Vitamin D deficiency is associated with cortical bone loss and fractures in the elderly

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Abstract

Introduction: The role of vitamin D on bone microarchitecture and fragility is not clear.

Objective: To investigate whether vitamin D deficiency (25(OH)D <20 ng/mL) increases cortical bone loss and the severity of fractures.

Design: Cross-sectional study of 287 elderly women with at least one prevalent low-impact fracture.

Methods: Biochemistry, X-rays to identify vertebral fractures (VFs) and to confirm non-vertebral fractures (NonVFs), and high-resolution peripheral quantitative computed tomography (HR-pQCT) to evaluate bone microstructure.

Results: Serum 25(OH)D levels were associated with body mass index (BMI: $r = -0.161$, $P = 0.006$), PTH ($r = -0.165$; $P = 0.005$), CTX ($r = -0.119$; $P = 0.043$) and vBMD at cortical bone (Dcomp: $r = 0.132$; $P = 0.033$) and entire bone (D100: $r = 0.162$, $P = 0.009$) at the distal radius, but not at the tibia. Age and PTH levels were potential confounding variables, but in the multiple linear regressions only BMI (95% CI: 0.11–4.16; $P < 0.01$), 25(OH)D (95% CI: -0.007 to 1.70 ; $P = 0.05$) and CTX (95% CI: -149.04 to 21.80 ; $P < 0.01$) predicted Dcomp, while BMI (95% CI: 1.13–4.18; $P < 0.01$) and 25(OH)D (95% CI: 0.24–1.52; $P < 0.01$) predicted D100. NonVFs predominated in patients with 25(OH)D <20 ng/mL ($P = 0.013$). Logistic regression analysis showed a decrease in the likelihood of presenting grade 2–3 VFs/NonVFs for every increase in 25(OH)D (OR = 0.962, 95% CI: 0.940–0.984; $P = 0.001$), BMI (OR = 0.932, 95% CI: 0.885–0.981; $P = 0.007$) and D100 at radius (OR = 0.994, 95% CI: 0.990–0.998; $P = 0.005$).

Conclusion: In elderly patients with prevalent fractures, vitamin D deficiency was associated with cortical bone loss and severity of fractures.

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Introduction

Vitamin D is an important regulator of skeletal health. Vitamin D content in diet is usually low and the main source of vitamin D in humans is the cutaneous conversion of 7-dehydroxycholesterol under ultraviolet light. In the elderly population, reduced sun exposure and atrophic skin impairs vitamin D synthesis (1). The consequences of vitamin D deficiency include a trend toward secondary hyperparathyroidism, increased bone turnover and bone loss (2, 3). Alterations in bone

density and microarchitecture and a propensity to fall also contribute to the occurrence of fragility fractures in these patients (1, 4). Some authors identify the hip as the site more susceptible to fractures associated with vitamin D deficiency and hyperparathyroidism (5, 6, 7, 8). Nevertheless, the impact of alterations in bone metabolism, bone microstructure and the type of fracture associated with vitamin D deficiency remains an open question.

Subjects and methods

Study population

This cross-sectional study included female patients from the Osteoporosis Prevention Study (OSTEOPREV) database that was held at the National Institute of Traumatology and Orthopedics and Federal University of Rio de Janeiro in Rio de Janeiro, Brazil. The study was performed with approval of the Institute Review Board (IRB no. 0005.0.305.000-09) and all participants provided written informed consent. The aim of the OSTEOPREV study was to evaluate patients at the age of 60 years and older who sustained a low-impact fracture at ≥ 50 years old, including VFs detected on radiological examination. After media advertising, 3200 people made a first contact by phone for a preliminary screening. From this total 893 met the inclusion criteria and were invited to attend a medical interview. One hundred forty-one volunteers did not meet the inclusion criteria on further examination and were thus excluded from the study. A total of 752 participants were referred for radiographic examination, and those with at least one grade 1 VF and/or one NonVF were submitted to laboratory tests. At the end of the screening process 340 patients (287 female) fulfilled the inclusion criteria (Fig. 1).

