Serum parathyroid hormone is associated with increased cortical porosity of the inner transitional zone at the proximal femur in postmenopausal women: The Tromsø Study

Marit Osima[1,2] MD
Tove T Borgen[3] MD
Marko Lukic[1] MD
Guri Grimnes[4,5] MD, PhD
Ragnar M Joakimsen[4,5] MD, PhD
Erik F Eriksen[6,7] MD, PhD
Åshild Bjørnerem[5,8] MD, PhD

[1]Department of Community Medicine, UiT The Arctic University of Norway, Tromsø, Norway
[2]Department of Orthopaedic Surgery, University Hospital of North Norway, Tromsø, Norway
[3]Department of Rheumatology, Vestre Viken Hospital Trust, Hospital of Drammen, Drammen, Norway
[4]Department of Medicine, University Hospital of North Norway, Tromsø, Norway
[5]Department of Clinical Medicine, UiT The Arctic University of Norway, Tromsø, Norway
[6]Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway
[7]Institute of Clinical Medicine, University of Oslo, Oslo, Norway
[8]Department of Obstetrics and Gynaecology, University Hospital of North Norway, Tromsø, Norway

Disclosures
Marit Osima, Tove Tveitan Borgen, Marko Lukic, Guri Grimnes, Ragnar M Joakimsen, Erik F Eriksen, and Åshild Bjørnerem declare that they have no conflict of interest.

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Corresponding author:
Marit Osima, MD
Department of Community Medicine, Faculty of Health Sciences,
UiT, The Arctic University of Norway, N-9037 Tromsø, Norway
Tel +47 99711178
Email: mos015@uit.no

Abstract

Summary

Serum parathyroid hormone (PTH) was associated with increased bone turnover markers, and cortical porosity of the inner transitional zone at the proximal femur. These results suggest that PTH through increased intracortical bone turnover leads to trabecularization of inner cortical bone in postmenopausal women.

Purpose

Vitamin D deficiency leads to secondary hyperparathyroidism and increased risk for fractures, whereas its association with cortical porosity is less clear. We tested (i) whether serum 25-hydroxyvitamin D (25(OH)D) and PTH were associated with cortical porosity, and (ii) whether the associations of 25(OH)D and PTH with fracture risk are dependent on cortical porosity.

Methods

This case-control study included 211 postmenopausal women, 54–94-years-old, with prevalent fractures and 232 controls from the Tromsø Study. Serum 25(OH)D, PTH, and bone turnover markers (procollagen type I N-terminal propeptide [PINP] and C-terminal cross-linking telopeptide of type I collagen [CTX]) were measured. Femoral subtrochanteric cortical and trabecular parameters were quantified using computed tomography, and femoral neck areal bone mineral density (FN aBMD) was quantified using dual-energy X-ray absorptiometry.

Results

Compared with controls, fracture cases exhibited reduced serum 25(OH)D and increased PTH, PINP, and CTX, increased femoral subtrochanteric cortical porosity and reduced cortical thickness and FN aBMD (all, $p < 0.05$). Serum 25(OH)D was not associated with cortical parameters (all, $p > 0.10$). PTH was associated with increased PINP, CTX, and cortical porosity of the inner transitional zone, and reduced trabecular bone volume/tissue volume, and FN aBMD (p ranging from 0.003 to 0.054). Decreasing 25(OH)D and increasing PTH were associated with increased odds for fractures, independent of age, height, weight, calcium supplementation, serum calcium, cortical porosity and thickness.

Conclusions

These data suggest that serum PTH, not 25(OH)D, is associated with increased intracortical bone turnover resulting in trabecularization of the inner cortical bone, nevertheless, decreasing 25(OH)D) and increasing PTH are associated with fracture risk, independent of cortical porosity and thickness.

Key Words

Bone turnover markers, cortical porosity, 25-hydroxyvitamin D, non-vertebral fracture, parathyroid hormone

Introduction

Sufficient vitamin D is important for normal development and maintenance of bone health [1-3]. Low serum levels of 25-hydroxyvitamin D (25(OH)D) are associated with secondary hyperparathyroidism, increased bone turnover, bone loss [4-6], and increased hip fracture risk in elderly women and men [5, 7, 8]. Supplementation of vitamin D, with or without calcium, reduces the fracture risk [9, 10]; however, the optimal levels required for bone health and the target treatment threshold levels remain unclear and continue to be debated [4, 11, 12].

