

Original Article

Does Vitamin D affects changes in volumetric bone mineral density and architecture in postmenopausal women after conservatively treated distal radius fractures?

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Abstract

Objective: We examined the role of vitamin D on volumetric bone mineral density (vBMD) and architecture during the first week's post-fracture in postmenopausal women (PMW) with distal radial fractures (DRF) treated conservatively using peripheral Quantitative Computed Tomography (pQCT). **Methods:** Patients were classified into 2 groups according to initial median 25(OH)D level; Group A (25(OH)D \geq 15 ng/ml) and group B (25(OH)D < 15 ng/ml). All patients were followed for 12 weeks at three visits: baseline, 6 weeks and 12 weeks post fracture. pQCT was performed at baseline in fractured and contralateral non-fractured radius and at 6th and 12th week on the fractured side. **Results:** 39 patients completed the protocol. Mean 25(OH)D levels were 15.60 ± 7.35 ng/ml (3.5-41.7). Trabecular (trab) bone mineral content (BMC) and trabvBMD increased at 6 wk. vs. baseline ($p < 0.001$). Cortical BMC, cortvBMD and cross-sectional area (CSA) progressively decreased ($p < 0.001$) during the 12 weeks. There was no interaction between baseline 25(OH)D levels and changes in trabecular and cortical BMC, vBMD and CSA. Advanced age and higher CTX and P1NP were associated with higher cortical bone loss. **Conclusion:** Vitamin D deficiency does not affect the early architectural changes after a DRF. Advanced age and higher bone remodeling were associated with higher cortical bone loss, probably related to immobilization and independent of vitamin D levels.

Keywords: Osteoporosis, Peripheral Quantitative Computed Tomography, Radial Fractures, Vitamin D

Introduction

Osteoporosis represents a silent, asymptomatic disease, characterized by low bone mass and abnormal bone structure, leading to low trauma fractures¹. The disease is

disproportionately observed in females, with an estimated prevalence of more than 1.5 million osteoporotic fractures per year in the US. In postmenopausal women distal radial fractures (DRFs) are among the most common types of low energy fractures, associated with pain, stiffness, reduced grip strength and other complications affecting daily activities and quality of life^{2,3}.

A high prevalence of vitamin D deficiency and insufficiency, defined as 25(OH)D levels below 10 ng/ml and 20 ng/ml, respectively, has been reported in numerous studies concerning post-menopausal women⁴. A recent study in Greece, reported that up to 54% of postmenopausal women had vitamin D insufficiency⁵. Of note, more than 77% of patients with DRFs have been reported with vitamin

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D insufficiency or deficiency⁶. The role of vitamin D in the pathogenesis and repair of DRF is generally accepted. However, limited data exist concerning the effect of vitamin D on bone architectural changes in the first weeks following a DRF, namely the early fracture healing period^{7,8}.

The purpose of this prospective study was to investigate the role of vitamin D on early changes in volumetric bone mineral density (vBMD) and bone architecture in postmenopausal women with distal radius fracture, using peripheral Quantitative Computed Tomography (pQCT).

Methods

This is a prospective observational study. The study group consisted of postmenopausal women (no menses for >12 months) aged 50 years or older, who presented to the Emergency Department of KAT Hospital with a DRF. All patients, conservatively treated, after manual traction, by cast immobilization were included in the study. In order to minimize the effect of cast on pQCT measurements, we used the Optima Cast casting tape (<http://joinenterprise.com/indexe.php>), a radiolucent material made of knitted fiberglass impregnated with polyurethane resin. The cast was applied only during the baseline measurement at the fractured site. Exclusion criteria were: any previous DRF, use of medications that might affect bone metabolism (bisphosphonates, denosumab, SERMS, menopausal hormone therapy, teriparatide, vitamin D analogues, aromatase inhibitors, corticosteroids), liver and renal disease, primary hyperparathyroidism and rheumatic disease. Patients treated operatively were also excluded. Based on the median 25(OH)D levels, patients were classified into 2 groups according to initial serum 25(OH)D level, Group A (25(OH)D \geq 15 ng/ml) and group B (25(OH)D <15 ng/ml). All patients were followed for 12 weeks, at three outpatient visits: baseline (visit 1), 6 weeks (visit 2) and 12 weeks (visit 3) post-fracture. During the study all patients were treated with 800 IU of cholecalciferol and 1000 mg of calcium carbonate. Compliance was also estimated, at each follow-up visit, when the prescription was given to the patients and they were asked if they had any remnant of the supplement. Patients were classified as non-compliant with 0-49% drug intake, partially compliant with 50-74% drug intake and compliant with 75-100% drug intake. A gentle reminder was also performed for the continuous receipt of the supplement. With the exception of three patients, all were over 75% compliant with drug intake. The clinical protocol (Supplementary Figure 1 and 2) was approved by the ethics committee of KAT hospital and written informed consent was obtained from all patients.