Patients with a fracture of the skull, facial bones, metacarpals, fingers, and toes, and fractures associated with high-energy trauma were not enrolled in the study. Exclusion criteria were alcoholism (>3 standard drinks per day), malignancy, chronic inflammatory diseases, AIDS, untreated hyperthyroidism or hypothyroidism, primary hyperparathyroidism, hypoparathyroidism, elevated aminotransferase levels and impaired renal function (estimated creatinine clearance <30 mL/min/1.73 m² as assessed by means of the CKD-EPI equation) (9). Current use of glucocorticoids, combined antiretroviral therapy for HIV-infection and anticonvulsants were also part of exclusion criteria. Anti-osteoporotic drugs (bisphosphonates, denosumab, strontium ranelate, teriparatide) should have been discontinued more than 1 year prior to study initiation. Patients with arterial hypertension and type 2 diabetes mellitus (T2DM) on adequate diet and in use of metformin as the only anti-diabetic drug were included in the study.

Height and weight were measured to calculate body mass index (BMI: weight/height²). Patients were assessed retrospectively for a history of falls in the last year (one or more falls per month was considered frequent falls).

X-rays

Frontal and lateral plain radiographs of thoracic and lumbar spine were performed in all participants in order to identify and classify VFs based on Genant's semiquantitative method according to deformities of vertebral bodies as grade 1 (mild: 20–25%), grade 2 (moderate: 25–40%) or grade 3 (severe: $>40\%$) (10). All radiographs were analyzed by two senior orthopedic surgeons (F P P and J A M G) and, in case of disagreement, the two authors reviewed the diagnosis to reach consensus. Previous NonVFs were also confirmed by new radiographs of the respective sites.

HR-pQCT

Volumetric bone density and bone microstructure were evaluated by high resolution peripheral quantitative computed tomography (HR-pQCT) using the X-TREME CT device (Scanco Medical AG, Bassedorf, Switzerland). The distal radius of the non-dominant arm and the ipsilateral distal tibia were used for analysis except in the case of a previous fracture in either region. From each site, 110 slices of 82 mm thickness (voxel dimension) were obtained and then digitized to generate a 3D representation of approximately 9 mm in the axial direction. The starting region for image acquisition was 9.5 mm proximal to a reference line positioned at the margin of the distal articular surface of the radius, perpendicular to the long axis of the diaphysis, and 22.5 mm proximal to a reference line positioned in the same way on the articular surface of the distal tibia. The parameters analyzed were total bone density (D100, mgHA/cm³), cortical bone density (Dcomp, mgHA/cm³), trabecular bone density (Dtrab, mgHA/cm³), cortical thickness (Ct.Th, mm), ratio of bone volume/tissue volume (BV/TV, 1), trabecular number (Tb.N, 1/mm), trabecular thickness (Tb.Th, mm), trabecular separation (Tb.Sp, mm), and standard deviation of trabecular number, which reflects the heterogeneity of the trabecular network (Tb.1/N.SD, mm). All HR-pQCT-derived bone values obtained at distal radius and distal tibia were considered for statistical analysis.

Biochemistry

Blood samples were drawn between 8:00 and 10:00 h after an overnight fast. Methods employed and reference values were as follows: calcium (RV: 8.5–10.1 mg/dL), phosphorus (RV: 3.0–4.5 mg/dL), albumin (RV: 3.5–5.5 g/dL) and creatinine (RV: 0.8–1.3 mg/dL) were measured by routine methods. The glomerular filtration rate (GFR) was

Figure 1

Flowchart of the selection strategy and inclusion/exclusion criteria in the study.

estimated by the Chronic Kidney Disease Epidemiology Collaboration equation (9). Serum 25(OH)D levels were assayed by electrochemiluminescence (Elecys kit 2010, Roche; RV: 30–100 ng/mL). According to the Endocrine Society guidelines, levels below 20 ng/mL (50 nmol/L)

indicated vitamin D deficiency, levels between 20 and <30 ng/mL identified vitamin D insufficiency, whereas values at or higher than 30 ng/mL corresponded to vitamin D sufficiency (1). Intact parathyroid hormone (PTH) levels were assayed by chemiluminescence (IMMULITE 2000 kit;

Siemens; RV: 11–65 pg/mL). Bone resorption was evaluated by serum levels of carboxyterminal telopeptide of type I collagen (CTX) measured by electrochemiluminescence (β -Cross Laps, Roche Diagnostics; RV for postmenopausal women=0.104–1.008 ng/mL).