Globally, Scandinavian countries have the highest rates of fragility fractures [13, 14], and 25(OH)D deficiency could be thought of as one possible reason. This could particularly apply to the northern part of Norway, located at latitude 65-71º N, where the sun is below the horizon for up to two months during the winter season, and where dermal vitamin D synthesis can be absent for up to 5 months [15, 16]. However, people in Scandinavia exhibit higher levels of 25(OH)D than do those in other European countries [7, 17]. Some reasons for this include the traditions of eating fatty fish and the use of fish oil or other vitamin D supplements during the winter [15]. The mechanisms behind fracture susceptibility in individuals with low 25(OH)D levels are reported to be mediated by hyperparathyroidism, leading to increased bone resorption and bone loss, or through poor or reduced muscle function and the associated risk of falls [1, 3, 18].

Increased cortical porosity is also associated with fracture risk in both women and men [19-22], and bone turnover markers are associated with cortical porosity and increased odds for fractures [20, 23, 24]. However, few studies have investigated whether vitamin D is associated with bone turnover markers and cortical porosity. One study reported that low serum 25(OH)D is associated with increased cortical porosity in elderly men (mean age, 80 years) [25], whereas 25(OH)D was not associated with cortical porosity in another study of women and men (mean age, 55 years) [26]. In individuals with primary hyperparathyroidism and very high serum parathyroid hormone (PTH) levels, cortical volumetric bone mineral density (BMD) was reduced due to increased cortical porosity. Others have reported that serum PTH associated with reduced cortical thickness [25, 27], but not cortical porosity [25]. Cortical thinning due to secondary hyperparathyroidism was suggested to lead to increased endocortical resorption and trabecularization of the inner part of the cortical bone, but cortical porosity was not studied [27]. The relationship of 25(OH)D and PTH levels with cortical porosity is, therefore, unclear.

There is increasing interest in the contribution of cortical parameters to bone strength and fracture risk, and we wanted to explore the potential link between 25(OH)D and PTH levels with cortical parameters of the proximal femur. We pooled data from a case-control study, consisting of women with non-vertebral fractures (largely of the wrist) and fracture-free controls from the general population of Tromsø, Norway, which is located at 70º N. In this study, we tested (i) whether serum 25(OH)D and PTH were associated with cortical porosity, and (ii) whether associations of 25(OH)D and PTH with fracture risk were dependent on cortical porosity.

Materials and methods

Study population

The Tromsø Study is a single-centre, population-based health study in Northern Norway, which conducted six surveys in 1974, 1979–1980, 1986–1987, 1994–1995, 2001–2002, and 2007–2008 [28]. During the Tromsø 4 survey (1994–1995), all 37,558 eligible inhabitants of Tromsø, older than 24 years, were invited to participate, and 27,158 (72%) did. All their non-vertebral fractures were registered from the x-ray archives of the University Hospital of North Norway, Tromsø, between J January 1994 and 1 January 2010 [29]. Participants with vertebral fractures were not included in this x-ray-based fracture registry, as few of these patients came to the hospital for x-rays.

In 2011, we designed a nested case-control study, and identified 1250 women who had participated in Tromsø 4 and who had suffered a fracture of the hip, wrist, or proximal humerus, after age 50 years, during the 15-year registry period (1994–95 to 2010) [20, 23, 24, 30, 31]. The 760 women who were still alive and living in Tromsø were invited to participate in this study. After excluding those who were premenopausal; received bisphosphonates for osteoporosis; or who had hip prostheses, metal screws, or pathological fractures, 264 women with fractures participated. Age-matched, fracture-free women who were within the same 5 year age groups were randomly selected from among the Tromsø 4 participants, 1186 were invited, and after using the same exclusion criteria, 260 controls attended. Of these 524 participants, we excluded 15 individuals who were receiving hormone replacement therapy and 66 with movement artefacts during computed tomography (CT) scanning. Thus, 443 women were included in the final analyses, including 232 controls and 211 fracture cases (4 hips, 181 wrists, and 26 proximal humeri). The median time that had elapsed since their most recent fracture was 6.6 (range, 1–25) years. All variables included in this study were obtained between November 2011 and January 2013; the data were analysed in a cross-sectional manner. All participants provided written informed consent; the study was approved by the Regional Committee of Research Ethics and was conducted in accordance with the World Medical Association Declaration of Helsinki.

