Association Between Serum 25-Hydroxyvitamin D Levels, Bone Geometry, and Bone Mineral Density in Healthy Older Adults

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Purpose. The association between serum 25-hydroxyvitamin D values and cortical/trabecular bone parameters in older adults has been incompletely explored. This study was designed to investigate the relationship between serum 25-hydroxyvitamin D levels and bone parameters for the tibia and radius using peripheral quantitative computed tomography in free-living healthy older adults.

Methods. The study involved 134 older adults attending a twice-weekly low-intensity fitness program. In addition to clinical history and serum parameters, we assessed fat-free mass using dual-energy X-ray absorptiometry, total bone and cortical bone cross-sectional areas, and trabecular and cortical bone mineral density for the tibia and radius by peripheral quantitative computed tomography.

Results. After applying multivariate linear regression models, adjusting for sex, age, body mass index, fat mass and fat-free mass, and creatinine, the association between 25-hydroxyvitamin D and bone parameters was significant for total bone and cortical bone cross-sectional areas in the radius (partial $R^2 = 0.05$ and 0.09, respectively) and for trabecular bone mineral density and cortical bone cross-sectional area in the tibia (partial $R^2 = 0.11$ and 0.02, respectively).

Conclusion. These findings support the idea that serum 25-hydroxyvitamin D levels and bone parameters are linked in older adults. Longitudinal studies are needed to establish whether vitamin D levels over time are associated with changes in these parameters.

Key Words: 25-OHD—Cortical bone area—Bone density—Older adults—pQCT.

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With aging, frailty fractures have become increasingly common worldwide. They now account for a large proportion of disability, death, and medical costs (1). Age-related changes in bone quality and geometry play an important part in causing frailty fractures. The bone loss related to aging is accompanied by a loss of trabeculae and fewer trabecular connections (2), more endocortical resorption and less periostial apposition (3,4). This is a key process in the onset of bone fragility in older age, especially in women (5).

Dual-energy X-ray absorptiometry (DXA) is now used routinely to assess bone mineral density (BMD) at different bone sites and to predict the risk of fracture. DXA is insensitive to changes in the quantitative and geometric distribution of trabecular and cortical bone tissues, however (6–8). Peripheral quantitative computerized tomography (pQCT) is a more reliable method for measuring volumetric BMD (mass/volume) because it distinguishes between cortical and trabecular bone components (5), which are known to respond differently to disease, loading and aging. pQCT also has the advantage of detecting bone geometry in distal segments of the limbs (9,10).

Among the factors influencing frailty fractures, serum 25-hydroxyvitamin D (25-OHD) level has received more attention recently (11,12). One of the most important functions of serum 25-OHD is to maintain skeletal calcium balance by promoting calcium absorption in the intestines; it promotes bone resorption by increasing the number of osteoclasts, it maintains the calcium and phosphate levels needed for bone formation, and it enables the parathyroid hormone to function properly in maintaining serum calcium levels.

Serum 25-OHD is important to bone and global health and in older adults because low serum levels of 25-OHD are common (13), predict reduced BMD (14) and a higher frailty
fracture risk (15), and are associated with an increased risk of disability (16) and cognitive decline (17,18).

Long-standing underdiagnosed serum 25-OHD deficiencies may already exist in earlier adulthood, exacerbating the negative effects of aging on bone.

Although there is a shortage of studies concerning the correlation between pQCT parameters and serum levels of 25-OHD, this association is well established from DXA studies. In a large cohort of more than 13,000 subjects, Heike and colleagues (19) found a significant positive association between 25-OHD and bone mineral density, with no gender-related differences. Focusing on older adults in particular, Von Muhlen and colleagues conducted a multiple linear regression analysis and found a positive association between serum 25-OHD levels and BMD in a large group of old women (20).

