CLINICAL TRIAL

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The Effect of Bolus Vitamin D_3 Supplementation on Distal Radius Fracture Healing: A Randomized Controlled Trial Using HR-pQCT

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ABSTRACT

Vitamin D is an important factor in bone metabolism. Animal studies have shown a positive effect of vitamin D_3 supplementation on fracture healing, but evidence from clinical trials is inconclusive. A randomized controlled trial was performed to assess the effects of vitamin D_3 supplementation on fracture healing using HR-pQCT–based outcome parameters. Thirty-two postmenopausal women with a conservatively treated distal radius fracture were included within 2 weeks postfracture and randomized to a low-dose ($N = 10$) and a high-dose ($N = 11$) vitamin D intervention group receiving a 6-week bolus dose, equivalent to 700 and 1800 IU vitamin D_3 supplementation per day, respectively, in addition to a control group ($N = 11$) receiving no supplementation. After the baseline visit 1–2 weeks postfracture, follow-up visits were scheduled at 3–4, 6–8, and 12 weeks postfracture. At each visit, HR-pQCT scans of the fractured radius were performed. Cortical and trabecular bone density and microarchitectural parameters and microfinite element analysis–derived torsion, compression, and bending stiffness were assessed. Additionally, serum markers of bone resorption (CTX) and bone formation (PINP) were measured. Baseline serum levels of $25OHD_3$ were <50 nmol/L in 33% of all participants and <75 nmol/L in 70%. Compared with the control group, high-dose vitamin D_3 supplementation resulted in a decreased trabecular number (regression coefficient β: −0.22; $p < 0.01$) and lower compression stiffness (B: −3.63; $p < 0.05$, together with an increase in the bone resorption marker CTX (B: 0.062; $p < 0.05$). No statistically significant differences were observed between the control and low-dose intervention group. In conclusion, the bolus equivalent of 700 U/day vitamin D_3 supplementation in a Western postmenopausal population does not improve distal radius fracture healing and an equivalent dose of 1800 IU/day may be detrimental in restoring bone stiffness during the first 12 weeks of fracture healing. © 2021 The Authors. *Journal of Bone and Mineral Research* published by Wiley Periodicals LLC on behalf of American Society for Bone and Mineral Research (ASBMR).

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Introduction

Fracture healing consists of a complex series of events aimed at restoring the mechanical function of the affected bone region.[1,2] Concepts such as the four-phase model, beginning with inflammation, followed by soft- and hard-callus formation ending with long-term remodeling have been used as a framework to understand these cellular and molecular processes.[3] In addition, the so-called diamond model has been developed to integrate the various therapeutic factors that affect fracture healing.[4,5] In this four-part model, osteogenic cells form new bone tissue in osteoconductive scaffolds (or biomaterials) under the influence of growth factors (or local anabolic mediators), a process shaped by mechanical stimuli from the local environment.

There is increasing interest in the effect of systemic medications such as bisphosphonates and PTH on fracture healing.[6] Although not a pharmacological drug in the strict sense, vitamin D_3 supplementation is widely used as part of the preventive and treatment strategies in osteoporosis[7] because of the high prevalence of low serum $25OHD_3$ levels and calcium insufficiency in the postmenopausal fracture population.[8,9] Supplementation of 800 to 1000 IU/day of vitamin D_3 for persons aged 60 years and over is advocated in international guidelines or when antiosteoporosis treatment is indicated.[7]

Besides being part of the fracture-prevention strategy, there is an interest in vitamin D_3 supplementation in fracture healing. Animal studies have reported a positive effect of vitamin D_3 on fracture healing as measured by radiographic imaging, histology, and mechanical testing.[10–13] In a clinical setting, retrospective studies have shown that patients with a delayed-union have lower 25OHD levels compared with normally healing fracture patients,[14] and that 25OHD deficiency is common in nonunion patients.[15] Furthermore, a clinical study investigating combined vitamin D_3 and calcium supplementation showed increased callus BMD in proximal humerus fractures.[16]

In this randomized controlled trial (RCT), the effect of early vitamin D_3 supplementation on distal radius fracture healing was assessed using HR-pQCT and microfinite element analysis (μFEA) as primary outcome measurement. These techniques have been shown to be capable of quantifying changes in bone density, structure, and biomechanical properties during distal radius fracture healing, while also being associated with clinical outcome,[17–19] facilitating a high-resolution and objective method of assessing the intervention.