Variables

A questionnaire was used to gather information concerning all fractures occurring after the age of 50 years, diseases, medication use, and lifestyle [20, 23, 24, 30]. Height and weight were measured in participants wearing light clothing, without shoes; the body mass index (BMI) was calculated as weight (kg)/height (m)^2. Femoral neck (FN) and total hip areal bone mineral density (aBMD) was measured at the non-dominant proximal femur using dual-energy x-ray absorptiometry (DXA, GE Lunar Prodigy, Lunar Corporation, Madison, WI, USA), with coefficients of variation (CV) of 1.7% and 1.2%, respectively [30]. Fasting blood samples were collected between 8 am and 10 am and assayed for serum 25(OH)D using mass spectrometry, PTH using Immulite 2000, and procollagen type I N-terminal propeptide (PINP) and C-terminal cross-linking telopeptide of type I collagen (CTX) using electrochemiluminescence immunoassays; (Elecsys 1010 Analytics, Roche Diagnostics, Germany), with CV of 3–8%. Creatinine was measured photometrically, with a CV of 3%. Kidney function was assessed using the estimated glomerular filtration rate (eGFR), which was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Corrected serum calcium was calculated as serum-calcium concentration + 0.0227 × (46 – serum-albumin concentration), with a CV of 2%.

CT scans (Siemens Somatom Sensation 16, Erlangen, Germany) of the non-dominant hip were performed at the Department of Radiology, University Hospital of North Norway [20]. The CT machine had an in-plane resolution of 0.74 mm and a slice thickness of 0.6 mm, and the hip was scanned from just above the femoral head to 2 cm below the lesser trochanter, with a radiation dose ~1.5 mSv [20]. The CT scans were performed at 120 kV, with a pitch of 0.75, and 90 mA prior to reconstruction using a fixed field of view of 120 mm [30]. Quality control was carried out by scanning a phantom containing rods of hydroxyapatite (HA) (QRM Quality Assurance in Radiology and Medicine GmbH, Moehrendorf, Germany). The CT images were sent to Melbourne, Australia and analysed by collaborators, blinded to the patient fracture status, using StrAx1.0 software (StraxCorp Pty Ltd, Melbourne, Australia) [32]. As cortices are thin at the most proximal femur (femoral head, neck, and trochanter), analyses were confined to a 3.7-mm subtrochanteric region-of-interest (ROI) with thicker cortices, which started at the tip of the lesser trochanter (Fig. 1).

The StrAx1.0 software is a non-thresholding method that automatically selects attenuation profile curves and segments the bone within the ROI into the compact-appearing cortex, outer (OTZ) and inner transitional zones (ITZ), and trabecular compartment [32]. This was achieved by quantifying the attenuation produced by the background (i.e., muscle) and fully mineralized bone matrix, which has a density of 1200 mg HA/cm^3) and was assigned a value of 100% [32, 33]. Voxels that were completely empty and had an attenuation equivalent to background were assigned a value of 0%. The volume fraction of a voxel that is void (i.e., porosity) was 100% minus the mineralized bone matrix fraction. Once deposited, osteoid is rapidly mineralized to become 'bone', reaching 80% of full mineralization (1200 mg HA/cm^3) within a few days. Voxels with attenuation values of 80% are unlikely to contain a pore or part of a pore, because porosity results in voxel attenuation values <80% of the maximum. Variations in attenuation within 80–100% of full mineralization likely reflect heterogeneity in secondary mineralization of the matrix; thus, these voxels were excluded from the calculation of porosity. Voxels with attenuation <80% may contain a pore or part of a pore [32].