Statistical analysis

In the descriptive analysis, categorical variables are expressed as percentages and their frequencies, whereas numerical variables are expressed as the mean $\pm$ s.d. A normal distribution was assessed using the Kolmogorov–Smirnov test. Student's *t*-test or the Mann–Whitney test was used to compare numerical variables, as appropriate. ANOVA or the Kruskal–Wallis test was used to compare numerical variables among three groups (25(OH)D <20 ng/mL, 20 to <30 ng/mL and $\geq$ 30 ng/mL), followed by Tukey's honestly significant difference *post hoc* test and a Bonferroni *post hoc* test, respectively. Correlations between numeric variables were analyzed using Pearson's correlation test. The χ^2 test or Fisher's exact test was applied to compare categorical variables. The Nagelkerke determination coefficient was used for the binary logistic regression model. A *P* value below 0.05 was considered statistically significant, and in the *post hoc* analysis, the *P* value was adjusted according to the analysis. Analyses were performed using the statistical program SPSS, version 20.0 for Mac (SPSS Inc.).

Results

Study population comprised 287 elderly women who had normal values of serum calcium (corrected for albumin) and phosphorus. GFR was estimated to

be $\geq$ 60 mL/min/1.73 m² in 250 patients and between 32 and 60 mL/min/1.73 m² in 37 patients. Serum PTH was >65 pg/mL in 44 patients (15.34%), and CTX was within the 0.104–1.008 ng/mL range in all patients. The characteristics of the sample are shown in Table 1.

Most patients diagnosed with a mild morphometric vertebral fracture were not aware of having osteoporosis, and thus, did not refer previous use of anti-osteoporosis drugs. Those who ever received alendronate or risedronate had it discontinued for more than 2 years before entering the study. None of the participants reported previous or current use of ibandronate, strontium ranelate, zoledronic acid, denosumab or teriparatide, or were receiving calcium and vitamin D supplements when they were allocated in the study.

Serum 25(OH)D was <20 ng/mL in 114 patients (D-deficient), between 20 and <30 ng/mL in 82 patients (D-insufficient), and $\geq$ 30 ng/mL in 91 patients (D-sufficient). Comparisons among these three subgroups showed that D-sufficient patients, as compared to those D-deficient, had significantly higher calcium (9.57 ± 0.56 vs 9.23 ± 0.68 mg/dL $P < 0.001$), higher phosphorus (3.70 ± 0.53 vs 3.53 ± 0.48 mg/dL; $P = 0.016$) and a trend toward higher D100 (285.00 ± 81.07 vs 262.45 ± 67.70 mgHA/cm³, $P = 0.07$) and higher Ct.Th (0.68 ± 0.22 vs 0.62 ± 0.20 mm, $P = 0.08$) at distal radius. D-sufficient patients, compared to those D-insufficient, also tended to have lower PTH values (42.01 ± 15.04 vs 48.66 ± 24.40 pg/mL, $P = 0.02$). Complete data of all comparisons are shown in Supplementary Table 1 (see section on [supplementary data](#) given at the end of this article).

Twenty-three patients with 25(OH)D below 20 ng/mL had PTH levels above normal limits. However, no significant differences in bone density or microstructure were observed when they were compared with D-deficient patients without secondary hyperparathyroidism.

Linear correlations are shown in Table 2. BMI had a negative influence on 25(OH)D levels, but a positive association with vBMD. 25(OH)D was inversely correlated with PTH and CTX. Finally, low 25(OH)D and high CTX were associated with decreased vBMD at the cortical and entire bone at the distal radius. No significant correlation was found with bone parameters measured at the distal tibia.