We hypothesized that a bolus equivalent of 700 IU/d of vitamin D_3 would enhance fracture healing and that a higher equivalent dose of 1800 IU/d could be even more beneficial, especially in vitamin D-deficient patients.

Participants and Methods

Study design

This single-blind RCT was conducted at the Maastricht University Medical Center (MUMC) in the Netherlands. Early supplementation of vitamin D_3 during fracture healing was compared with a control group receiving no intervention. Study visits were scheduled 1–2 weeks (visit 1), 3–4 weeks (visit 2), 6–8 weeks (visit 3), and 12 weeks (visit 4) postfracture. The study protocol, approved by the institutional medical ethics committee (file number NL33512.068.10), was submitted to the Dutch Trial Register (NTR) and filed under registration number NTR3821. All participants provided written informed consent before enrolling in the study.

Participants

Women aged 50 years and older presenting at the emergency room of the MUMC with a distal radius fracture, receiving cast immobilization, were screened for inclusion. Patients requiring surgical treatment were excluded because of the effect of metal implants on the primary outcome measures.[20] Patients with a known systemic or metabolic bone disorder, such as hyperthyroidism, hyperparathyroidism, chronic kidney disease (with an estimated glomerular filtration rate <30 mL/min/1.73 m^2), sarcoidosis, or an active inflammatory disease (rheumatoid arthritis, inflammatory bowel disease) were also excluded. Final exclusion criteria were use of oral glucocorticoids in the past 12 months, malignant disease in the past 12 months, previous (bone) surgery at the current fracture site, a neuromuscular or neurosensory condition, severe concurrent joint involvement, or the inability to provide informed consent.

In addition to the study procedures described below, all study participants were invited to participate in the screening program for osteoporosis at the local fracture liaison service, as per the national guidelines.[21] This screening included laboratory tests for metabolic bone disorders and DXA of the lumbar spine and femur.

Intervention

Clinically, vitamin D status is determined by the measurement of serum 25-hydroxyvitamin D (25OHD) concentration.[22] Previous studies have shown a comparable effect of daily versus monthly doses of vitamin D_3 (cholecalciferol) on serum 25OHD levels,[23,24] although it is still unknown if a sudden increase of 25OHD serum levels could, independent of absolute serum levels, contribute to the negative effects observed by others with a 500,000 IU bolus dose.[25]

To achieve full compliance, liquid vitamin D_3 50,000 IU/mL (Fagron BV), was administered orally at the first and third study visits in two bolus doses. Participants enrolled in the low- dose group received 0.6 mL or 30,000 IU once per 6 weeks (equivalent to 700 IU daily) versus 1.5 mL or 75,000 IU once every 6 weeks (equivalent to 1800 IU daily) in the high-dose group.

If there was an indication for antiosteoporosis treatment, standard supplementation with 800 IU vitamin D_3 daily was started after the end of this study.

Randomization

Block randomization was performed by an independent member of the orthopedic trial bureau using a computer-generated randomization list (SPSS 16.0; IBM Corp). Patients were allocated using sequentially numbered envelopes to either a control group ($N = 10$) receiving no early vitamin D_3 supplementation, a low-dose intervention group (vitamin D_3 700 IU daily), or a high-dose intervention group (vitamin D_3 1800 IU daily). No placebo was used in the control group. Participants were asked to report any use of over-the-counter vitamin D supplements.

During data collection and image and statistical analyses, the investigator performing these tasks was blinded for the intervention assignment.

Measurements

HR-pQCT scanning

At each visit, the fractured radius was scanned using a first-generation HR-pQCT scanner (XtremeCT; Scanco Medical AG). In addition, the contralateral side was scanned at the first and last visit using the same scan protocol to detect an effect of the intervention on nonfractured bone. This protocol, similar to previous studies,[17,18,26] consisted of two consecutive stacks of 9 mm using the standard in vivo settings as prescribed by the manufacturer (82-μm isotropic voxel size, X-ray tube voltage 60 kVp and tube current 0.900 mA, 100 ms integration time, 750 projections/180°). The acquired image thus comprised an 18 mm/220 slices long region of the distal radius. Use of the standard reference point on the articular surface of the distal radius is not feasible in case of a fracture. Therefore, scan offset was set at 3.0 mm from the proximal edge of the lunate (Fig. 1).[17]

The cast was usually removed at or shortly before the third study visit, 6–8 weeks postfracture. To avoid bias of the subsequent scans,[27] the cast was preserved and temporarily replaced around the wrist during the later scans. To facilitate the lower arm with cast and minimize motion artifacts, a custom carbon holder with inflatable cushion (Pearltec AG, Schlieren, Switzerland) was used.[17]

All scans were quality-graded according to Pialat and colleagues.[28] Scans with significant motion artifacts (i.e., grade 4 or 5) were repeated once. Only scans with grade 1–3 were used for the analyses.