Biochemical measurements

At baseline, fasting venous blood samples were collected for the determination of calcium, phosphate, creatinine, alkaline phosphatase, parathyroid hormone (iPTH), 25(OH) D, C-terminal cross-linking telopeptide of type I collagen (β -CTX), total procollagen type I N-terminal propeptide

(P1NP). All assays were measured by a second generation electrochemiluminescence immunoassay (ECLIA) according to the manufacturer's instructions. Samples for β -CTX and P1NP were centrifuged within one hour from collection (at 3000 rpm for 10 min), aliquoted and stored at -80°C until tested in a single batch. The measurement range and the total analytical imprecisions in our laboratory for the measured bone parameters are: iPTH 1.2-5000 pg/ml and <4%, respectively; β -CTX: 10-6000 ng/L and <3.5%, respectively; P1NP 5-1200 ng/ml and <4.5%, respectively; 25(OH)D 3-100 ng/ml and <4.7%, respectively.

Areal BMD measurements by DXA

At baseline, dual-energy X-ray absorptiometry (DXA) was performed on the non-dominant hip [femoral neck (FN), total proximal femur (TH)] and the lumbar spine (LS) (L1-L4) using Lunar Prodigy Pro (GE Lunar Corp., Madison, WI, USA). Data are reported for both absolute aBMD values (as g/cm²) and T-scores (SD values from the mean for a young reference population). The long-term precision (% CV) was 0.27% (LS), 1.3% (FN), 1.1% (TH).

pQCT scan measurements

pQCT measurements (XCT-2000; Stratec Medizintechnik, Pforzheim, Germany) were performed at baseline in fractured and contra-lateral non-fractured distal radius, visit 2 (6th week) and visit 3 (12th week) on the fractured side. Baseline pQCT was performed with one-week since the fracture. Measurements were taken while forearm was supinated and elbow flexed at 90°. Image analysis was performed using integrated software (STRATEC XCT-2000, version 5.4). A single-energy X-ray source was used. All computed tomography scans had a slice thickness of 2.4 mm and a voxel size of 0.5 mm³. The distal end of the wrist joint was used as an anatomical marker. The bone cross-sectional area (CSA) was imaged at 4% (trabecular) and 20% (cortical) of the radius length, proximal to this point. Analyzing each slice, volumetric bone mineral density (vBMD) (mg/cm³), corresponding bone mineral content (BMC) (mg), and CSA (mm²) of bone section, as well as cortical thickness (mm) (cort THICK), endosteal (ENDO C) (mm) and periosteal circumference (PERI C) (mm) and polar stress strength index in torsion (SSIp) (mm³), were estimated. Trabecular vBMD was measured, with the outer bone contour of the radius detected at a threshold of 280 mg/cm³. Cortical vBMD was measured with a threshold of 710 mg/cm³. The long-term precision (%CV) of the pQCT was 0.35% for trabecular vBMD and 0.26% for cortical vBMD⁹.

Statistical analysis (Supplementary Figure 1)

Data are presented as mean \pm SD or mean \pm SE, for two-way ANOVA model results. Normality was tested using the Kolmogorov-Smirnov test. Two-way mixed ANOVA model with factors time (baseline vs. 6 weeks vs. 12 weeks) and 25(OH)D level (<15 ng/ml vs. \geq 15 ng/ml) was used to

Table 1. Baseline characteristics of the study group.

Parameter	All (n=39)	Group A (n=20)	Group B (n=19)	p
Age (yrs.)	66.69±10.12	67.4 ± 9.48	65.94±10.96	0.66
Weight (Kg)	67.63±12.6	65.11±9.52	70.66 ±15.33	0.21
Height (cm)	156.15±6.39	155.5±6.83	156.9±5.95	0.53
BMI (Kg/m ²)	27.74±4.92	29.99±4.11	28.63±5.77	0.65
Calcium (mg/dl)	9.35±0.47	9.32±0.48	9.39±0.48	0.67
Phosphate (mg/dl)	3.45±0.43	3.61±0.38	3.28±0.43	0.03
Creatinine (mg/dl)	0.74±0.09	0.77±0.08	0.71±0.10	0.07
ALP (IU/L)	67.20±21.01	62.38±15.81	72.62±25.08	0.15
PTH (pg/ml)	47.53±20.32	45.01±17.29	50.37±23.52	0.45
25(OH)D (ng/ml)	15.60±7.35	20.47±6.63	10.47±3.68	<0.001
OC (ng/ml)	19.82±7.4	17.73±7.15	21.04±7.72	0.37
β-CTX (ng/ml)	0.35±0.17	0.327±0.17	0.378±0.18	0.41
P1NP (ng/ml)	40.18±14.11	37.42±11.88	43.28±16.08	0.23
BMD FN (mg/cm ²)	0.795±0.16	0.747±0.16	0.837±0.15	0.11
FN T-score	-1.51±1.37	-1.88±1.41	-1.18±1.28	0.14
BMD TH (mg/cm ²)	0.850±0.15	0.814±0.14	0.882±0.16	0.21
TH T-score	-1.23±1.32	-1.55±1.25	-0.95±1.35	0.19
BMD LS (mg/cm ²)	0.997±0.16	0.972±0.17	1.022±0.16	0.38
LS T-score	-1.52±1.39	-1.74±1.4	-1.31±1.39	0.30

Data are presented as mean ± SD. Group A (25(OH)D ≥15 ng/ml) and group B (25(OH)D <15 ng/ml). 25(OH)D: 25 (OH) vitamin D, BMI: Body Mass Index. PTH: Parathyroid Hormone, ALP: alkaline phosphatase, OC: Osteocalcin, β-CTX: C-terminal cross-linking telopeptide of type I collagen, P1NP: total procollagen type 1 N-terminal propeptide. BMD: Bone Mineral Density, FN: Femoral Neck, TH: Total Hip, LS: Lumbar Spine