Porosity within the total cortex, as well as within each cortical compartment, was quantified automatically throughout the ROI using the StrAx1.0 software [20]. The porosity quantified by this algorithm is the proportion of emptiness within each voxel or the fraction of the bone that is void, with CV of 0.3–2.3% [20]. Of the total cortex at this subtrochanteric site, 70.0% was compact-appearing cortex, while 22.3% and 11.7% were OTZ and ITZ, respectively. StrAx1.0 quantifies porosity in low-resolution images [20, 33], as in high-resolution images [32, 34], even though pores are not visible. This is a density-based, indirect measure of porosity, and the size and number of pores are not determined [20, 21, 23, 24, 32, 34]. The agreement (R^2) between CT and high-resolution peripheral quantitative computed tomography (HR-pQCT) ranged from 0.86 to 0.96 for porosity quantification (range, 40–95%), at the same femoral subtrochanteric site [20, 33]. StrAx1.0 software quantifies porosity as a fraction of void, regardless of pore size, and indirectly captures porosity produced by large and small pores. It also accounts for partial volume effects by including not only voids within completely empty voxels, but also within partly empty voxels [32]. StrAx1.0 software quantifies porosity of the compact cortex and the transitional zone, making it more inclusive than traditional measurements, and yielding a higher porosity than that reported using other methods [21, 32].

Statistical methods

Differences between fracture cases and controls were assessed using analysis of variance (ANOVA) and were adjusted for age. The data were pooled, analysed as a single cohort, and adjusted for fracture status to avoid confounding due to differences between cases and controls. The participants were divided into quartiles according to either serum 25(OH)D or serum PTH levels. Differences between women in each of the 25(OH)D and PTH quartiles were compared using ANOVA, and adjusted for age, height, weight, and fracture status. Due to multiple comparisons, we adjusted all p-values in these analysis by controlling the false discovery rate using the Benjamini-Hochberg method [35]. Linear regression analysis was used to identify associations between serum 25(OH)D and

PTH levels with bone turnover markers and bone parameters, adjusted for age, height, weight, fracture status, calcium supplementation, corrected serum calcium levels, and season during which blood sampling occurred. We used standardized regression coefficients to facilitate comparing the strengths of the associations between the exposure and endpoints. The odds ratios (OR) for fractures per standard deviation (SD) change in serum 25(OH)D and PTH levels were calculated using logistic regression analysis, and were adjusted for age, height, weight, calcium supplementation, corrected serum calcium levels, oral glucocorticoid use, ulcerative colitis or Crohn's disease, and season of blood sampling. They were also mutually adjusted for 25(OH)D and PTH levels, and further adjusted for cortical porosity, cortical thickness, and FN aBMD. To evaluate whether the association of 25(OH)D and PTH levels with the odds for fractures was modified by season; we included interaction terms between both 25(OH)D and PTH levels and the season of blood sampling (summer vs. winter); summer months were defined as May–September. Analyses were performed using STATA Software (Stata 13.0, Stata Corp, College Station, TX, USA) and SAS Software, v9.4 (SAS Institute, Cary, NC, USA).

Results

Fracture cases compared with controls

Compared with controls, women with fractures exhibited lower mean serum levels of 25(OH)D (76.4 vs 82.9 nmol/L) and corrected calcium level (2.43 vs. 2.45 mmol/L), but higher mean levels of PTH (4.58 vs. 4.13 pmol/L), PINP (49.7 vs. 43.5 ng/mL), and CTX (0.49 vs. 0.44 ng/mL) (all, $p < 0.05$; Table 1). They also exhibited increased femoral subtrochanteric cortical porosity (43.8 vs. 41.7%) and reduced cortical thickness (4.06 vs. 4.36 mm) and FN aBMD (794 vs. 860 mg/cm^2) (all, $p < 0.05$). Fracture cases did not differ from controls in terms of weekly hours of physical activity, smoking, alcohol intake, eGFR, proportion with hyperthyroidism, or self-reported good health. Compared with controls, those with fractures more frequently reported ulcerative colitis or Crohn's disease (5.7% vs. 2.2%, $p = 0.054$), oral glucocorticoid use (3.8% vs. 0.9%. $p = 0.023$), calcium supplementation (20.9% vs. 12.1%, $p = 0.007$) and vitamin D supplementation (77.3% vs. 71.6%, $p = 0.278$). In women with fractures, only one had a 25(OH)D level <25 nmol/L; 23 (10.9%) had levels <50 nmol/L. None of the controls had 25(OH)D <25 nmol/L, and 27 women (11.6%) had levels <50 nmol/L ($p = 0.807$). In winter, the mean 25(OH)D levels were lower than in summer (76.9 vs 82.7 nmol/L, $p = 0.018$).