Image analyses

HR-pQCT images were analyzed by a single investigator blinded for the randomization using μFEA in addition to the standard evaluation method, both provided by the manufacturer (Scanco Medical AG) as described previously.[17] Briefly, after semiautomatic contouring of the periosteal boundary, segmentation of the mineralized tissue was achieved using a Laplace-Hamming filter (epsilon 0.5 and cutoff frequency 0.4) with normalization (range, 0–1000) and global thresholding (threshold 400). BMDs (mgHA/cm^3) were determined for the trabecular, cortical, and total region of interest. A 3D ridge extraction method was used to assess trabecular number (1/mm) and derive trabecular thickness (mm) and separation (mm).[29]

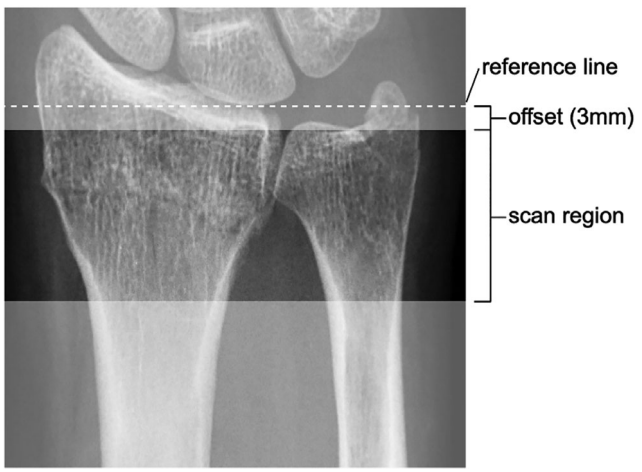

Fig 1. Graphical representation of the scan region. The reference line was set on the proximal edge of the os lunatum. The scan region was determined as an 18-mm section, starting 3-mm proximal of the reference line.

Microfinite element analyses

Segmented images were used to create μFE models with brick elements similar in size to the 82-μm isotropic voxels. Assigned material properties were a Young's modulus of 10 GPa and a Poisson's ratio of 0.3.[30] The μFE models were subjected to four virtual load cases to assess bone stiffness: a high-friction compression test (prescribed displacement of 1% length change in axial direction), a rotation test around the longitudinal axis (0.01 rad), and two rotational tests around the sagittal and transversal axis (0.01 rad). The latter two were combined to calculate estimated bending stiffness.[17]

Bone turnover makers

Nonfasting venous blood samples were taken in the morning (before noon) at each visit and before administering the cholecalciferol at visits 1 and 3. From these samples, the serum marker of bone formation PINP and serum marker for bone resorption CTX were measured using chemiluminescence immunometric assays on the IDS-iSYS instrument (Immunodiagnostic Systems, PLC).

Functional outcome: PRWE

Study participants completed the Dutch version of the Patient-Rated Wrist Evaluation (PRWE)[31,32] at each visit. This 15-item questionnaire has been validated to assess functional outcome after distal radius fractures.[33] The PRWE consists of a pain domain (5 questions) and a function domain (10 questions), resulting in a combined score ranging from 0 (no pain/disabilities) to 100 (worst pain/disabilities).

Additional data

Medical history (previous surgeries and/or fractures, medication) and baseline characteristics (age, height, and weight) were collected at the first visit. In addition, data from the fracture liaison service were extracted: daily calcium intake (including supplements), smoking status and alcohol intake, DXA results (lumbar spine and proximal femur BMD and *T* scores), and baseline 25OHD serum levels. From the blood sample collected at visit 4, 25OHD response after the intervention was also measured using a chemiluminescence immunometric assay on the IDS-iSYS instrument (Immunodiagnostic Systems, PLC).

Sample size

PS power and sample size calculator software (version 3.0.14) was used to calculate sample size (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). Limited data on clinical fracture healing trials using HR-pQCT and μFEA were available at the time of study inception. It was estimated that 0.5 kN constituted a clinically relevant difference between control and intervention groups. A power of 80% and a significance level $\alpha = 0.05$ yielded a sample size of 10 subjects per group: 30 in total.