When the relationships between age, BMI, PTH, 25(OH)D, CTX and all HR-pQCT derived bone parameters were examined in a multiple linear regression, CTX (95% CI -149.04 – 21.80 ; $P < 0.01$), BMI (95% CI 0.11 – 4.16 ; $P < 0.01$), and 25(OH)D (95% CI -0.007 to 1.70 ; $P = 0.05$)

Table 1 Anthropometric and biochemical characteristics of the whole population of elderly women with prevalent low-impact fractures. Values are expressed as mean $\pm$ S.D.

Parameters	Mean $\pm$ s.d.	Reference values
Age (years)	70.8 $\pm$ 6.7	
Body mass index (kg/m ²)	29.4 $\pm$ 5.6	20–27
Serum calcium (mg/dL)	9.4 $\pm$ 0.6	8.5–10.1
Serum phosphorus (mg/dL)	3.6 $\pm$ 0.5	3.0–4.5
Serum albumin (g/dL)	4.1 $\pm$ 0.5	3.5–5.5
GFR (mL/min/1.73 m ²)	76.6 $\pm$ 15.0	$\geq$ 60
25(OH)D (ng/mL)	24.7 $\pm$ 12.9	30–100
Serum PTH (pg/mL)	46.5 $\pm$ 20.3	11–65
Serum CTX (ng/mL)	0.335 $\pm$ 0.188	0.104–1.008

Reference values considered the elderly/osteoporotic population. CTX, C-terminal telopeptide of type I collagen; GFR, glomerular filtration rate was estimated by the formula CKD-EPI; PTH, parathyroid hormone.

Table 2 Significant associations between anthropometry, biochemistry and HR-pQCT parameters at the distal radius using Pearson correlation test.

	25(OH)D	PTH	D100-R	Dcomp-R	Dtrab-R
25(OH)D	1				
<i>r</i>		-0.165	0.162	0.132	
<i>P</i>		0.005	0.009	0.033	
CTX					
<i>r</i>	-0.119	0.220	-0.148	-0.198	
<i>P</i>	0.043	<0.001	0.016	0.001	
BMI					
<i>r</i>	-0.161	0.280	0.186		0.129
<i>P</i>	0.006	<0.001	0.002		0.037

BMI, body mass index; CTX, C-telopeptide of type I collagen; D100, Dcomp and Dtrab, volumetric bone mineral density at entire bone, cortical and trabecular compartments, respectively, measured at the distal radius.

significantly predicted cortical density, and 25(OH)D (95% CI 0.24–1.52; $P < 0.01$) and BMI (95% CI 1.13–4.18; $P < 0.01$) significantly predicted total bone density at the distal radius.

Forty-three patients had previous NonVFs (wrist, ribs, shoulder, pelvis and femoral neck); in 22 of these patients, VFs were also detected by X-ray examination. The remaining 234 patients had only VFs. In the whole population, 556 fractures were confirmed (477 VFs and 79 NonVFs, 21 of which in the femoral neck).

To investigate the influence of vitamin D deficiency on fractures, patients with 25(OH)D blood levels < 20 ng/mL were compared with patients with levels ≥ 20 ng/mL. The prevalence of type 2 diabetes mellitus in patients considered D-deficient (15.8%) and all others (15.0%) was not significantly different ($P = 0.895$). Similarly, the prevalence of arterial hypertension in D-deficient patients (52.6%) and all others (53.1%) was not significantly different ($P = 0.913$). NonVFs were more likely to occur in patients with 25(OH)D < 20 ng/mL, whereas patients with vitamin D levels > 20 ng/mL had less severe fractures, classified as grade 1 VFs (Table 3). Using 30 ng/mL as the cutoff, the differences in fractures lost significance.

A binary logistic regression model was conducted to assess whether BMI, 25(OH)D, and D100 at the radius significantly predicted if the patient presented only a grade 1 VF or more severe fractures (grade 2–3 VF and/or NonVF). Considering the variables (BMI, 25(OH)D, and D100 at radius) that were statistically significant in the bivariate analysis, the model was statistically significant for predicting fracture grade. In fact, there was a decrease in the likelihood of presenting with a grade 2–3 VF/NonVF for every increase in BMI (OR = 0.932, 95% CI: 0.885–0.981; $P = 0.007$), 25(OH)D level (OR = 0.962, 95% CI: 0.940–0.984;

$P = 0.001$), and D100 at the radius (OR = 0.994, 95% CI: 0.990–0.998; $P = 0.005$). The Omnibus Tests of Model Coefficients indicates that when all three predictors are considered together, the equation is significant ($\chi^2 = 32.27$; $df = 3$; $n = 263$; R^2 Nagelkerke = 0.16; $P < 0.001$).