Hence, our interest in identifying any relationship between serum 25-OHD levels and bone parameters (measured using a reference method such as pQCT) in older adults. So far, only two studies have used pQCT to show an association between serum levels of vitamin D and bone parameters in older adults (2,21), but the information they provide on this relationship is incomplete. In fact, the InChiantI study found a relationship between 25-OHD and the cortical BMD of the tibia, but only in older women (2). Barbour and colleagues (21) found a positive association between 25-OHD levels and bone parameters concerning the radius and tibia in older Caucasian men, but they provided no data on older women.

Based on the hypothesis that higher serum 25-OHD levels would help to preserve bone structure in older adults, we used pQCT to investigate the relationship between serum 25-OHD levels and bone parameters for the tibia and radius in free-living, healthy older men and women.

**Materials and Methods**

*Subjects*

This cross-sectional cohort study was conducted at the Geriatrics Department of Padova University. Individuals older than 65 years who attended a twice-weekly low-intensity fitness program of aerobic and/or resistance exercises at public gyms in Padova were recruited on a voluntary basis. Trained medical personnel established that participants were healthy according to their clinical history, clinical examination, and biochemical test results.

The level of physical activity was explored using the NASA/Johnson Space Center Physical Activity Rating (PA-R) questionnaire, which contains seven categories of questions on a person’s regular exercising habits, scored according to the time spent on the physical activity and the type of activity (22). According to this scale, all participants were in class B, meaning they engaged regularly in a moderate physical activity.

Individuals with severe cardiovascular or pulmonary diseases, renal impairment (serum creatinine > 130 μmol/L), uncontrolled metabolic disease (diabetes, anemia, or thyroid disease), electrolyte abnormalities, cancer, or inflammatory conditions were excluded. Any use of drugs (corticosteroids, hormones, etc.) that might interfere with bone and body composition was also considered a reason for exclusion. A hundred and thirty-four older adults (86 women and 48 men) met the inclusion/exclusion criteria and were enrolled in this study.

Using the variance estimates for each bone characteristic obtained by pQCT in earlier studies conducted at our clinic, we performed a power analysis to assess the adequacy of the sample size in our statistical analysis. The sample size of 134 enabled us to estimate the bone characteristics to be within 4%, based on two-sided 95% confidence intervals. It also had a power of 82% in detecting an incremental R-squared value of 0.05 attributed to one independent variable in a multiple regression model, after controlling for six independent variables with a total R-squared of at least 0.15.

The study was designed in accordance with the Helsinki Declaration. All participants were fully informed about the study’s design and purpose, procedures, and risks, and they gave their informed consent.

**Study Design**

All patients underwent the following tests:

**Blood assays:** Fasting venous blood samples were collected for all patients to measure 25-OHD, parathyroid hormone (PTH), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and creatinine. 25-OHD was tested by RIA (Incestar Corp, Stillwater, MN), PTH by IRMA (Allegro TM Intact, Nicholas Institute, S. Juan Capistrano, CA), and IL-6 and TNF-α by IMMUNOLITE one (Medical System S.p.A., Genova, Italy), a solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay.

**Anthropometric measurements:** Body weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm using a standard balance and stadiometer (Seca, Germany), with participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as their weight in kilograms divided by their height in square meters.

**Bone measurements:** pQCT was performed on the dominant forearm and right tibia using the Norland/Stratec XCT-3000 scanner (Stratec Medizintechnik GmbH, Pforzheim, Germany), following a standardized patient positioning and scanning protocol. Forearm length was measured from the olecranon to the ulna styloid process, and tibia length from the medial malleolus to the medial condyle. A pQCT scout view was obtained to establish an anatomical reference line bisecting the medial edge of the end of the distal radius or tibia. Starting from this reference line, scans were obtained at sites 4% and 66% along the
length of the shaft for the radius and at sites 4%, 14%, 38%, and 66% along the length of the tibia. The following bone parameters were derived from the pQCT images:

Trabecular volumetric BMD (BMDt), that is, the mean density of the trabecular bone area detected at the 4% site on the radius and tibia.

Cortical volumetric BMD (BMDc), that is, the mean density of the cortical bone measured at the 66% and 38% sites on the radius and tibia, respectively.

