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Low serum vitamin D is associated with higher cortical porosity in elderly men

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Abstract. Sundh D, Mellström D, Ljunggren Ö, Karlsson MK, Ohlsson C, Nilsson M, Nilsson AG, Lorentzon M (Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg; Center for Bone and Arthritis Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg; Uppsala University, Uppsala; Lund University, Lund; Skåne University Hospital, Malmö). Low serum vitamin D is associated with higher cortical porosity in elderly men. *J Intern Med* 2016; **280**: 496–508.

Background. Bone loss at peripheral sites in the elderly is mainly cortical and involves increased cortical porosity. However, an association between bone loss at these sites and 25-hydroxyvitamin D has not been reported.

Objective. To investigate the association between serum levels of 25-hydroxyvitamin D, bone microstructure and areal bone mineral density (BMD) in elderly men.

Methods. A population-based cohort of 444 elderly men (mean ± SD age 80.2 ± 3.5 years) was investigated. Bone microstructure was measured by high-resolution peripheral quantitative computed tomography, areal BMD by dual-energy X-ray absorptiometry and serum 25-hydroxyvitamin D and parathyroid hormone levels by immunoassay.

Results. Mean cortical porosity at the distal tibia was 14.7% higher (12.5 ± 4.3% vs. 10.9 ± 4.1%, $P < 0.05$) whilst cortical volumetric BMD, area, trabecular bone volume fraction and femoral neck areal BMD were lower in men in the lowest quartile of vitamin D levels compared to the highest. In men with vitamin D deficiency (<25 nmol L^{-1}) or insufficiency [25–49 nmol L^{-1}, in combination with an elevated serum level of parathyroid hormone (>6.8 pmol L^{-1})], cortical porosity was 17.2% higher than in vitamin D-sufficient men ($P < 0.01$). A linear regression model including age, weight, height, daily calcium intake, physical activity, smoking vitamin D supplementation and parathyroid hormone showed that 25-hydroxyvitamin D independently predicted cortical porosity (standardized β = −0.110, R^2 = 1.1%, P = 0.024), area (β = 0.123, R^2 = 1.4%, P = 0.007) and cortical volumetric BMD (β = 0.125, R^2 = 1.4%, P = 0.007) of the tibia as well as areal BMD of the femoral neck (β = 0.102, R^2 = 0.9%, P = 0.04).

Conclusion. Serum vitamin D is associated with cortical porosity, area and density, indicating that bone fragility as a result of low vitamin D could be due to changes in cortical bone microstructure and geometry.

Keywords: cortical porosity, high-resolution peripheral computed tomography, vitamin D.

Introduction

Serum calcium is tightly regulated, and vitamin D is a key player in the regulation of calcium homoeostasis as it stimulates the intestinal absorption of calcium. In patients with vitamin D deficiency, insufficient absorption of dietary calcium will lead to an elevation of serum parathyroid hormone (PTH) levels and a negative calcium balance in the bone tissue to maintain serum calcium levels [1]. Vitamin D deficiency can therefore contribute to the development of osteoporosis and, if severe, results in mineralization defects and rickets in children and osteomalacia in adults [2]. Serum levels of 25-hydroxyvitamin D (25-OH-vitamin D) reflect the dietary intake and the skin

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production of vitamin D and can be measured to assess vitamin D status [3].

Osteoporosis is a disease characterized by low areal bone mineral density (aBMD) measured by dual-energy X-ray absorptiometry (DXA). Using high-resolution peripheral quantitative computed tomography (HR-pQCT), bone microstructure can now be assessed noninvasively at peripheral sites [4]. Bone loss at older ages has been shown to occur predominantly in cortical bone and is associated with declining oestradiol levels [5]. Such bone loss is due to larger accessible areas for bone resorption in cortical compared to trabecular bone, which results in cortical remnants and lower bone strength [6]. Cortical porosity has previously been associated with prevalent fracture in men and women [7, 8].

A positive relationship between serum levels of 25-OH-vitamin D and aBMD has been found in some cohorts [9, 10], whilst the association with bone microstructure remains unclear. In one study, an association was found between higher trabecular number at the tibia and higher levels of 25-OH-vitamin D in younger (<65 years) but not in older (≥65 years) men, whilst a higher level of PTH was negatively correlated with cortical thickness and trabecular density and thickness [11]. Recently, Boyd *et al.* [12] reported a lack of association between 25-OH-vitamin levels and most bone microstructural parameters in a cohort of men and women receiving high-dose vitamin D supplementation.

A 25-OH-vitamin D level above 50 nmol L^{-1} has been reported to be sufficient for the vitamin D requirements for at least 97.5% of the population [13]. This concentration has also been suggested as a threshold value, where levels of bone resorption markers start to reach a plateau [14]. Nevertheless, there is no consensus regarding when to treat patients for vitamin D insufficiency. In a recent review by Rizzoli *et al.* [15], levels were defined for vitamin D deficiency (<25 nmol L^{-1}) and insufficiency (25–50 nmol L^{-1}). Complications of vitamin D deficiency include potential mineralization defects; for insufficiency, complications include increased bone turnover and/or PTH [15].

The aim of this study was to investigate whether low 25-OH-vitamin D is associated with high cortical porosity and, if so, whether this association also applies to a subcategory of men eligible for vitamin D supplementation.

Methods

Study subjects

The Osteoporotic Fractures in Men Study (MrOS) is a prospective multicentre study, as previously described [16]. This study consists of a subsample, as previously reported [8], of men included in Gothenburg (n = 1010) as part of the Swedish MrOS cohort (n = 3014) [17, 18]. Study subjects (men aged 69–81 years old at baseline) were randomly selected via the Swedish Population Register. Subjects were asked to participate by letter and telephone. To be entitled to participate in the study, subjects had to be able to walk without aid, sign an informed consent form and complete a questionnaire. At baseline for the Swedish part of the MrOS study, the inclusion rate was 45% [19].

