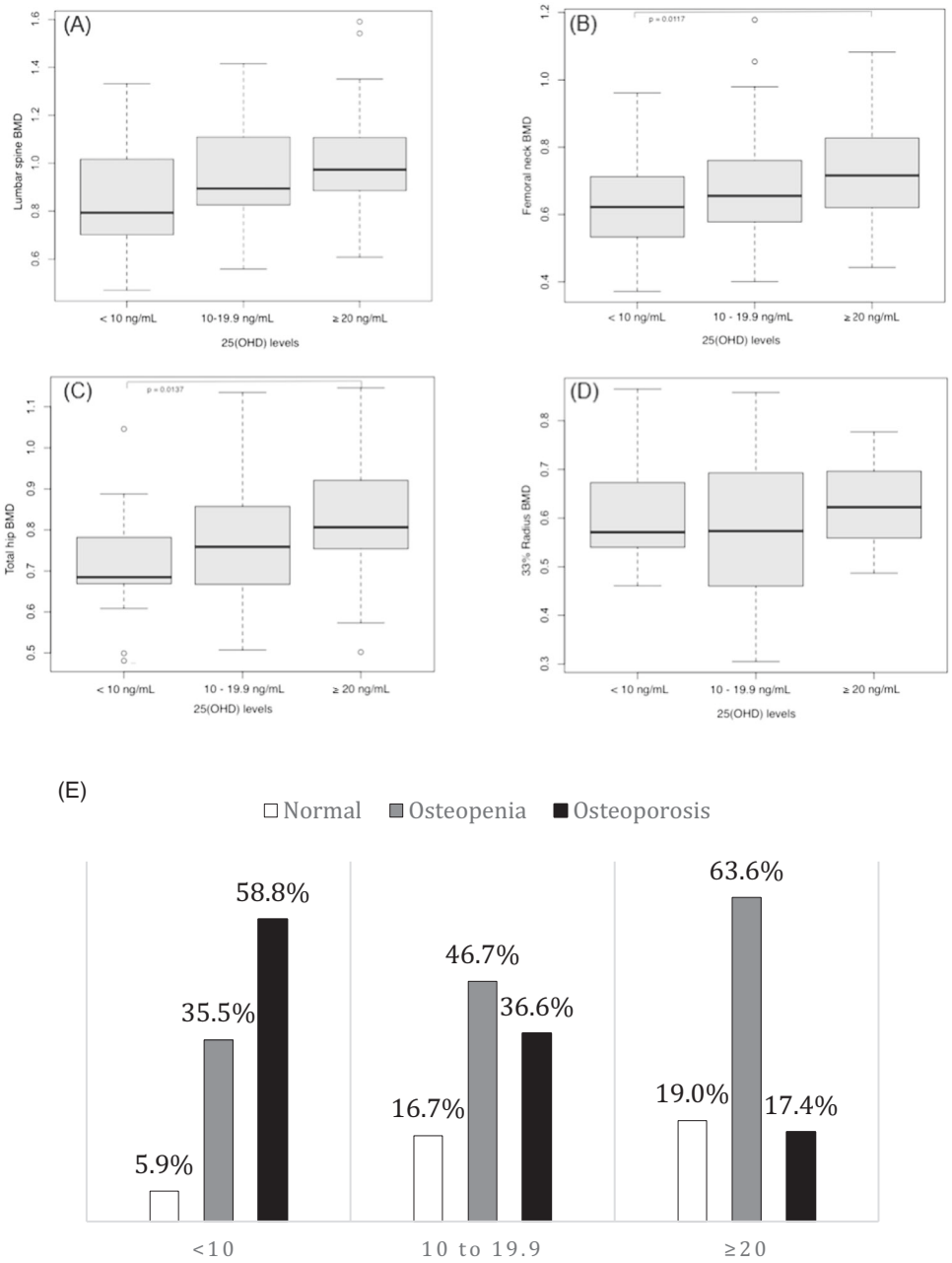

Fig. 2 Correlations between the ranges of serum 25(OH) D < 10 ng/mL, 10 to 19.9 ng/mL and ≥20 ng/mL and lumbar spine (**A**), femoral neck (**B**), total hip (**C**) and 33% radius BMD (**D**) and densitometric diagnosis (**E**)

effect, and others even showing harmful outcomes [45–48]. Explanations for these differences may include bolus administration of vitamin D [47], co-administration of vitamin D with other nutrients such as calcium [45, 48], and different baseline serum 25(OH)D concentrations, with a tendency to benefit those who initially have lower baseline concentrations [48].

The negative correlation between serum levels of 25(OH)D and PTH, although of small magnitude, has been consistently reproduced in the literature [6, 38, 39]. This could be explained by the fact that 25(OH)D acts directly on parathyroid cells, converting itself to its active form, forming the complex with its receptor and suppressing the PTH gene transcription, reducing the prevalence of secondary hyperparathyroidism [49]. The treatment of vitamin D deficiency usually decreases PTH concentrations and, consequently, the bone turnover [49].