Total bone cross-sectional area (CSA), that is, the area within the circumference delimiting all cortical bone tissues with a density higher than 180 mg/cm², measured at the 4% site on the radius and tibia (this is a measure of bone size).

Cortical bone cross-sectional area (CSAc), assessed as the cross-sectional area of the voxels with a density higher than 710 mg/cm², measured at the 38% site on the tibia and at the 66% point on the radius.

Body composition: We measured fat-free mass (FFM) and fat mass (FM) with Dual Energy X-ray Absorptiometry (DXA) using fan-beam technology (Hologic QDR 4500W, Inc.). The DXA method has a good reproducibility in determining soft tissue composition, achieving consistent results in older adults too (23).

Illness: The Cumulative Illness Rating scale (CIRS) assesses clinical status, comorbidities, and illness severity levels, classifying comorbidities among 13 organ systems and grading each condition from 1 (no problem) to 5 (severely incapacitating or life-threatening conditions). The comorbidity index (CI) is given by the number of conditions graded for severity as ≥3 (24). The Illness Severity Index (SI) is given by the average of all CIRS items.

Statistical Analysis

Statistical analyses were performed using the SPSS for Windows, rel. 17.0 (SPSS Inc. Chicago). For the data analysis, serum 25-OHD was treated as both a quantitative and a categorical variable. Four levels of serum 25-OHD values were classified as follows: ≤25; 25–50; 50–75; >75 nmol/L. These cut-offs have been amply used in the literature and roughly corresponded to quartiles in our sample population (Q1 = 28 nmol/L, Q2 = 52 nmol/L, Q3 = 75 nmol/L).

To choose the best data analysis approach, analysis of variance was used to test the interaction between sex and 25-OHD levels. Because the interaction term was never statistically significant, all statistical methods were applied to the whole sample and by 25-OHD level, whereas sex was included as a covariate in the multivariate analyses. Only the results regarding the bone characteristics measured by pQCT at the different sites were performed on the whole sample and by gender.

In the descriptive analyses, the prevalence of specific conditions was expressed as a percentage, and differences among groups were assessed using the χ² test. Normally distributed variables were reported as means ± standard deviations. The median and the first and third quartiles were calculated for ordinal variables (scores). Differences among mean values were assessed using Student’s unpaired t test, whereas differences among median values were analyzed using the unpaired Mann–Whitney test. The means of the quantitative variables for the different 25-OHD levels were compared by analysis of variance for unbalanced groups. The same procedure was used to obtain sex- and age-adjusted p values.

Pearson’s product-moment correlation coefficient (r) was applied to measure simple linear associations between pQCT parameters and serum 25-OHD values, age, BMI, FFM, FM, PTH, creatinine, albumin, IL6 and TNFα, comorbidity, severity of diseases, and physical activity score.

Multiple linear regression models were used with a step-wise forward procedure to assess the independent association among the bone parameters at each site and the 25-OHD values, including the factors found significantly associated with the previous simple linear analysis as covariates (ie, age, BMI, FM, FFM, serum creatinine, and sex). Partial and total R² coefficients were estimated, and R² was used as a fit criterion. A variance inflation factor was computed for each independent variable in the model to test for multicollinearity: Values more than 2 were used to indicate a multicollinearity problem in the model.

In all analyses, a p value less than .05 was considered statistically significant.

RESULTS

Table 1 shows the descriptive statistics for the anthropometric measurements, body composition, and serum parameters, for the whole sample and stratified by serum 25-OHD levels.

Participants averaged approximately 73 years of age, with a BMI around 26 and a mean albumin level of 44 g/L. Hypovitaminosis D (<75 nmol/L) was identified in about 75% of the sample.

Basically, there were no significant differences among the groups divided by 25-OHD level in terms of their general and clinical characteristics, except for age (p = .03) and height (sex- and age-adjusted p = .004).