All living men from the baseline study were contacted by letter followed by telephone and asked to participate in the follow-up examination. Of the original 1010 study subjects from the Gothenburg cohort, 600 men participated in the follow-up examination leading to an inclusion rate of 59.4%. The last 478 men included in the follow-up study were examined using HR-pQCT [8]. A total of 456 subjects had HR-pQCT measurements of high enough quality to allow their use in this study (see below). This study is solely based on results from 444 (44.0% of the original baseline cohort) of these men in whom serum 25-OH-vitamin D was also analysed. These men were younger at baseline (mean ± SD age 74.5 ± 3.0 years) than those who did not participate in the follow-up study (n = 566; 75.9 ± 3.2 years, $P < 0.001$). No significant differences could be seen in height and weight (176.0 ± 6.4 vs. 175.5 ± 6.4 cm, and 81.3 ± 11.2 vs. 80.8 ± 13.0 kg, respectively). The study was approved by the Ethical Review Board at the University of Gothenburg. All participants provided written informed consent prior to inclusion.

Anthropometric measurements and questionnaires
Standardized equipment was used for anthropometric measurements [8]. Information about smoking habits, calcium intake, medical history, medications, previous fracture (after 50 years of age) and physical activity was collected using a standardized questionnaire. The Physical Activity Scale for the Elderly (PASE), a validated self-reported questionnaire designed to measure physical activity in individuals aged 65 years or older [20, 21], was used to assess current physical

activity. A questionnaire regarding calcium-containing foods (e.g. diary products and vegetables) and supplements was used to calculate daily intake of calcium.

Assessment of serum levels of 25-OH-vitamin D and PTH

At the follow-up examination, levels of 25-OH-vitamin D were measured from blood samples with competitive radioimmunoassay [DiaSorin, Stillwater, MN, USA; intra-assay coefficient of variation (CV) 6%, interassay CV 15–16%] at a single laboratory. Serum PTH was analysed in 443 men using the Immulite 2000 intact PTH assay (reference range 1.1–6.9 pmol L^{-1}; intra-assay CV 5%, interassay CV 9%; Diagnostic Products, Los Angeles, CA, USA).

Assessment of BMD

aBMD (g cm^{-2}) was measured at the hip, femoral neck and lumbar spine (L_1–L_4) using the QDR 4500/A-Delphi DXA system (Hologic, Waltham, MA, USA). The CV for the performed measurements ranged from 0.5% to 3.0% in one young man (19 years old) for three consecutive measurements with repositioning between scans. In addition, two consecutive measurements with repositioning between scans were performed in 30 older women (75–80 years), and CVs ranged from 0.7% to 1.3%.

Assessment of bone microarchitecture

With the use of a high-resolution 3D HR-pQCT device (XtremeCT; Scanco Medical AG, Brüttisellen, Switzerland), volumetric BMD (vBMD) and bone microarchitecture at the distal tibia were investigated using a previously described protocol [22]. In short, a reference line was placed manually by the operator at the distal articular plateau. The first slice, of a total 110 parallel images, was captured 22.5 mm proximal to the established reference line. Images were obtained with an isotropic resolution of 82 μm, resulting in a 3D representation of a 9.02 mm section of the tibia. The effective dose generated was about 3 μSv, and the scan time was approximately 3 min.

Obtained images were processed with a threshold method, as previously described [23], resulting in separate compartments for trabecular and cortical bone. Cortical bone was separated from trabecular bone with the use of a threshold value of one-third of the apparent cortical vBMD (mg cm^{-3}) [4]. Mean cortical thickness (mm) was derived by dividing mean cortical volume by the outer bone surface [4]. Trabecular bone volume fraction (BV/TV, %) was derived by dividing measured trabecular BMD from the trabecular bone compartment by fully mineralized bone (1200 mg cm^{-3}) [23]. Trabecular number (TbN, mm^{-1}) was taken as the inverse of the mean spacing between the ridges using a distance transformation method [24]. Standard deviation of 1/TbN was used to assess trabecular network inhomogeneity. Trabecular thickness (TbTh, mm) and trabecular separation (TbSp, mm) were derived from BV/TV and TbN [i.e. TbTh = (BV/TV) / TbN and TbSp = (1 − BV/TV) / TbN] using standard histomorphometric methods [25]. The obtained parameters had CVs, with repositioning three times, that ranged from 0.1% to 1.6% for the tibial measurements in one middle-aged woman. These parameters had a CV of 0.2% to 4.5% measured twice consecutively, with repositioning, in six older women (75–80 years). To ensure high quality, obtained images were graded according to the manufacturer's recommendations (Scanco Medical AG). Grade 1 corresponds to the highest quality, 2 and 3 are regarded as acceptable quality, and 4 and 5 are of unacceptable quality. Of a total of 478 images, four were excluded due to incorrectly placed scout views and 18 were excluded due to low quality. There was no difference in levels of vitamin D between men excluded, due to low image quality, and included in the analyses (65.0 ± 15.7 vs. 62.4 ± 17.6 nmol L^{-1}; $P = 0.52$, independent samples *t*-test).

Cortical evaluation

Images were further processed using a customized version of Image Processing Language (IPL v5.08b Scanco Medical AG) as previously described [26]. Briefly, a contour was automatically placed around the bone to delineate the periosteal surface from extra-osseal soft tissue. Another contour was automatically placed on the endosteal side to separate trabecular from cortical bone. All contours (both periosteal and endosteal) were carefully investigated and areas in which they were misplaced were manually corrected. Such correction could be soft tissue within the periosteal contour. Two operators performed the periosteal analyses, and all the endosteal analyses were performed by only one of the two operators. When every contour had been defined, cortical porosity was defined within both contours. Then, all artefacts were excluded such as surface roughness and transcortical foramen or erosions. Finally, combining the segmented and the cortical porosity images produced a more defined cortical compartment. This method can be used to acquire parameters including cortical pore diameter, cortical pore volume (Ct.Po.V) and

cortical bone volume (Ct.BV). Using this segmentation procedure, cortical porosity (Ct.Po, %) can be calculated [26, 27]: Ct.Po (%) = Ct.Po.V / (Ct.Po.V + Ct.BV). The CV for cortical porosity was obtained using the same images as for the standard analyses. All CV measurements were performed with repositioning. The CV for cortical porosity was 5.5% in one middle-aged woman and 0.9% for the measurements performed in six older women (75–80 years).