According to the structured questionnaire, 39.6% of the patients reported frequent accident falls in routine everyday activities. The influence of 25(OH)D levels on frequent falls was also tested, but there was no significant influence considering a cutoff value of 20 ng/mL ($P = 0.79$).

Discussion

This study in elderly women with osteoporosis and at least one low trauma fracture suggested that vitamin D deficiency has a negative impact on cortical bone density and is associated with the occurrence of severe vertebral and NonVFs.

Vitamin D plays an important role in skeletal homeostasis throughout life. The definition of vitamin D adequacy is still a matter of debate and varies according to the patient's age and overall health. Serum levels of 25(OH)D near 20 ng/mL (50 nmol/L) may be adequate for a large population (11), but many authors consider 30 ng/mL (75 nmol/L) as the minimum concentration to guarantee bone health in elderly people, especially in those with osteoporosis and fragility fractures (1, 12, 13).

In the elderly population with prevalent nontraumatic fractures included in this study, 25(OH)D levels were below 30 ng/mL in 196 patients (68.3%). Even considering

Table 3 Comparisons of vertebral and non-vertebral fractures between subgroups according to 25(OH)D levels. Values expressed as absolute number of fractures (number of fractures for 100 patients). Other non-vertebral fractures included wrist (Colles), ribs, shoulder and pelvis).

	25(OH)D		<i>P</i>
	<20 ng/mL (<i>n</i> = 114)	≥ 20 ng/mL (<i>n</i> = 173)	
Vertebral grade 1	100 (88)	205 (119)	0.031
Vertebral grade 2	41 (36)	58 (34)	0.811
Vertebral grade 3	28 (25)	45 (26)	0.889
Vertebral fractures (all grades)	169 (148)	308 (178)	0.097
Femoral neck	13 (11)	8 (5)	0.134
Other non-vertebral	31 (27)	27 (16)	0.093
Non-vertebral fractures	44 (39)	35 (20)	0.013
All fractures	213 (187)	343 (198)	0.575

20 ng/L as a cutoff, 114 patients (39.7%) were found to be vitamin D-deficient. Aging *per se* contributes to decreased vitamin synthesis in the skin, the main source of vitamin D in humans (2). The sequestration of vitamin D by adipose tissue is a possibility to be considered (14) as BMI was inversely correlated with 25(OH)D levels. The inverse correlation between PTH and 25(OH)D levels is possibly associated to low levels of dietary calcium previously described in the Brazilian population (15, 16), though not evaluated in this study.

Regardless of the mechanism, it is quite likely that the hormonal imbalance contributed significantly to the increase of the turnover and of the bone loss. This is consistent with findings of elderly populations (2, 3, 4, 7, 17) including a population survey in Brazil (18, 19).

Reports on the association of vitamin D deficiency and hip fracture (5, 6) suggest a preferential bone loss at the cortical compartment. Approximately 85% of the human skeleton is composed of compact (cortical) bone, demanding a great amount of calcium to mineralize newly formed bone matrix, which is provided by vitamin D-dependent intestinal calcium absorption. PTH hypersecretion has also been related to cortical bone loss in a study based on bone biopsies from patients with primary hyperparathyroidism (20). Although compact bone has less surface exposure for remodeling than trabecular bone, studies of bone samples taken from cadavers showed a progressive increase in intracortical bone resorption with aging, leading to increased cortical porosity (21). These changes contribute significantly to overall bone fragility, including at the vertebrae, where trabecular bone predominates (22).

The possibility of evaluating bone microstructure by a noninvasive method such as HR-pQCT greatly increased our knowledge of bone changes during the normal aging process (23, 24). Despite a modest correlation with microstructure parameters measured by histomorphometry (25), HR-pQCT is considered a reliable method to evaluate volumetric bone density and microarchitecture at the trabecular and cortical compartments. The measurement of cortical porosity and estimation of bone strength by Finite Element Analysis improve the ability to identify people at risk for fractures (26, 27, 28, 29, 30, 31).