The findings regarding comorbidities (CI) and severity of diseases (SI), as expressed by the CIRS, consistently confirmed that our study population was in substantially good health (the first and the third quartiles ranged from 0 to 2 for CI and from 1.10 to 1.35 for SI), with no significant differences among the 25-OHD groups.

The NASA scale also confirmed the sample’s homogeneity in terms of the participants’ physical activity scores, which ranged between 3 and 4.

Figure 1 shows the mean bone features identified by pQCT by sex. As expected, statistically significant
differences emerged among the genders for all bone characteristics, with significantly higher mean values in men ($p = .002$ and .02 for BMDe at the radius and tibia, respectively; $p < .0001$ for the other comparisons at both sites).

Table 2 shows the pQCT results for the bone characteristics in our whole sample and by 25-OHD level. After adjusting for sex and age, there were statistically significant differences for CSA ($p = .0004$) and CSAc ($p < .0001$) at the radius, and BMDe ($p = .04$), CSA ($p = .02$) and CSAc ($p = .01$) at the tibia.

Table 3 shows the simple correlations between the bone characteristics and a number of potential covariates. 25-OHD levels were linearly associated with all bone characteristics at the radius and tibia. Other covariates

Table 1. Mean Values of Anthropometry, Body Composition, Biochemical Parameters, Comorbidity Indices (CIRS), and Physical Activity Level (NASA) in the Sample as a Whole and by 25-OHD Levels

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total Sample, N = 134</th>
<th>≤25, N = 27, Mean ± SD</th>
<th>25–50, N = 36, Mean ± SD</th>
<th>50–75, N = 38, Mean ± SD</th>
<th>&gt;75, N = 33, Mean ± SD</th>
<th>Unadjusted p*</th>
<th>Sex–Age, Adjusted p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-OHD (nmol/L)</td>
<td>53.2 ± 28.1</td>
<td>19.6 ± 5.0</td>
<td>36.4 ± 7.5</td>
<td>63.5 ± 7.5</td>
<td>93.1 ± 14.3 (77–152)</td>
<td>&lt;.0001</td>
<td>—</td>
</tr>
<tr>
<td>Sex f (%)</td>
<td>64.2</td>
<td>70.4</td>
<td>66.7</td>
<td>52.6</td>
<td>69.7</td>
<td>n.s</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>72.9 ± 5.2</td>
<td>74.5 ± 5.3</td>
<td>73.7 ± 6.1</td>
<td>72.8 ± 4.3</td>
<td>70.9 ± 4.6</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.4 ± 9.0</td>
<td>156.8 ± 7.4</td>
<td>161.6 ± 9.0</td>
<td>164.6 ± 8.7</td>
<td>161.4 ± 9.2</td>
<td>.006</td>
<td>.004</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.7 ± 11.1</td>
<td>64.6 ± 11.5</td>
<td>67.9 ± 12.7</td>
<td>69.2 ± 8.2</td>
<td>68.3 ± 11.9</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9 ± 3.2</td>
<td>26.2 ± 3.9</td>
<td>25.9 ± 3.2</td>
<td>25.6 ± 2.6</td>
<td>26.1 ± 3.4</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>44.3 ± 2.3</td>
<td>44.2 ± 2.4</td>
<td>43.6 ± 2.9</td>
<td>44.7 ± 2.0</td>
<td>44.4 ± 1.9</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>20.7 ± 6.1</td>
<td>21.1 ± 7.1</td>
<td>20.6 ± 6.1</td>
<td>20.5 ± 6.2</td>
<td>20.6 ± 5.4</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>46.3 ± 9.1</td>
<td>42.6 ± 7.4</td>
<td>46.3 ± 9.3</td>
<td>48.6 ± 8.6</td>
<td>46.8 ± 10.1</td>
<td>.07</td>
<td>n.s</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>78.5 ± 17.4</td>
<td>77.1 ± 20.1</td>
<td>81.8 ± 19.7</td>
<td>78.5 ± 12.9</td>
<td>75.3 ± 17.2</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>3.2 ± 2.2</td>
<td>3.9 ± 3.3</td>
<td>3.0 ± 1.6</td>
<td>3.4 ± 2.6</td>
<td>2.7 ± 1.0</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>TNF (ng/L)</td>
<td>10.8 ± 5.0</td>
<td>10.9 ± 7.2</td>
<td>10.8 ± 4.1</td>
<td>10.0 ± 2.9</td>
<td>11.6 ± 5.6</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>PTH (ng/L)</td>
<td>88.2 ± 31.3</td>
<td>92.1 ± 32.8</td>
<td>86.5 ± 33.4</td>
<td>90.1 ± 26.9</td>
<td>88.2 ± 31.3</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>Comorbidity index, median (Q1–Q3)</td>
<td>1 (0–2)</td>
<td>1 (1.2)</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
<td>1 (0–2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Illness severity index, median (Q1–Q3)</td>
<td>1.21 (1.10–1.35)</td>
<td>1.21 (1.14–1.42)</td>
<td>1.28 (1.14–1.36)</td>
<td>1.14 (1.04–1.32)</td>
<td>1.21 (1.07–1.29)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Notes: BMI = body mass index; PTH = parathyroid hormone; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha; 25-OHD = 25-Hydroxyvitamin D; NASA = NASA/Johnson Space Center Physical Activity Rating (PA-R) questionnaire.