Statistical analysis

Study subjects were divided into quartiles according to levels of 25-OH-vitamin D and PTH. Differences between mean values for bone variables and covariates between men in each quartile (for both 25-OH-vitamin D and PTH) were compared using analysis of variance (ANOVA) for continuous variables and chi-squared test for categorical variables (percentage). Significant results for the continuous variables were tested with the least significant difference (LSD) *post hoc* test.

Linear regression models were used to test independent associations and presented with standardized beta values calculated for each bone variable as (i) crude, (ii) with covariates (age, height, weight, smoking, physical activity, daily intake of calcium, vitamin D supplementation and log PTH) for vitamin D and with the addition of comorbidities (stroke, diabetes, angina pectoris and heart failure) for PTH and (iii) as complete models. Change in R^2 for the hierarchical linear regression model with and without vitamin D or log PTH was calculated and presented as percentage.

Bone variables were compared between vitamin D-sufficient (≥50 nmol L^{-1}) men and those with either vitamin D deficiency (<25 nmol L^{-1}) or insufficiency (25–49 nmol L^{-1}) combined with a high level of PTH (>6.8 pmol L^{-1}). *P*-values below 0.05 were considered significant, and all analyses were performed using SPSS (version 23; SPSS, Inc., Chicago, IL, USA).

Results

The mean age of men included in the study was 80.2 ± 3.5 years at the time of the investigation.

Cohort characteristics according to Vitamin D levels

The included men were divided into quartiles (Q1–Q4) according to their vitamin D status (mean ± SD vitamin D levels: Q1, 41.0 ± 8.2; Q2, 55.8 ± 3.3; Q3, 67.6 ± 3.7; Q4, 85.2 ± 10.6 nmol L^{-1}). Men in Q4 were younger than those in Q1 (Table 1). Men in Q2, Q3 and Q4 were more physically active and had lower levels of PTH than subjects in Q1 (Table 1). There were no significant differences in weight between the quartiles, but percentage of fat was lower in men in Q2, Q3 and Q4 than in those in Q1, as well as in Q4 versus Q2 (Table 1).

Comparison of bone measurements according to Vitamin D levels

Bone measurements were compared between subjects in the different quartiles of vitamin D. Men in Q4 had higher aBMD at the femoral neck (5.4%) and total hip (5.5%) than men in Q1 (Table 1).

With regard to geometric and microstructural measurements, men in Q4 had lower tibial cortical porosity (−12.8%) (Table 1) but greater trabecular thickness (8.3%), cortical vBMD (4.6%), cortical area (11.7%) and cortical thickness (12.1%) than men in Q1 (Table 1).

Linear regression models for vitamin D and DXA-derived bone variables

A linear regression model was used to investigate the predictive ability of vitamin D for bone variables. Unadjusted models showed an association between the vitamin D levels and aBMD at the total hip and femoral neck (Table 2). A hierarchical linear regression model was used to evaluate whether vitamin D could explain the variation in DXA-derived bone variables, independently of covariates (age, height, weight, daily intake of calcium, smoking, log PTH, vitamin D supplementation and physical activity). In this analysis, vitamin D independently predicted femoral neck and total hip aBMD. Investigation of the R^2 change (%) showed an increased explanation of the variation in both the femoral neck (0.9%) and the total hip (1.2%) aBMD. The complete model for femoral neck and total hip aBMD explained 22.5% and 26.7%, respectively, of the variability in aBMD (Table 2).

Linear regression models for vitamin D and HR-pQCT-derived bone variables

The same linear regression models as for the DXA-derived bone variables above were used to investigate the ability of vitamin D to predict bone geometric and microstructural measurements.

Table 1 *Participant characteristics and comparison of aBMD, geometric and microstructural measurements by quartiles of vitamin D levels in older men*