Table 2. Comparison of pQCT measurements during the observation period controlling for 25(OH)D levels

	Baseline	6 wks.	12 wks.	P _{interaction}	P _{time}	P _{25(OH)D}
Trab BMC (mg)	36.11±1.73	45.02±2.04 ^a	43.68±2.66 ^a	0.087	<0.001	0.125
Trab CSA (mm ²)	185.17±3.44	184.63±5.29	181.88±5.76	0.558	0.510	0.101
Trab vBMD (mg/cm ³)	194.73±7.95	242.83±8.44 ^a	236.61±11.20 ^a	0.205	<0.001	0.420
Cort BMC (mg)	56.82±2.45	53.74±2.69 ^a	48.19±2.73 ^{ac}	0.205	<0.001	0.725
Cort CSA (mm ²)	55.08±1.91	52.62±2.21 ^a	47.97±2.28 ^{ac}	0.226	<0.001	0.935
Cort vBMD (mg/cm ³)	1018.19±12.45	1005.18±12.3 ^a	984.13±13.5 ^{ac}	0.209	<0.001	0.565
Cort THICK (mm)	1.54±0.06	1.48±0.07 ^b	1.35±0.07 ^{ac}	0.285	<0.001	0.704
Endo C (mm)	31.33±0.63	31.43±0.71	34.74±0.69	0.586	0.411	0.348
Peri C (mm)	41.00±0.48	40.71±0.52	40.24±0.48	0.128	0.318	0.334
SSIp (mm ³)	162.49±6.95	149.69±6.96 ^a	132.77±7.03 ^{ac}	0.207	<0.001	0.825

Data are presented as mean ± SE derived from two-way mixed ANOVA model. Trab: trabecular, BMC: Bone mineral Content, CSA: Cross-sectional area, vBMD: volumetric Bone Mineral Density, Cort: Cortical, Cort THICK: cortical thickness, Endo C: endosteal circumference Peri C: periosteal circumference, SSIp: polar stress strength index in torsion^a: p<0.001 vs. baseline, ^b: p<0.05 vs. baseline, ^c: p<0.005 vs. 6 wks.

examine the differences of pQCT measurements during the observation period controlling for the influence of 25(OH) D levels, by examining the interaction term between time and 25(OH)D level. Pairwise comparisons between time points were made by Bonferroni test. We created a new variable PDFrNonFr (%), the percentage (%) difference in

pQCT derived variables (v) between the fractured and non-fractured radius calculated as follows: PDFrNonFr(%)=[(VFr-VNonFr)/Vnon-Fr]*100. Two-way mixed ANOVA model with factors Time (baseline vs. 6 weeks vs. 12 weeks) and dominant radius (dominant vs. non-dominant) was used to examine the differences of PDFrNonFr variables during the

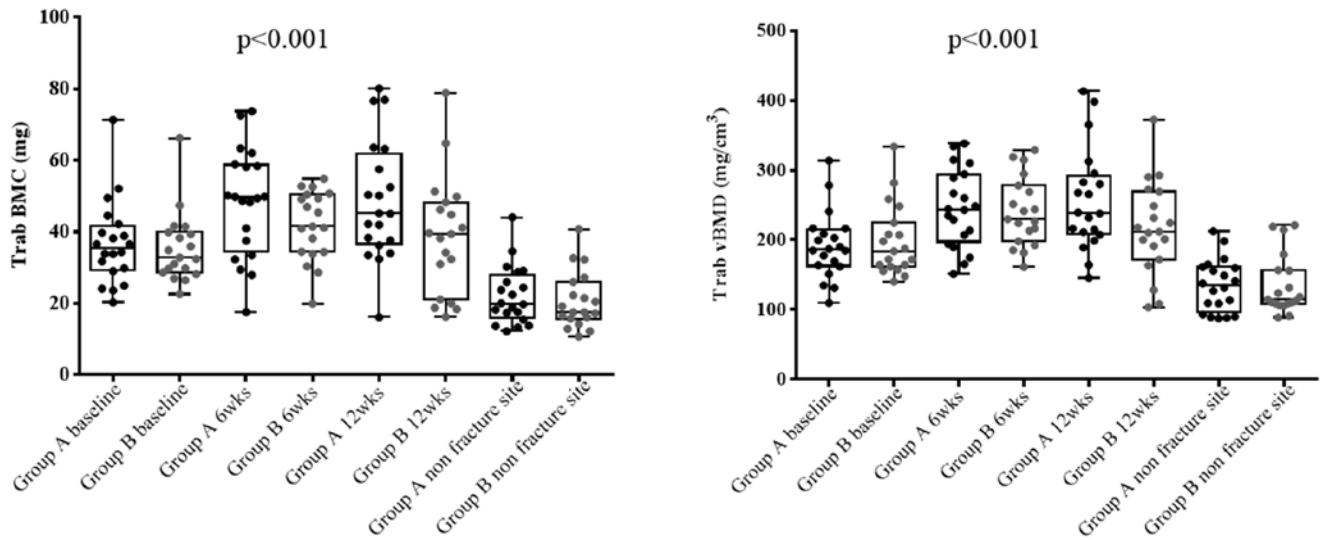


Figure 1. Trabecular BMC and vBMD (4% from the distal end) in the two groups. BMC: Bone Mineral content, vBMD: volumetric Bone Mineral Density. Group A: (25(OH)D ≥ 15 ng/ml), Group B: (25(OH)D < 15 ng/ml). p: Two-way mixed ANOVA model with factors time (baseline vs. 6 weeks vs. 12 weeks) and 25(OH)D level (< 15 ng/ml vs. ≥ 15 ng/ml) was used to examine the differences of trabecular BMC and trabecular vBMD during the observation period.