We observed that the oldest olds with serum 25(OH)D concentrations in the range ≥20 ng/mL had a lower proportion of osteoporosis, higher BMD of total hip and femoral neck, lower PTH levels and a tendency to present greater appendicular lean mass by the Baumgartner index

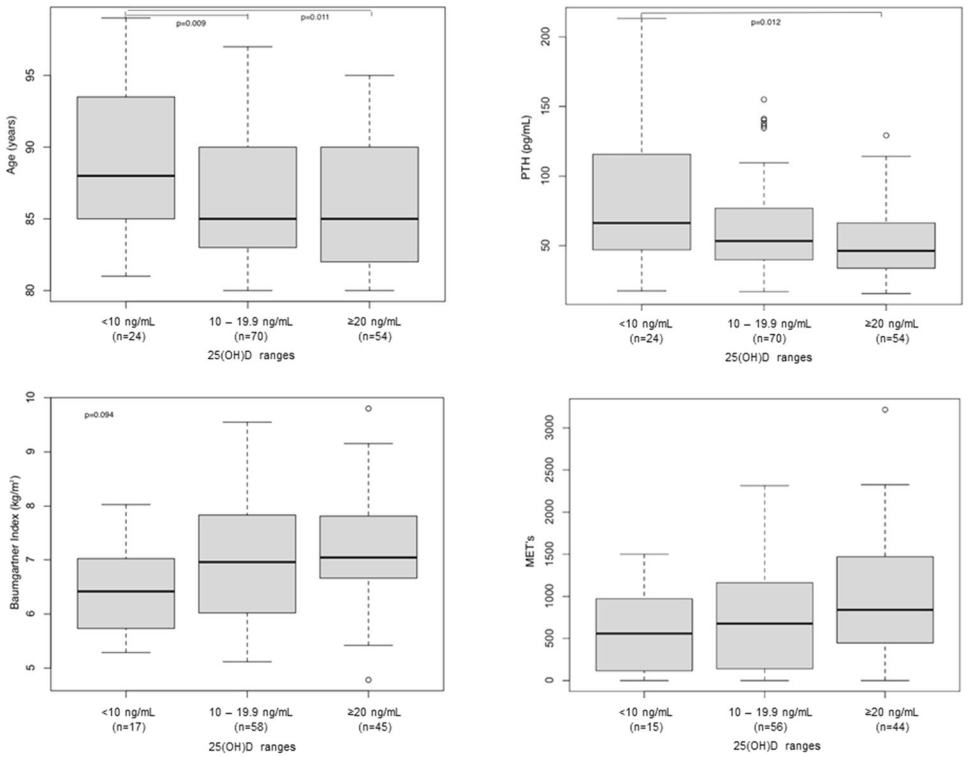

Fig. 3 Differences between the mean values of age, PTH, Baumgartner index and energy expenditure (MET) in the three 25(OH)D ranges

and greater energy expenditure (MET's). The range of 25(OH)D < 10 ng/mL, on the other hand, tended to have a higher frequency of individuals with 2 or more falls in the previous year ($p = 0.068$). Some studies support the characterization of low serum concentrations of 25(OH)D as a risk factor for elderly people in the community to present 2 or more falls in 1 year [6, 50].

One or more falls in the previous year were reported by 49.4% of evaluated participants, similar to the nearly 40% obtained in community elders aged 80 and over by Prudham [51]. Two or more falls were reported in 27.7%, similar to the 24.1% observed by Tromp in women aged 80 and over in the community [52]. Observational and experimental studies are conflicting in supporting vitamin D's protective effect on falls [41, 53, 54]. Whereas some authors found no decrease in the risk of falls with daily vitamin D supplementation in the elderly [53], others found it only in those elderly with 25(OH)D levels lower than 20 ng/mL [54]. Increased risk of falls was also described when high doses of cholecalciferol were given in bolus, monthly or annually [47, 55]. Although we found no association with serum 25(OH)D concentrations and falls in the previous year, we found a positive correlation with dynamic balance (functional range test). Dhesi et al. also observed a beneficial effect of vitamin D supplementation in 25(OH)D deficient elderly fallers only for reaction time and balance, but not for muscle strength [56]. Previous studies have also observed an association between 25(OH)D levels and dynamic balance [57, 58], suggesting that vitamin D may be related to improved neuromuscular function.

In the multiple linear regression analysis, age and osteoporosis diagnosis remained negatively and directly associated with serum concentrations of 25(OH)D, while the use of cholecalciferol was directly and positively associated with it. Thus, in this sample of octogenarians and nonagenarians, the processes related to aging exerted a direct influence on the decrease in 25(OH)D concentrations, which in turn may have caused damage to bone mass, increasing the diagnosis of osteoporosis. On the other hand, the use of cholecalciferol may have mitigated this effect.

Our study has several limitations, including its cross-sectional design, which prevents us from evaluating associations with future outcomes. In the absence of a control group, the findings of this study in independent very elderly individuals cannot be extrapolated to other younger or frail elderly populations. In addition, we did not have access to dietary information such as the average intake of calcium and vitamin D in the diet, nor the average exposure to the sun of these individuals. Blood samples were collected throughout the year, without reference to the season. The fractures were the result of historical reports and were not necessarily documented. Despite these limitations, the findings of our study are of great relevance in the current context, as life expectancy has grown exponentially around the world and data on this peculiar population of very elderly individuals are still scarce.

In conclusion, our study found a high prevalence of vitamin D inadequacy (13% <10 ng/mL, 56% <20 ng/mL and 85.5% <30 ng/mL) in this particular population. We observed that in these oldest old, serum concentrations of 25(OH)D were positively associated with bone mass and dynamic balance and negatively associated with PTH levels and osteoporosis diagnosis. Additionally, 25(OH)D ≥ 20 ng/mL was associated with better bone mass and lower PTH. Interventional studies should be carried out to confirm this data and to define the optimal vitamin D levels for this population.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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