Statistical analysis

Baseline characteristics were presented as median with interquartile range (IQR; continuous data) or number with percentage (dichotomous data). Outcome measures during follow-up were compared between the control and intervention groups using generalized estimating equations (GEEs).[34] This statistical model is able to handle the longitudinal nature of this study, as well as the missing data as a result of discarding the scans of insufficient quality. The GEE model was adjusted for baseline serum 25OHD and for the baseline measurement of each respective outcome: The follow-up measurements of trabecular number were corrected for the baseline trabecular number. The two HR-pQCT measurements of the contralateral wrist were compared using the Wilcoxon signed rank test. All statistical analyses were performed in SPSS 24.0 (IBM Corp).

Results

From June 2013 until May 2016, 32 participants were enrolled in the study (Fig. 2). A significant part of the screened patients ($N = 54$) were not included because of unforeseen reasons: A number of patients were from different regions and received further treatment and follow-up elsewhere, some had accompanying fractures requiring surgery (e.g., hip fractures) or were judged to be unable to participate successfully in the scans and follow-up schedule because of tremors (Parkinson disease) or the high amount of eldercare they received.

From the included patients, one withdrew informed consent after the first study visit, and another dropped out following surgical intervention because of a secondary dislocation of the fracture. Both patients were replaced with additional inclusions in accordance with the amended study protocol, where they were assigned to the group randomization from the participant they replaced, to realize 10 subjects per group.

Baseline characteristics of the groups were similar (Table 1), with the exception of the number of patients with a fracture requiring reduction. Serum levels of 25OHD were <50 nmol/L in 30%–40% and <75 nmol/L in 60%–90% of patients (see Table 1 for the specific values for each group). None of the study participants reported over-the-counter vitamin D supplement use during participation.

Microarchitecture and BMD

The HR-pQCT-based BMD and microarchitectural parameters are presented in Fig. 3. Despite randomization, a baseline difference