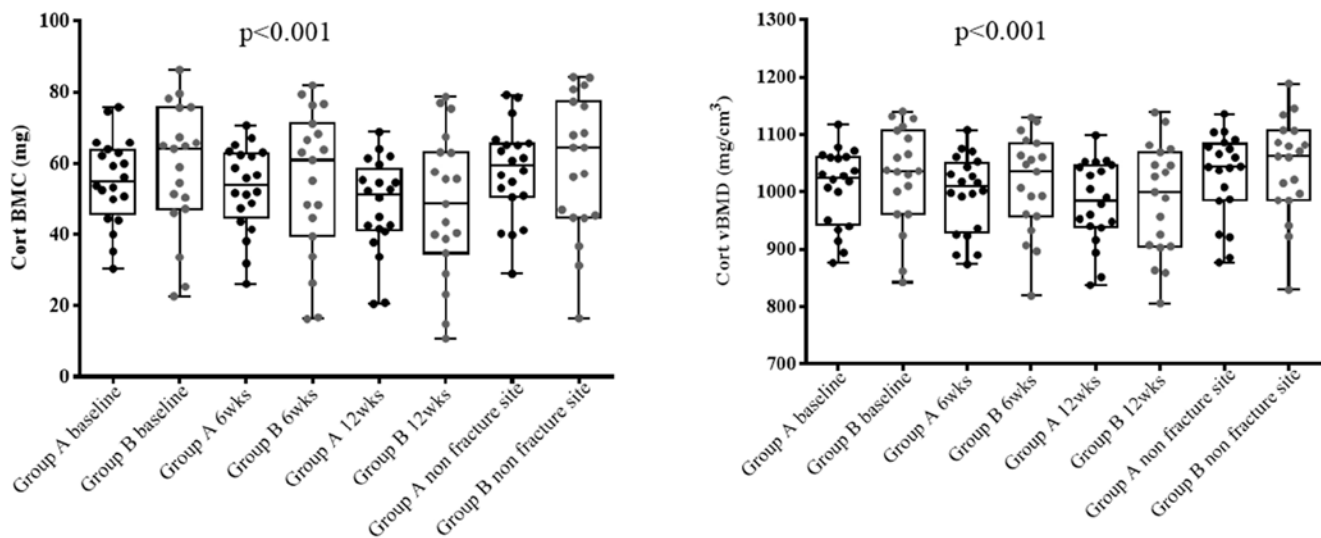


Figure 2. Cortical BMC and vBMD (20% from the distal end) in the two groups. BMC: Bone Mineral Content, vBMD: volumetric Bone Mineral Density. Group A: (25(OH)D ≥ 15 ng/ml), Group B: (25(OH)D < 15 ng/ml). p: Two-way mixed ANOVA model with factors time (baseline vs. 6 weeks vs. 12 weeks) and 25(OH)D level (< 15 ng/ml vs. ≥ 15 ng/ml) was used to examine the differences of Cortical BMC and Cortical vBMD during the observation period.

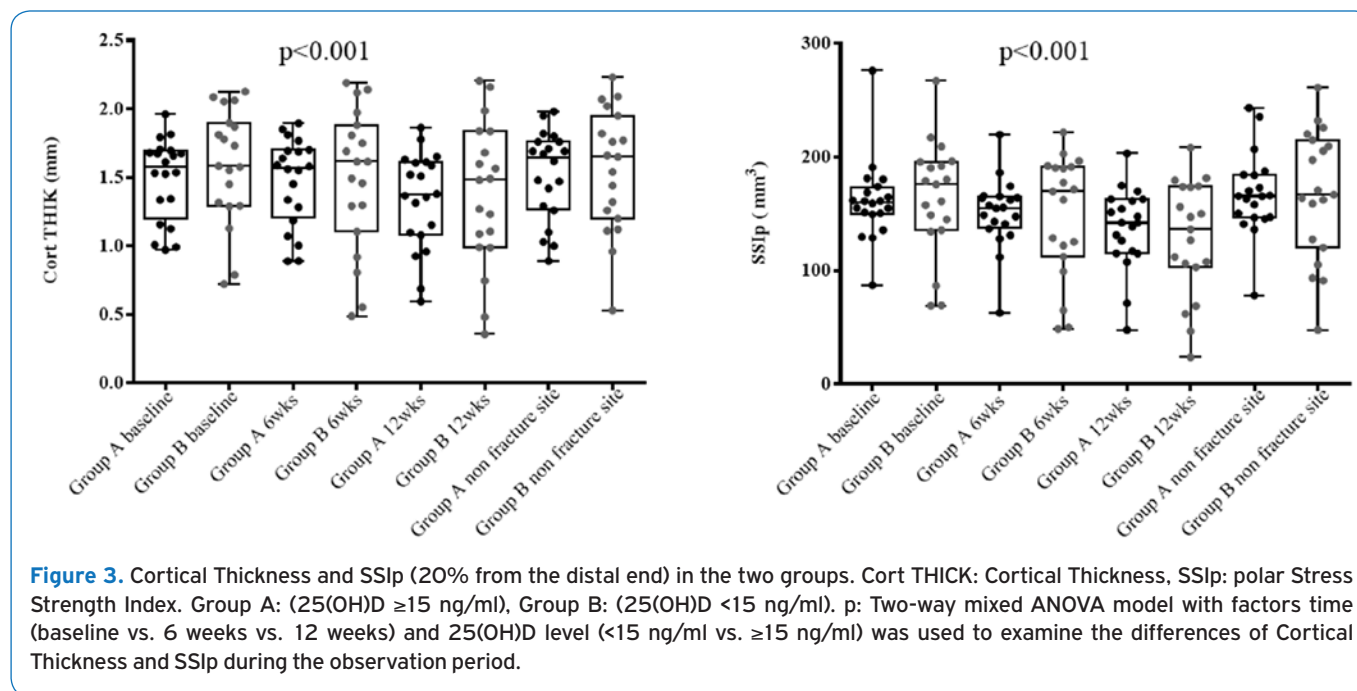
observation period controlling for the influence of dominant radius by examining the interaction term between time and dominant radius. Pairwise comparisons between time points were made by Bonferroni test. Correlation analysis was performed using the Spearman correlation coefficient. Due to the exploratory nature of the study, we performed post-