	Q1	Q2	Q3	Q4	*P*
Number of subjects	109	113	113	109	
Measured levels of vitamin D, nmol L^{-1}	41.0 ± 8.2	55.8 ± 3.3	67.6 ± 3.7	85.2 ± 10.6	–
Age, years	80.9 ± 3.2	80.3 ± 3.5	80.0 ± 3.7	79.6 ± 3.5[b,f]	**0.04**
Height, cm	175.1 ± 6.8	174.6 ± 6.2	174.9 ± 6.0	175.6 ± 7.0	0.69
Weight, kg	81.9 ± 11.7	79.8 ± 11.4	78.3 ± 11.9	78.3 ± 9.9	0.06
Fat mass, %	29.6 ± 5.0	27.9 ± 5.1[a,d]	27.1 ± 5.1[c,e]	26.4 ± 4.4[c,f,a,g]	**<0.001**
Calcium intake, mg day^{-1}	918 ± 426	918 ± 452	955 ± 452	989 ± 405	0.57
PTH, pmol L^{-1}	6.19 ± 3.3	4.84 ± 2.1[c,d]	4.64 ± 2.4[c,e]	4.51 ± 2.4[c,f]	**<0.001**
Current physical activity, (PASE score)	326 ± 209	395 ± 224[a,d]	410 ± 218[b,e]	420 ± 252[b,f]	**0.01**
Vitamin D supplementation, % (*n*)	1.8 (2)	5.3 (6)	8.0 (9)	8.3 (9)	0.15
Smoking, % (*n*)	4.6 (5)	2.7 (3)	3.5 (4)	6.4 (7)	0.55
Previous fracture, % (*n*)	20.2 (22)	21.2 (24)	17.7 (20)	17.4 (19)	0.86
Stroke, % (*n*)	11.9 (13)	12.5 (14)	8.8 (10)	7.3 (8)	0.53
Rheumatoid arthritis, % (*n*)	1.8 (2)	1.8 (2)	4.4 (5)	2.8 (3)	0.59
Diabetes, % (*n*)	18.3 (20)	10.7 (12)	7.1 (8)	13.8 (15)	0.07
Angina pectoris, % (*n*)	14.8 (16)	14.3 (16)	10.6 (12)	11.0 (12)	0.70
Heart failure, % (*n*)	11.9 (13)	9.8 (11)	5.3 (6)	11.9 (13)	0.29
Chronic bronchitis, asthma or emphysema, % (*n*)	9.2 (10)	8.0 (9)	7.1 (8)	3.7 (4)	0.42
Colon cancer, % (*n*)	4.6 (5)	2.7 (3)	– (0)	3.7 (4)	0.17
Prostate cancer, % (*n*)	10.1 (11)	9.8 (11)	5.3 (6)	9.2 (10)	0.55
DXA					
Number of subjects	104	100	103	104	–
Total hip aBMD, g cm^{-2}	0.91 ± 0.13	0.96 ± 0.16[a,d]	0.98 ± 0.16[b,e]	0.96 ± 0.15[a,f]	**0.02**
Femoral neck aBMD, g cm^{-2}	0.74 ± 0.12	0.78 ± 0.15[a,d]	0.80 ± 0.12[b,e]	0.78 ± 0.14[a,f]	**0.01**
Lumbar spine aBMD, g cm^{-2}	1.08 ± 0.19	1.05 ± 0.16	1.07 ± 0.21	1.07 ± 0.19	0.79
HR-pQCT					
Number of subjects	109	113	113	109	–
Trabecular BV/TV, %	14.4 ± 2.7	15.0 ± 2.5	14.9 ± 3.1	15.3 ± 2.7	0.11
Trabecular number, mm^{-1}	2.02 ± 0.34	1.97 ± 0.30	1.94 ± 0.31	1.97 ± 0.26	0.29
Trabecular thickness, mm	0.072 ± 0.01	0.077 ± 0.01[b,d]	0.077 ± 0.01[c,e]	0.078 ± 0.01[c,f]	**<0.001**
Trabecular separation, mm	0.44 ± 0.09	0.44 ± 0.08	0.45 ± 0.09	0.44 ± 0.07	0.58
Trabecular inhomogeneity of network, mm	0.20 ± 0.08	0.20 ± 0.05	0.21 ± 0.06	0.20 ± 0.04	0.67
Cortical area, mm^2	111 ± 34.1	119 ± 34.4	123 ± 34.9[a,e]	124 ± 36.6[b,f]	**0.03**
Cortical thickness, mm	0.91 ± 0.30	1.00 ± 0.32	1.02 ± 0.30[a,e]	1.02 ± 0.32[a,f]	**0.04**

Table 1 (*Continued*)

	Q1	Q2	Q3	Q4	*P*
Cortical porosity, %	12.5 ± 4.3	11.8 ± 4.0	11.9 ± 4.0	10.9 ± 4.1[b,f]	**0.03**
Cortical vBMD, mg cm^{-3}	758 ± 74.6	776 ± 79.3	786 ± 69.6[b,e]	793 ± 73.8[c,f]	**0.003**

PTH, parathyroid hormone; aBMD, areal bone mineral density; DXA, dual-energy X-ray absorptiometry; HR-pQCT, high-resolution peripheral quantitative computed tomography; BV/TV, trabecular bone volume fraction; vBMD, volumetric bone mineral density.
Values for age, height, weight, calcium intake and current physical activity are presented as mean ± SD and compared for significance with ANOVA with LSD *post hoc* test. Smoking, vitamin D supplementation (>200 IU day^{-1}), previous fracture and comorbidities (stroke, rheumatoid arthritis, diabetes, angina pectoris, heart failure, chronic bronchitis, asthma, emphysema and colon or prostate cancer) are presented as percentage (number of subjects). Differences in proportions were tested with chi-squared test. Significant *P*-values are presented in bold. Numbers of subjects are presented as maximum participants in one of the variables for each quartile. Differences between different quartiles: [d]Q2 versus Q1, [e]Q3 versus Q1, [f]Q4 versus Q1, [g]Q4 versus Q2.
[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

Table 2 *Linear regression models of associations between vitamin D, DXA and HR-pQCT-derived bone variables*

	Unadjusted model			Adjusted model			
DXA	β	R^2 (%)	*P*	β	R^2 change (%)	R^2 (%)	*P*
Femoral neck aBMD, g cm^{-2}	0.116	1.4	**0.02**	0.102	0.9	22.5	**0.04**
Total hip aBMD, g cm^{-2}	0.120	1.4	**0.02**	0.117	1.2	26.7	**0.014**
Lumbar spine aBMD, g cm^{-2}	0.040	0.2	0.42	0.082	0.6	16.4	0.09
HR-pQCT							
Trabecular BV/TV, %	0.137	1.9	**0.004**	0.174	2.7	10.9	**<0.001**
Trabecular number, mm^{-1}	−0.038	0.1	0.42	0.049	0.2	19.3	0.282
Trabecular thickness, mm	0.20	4.0	**<0.001**	0.163	2.4	13.9	**<0.001**
Trabecular separation, mm	−0.009	0.001	0.85	−0.095	0.8	17.7	**0.04**
Trabecular inhomogeneity of network, mm	−0.031	0.1	0.51	−0.103	1.0	13.7	**0.03**
Cortical thickness, mm	0.125	1.6	**0.01**	0.109	1.1	20.0	**0.017**
Cortical porosity, %	−0.126	1.6	**0.01**	−0.110	1.1	9.2	**0.024**
Cortical area, mm^2	0.141	2.0	**0.003**	0.123	1.4	19.4	**0.007**
Cortical vBMD, mg cm^{-3}	0.161	2.6	**<0.001**	0.125	1.4	18.8	**0.007**

25-OH-D, 25-hydroxyvitamin D; aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; DXA, dual-energy X-ray absorptiometry; HR-pQCT, high-resolution peripheral quantitative computed tomography; BV/TV, trabecular bone volume fraction.
Unadjusted model: bivariate linear regression model for 25-OH-D with DXA and HR-pQCT-derived bone variables. Adjusted model: effect of adding vitamin D to the adjusted (age, height, weight, physical activity, calcium intake, smoking, log PTH and vitamin D supplementation) hierarchical linear regression model. β represents the standardized coefficient of 25-OH-D. R^2 change, presented in percentage, represents the amount of variance explained in the dependent variable by adding 25-OH-D. The *P*-value represents significance in R^2 change (for vitamin D), and R^2 shows the amount of variance explained by the complete model. Significant *P*-values are presented in bold.