*p values obtained from a general linear model (F test) with 25-OHD levels included in the model as a categorical variable.

Figure 1. Mean values of bone characteristics by site and gender (vertical lines indicate the upper limit of the 95% confidence interval).
significantly associated with at least one bone characteristic were BMI, FFM, FM, and creatinine. As expected, given the homogeneity of our sample population, the comorbidity, disease severity, and physical activity indexes did not correlate with bone characteristics.

Table 4 shows the results of the multivariate linear regression models evaluating the independent association between the 25-OHD levels and the bone characteristics as dependent variables, adjusting for sex, age, BMI, FFM and FFM, and creatinine. The association between 25-OHD levels and bone parameters was significant for CSA and CSAc at the radius (partial $R^2 = 0.05$ and 0.09, respectively), and for BMDt and CSAc at the tibia (partial $R^2 = 0.11$ and 0.02, respectively).

**DISCUSSION**

This study identified a strong correlation between serum 25-OHD levels and bone parameters assessed by pQCT, suggesting an important but still incompletely elucidated role for vitamin D in contributing to bone density and bone area in older adults.

The high prevalence of older adults with low basal serum levels of 25-OHD in our study population has already been reported in many other publications (2, 25) and is attributable to an inadequate intake, scarce exposure to sunlight, and a declining vitamin D synthesis in the skin (25).

Our sample’s mean BMI corresponded to the 50th percentile of a reference older adult population in the ILSA study (26). Our exclusion criteria meant that our older adults’ total cumulative illness rating scores were low, indicating minimal comorbidities, so our participants could be seen as a sample of healthy older adults with a good nutritional status.

The association between 25-OHD and bone density was only evident for the trabecular bone regions in the tibia. Previous findings in rats showed that vitamin D deficiency results in a significant decline in trabecular bone density, but the reasons why 25-OHD is differently associated with

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**Table 2. Bone Mean Values of the Total Sample and by 25-OHD Levels by Peripheral Quantitative Computerized Tomography**