hoc power analysis concerning between time measurements of trab vBMD and cort vBMD. It was calculated that a sample size of 39 patients had 100% probability to demonstrate a between time difference of an effect size f equals to 0.656 and 0.676 for trab vBMD and cort vBMD respectively, with a two tailed significance of $p < 0.05$. All tests were two-sided,

Table 3. Percentage (%) difference in pQCT derived variables between the fractured and non-fractured radius during the observation period controlling for dominance.

	Baseline	6 wks.	12 wks.	P _{interaction}	P _{time}	P _{dominance}
Trab BMC (mg)	85.87±11.6	133.64±17.1 ^a	128.89±20.12 ^a	0.219	<0.001	0.219
Trab CSA (mm ²)	20.17±3.10	19.56±4.00	17.69±4.05	0.088	0.586	0.731
Trab vBMD (mg/cm ³)	51.56±7.37	89.68±9.19 ^a	88.75±12.87 ^a	0.501	<0.001	0.485
Cort BMC (mg)	-1.94±1.70	-8.63±1.97 ^a	-18.89±2.23 ^{a,c}	0.601	<0.001	0.015
Cort CSA (mm ²)	-0.38±1.58	-5.93±1.97 ^a	-14.87±2.17 ^{a,c}	0.691	<0.001	0.015
Cort vBMD (mg/cm ³)	-1.64±0.35	-2.88±0.37 ^a	-4.95±0.52 ^{a,c}	0.352	<0.001	0.336
Cort THICK (mm)	1.42±2.31	-3.63±2.72 ^b	-12.92±2.63 ^{a,c}	0.598	<0.001	0.121
Endo C (mm)	-1.36±1.22	-1.13±1.61	-0.19±1.34	0.517	0.493	0.600
Peri C (mm)	-1.17±0.68	-1.86±0.88	-2.95±0.81 ^a	0.897	0.016	0.067
SSIp (mm ³)	-1.20±2.18	-10.22±2.09 ^a	-21.16±2.50 ^{a,c}	0.725	<0.001	0.277

Data are presented as mean±SE derived from two-way mixed ANOVA model. Trab: trabecular, BMC: Bone Mineral Content, CSA: Cross-sectional area, vBMD: volumetric Bone Mineral Density, Cort: Cortical, Cort THICK: cortical thickness, Endo C: endosteal circumference Peri C: periosteal circumference, SSIp: polar stress strength index in torsion. ^a: p<0.001 vs baseline, ^b: p<0.05 vs baseline, ^c: p<0.005 vs. 6 wks.



and statistical significance was set at p<0.05. All analyses were carried out using the statistical package SPSS v21.00 (IBM Corporation, Somers, NY, USA).

Results

Patients' characteristics (Table 1, Supplementary Figure 2)

During the 19-month period we identified 57 women meeting the inclusion criteria. In the course of the study 18 women were excluded: 16 did not attend the 2nd (n=11)

or 3rd (n=5) visit and in 2 patients the pQCT scan could not be interpreted due to movement artifacts (Supplementary Figure 2). Thus, 39 women completed the protocol. The mean age was 66.69±10.12 yrs. (range: 51-88). 51.3% (n=20) of the fractures occurred at the right radius, while 51.3% (n=20) at the dominant side. At baseline, mean 25(OH)D levels were 15.60±7.35 ng/ml (3.5-41.7). 17.9% of the patients (n=7) had 25(OH)D levels below 10 ng/ml and 20.5% (n=8) higher than 20 ng/ml. 28.2% (n=11) had LS T-score below -2.5 and 25.6% (n=11) had T-score below -2.5 at the FN or TH.