Unadjusted models showed an association between vitamin D and trabecular bone variables including trabecular bone volume fraction and thickness (Table 2). Amongst cortical measurements, cortical area, thickness, vBMD and porosity were associated with vitamin D levels (Table 2).

Hierarchical linear regression models investigated the effect of adding vitamin D to a model adjusted for covariates (age, height, weight, daily calcium intake, smoking, log PTH, vitamin D supplementation and physical activity). By adding vitamin D to the model, an R^2 change was seen for trabecular

bone volume fraction (2.7%), trabecular thickness (2.4%), trabecular separation (0.8%) and trabecular network inhomogeneity (1.0%) (Table 2). For cortical parameters, the pattern was similar for cortical area (1.4%), thickness (1.1%), vBMD (1.4%) and porosity (1.1%) (Table 2). The complete model, including vitamin D, could explain 9.2% of the total variation in cortical porosity (Table 2).

Cohort characteristics according to PTH levels

The cohort was also divided into quartiles based on serum PTH (mean ± SD serum PTH levels: Q1, 2.46 ± 0.6; Q2, 3.85 ± 0.3; Q3, 5.21 ± 0.6; Q4, 8.59 ± 2.6 pmol L^{-1}). Men in Q3 and Q4 were older than those in Q1 (Table 3). Men in Q4 were also less physically active than those in all other groups (Q1, Q2 and Q3). Absolute levels of vitamin D were significantly lower amongst men in Q3 and Q4 than those in Q1 and Q2 (Table 3). The distributions of stroke, diabetes, angina pectoris and heart failure varied between quartiles (Table 3).

Comparison of bone measurements according to PTH levels

To compare bone measurements with regard to serum levels of PTH, the same ANOVA analysis was used as for the comparison above of quartiles of serum vitamin D. Men in Q4 had lower total hip (−6.2%) and femoral neck (−7.5%) aBMD (Table 3). With regard to the microstructural measurements, men in Q4 had lower cortical area (−11.4%), thickness (−11.8%) and vBMD (−4.1%). Cortical porosity was higher in men in Q3 and Q4 than in those in Q2 (11.0% and 13.8%, respectively) (Table 3).

Linear regression models for log PTH and DXA-derived bone variables

Using a linear regression model, log PTH could predict aBMD at the femoral neck and total hip (Table 4). The contribution of log PTH in a hierarchical linear regression model could be seen in an increase in R^2 change at the femoral neck (2.0%) and total hip (2.5%) (Table 4). The complete model, including log PTH, could predict femoral neck (24.6%) and total hip aBMD (27.6%) (Table 4).

Linear regression models for log PTH and HR-pQCT-derived bone variables

With regard to microstructural measurements, log PTH predicted cortical area, thickness and vBMD but none of the trabecular bone traits (Table 4). An increase in R^2 was seen for cortical thickness (1.2%), area (1.7%) and vBMD (0.8%). No independent contribution to R^2 could be seen for cortical porosity by adding log PTH to the model. In the complete model, log PTH predicted cortical thickness, area and vBMD but was not associated with cortical porosity (Table 4).

Comparing men with low or insufficient levels of vitamin D combined with high levels of PTH

An extended analysis was performed in men who were vitamin D deficient (<25 nmol L^{-1}) or had insufficient vitamin D concentrations (25–49 nmol L^{-1}) in combination with a high serum level of PTH (>6.8 pmol L^{-1}). These men were less physically active and had higher rates of previous fracture, stroke and heart failure than vitamin D-sufficient men (Table 5). Men with vitamin D deficiency, or insufficiency with higher levels of PTH, had lower femoral neck and total hip aBMD (Table 5). With regard to trabecular measurements, only trabecular thickness differed between the groups (Table 5). For cortical measurements, vitamin D deficiency and insufficiency with high PTH were associated with a difference in cortical area, thickness and vBMD (Table 5). Further, cortical porosity was markedly elevated (17.2%) in men with vitamin D deficiency or insufficiency with elevated PTH levels (Table 5).

Discussion

Our results revealed an association between vitamin D levels and cortical porosity in elderly men. To our knowledge, this is the first report of this association.

We found that higher vitamin D levels were associated with higher aBMD at both the femoral neck and total hip. This confirms previous findings [9, 10], but whether vitamin D has any effect on bone microstructure remains unclear. Cortical bone has been shown to be of great importance for bone strength [28], especially in the elderly in whom a large proportion of the trabecular bone has been lost and the loss of cortical bone has resulted in increased porosity, and thus decreased bone strength [6]. Even though only large pores (over 130 μm) can be detected [7] and cortical porosity therefore is underestimated by our method, it may capture interindividual differences in porosity. In our study, this between-subject variability was dependent on vitamin D levels.