Studies using peripheral computed tomography (pQCT and HR-pQCT) confirmed cortical derangement in primary hyperparathyroidism, but also showed that trabecular bone is not spared in this disease (32, 33). Few publications address the deleterious effect of vitamin D

deficiency on bone microstructure, and the participation of secondary hyperparathyroidism cannot be ruled out (34, 35). In this study, cortical bone loss was associated with bone turnover and vitamin D levels, but not with PTH. Furthermore, no difference was found between patients with 25(OH)D below 20 ng/mL with ($n=23$) or without ($n=91$) associated secondary hyperparathyroidism regarding bone density and microstructure. These findings are in agreement with the results of univariate and multivariate analyses. Also, a direct beneficial effect of vitamin D supplementation on bone microstructure and strength, rather than an effect mediated through lowering of PTH levels, was recently reported (36). Obesity may be beneficial for weight-bearing bones, although the positive effects on bone microstructure seem mostly related to lean mass, rather than to fat mass (37). In our study, BMI and 25(OH)D were independent predictors of cortical and entire bone vBMD, and the analysis of subgroups suggest that vitamin D levels ≥ 30 ng/mL may be of greater benefit for calcium-phosphorus homeostasis and bone microstructure.

All patients included in this study were selected based on at least one morphometric vertebral fracture; thus, we cannot associate hormone and bone derangements with the occurrence of fractures. For this reason, we chose to investigate the eventual contribution to the type of fragility fracture. The finding of a higher proportion of NonVFs in our patients with very low 25(OH)D levels seems consistent with more severe cortical bone loss, supported by the association of vitamin D and cortical bone density. Otherwise, less severe fractures, such as grade 1 VFs, were more common in those patients with higher vitamin D levels, who tended to have better bone density (D100) and cortical microstructure (Ct.Th). Cortical porosity has been identified as the main contributor to skeletal fragility at appendicular sites (38) and to the severity of VFs (39) in postmenopausal women. Cortical porosity was not measured in the current study, but our findings are consistent with studies describing this association (31, 40, 41, 42).

The increased bone fragility described in patients with type 2 diabetes mellitus (T2DM) is multifactorial and displays a preference for cortical bone (43, 44). Approximately 15% of the whole population presented T2DM, which might have had a negative impact on bone. However, T2DM was equally distributed in the subgroups of patients with 25(OH)D <20 and ≥ 20 ng/mL and thus could not explain the differences in bone microstructure and the increased frequency of NonVFs.

Arterial hypertension is also considered a risk factor for fractures, although treatment with anti-hypertensive drugs seems to counterbalance the deleterious effects on bone (45). Approximately half of the patients was treated for arterial hypertension but equally distributed in the vitamin D subgroups, reducing possible interference with our findings.

Vitamin D deficiency can also contribute to sarcopenia and falls, increasing the risk for fractures (46). In our study, muscle mass and function were not investigated, and we did not observe an association between vitamin D levels and falls.

The limitations of our study include the measurement of serum 25(OH)D by electrochemiluminescence, rather than using the gold standard method that is isotope dilution liquid chromatography-mass spectrometry, the cross-sectional design which limits defining causality, and the lack of comparison with age-matched patients without fractures to better investigate the impact of vitamin D deficiency on bone microstructure and fractures. A therapeutic intervention with vitamin D supplementation targeting levels at or higher than 30 ng/mL could help identify improvements in bone microstructure and incident fractures in this elderly population with prevalent fractures.

The main conclusion of this cross-sectional study is that vitamin D deficiency and consequent disruptions to calcium-PTH homeostasis, bone turnover, and bone microstructure, especially in the cortical compartment, are associated with the occurrence of severe VFs and NonVFs. These results support the biochemical evaluation of serum levels of vitamin D and supplementation in elderly patients at high risk for fractures. This is in accordance with recent guideline proposals from the European Calcified Tissue Society (47).

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-19-0197>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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