Changes of vBMD and geometry at the fracture site Trabecular site (4%) (Figure 1, Tables 2 and 3)

At the peripheral trabecular site, there was no interaction between baseline 25(OH)D levels and changes in trab BMC, vBMD and CSA (p: ns). In addition, there were no differences concerning trab BMC, vBMD and CSA at any of the three time points between group A vs. group B. Concerning within group changes, trab BMC and trab vBMD increased at 6 wk. vs. baseline ($p < 0.001$), with no further change until 12 wks. post-fracture. Specifically, for trab BMC, % changes over the previous measurement were 28.32% and -2.72% at 6 and 12 wks., respectively, while for trab vBMD the corresponding % changes were 27.75% and -1.58%. Trab CSA did not change (p: ns) (Figure 1). Concerning % differences with the non-fractured radius (PDFrNonFr) (Table 3), trab BMC and trab vBMD % difference at 6 wks and 12 wks increased as compared with baseline.

Cortical site (20%) (Figures 2 & 3, Tables 2 and 3)

At the cortical site, there was no interaction between baseline 25(OH)D levels and changes in cort BMC, vBMD and CSA (p: ns). In addition, there were no differences concerning cort BMC, vBMD and CSA at any of the three time points between group A vs. group B. Concerning within group changes, cort BMC, cort vBMD and cort CSA progressively decreased ($p < 0.001$) during the 12 wks., with each variable being significantly lower vs. the previous one. For cort BMC, % changes over the previous measurement were -6.70% and -11.06% at 6 and 12 wks., respectively, for cort vBMD -1.25% and -2.1% and for cort CSA -5.46% and -9.21%, respectively. Concerning PDFrNonFr (Table 3), cort BMC, cort CSA and cort vBMD % differences at 6 wks and 12 wks progressively and significantly decreased as compared with baseline, indicating cortical bone loss at the fractured site.

Concerning bone geometry indices, there was no interaction between baseline 25(OH)D levels and changes in cort THICK, PERI C, Endo C and SSIP (p: ns). Moreover, there was no difference in any bone geometry indices at any of the three time points between group A vs. group B. Concerning within group changes, cort THICK progressively decreased ($p < 0.001$), with the effect being most evident at 12 wks. The corresponding % changes over the previous measurement were -4.88% and -8.92%, respectively. SSIP decreased significantly ($p < 0.001$) during the 12 wks., with each value being significantly lower vs. the previous one. The corresponding % changes over the previous measurement were -8.67% and -11.85%, respectively. Concerning PDFrNonFr (Table 3), cort THICK and SSIP % difference at 6wks and 12wks progressively and significantly decreased as compared with the baseline difference, indicative of loss of bone strength.

Correlation Analysis (Supplementary Table 1)

Correlation analysis revealed significant negative association between age with % changes in cort

BMC ($r_{6wk} = -0.43$ and $r_{12wk} = -0.44$ for 6wk and 12wk respectively, all $p < 0.01$), % changes in cort CSA and cort THICK (CSA: $r_{6wk} = -0.50$ and $r_{12wk} = -0.46$, THICK: $r_{6wk} = -0.51$ and $r_{12wk} = -0.47$, all $p < 0.01$) and % changes in SSIP ($r_{6wk} = -0.42$ and $r_{12wk} = -0.37$, $p < 0.01$ and $p < 0.05$, respectively), indicating that elderly patients had higher bone loss and loss of strength during the post-fracture period. Similar negative association was observed between bone turnover markers (osteocalcin, β -CTX and PINP) and % changes in cort BMC, cort CSA, cort THICK and SSIP, indicating that higher bone remodeling is associated with higher cortical bone loss in the post-fracture period. There was no correlation between % changes in trabecular indices with anthropometric and biochemical variables.

Discussion

Our main finding is that vitamin D deficiency does not affect the short-term changes in vBMD and architecture after a DRF. Cortical vBMD, cortical thickness and the corresponding strength index, assessed away from the fracture site, significantly decreased (-2.1%, -8.9% and -11.8% respectively), probably related to immobilization, while at the site of the fracture, mainly composed by trabecular bone, BMC and vBMD increased (28.3% and 27.7%, respectively) reflecting the early phases of fracture healing.

The first finding of this study was that at the cortical site of the radius, vBMD, cort CSA, cort THICK and ultimately bone strength as expressed by SSIP progressively decrease, with the effect being most evident at 12 wks. These findings are concordant with several human studies of limb immobilization, where significant bone loss of the immobilized limb was observed¹⁰⁻¹². Furthermore, animal studies demonstrate similar results of rapid bone loss following immobilization, ranging from 22 to 41%¹³⁻¹⁵. According to Wolff's law, bone's internal structure and shape adapts to the mechanical loading conditions imposed on it¹⁶. Thus, extended periods of inactivity, similar to those of patients suffering from DFR, cause substantial bone loss and concomitant deterioration of the musculoskeletal system¹⁷. Osteocytes orchestrate the response of bone to declined mechanical stimuli, like conditions of limb unloading, as in DRF early post traumatic period. The direct effect of immobilization on osteocyte lacunar properties remains controversial. Experimental studies in mice and rodents¹⁸ have demonstrated that mechanical unloading leads to osteocytic osteolysis in cortical bone. A possible explanation of this phenomenon is that after osteocytes directly sense unloading, this results in increased expression of inhibitors of bone formation and stimulators of bone resorption, like sclerostin and RANKL¹⁹⁻²¹. These observations indicate that limb immobilization leads to accelerated osteoclast - induced bone resorption and inhibition of osteoblast mediated bone formation and probably increased osteocytic osteolysis²².