Table 3 *Participant characteristics and comparison of aBMD, geometric and microstructural measurements by quartiles of PTH in older men*

	Q1	Q2	Q3	Q4	*P*
Number of subjects	110	109	113	111	
PTH, pmol L^{-1}	2.46 ± 0.6	3.85 ± 0.3	5.21 ± 0.6	8.59 ± 2.6	–
Measured levels of vitamin D, nmol L^{-1}	67.9 ± 18.0	64.5 ± 14.0	59.6 ± 17.2[c,d,a,f]	57.9 ± 19.0[c,e,b,g]	**<0.001**
Age, years	79.5 ± 3.1	79.8 ± 3.5	80.5 ± 3.8[a,d]	80.9 ± 3.4[b,e,a,g]	**0.01**
Height, cm	175.1 ± 6.3	175.0 ± 7.3	175.0 ± 6.2	175.2 ± 6.2	0.99
Weight, kg	78.8 ± 11.6	79.8 ± 12.4	79.2 ± 11.3	80.3 ± 10.0	0.78
Fat mass, %	26.8 ± 5.2	27.6 ± 5.0	27.9 ± 5.1	28.6 ± 4.8	0.08
Calcium intake, mg day^{-1}	963 ± 456	999 ± 471	926 ± 414	897 ± 390	0.33
Current physical activity, PASE score	436 ± 229	399 ± 222	391 ± 229	330 ± 223[c,e,a,g,a,h]	**0.01**
Vitamin D supplementation, % (*n*)	7.3 (8)	6.4 (7)	4.4 (5)	5.4 (6)	0.82
Smoking, % (*n*)	6.4 (7)	4.6 (5)	4.4 (5)	1.8 (2)	0.42
Previous fracture, % (*n*)	12.7 (14)	21.1 (23)	18.6 (21)	24.3 (27)	0.16
Stroke, % (*n*)	5.5 (6)	12.8 (14)	6.2 (7)	15.5 (17)	**0.03**
Rheumatoid arthritis, % (*n*)	2.7 (3)	2.8 (3)	1.8 (2)	3.6 (4)	0.87
Diabetes, % (*n*)	20.9 (23)	7.3 (8)	8.8 (10)	12.7 (14)	**0.01**
Angina pectoris, % (*n*)	5.5 (6)	13.8 (15)	12.5 (14)	18.2 (20)	**0.04**
Heart failure, % (*n*)	1.8 (2)	8.3 (9)	8.8 (10)	19.1 (21)	**<0.001**
Chronic bronchitis, asthma or emphysema, % (*n*)	9.1 (10)	4.6 (5)	6.2 (7)	8.2 (9)	0.56
Colon cancer, % (*n*)	2.7 (3)	0.9 (1)	4.4 (5)	2.7 (3)	0.46
Prostate cancer, % (*n*)	10.0 (11)	8.3 (9)	8.0 (9)	8.2 (9)	0.95
DXA					
Number of subjects	102	102	103	103	–
Total hip aBMD, g cm^{-2}	0.97 ± 0.16	0.98 ± 0.16	0.94 ± 0.13[a,f]	0.91 ± 0.14[b,e,b,g]	**0.01**
Femoral neck aBMD, g cm^{-2}	0.80 ± 0.15	0.80 ± 0.14	0.77 ± 0.12	0.74 ± 0.12[b,e,b,g]	**0.01**
Lumbar spine aBMD, g cm^{-2}	1.06 ± 0.19	1.10 ± 0.19	1.05 ± 0.18	1.08 ± 0.19	0.27
HR-pQCT					
Number of subjects	110	109	113	111	–
Trabecular BV/TV, %	14.7 ± 3.0	15.1 ± 2.9	14.8 ± 2.5	15.0 ± 2.7	0.81
Trabecular number, mm^{-1}	1.93 ± 0.30	1.97 ± 0.31	1.97 ± 0.32	2.01 ± 0.29	0.33
Trabecular thickness, mm	0.077 ± 0.01	0.077 ± 0.01	0.076 ± 0.01	0.075 ± 0.01	0.62
Trabecular separation, mm	0.45 ± 0.09	0.44 ± 0.09	0.44 ± 0.08	0.43 ± 0.08	0.33
Trabecular inhomogeneity of network, mm	0.21 ± 0.06	0.20 ± 0.06	0.20 ± 0.06	0.20 ± 0.06	0.79
Cortical area, mm^2	123 ± 33.7	128 ± 34.5	118 ± 36.5[a,f]	109 ± 33.9[b,e,c,g]	**<0.001**
Cortical thickness, mm	1.02 ± 0.30	1.06 ± 0.30	0.97 ± 0.33[a,f]	0.90 ± 0.30[b,e,c,g]	**0.002**

Table 3 (*Continued*)

	Q1	Q2	Q3	Q4	*P*
Cortical porosity, %	11.6 ± 3.8	10.9 ± 4.2	12.1 ± 4.1[a,f]	12.4 ± 4.3[b,g]	**0.03**
Cortical vBMD, mg cm^{-3}	788 ± 65.4	798 ± 65.9	772 ± 73.1[b,f]	756 ± 88.9[b,e,c,g]	**<0.001**

PTH, parathyroid hormone; aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; DXA, dual-energy X-ray absorptiometry; HR-pQCT, high-resolution peripheral quantitative computed tomography; BV/TV, trabecular bone volume fraction.
Values for age, height, weight, calcium intake and current physical activity are presented as mean ± SD and compared for significance using ANOVA with LSD analysis. Smoking, vitamin D supplementation (>200 IU day^{-1}), previous fracture and comorbidities (stroke, rheumatoid arthritis, diabetes, angina pectoris, heart failure, chronic bronchitis, asthma, emphysema and colon or prostate cancer) are presented as percentage (number of subjects). Differences in proportions were tested by a chi-squared test. Significant *P*-values are presented in bold. Numbers of subjects are presented as maximum participants in one of the variables for each quartile.
Differences between different quartiles: [d]Q3 versus Q1, [e]Q4 versus Q1, [f]Q3 versus Q2, [g]Q4 versus Q2, [h]Q4 versus Q3.
[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

Low levels of vitamin D (<25 nmol L^{-1}) have previously been shown to be associated with lower aBMD [9, 29]. PTH levels need to be taken into account in interpreting the impact of a particular vitamin D level on calcium balance in an individual. We therefore identified a subgroup of individuals based on their levels of vitamin D and PTH. This group with vitamin D deficiency, or insufficiency with elevated PTH, had lower aBMD at the hip and the femoral neck, sites with more cortical bone. Similar results were seen for the geometric and microstructural measurements for which the cortical parameters differed most. Men with low vitamin D and high PTH had lower cortical vBMD, thickness and area, and substantially higher cortical porosity.