Calciotropic hormones, bone turnover markers, and bone parameters

Compared to those in the upper quartile, women in the lowest quartile of serum 25(OH)D had higher PTH and BMI, and a larger proportion had prevalent fractures, after adjustment for age, height, weight, and fracture status (all, $p < 0.05$; Table 2). Women in the upper PTH quartile had lower 25(OH)D and were older than those in the lowest quartile (all, $p < 0.05$; Table 2). Serum 25(OH)D was not significantly associated with the bone turnover markers (CTX and PINP), femoral subtrochanteric parameters, or FN aBMD, but the estimates pointed toward higher porosity and thinner cortices by decreasing 25(OH)D (Table 3). Each SD higher PTH was associated with 0.10–0.14 SD increase in PINP and CTX, 0.10 SD increase in porosity of the ITZ, and 0.09–0.10 SD decrease in trabecular bone volume/tissue volume (BV/TV) and FN aBMD, ($p = 0.003$–0.054; Table 3). All results were adjusted for age, height, weight, fracture status, calcium supplementation, corrected serum calcium, and season of blood sampling (winter vs. summer). PTH accounted for 2% of the variance in CTX, 1% of the variance in porosity of the ITZ, 1% of the variance in trabecular BV/TV, and 1% of the variance in femoral neck aBMD.

Calciotropic hormones and odds for fractures

In the univariate analysis, height, BMI, oral glucocorticoid use, PINP, CTX, FN aBMD, femoral subtrochanteric cortical porosity and thickness, 25(OH)D and PTH were associated with increased odds for fracture (Tables 4–5). Each SD decrease in 25(OH)D (odds ratio [OR], 1.27; 95% confidence interval [CI], 1.00-1.61) and each SD increase in PTH (OR, 1.29; 95% CI, 1.01–1.63) was associated with increased odds for fracture, after adjustment for age, height, weight, calcium supplementation, corrected serum calcium, glucocorticoid use, ulcerative colitis or Crohn's disease, season of blood sampling, cortical porosity, and cortical thickness (Table 5). Serum 25(OH)D, but not PTH remained associated with odds for fractures after further adjustment for FN aBMD.

In additional analyses, after excluding those with reduced kidney function (eGFR <60 mL/min/1.73 m^2, n = 47), the association of 25(OH)D with odds for fractures remained unchanged (p ranging from 0.003 to 0.024), while the association of PTH with odds for fractures was attenuated (p ranging from 0.052 to 0.624). After excluding those with malabsorption (n = 17) or hyperthyroidism (n = 14), the association of 25(OH)D with fractures was attenuated and mostly non-significant, while the association of PTH with fractures remained similar. None of the women had severe reductions in kidney function (eGFR <30 mL/min/1.73 m^2), hypocalcaemia, hypoparathyroidism, only 4 women had primary hyperparathyroidism, and 48 had secondary hyperparathyroidism. These results remained similar following additional analysis that excluded those taking calcium supplementation. Results did not change after additional adjustment for eGFR, hyperthyroidism, self-reported health, weekly hours

of physical activity, or number of falls during the preceding 12 months. There was no interaction between serum 25(OH)D and PTH, between 25(OH)D or PTH and season of blood sampling on odds for fractures (all, $p > 0.10$).

Discussion

We report that women with fractures had lower serum 25(OH)D levels, higher levels of PTH and bone turnover markers, greater cortical porosity, and thinner cortices than controls. Increasing PTH was associated with increased bone turnover markers, increased cortical porosity of the ITZ, and lower FN aBMD, but 25(OH)D was not associated with either. The latter may reflect a lack of statistical power as the non-significant estimates pointed towards an increase in porosity and thinner cortices by decreasing 25(OH)D. These results suggest that increasing PTH increased bone remodelling on the intracortical surfaces of the inner cortex where porosity is high, with more bone surfaces per unit of bone matrix volume available for bone remodelling than in the more compact outer cortex, where porosity is low [23, 24, 34, 36-37]. Nevertheless, both decreasing 25(OH)D and increasing PTH were associated with increased odds for fractures, independent of each other and independent of cortical porosity and thickness. Moreover, 25(OH)D, not PTH, was associated with increased odds for fractures, independent of FN aBMD. These results suggest there may be some differences in the mechanisms behind the effects of 25(OH)D and PTH on fracture risk. However, most of these associations were weak, with 27–41% increases in the odds for fracture, after accounting for many well-known risk factors for fracture in the multivariable models.