This short-term maladaptive response of cortical bone in the vicinity of the DRF was independent of vitamin D

status and seemed to be more pronounced in the elderly and in states of higher bone remodeling. The number of studies addressing the impact of vitamin D deficiency on immobilization induced bone loss is limited. The study of Melhus et al in rats sustaining tibia fracture reported no significant difference neither in cortical bone loss, nor in the mechanical strength in the midshaft relative to vitamin D level²³. On the contrary, the studies of Fischer et al and Dirschl et al reported significant correlation of bone loss with low vitamin D levels^{24,25}. In any case, correction of vitamin D (and calcium) deficiency in the early post fracture healing period may prevent systematic cortical bone loss after fracture and diminish the increased risk for new fractures in elderly patients. Concerning the impact of aging on the skeletal response in disuse osteoporosis, limited data in animals indicate further decrease in cortical BMD via induction of new cortical pores and enlargement of existing ones²⁶. Given that the time needed for full recovery of bone strength might be up to two years, while in clinical practice the cast is removed after 3-5 wks., a time point, that based on the present study, cortical bone strength is waning, a closer follow-up, at least in elderly patients, might be prudent^{15,27}.

Contrary to the cortical site, at the peripheral trabecular site of the radius we observed an increase in trab BMC and trab vBMD during the early post-fracture period. This result is in agreement with the studies of Meyer et al and de Jong et al using hrpQCT, reporting 16,2% increase of trab vBMD at 4 wks. post-fracture, associated with increase in trabecular thickness^{12,27}. In our study the increase in bone parameters was observed in the first 6 weeks, with no further change until 12 weeks post-fracture. This early increase in trabecular vBMD probably represents the early stages of hard callus formation. This finding was also recorded by Han et al in mice, where, at the 7th day post fracture, the trabecular width of cancellous fracture zone was 39.4% thicker than the normal counterpart, indicating that the recovery of cancellous bone micro-architecture may first require an "over-ossification" in the bone marrow cavity²⁸.

This increase of trabecular bone parameters in callus area was independent of vitamin D status. There is a scarcity of studies addressing the impact of vitamin D deficiency on the callus formation at the fracture, while most existing relative low-quality studies focus in vitamin D and calcium, rather than evaluating the importance of vitamin D as a sole entity. Our results are concordant with Meyer's study presenting no correlation between changes in bone microarchitecture in early post-traumatic period and patient's vitamin D level¹². On the contrary, Doetsch et al reported a positive impact of vitamin D levels on the BMD of callus area in conservatively treated humerus fracture⁸. Concerning the experimental studies, the majority report a positive effect of vitamin D on fracture healing, callus mechanical strength and bone mineralization²⁹⁻³¹.

Regarding the effect of vitamin D supplementation on fracture healing, few data from randomized clinical studies exist. All these studies included elderly patients with fragility fractures and report positive effects of

supplementation on vitamin D levels, but none reported clear fracture healing outcomes. It is of note, that Haines et al reported a lower non-union rate of 2.3% in the intervention group as compared to 6.7% in the control group, while studies of Doetch et al, Harwood et al and Kolb et al described a positive effect of supplementation on BMD^{8,32-34}. These studies used a wide range of dosing regimens (800-100.000 IUs) and their conclusions suggest that larger doses of vitamin D supplements may be more efficacious³⁵. It is clear that well designed studies are needed to define the optimal dosing regimen.

The presence of polyester cast at baseline is an important concern in the interpretation of the results. Although the application of a conversion equation would, to a point, improve the accuracy of our results, unfortunately we do not have data from volunteers scanned with and without cast to develop such an equation. However, a recent study³⁶ using an *in vitro* and *in vivo* protocols, reported that the synthetic polyester cast, alters pQCT results statistically significantly, but less than 2.5% for the trabecular site and less than 7.5%, which was not significant, for the cortical sites. This effect is by far less than the changes we observed concerning trab vBMD (27.75%), while for the cortical site, pQCT measurements were significantly lower at 12 wks. vs. 6 wks, where no cast was applied. Moreover, post hoc power analysis indicated that our study had 100% probability of detecting a between time difference of an effect size f equals to 0.656 and 0.676 trab vBMD and cort vBMD respectively, with a two tailed significance of $p < 0.05$. Thus, we believe that the application of polyester cast essentially did not affect our findings.

The present study has limitations. First, due to the exploratory nature of the study, a relatively small number of patients was included. Furthermore, given that specific cutoffs are used in the literature for 25(OH)D to guide patients and clinicians, we used the median 25(OH)D levels to categorize the patients and not the classic cutoffs such as 10 ng/ml and 20 ng/ml. However, the analysis of our data indicated that there was no correlation between baseline 25(OH)D levels with pQCT indices at either baseline or follow-up, while in two-way mixed ANOVA with 25(OH)D as covariate (continuous variable) and not as factor (categorical variable), there was no interaction between 25(OH)D and changes in pQCT variables. In any case given that the number of patients having severe vitamin D deficiency (<10 ng/ml) was small, we cannot exclude the possibility that severe long-standing vitamin D deficiency adversely affects the early post-fracture changes. Moreover, we did not measure 25(OH)D at the end of the study. Lastly, our study was short-term and thus we did not examine whether the observed changes were restored at a longer period post fracture.