<table>
<thead>
<tr>
<th>Bone Characteristics</th>
<th>Total Sample, $N = 134$</th>
<th>$≤25, N = 27$</th>
<th>$25–50, N = 36$</th>
<th>$50–75, N = 38$</th>
<th>$&gt;75, N = 33$</th>
<th>Sex–Age, Unadjusted, $p^*$</th>
<th>Adjusted $p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radius</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BMDc (mg/cm$^2$)</td>
<td>1081.5 ± 47.7</td>
<td>1060.1 ± 48.2</td>
<td>1077.4 ± 45.3</td>
<td>1090.3 ± 47.9</td>
<td>1093.8 ± 44.8</td>
<td>.03</td>
<td>n.s</td>
</tr>
<tr>
<td>BMDt (mg/cm$^2$)</td>
<td>167.8 ± 54.6</td>
<td>145.9 ± 44.3</td>
<td>166.5 ± 66.3</td>
<td>178.0 ± 52.8</td>
<td>175.8 ± 46.8</td>
<td>.09</td>
<td>n.s</td>
</tr>
<tr>
<td>CSA (mm$^2$)</td>
<td>409.6 ± 86.2</td>
<td>350.0 ± 82.8</td>
<td>392.3 ± 81.7</td>
<td>435.6 ± 88.3</td>
<td>433.6 ± 76.0</td>
<td>.004</td>
<td>.0004</td>
</tr>
<tr>
<td>CSAc (mm$^2$)</td>
<td>74.1 ± 22.2</td>
<td>50.2 ± 16.0</td>
<td>70.1 ± 17.6</td>
<td>80.1 ± 18.4</td>
<td>85.0 ± 22.0</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td><strong>Tibia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMDc (mg/cm$^2$)</td>
<td>1051.6 ± 99.3</td>
<td>1016.6 ± 49.6</td>
<td>1042.7 ± 51.3</td>
<td>1051.4 ± 53.5</td>
<td>1088.9 ± 173.4</td>
<td>.02</td>
<td>n.s</td>
</tr>
<tr>
<td>BMDt (mg/cm$^2$)</td>
<td>192.1 ± 41.0</td>
<td>172.9 ± 35.7</td>
<td>191.1 ± 43.6</td>
<td>192.9 ± 37.8</td>
<td>207.6 ± 41.6</td>
<td>.05</td>
<td>.04</td>
</tr>
<tr>
<td>CSA (mm$^2$)</td>
<td>1219.6 ± 193.9</td>
<td>1144.1 ± 201.6</td>
<td>1160.6 ± 165.8</td>
<td>1264.0 ± 204.1</td>
<td>1267.3 ± 188.1</td>
<td>.06</td>
<td>.02</td>
</tr>
<tr>
<td>CSAc (mm$^2$)</td>
<td>268.4 ± 59.2</td>
<td>229.2 ± 47.2</td>
<td>259.6 ± 55.8</td>
<td>284.8 ± 55.6</td>
<td>279.4 ± 63.5</td>
<td>.02</td>
<td>.01</td>
</tr>
</tbody>
</table>

**Notes:** BMDc = cortical bone mineral density; BMDt = trabecular bone mineral density; CSA = total cross-sectional area; CSAc = cortical cross-sectional area.

$^*$p values obtained by general linear models $F$ test, with 25-OHD level included in the model as categorical variable.

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**Table 3. Simple Correlation Between Bone Parameters at Radius and Tibia Sites and Independent Variables**

<table>
<thead>
<tr>
<th></th>
<th>Radius</th>
<th>Tibia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BMDc</td>
<td>BMDt</td>
</tr>
<tr>
<td>Age</td>
<td>-0.29**</td>
<td>-0.18*</td>
</tr>
<tr>
<td>BMI</td>
<td>0.26**</td>
<td>-0.02</td>
</tr>
<tr>
<td>FFM</td>
<td>0.25*</td>
<td>0.46***</td>
</tr>
<tr>
<td>FM</td>
<td>-0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>PTH</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.07</td>
<td>0.20*</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>IL6</td>
<td>-0.10</td>
<td>0.11</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.02</td>
<td>0.14</td>
</tr>
<tr>
<td>CI</td>
<td>-0.09</td>
<td>-0.11</td>
</tr>
<tr>
<td>SI</td>
<td>-0.10</td>
<td>-0.10</td>
</tr>
<tr>
<td>NASA</td>
<td>0.07</td>
<td>0.02</td>
</tr>
<tr>
<td>25-OHD</td>
<td>0.20*</td>
<td>0.22*</td>
</tr>
</tbody>
</table>

**Notes:** BMDc = trabecular bone mineral density; BMDt = cortical bone mineral density; CSA = total cross-sectional area; CSAc = cortical cross-sectional area; FFM = fat-free mass; FM = fat mass; BMI = body mass index; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-alpha; 25-OHD = 25-Hydroxyvitamin D; CI = Comorbidity Index; SI = Severity index; NASA = NASA/Johnson Space Center Physical Activity Rating (PA-R) questionnaire.