Women with 25(OH)D in the upper quartile did not have significantly lower femoral subtrochanteric cortical porosity than did those in the lowest quartile (42.0% vs. 43.0%), in the current study. One reason for this lack of association could be that we included a relatively young (mean age, 68 years) and healthy cohort of postmenopausal women, with serum 25(OH)D mainly in the normal range, similar to the Boyd et al. study [26]. They reported the absence of an association between 25(OH)D and distal radius or distal tibia cortical porosity in women and men (mean age, 55 years) receiving vitamin D supplementation [26]. Those with low levels of 25(OH)D (<75 nmol/L) did not have significantly higher cortical porosity of distal tibia than did those with high levels (>175 nmol/L) (6.5% vs. 6.1%). In another study, no association between 25(OH)D and cortical parameters (density and thickness) at distal radius or distal tibia were identified in men aged 20–87 years, not even in those with 25(OH)D <10 ng/mL [27]. However, Sundh et al reported that serum 25(OH)D was inversely associated with distal tibia cortical porosity in elderly men (mean age, 80 years) [25]. Cortical porosity was slightly higher in men with 25(OH)D in the lowest quartile, compared to those having 25(OH)D in the upper quartile (12.5% vs. 10.9%).

In the current study, >70% of participants (cases and controls) were receiving vitamin D supplementation, as previously reported [1]. More fracture cases than controls reported calcium supplementation (21% vs. 12%), which likely began after the fracture; still, serum calcium was lower and PTH was higher in fracture cases than in controls. We could not identify any association of serum 25(OH)D with aBMD of the femoral neck or total hip, assessed using DXA, or femoral subtrochanteric vBMD quantified in clinical CT images. In other studies, vitamin D and calcium supplementation was reported to decrease the synthesis of PTH and increase lumbar spine and hip aBMD [6, 38]. The normal to high levels of 25(OH)D, and paucity of low levels in the participants, may partly explain why 25(OH)D levels were not associated with cortical porosity or other bone parameters, in the current study. Other reasons may include a lack of statistical power or that little of the variance in porosity is explained by serum 25(OH)D [39, 40]. Further work is needed to clarify whether individuals with vitamin D deficiency (<25 nmol/L) have increased cortical porosity.

We confirmed that an increased odds for fractures is associated with decreasing 25(OH)D [5, 7, 10]. 25(OH)D was associated with odds for fractures independent of cortical porosity, cortical thickness and FN aBMD; thus, the effect of low 25(OH)D may involve other mechanisms, such as muscle function and balance [41]. The fracture cases were not less healthy or less active than the controls, and did not differ in terms of other lifestyle factors (e.g., smoking and alcohol intake). The absence of such differences could be due to the fact that most of the cases had wrist fractures, and few had hip fractures. Moreover, the observations were independent of the season of blood collection. Women with high serum 25(OH)D have a lower risk for hip fractures than those with low levels, independent of frailty, physical function and falls [7, 8]. Moreover, vitamin D and calcium supplementation provides better fracture prevention than only calcium supplementation, especially in those with inadequate levels of 25(OH)D [12].

Our finding of higher PTH being associated with increased porosity of the ITZ, in relatively healthy women, could be due to increased remodelling on the intracortical surfaces of the inner cortex, where porosity is higher with more surface area than of the outer cortex [27, 42]. This agrees with the findings of Vu et al. who reported that, in untreated patients with primary hyperparathyroidism and very high PTH (13 pmol/L), the cortical vBMD was reduced due to increased cortical porosity of the compact cortex, OTZ, and ITZ as well as to reduced tissue mineralization density [42]. Others have reported that PTH is associated with reduced cortical thickness [25, 27], but not cortical porosity [25]. This cortical thinning is suggested to be due to secondary hyperparathyroidism, leading to increased endocortical resorption and trabecularization of the inner cortical bone [27]. Moreover, the association of increasing PTH with reduced trabecular BV/TV and FN aBMD, in this study, might be due to

increased remodelling on the trabecular surfaces, leading to loss of trabeculae, as also reported by Chaitou et al. [27].