In conclusion our data indicate that vitamin D deficiency does not affect the early changes in vBMD and bone architecture after a DRF. Advanced age and higher bone remodeling were associated with higher cortical bone loss, probably related to immobilization and independent of vitamin D levels.

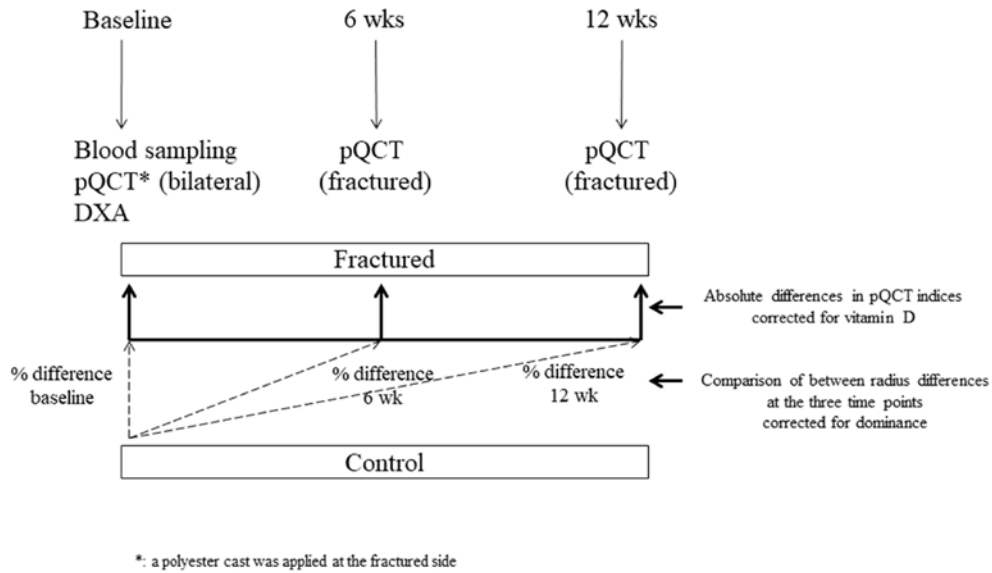
Acknowledgements

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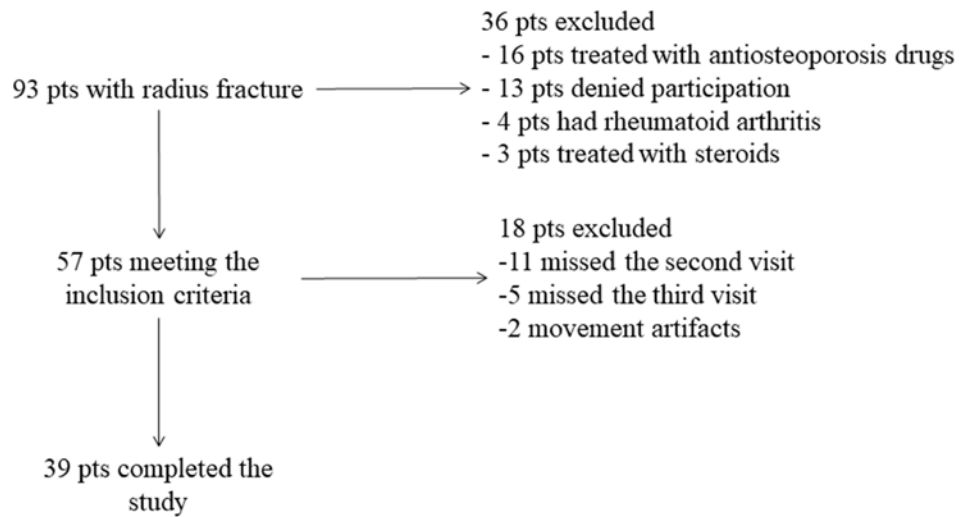
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Supplementary Figure 1. Study design.



Supplementary Figure 2. Screening and follow-up of patients through the end of the study period.

Supplementary Table 1. Correlation of % changes over baseline of cortical pQCT derived indices with age and bone turnover markers.

	Age	OC	β -CTX	P1NP
% Cort BMC6wk	-0.43 ^a	-0.44 ^a	-0.45 ^a	-0.45 ^a
% Cort BMC12wk	-0.41 ^a	-0.32	-0.37 ^b	-0.33
% Cort CSA6wk	-0.50 ^a	-0.38 ^b	-0.38 ^b	-0.42 ^b
% Cort CSA12wk	-0.46 ^a	-0.32	-0.37 ^b	-0.35 ^b
% Cort THICK6wk	-0.51 ^a	-0.36 ^b	-0.37 ^b	-0.38 ^b
% Cort THICK12wk	-0.47 ^a	-0.26	-0.32	-0.32
% SSIp6wk	-0.42 ^a	-0.26	-0.36 ^b	-0.31
% SSIp12wk	-0.37 ^b	-0.15	-0.23	-0.20

Cort: Cortical, BMC: Bone Mineral Content, CSA: Cross-sectional Area, Cort THICK: Cortical Thickness, SSIp: polar Stress Strength Index in torsion ^a: $p < 0.01$, ^b: $p < 0.05$