One previous study showed associations between the trabecular bone microstructure at the tibia and vitamin D levels in younger subjects (<65 years) [11]. Vitamin D levels were not associated with cortical parameters in these men. Of interest, cortical parameters were not altered in men with vitamin D deficiency (<10 ng mL^{-1}) compared to vitamin D-sufficient men. Amongst older men (≥65 years) in the same study, no association was seen between geometric or microstructural bone measurements and vitamin D levels [11]. By contrast, in the present study we revealed an association between vitamin D and trabecular as well as cortical bone at the tibia in elderly men. Possible reasons for this discrepancy could be the narrower and, in particular, the higher age range in our study. Age affects most of the studied bone parameters and with a much wider age range in the study by Chaitou *et al.* [11] we speculate that age is relatively more important for the variation in bone traits, reducing the explanatory role of vitamin D in such a context. Furthermore, vitamin D decreases with age [11] and may be more important for bone microstructure at older ages.

In a small study of men and women who had been treated with high doses of vitamin D for varying periods of time, Boyd *et al.* reported fewer but thicker trabeculae in individuals with higher vitamin D levels but no difference in cortical parameters [12]. Less than 10% of these subjects had serum levels of 25-OH-vitamin D below 75 nmol L^{-1}, a level generally considered to be sufficient, or even excessive. Thus, these findings are difficult to compare with our results that were obtained in a population with a low rate of vitamin D supplementation.

PTH was lower in subjects in the higher quartiles of vitamin D in our study, as expected. Low PTH levels could be a reason for reduced cortical porosity in those with higher vitamin D. However, vitamin D predicted cortical porosity after adjustment for PTH, whereas PTH levels did not independently predict cortical porosity. It therefore seems possible that vitamin D could have direct effects on the regulation of bone microstructure. This is plausible as there are receptors for vitamin D in bone cells and, as for PTH, previous studies have shown both anabolic and catabolic effects of active vitamin D on bone [30]. This possibility is also consistent with the findings of Martin *et al.* showing an association between 25-OH-vitamin D and femoral vBMD independent of PTH levels in the US MrOS [31]. PTH secretion is pulsatile and shows circadian

Table 4 *Linear regression model for log PTH with DXA and HR-pQCT-derived bone variables*

	Unadjusted model			Adjusted model			
DXA	β	R^2 (%)	*P*	β	R^2 change (%)	R^2 (%)	*P*
Femoral neck aBMD, g cm^{-2}	−0.174	3.0	**<0.001**	−0.155	2.0	24.6	**0.002**
Total hip aBMD, g cm^{-2}	−0.183	3.3	**<0.001**	−0.174	2.5	27.6	**<0.001**
Lumbar spine aBMD, g cm^{-2}	0.002	0.00	0.975	0.002	0.00	17.3	0.968
HR-pQCT							
Trabecular BV/TV, %	−0.021	0.00	0.655	−0.009	0.00	11.5	0.852
Trabecular number, mm^{-1}	0.076	0.6	0.112	0.005	0.00	19.3	0.918
Trabecular thickness, mm	−0.091	0.8	0.056	−0.006	0.00	14.3	0.904
Trabecular separation, mm	−0.065	0.4	0.173	−0.007	0.00	17.8	0.882
Trabecular inhomogeneity of network, mm	−0.037	0.1	0.435	0.014	0.00	14.0	0.771
Cortical thickness, mm	−0.182	3.3	**<0.001**	−0.117	1.2	20.4	**0.014**
Cortical porosity, %	0.079	0.6	0.097	0.013	0.01	9.9	0.791
Cortical area, mm^2	−0.196	3.9	**<0.001**	−0.140	1.7	19.8	**0.003**
Cortical vBMD, mg cm^{-3}	−0.182	3.3	**<0.001**	−0.096	0.8	19.2	**0.043**

PTH, parathyroid hormone; DXA, dual-energy X-ray absorptiometry; HR-pQCT, high-resolution peripheral quantitative computed tomography; aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; BV/TV, trabecular bone volume fraction.

Unadjusted model: bivariate linear regression model for log PTH with DXA and HR-pQCT-derived bone variables. Adjusted model: the effect of adding log PTH to the adjusted (age, height, weight, physical activity, calcium intake, smoking, vitamin D levels, vitamin D supplementation and comorbidities (stroke, diabetes, angina pectoris and heart failure) hierarchical linear regression model. β represents the standardized coefficient for log PTH. R^2 change, presented as percentage, represents the amount of variance explained in the dependent variable by adding log PTH. The *P*-value represents significance in R^2 change (for PTH), and R^2 shows the amount of variance explained by the complete model. Significant *P*-values are presented in bold.

variability, resulting in larger variations in serum levels in comparison with vitamin D levels [32]. Thus, the stronger associations found between vitamin D and cortical porosity could be due to more stable serum levels of vitamin D than of PTH. The response in PTH secretion to low vitamin D is highly individual with apparently different thresholds [33]. A high level of dietary calcium could inhibit or prevent the mineralization defect seen in vitamin D deficiency [34], whereas a low intake could contribute to the development of secondary hyperparathyroidism [35]. The increased cortical porosity with lower levels of vitamin D observed in our study could be due to increased cortical osteoids, being falsely perceived as bone void volume, because the porosity measurement uses a BMD algorithm to define what is bone or pores. Both cortical vBMD and porosity were associated with vitamin D levels, but vitamin D could explain a larger percentage of the variation in cortical vBMD than in porosity (1.4% vs. 1.1%), indicating that vitamin D levels primarily reflect cortical mineralization.