We report that both 25(OH)D and PTH were independently associated with increased odds for fractures; however, after mutual adjustment, both associations were attenuated. This suggests that 25(OH)D and PTH are partly dependent factors that contribute to the risk for fractures. As 25(OH)D remained associated with fracture risk, after accounting for cortical porosity, cortical thickness, and FN aBMD, 25(OH)D may have an independent effect via other mechanisms. Because PTH levels did not remain associated with fracture risk independent of FN aBMD, its effect may be mediated via aBMD. Despite robust evidence that serum PTH is inversely associated with 25(OH)D, as we confirmed, there are few studies describing the association between serum PTH and risk for fracture. One study reported no association of serum PTH with hip or other non-vertebral fractures [43].

By using StrAx1.0 software, porosity was quantified as a fraction of void, regardless of pore size, and it indirectly capture porosity produced by pores larger and smaller than 100 µm in diameter. The benefit and novelty of using this non-threshold based method lies in how it is different from threshold-based methods for measuring porosity [32]. This method is more inclusive because it encompasses porosity of both the compact cortex and the transitional zone. Further, it accounts for partial volume effects, including voids within totally empty and partly empty voxels [32], and the values of porosity are higher than those obtained using other methods [21, 32]. Other studies that used HR-pQCT to quantify porosity, presented low values of porosity (1–15%) because it quantifies only porosity of the compact cortex and only pores >100 µm in diameter [25-27], although 60% of cortical pores are <100 µm in diameter [32, 36, 44].

The strength of this nested case-control study is that it is based on a general population and uses a validated fracture registry (30) with updated and detailed information on diseases, medications, and lifestyle. Additionally, serum 25(OH) was measured using mass spectrometry. The StrAx software for quantification of bone architecture was validated by confirming strong correlations between measurements using CT scans and HR-pQCT, and by rescanning a human hip phantom using the same standard CT machines used for study participants and showing good reproducibility. The measurements were obtained from the proximal femur, which is a central site and a common site of the most serious fragility fractures.

This study has several limitations. Due to the cross-sectional design, we could only test associations, and the direction of the associations or causations could not be determined. The retrospective case-control design may have introduced selection bias, and the index fractures occurred at a median of 6.6 years before the women underwent 25(OH)D, PTH, and bone parameter measurements. Moreover, single measurements of 25(OH)D and PTH levels may not reflect actual 25(OH)D and PTH levels throughout the study period. Adding to the variability of these associations is the fact that the threshold for 25(OH)D, at which any given individual develops secondary hyperparathyroidism varies widely [45]. Among those invited, some women indicated that they were not well enough to participate. Therefore, the strength of the associations may be somewhat underestimated due to a "healthy" selection bias. Fracture cases who were taking anti-osteoporotic drugs (AOD) may differ from untreated participants. However, in Norway, only about 11–15% of women are treated with AOD after a hip or wrist fracture [46, 47]. The reasons why 25(OH)D was not associated with cortical porosity may be that few participants had low 25(OH)D levels or 25(OH)D deficiencies and lack of statistical power due to the moderate sample size. Furthermore, the StrAx software used to assess cortical bone parameters is sensitive to movement artefacts.

In conclusion, these data suggest that calciotropic hormones are weakly associated with bone turnover markers, bone parameters, and increased fracture risks. Increasing PTH was associated with increased porosity of the inner transitional zone even in these relatively healthy postmenopausal women. Both serum 25(OH)D and PTH were independently associated with fractures, after mutual adjustment, and were independent of cortical porosity, cortical thickness, and many other well-known risk factors for fracture. Further work is needed to determine the role of serum 25(OH)D and PTH in individuals with low 25(OH)D levels and in a larger sample of individuals, to better understand how this may influence cortical bone architecture and the risk for fractures.

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Authors' roles: Study concept and design, funding procurement, and study execution: MO, TTB, ML, RMJ, ÅB. Statistical analysis: MO, ML, ÅB. Drafting manuscript: MO, TTB, ML, GG, RMJ, EFE, ÅB. Data interpretation and critical revision of the manuscript for important intellectual content, writing of the report, and approval of the final version: MO, TTB, ML, GG, RMJ, EFE, ÅB. ÅB takes responsibility for the integrity of the data analyses.

Disclosures

All authors state that they have no conflict of interest.

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