$^p < .05; ^**p < .01; ^***p < .001; ^****p < .0001.$
Previous studies have shown that vitamin D supplementation has the effect of increasing bone parameters. For example, a pQCT analysis demonstrated a close relationship between cortical CSA and bending fracture load, suggesting that vitamin D may increase bone strength, at least partially by increasing bone mass and a greater gravitational load, contributing to determining bone parameters at the tibial site (especially in men) and that the effects of vitamin D are not actually site and gender related, but its action may be masked by other factors at some sites (such as the tibia).

Cortical bone area is a good measure of total cortical bone mass and a valid marker of bone resistance to compression and tensile loads (27). It has also been demonstrated in rats that vitamin D supplementation has the effect of increasing a bone’s size rather than its density (28). This was confirmed by a pQCT analysis that demonstrated a close relationship between cortical CSA and bending fracture load to x and y axes ($R^2$ ranged from 0.50 to 0.60 at both sites and in both genders; data not shown).

This unexpected association between vitamin D and several bone components measured using pQCT in older adults prompts several considerations. The lack of data in the literature on the relationship between 25-OHD and the bone parameters assessed by pQCT in older adults make our results difficult to compare. Chronic hypovitaminosis D would most likely have a negative impact on bone, and findings based on a single measurement may not reflect an individual’s real long-term exposure. On the other hand, we had measured 25-OHD levels in a subgroup of 92 individuals 3 years before the present study, and there was no difference between the two values (concordance coefficient = 0.85; unpublished data).

An expected outcome of our study was the finding that FFM and FM correlated with some of the bone parameters in our participants. Using the pQCT method enabled us to investigate the influence of body composition on cortical and trabecular bones, better clarifying the different effects of FFM and FM on the bone parameters considered. In our sample, FFM correlated particularly with cortical bone, emphasizing the effect of mechanical stress on cortical bone, on which the muscles exert their action through their insertion in the bone.

The effect of FM on bone seems to derive from a different mechanism (29–34). Adipose tissue is a possible source of estrogen, which may help to prevent bone mineral loss. Estrogen appears to be a major regulator of bone metabolism in men and in women (35), through its effect on RANKL and on inflammatory cytokines such as TNF-α and IL-1β, and possibly on the Wnt inhibitor, sclerostin (35). This study has several limitations. First, our participants were volunteers, not a random sample of older adults, so our findings may not be generally applicable to more frail, nonambulatory older adults in nursing homes. In addition, we assessed the relationship between vitamin D and bone strength based on bone density and geometry, not the real fracture risk. The study’s cross-sectional design also prevents us from attempting any causal inferences on the effect of 25-OHD levels on the way in which bone parameters change during an individual’s life span. On the other hand, our study has the advantage of being the first (to the best of our knowledge) to provide a complete picture of the correlation between vitamin D levels and bone parameters regarding the tibia and radius of healthy older adults.

In conclusion, vitamin D seems to have a marked influence on bone parameters, and 25-OHD levels could be used as a simple marker of bone structure and strength. Longitudinal studies on 25-OHD levels might help to predict changes in bone parameters and would make a valuable contribution to the literature, shedding light on the effect of chronic exposure to low or high vitamin D levels and extending this investigation to more specific outcomes, such as fracture risk.

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**Authors’ roles:** study design: GS and MM; conduction of the study: MM, AC, GS, LB; data collection: SS, AL, MM, FB, and GR; data analysis: SS, MM, EP; data interpretation: GS, MM, AC; drafting of the manuscript: MM GS; revision of manuscript content: GS; approval of the final version: MM GS EP EM; GS, AC, and EM take responsibility for the integrity of the data analysis.
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