Several limitations of our study should be considered. First, the cross-sectional design meant that it was not possible to investigate the effect of vitamin D on bone microstructure over time. Secondly, the proportion of variance of cortical porosity explained (R^2) by vitamin D levels was rather low and is therefore probably of little clinical usefulness. Thirdly, results were obtained in elderly men and cannot be generalized to women or younger individuals. Fourthly, kidney function influences levels of PTH and vitamin D, and measures of glomerular filtration were not available in the present study. However, in only one subject included in the follow-up study was glomerular filtration rate (GFR) severely reduced at baseline in the original MrOS study; in addition, there were insufficient data to determine GFR for five men. Furthermore, if the GFR at baseline was included in our linear regression models, the results were not different (data not shown). Finally, although most of the bone variables investigated were at least weakly to moderately intercorrelated (e.g. femoral neck aBMD and spine aBMD, r = 0.57;

Table 5 *Participant characteristics, aBMD and bone geometry and microstructure according to vitamin D status*

	Vitamin D sufficiency	Vitamin deficiency or insufficiency with elevated PTH	*P*
Number of subjects	409	34	
Serum levels of vitamin D, nmol L^{-1}	64.5 ± 16.5	37.5 ± 8.8	**<0.001**
Age, years	80.1 ± 3.5	81.3 ± 3.3	0.07
Height, cm	175.0 ± 6.5	175.8 ± 6.2	0.48
Weight, kg	79.3 ± 11.3	82.4 ± 10.6	0.13
Calcium intake, mg day^{-1}	943 ± 438	975 ± 386	0.68
PTH, pmol L^{-1}	4.67 ± 2.3	9.52 ± 2.9	**<0.001**
Current physical activity, PASE score	398 ± 225	277 ± 236	**0.003**
Vitamin D supplementation, % (*n*)	6.1 (25)	2.9 (1)	0.45
Smoking, % (*n*)	4.2 (17)	5.9 (2)	0.64
Previous fracture, % (*n*)	18.1 (74)	32.4 (11)	**0.04**
Stroke, % (*n*)	9.1 (37)	20.6 (7)	**0.03**
Rheumatoid arthritis, % (*n*)	2.5 (10)	5.9 (2)	0.24
Diabetes, % (*n*)	12.3 (50)	14.7 (5)	0.68
Angina pectoris, % (*n*)	12.3 (50)	14.7 (5)	0.68
Heart failure, % (*n*)	8.3 (34)	23.5 (8)	**0.004**
Chronic bronchitis, asthma or emphysema, % (*n*)	6.9 (28)	8.8 (3)	0.67
Colon cancer, % (*n*)	2.5 (10)	5.9 (2)	0.24
Prostate cancer, % (*n*)	7.8 (32)	17.6 (6)	0.05
DXA			
Number of subjects	378	32	
Total hip aBMD, g cm^{-2}	0.96 ± 0.15	0.88 ± 0.14	**0.006**
Femoral neck aBMD, g cm^{-2}	0.78 ± 0.13	0.70 ± 0.12	**0.001**
Lumbar spine aBMD, g cm^{-2}	1.07 ± 0.19	1.09 ± 0.20	0.54
HR-pQCT			
Number of subjects	409	34	
Trabecular BV/TV, %	15.0 ± 2.8	14.1 ± 2.8	0.08
Trabecular number, mm^{-1}	1.97 ± 0.30	2.01 ± 0.34	0.48
Trabecular thickness, mm	0.076 ± 0.01	0.071 ± 0.01	**0.004**
Trabecular separation, mm	0.44 ± 0.08	0.44 ± 0.09	0.88
Trabecular inhomogeneity of network, mm	0.20 ± 0.06	0.20 ± 0.06	0.84
Cortical area, mm^2	121 ± 34.9	97.6 ± 32.8	**<0.001**
Cortical thickness, mm	1.00 ± 0.31	0.80 ± 0.30	**<0.001**
Cortical porosity, %	11.6 ± 4.1	13.6 ± 4.2	**0.006**
Cortical vBMD, mg cm^{-3}	782 ± 73.7	730 ± 80.0	**<0.001**

PTH, parathyroid hormone; DXA, dual-energy X-ray absorptiometry; HR-pQCT, high-resolution peripheral quantitative computed tomography; aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; BV/TV, trabecular bone volume fraction.

Men with vitamin D deficiency (<25 nmol L^{-1}) or insufficiency (25–49 nmol L^{-1}) with high PTH values (>6.8 pmol L^{-1}) were compared to vitamin D-sufficient men (including men with vitamin D ≥ 50 nmol L^{-1} and those with levels of 25–49 nmol L^{-1} with normal PTH). Continuous variables are presented as mean ± SD, and differences were compared using the t-test. For dichotomous variables, a chi-squared test was used and data are presented as percentage (number of subjects). Significant *P*-values are presented in bold. Numbers of subjects are presented as maximum participants in one of the variables for each group.

cortical vBMD and BV/TV, r = 0.27; BV/TV and spine aBMD, r = 0.45), multiple comparisons have been performed which may have given rise to associations by chance. This should be kept in mind when interpreting the reported associations.

Experimental studies in animals would be beneficial to further determine what is truly captured by cortical porosity in this study. In animal studies, high-resolution images can be compared with histological analysis, which minimizes the problem of edge detection when measuring pores. Such studies could result in a more detailed view of how intracortical porosity might be associated with vitamin D. The definition of vitamin D deficiency and insufficiency also has limitations. In this study, we defined deficiency based on low levels of vitamin D (<25 nmol L^{-1}) or insufficient levels (25–49 nmol L^{-1}) combined with elevated PTH levels (>6.8 pmol L^{-1}). We acknowledge that using a single measurement of PTH, considering its circadian variation [36] and short half-life [37], is not optimal for defining vitamin D deficiency. Repeated measurements of PTH and bone turnover markers were not available in this cohort but could have improved the selection of vitamin D-deficient men.

In conclusion, we found that low levels of vitamin D are associated with increased cortical porosity independently of confounding factors and PTH levels. Cortical porosity was markedly higher in men with vitamin D deficiency, or insufficiency and elevated PTH levels, indicating that increased cortical porosity could contribute to skeletal fragility associated with poor vitamin D status. Future prospective studies are needed to investigate whether there is an association between vitamin D and cortical porosity over time and, if so, whether vitamin D supplementation in deficient subjects can reduce cortical porosity.

Author contributions

DS, ML, DM, AGN, ÖL, MKK, CO and MN (i) made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (ii) participated in drafting the manuscript or revising it critically for important intellectual content; (iii) approved the final version of the submitted manuscript; and (iv) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest statement

All authors state that they have no conflict of interests.

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