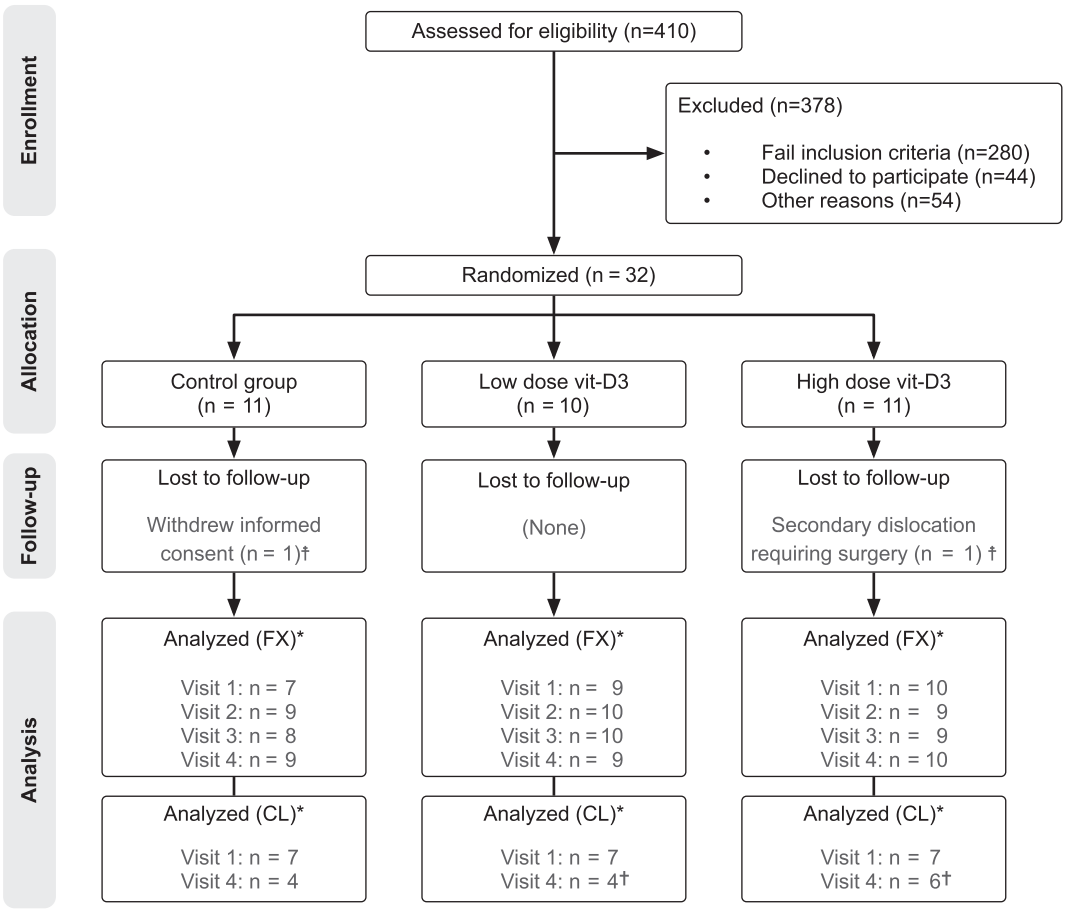

Fig 2. Diagram describing participant flow. ‡Patients lost to follow-up were replaced, resulting in 11 randomized subjects in the control and high-dose groups; *Only scans of sufficient quality (i.e., motion-grade 1–3) were used for analyses; †Because of equipment malfunction, contralateral scans were not completed for the fourth visit for two subjects (one in each intervention group). CL, contralateral side; FX, fracture side; vit, vitamin.

TABLE 1. Baseline Characteristics

	Control group		Low-dose group		High-dose group	
	Median	(IQR)	Median	(IQR)	Median	(IQR)
Age at time of fracture (y)	65	(61–66)	64	(58–75)	65	(61–70)
Height (cm)	166	(158–172)	163	(158–165)	161	(157–165)
Weight (kg)	66	(63–76)	69	(61–71)	68	(55–80)
BMI (kg/m^2)	25.8	(22.8–26.3)	25.7	(24.2–27.4)	26.2	(22.1–29.4)
Daily calcium intake (mg)	748	(569–966)	660	(350–1050)	738	(519–995)
25OHD level (nmol/L)	64	(36–76)[b]	60	(44–89)	56	(35–67)
PTH (pmol/L)	2.9	(2.6–5.4)	2.6	(1.7–4.0)	2.6	(1.6–5.0)
T-score total hip	−0.8	(−1.1 to −0.7)[a]	−1.4	(−2.1 to −0.9)[a]	−1.1	(−1.5 to 0)
T-score femoral neck	−1.1	(−1.7 to −0.3)[a]	−1.9	(−2.3 to −0.9)[a]	−1.4	(−1.8 to −0.5)
T-score lumbar spine	−1.3	(−2.3 to −0.6)[a]	−2.5	(−3.3 to −1.4)[a]	−1.7	(−2.4 to −0.1)
	N	%	*N*	%	*N*	%
25OHD level <50 nmol/L	3	33[b]	3	30	4	40
25OHD level 50–75 nmol/L	3	33[b]	3	30	5	50
25OHD level >75 nmol/L	3	33[b]	4	40	1	10
Intra-articular fracture	6	60	7	70	6	60
Fracture required reduction	1	10	5	50	5	50
Prior osteoporosis medication use	0	0	0	0	0	0
Start osteoporosis medication after last visit	0	0	5	50	3	30

[a]One patient in the control group and one in the low-dose group did not participate in the screening for osteoporosis.
[b]One patient refused to provide blood samples.
Abbreviation: IQR, interquartile range.

was observed that was considered clinically relevant, e.g., a mean baseline trabecular density of 179 ± 1.8 (mean with SE) for the control group versus 156 ± 9.6 and 162 ± 8.0 for the low- and high-dose groups, respectively. The baseline was incorporated in the statistical models for adjustment.

Longitudinal changes showed first an increase in trabecular BMD during fracture healing, followed by a decrease. No statistically significant differences were observed with regard to microarchitectural or BMD parameters between the control group and the low-dose intervention group (all *p* values >0.05). In the high-dose vitamin D_3 group, a decreased trabecular number was observed (β coefficient = −0.22; 95% CI, −0.36 to −0.08; *p* value = 0.002) and a correspondingly increasing trabecular separation (β coefficient = 0.05; 95% CI, 0.009 to 0.096; *p* value = 0.018), compared with the control group.

Adjustment for BMD at the lumbar spine, femoral neck, or total hip as assessed with DXA did not change these results.

Microfinite element analyses

No statistically significant differences were observed between the control group and the low-dose intervention group (*p* values between 0.48 and 0.85; Table 2). In the high-dose group a decreased compression stiffness was observed compared with the control group (β coefficient = −3.63; 95% CI, −6.76 to −0.50; *p* value = 0.023).

Measurements of the contralateral radius revealed no changes between baseline and 12 weeks postfracture in the nonfractured distal radius (*p* values between 0.1 and 1), although the number of usable scans was limited caused by the presence of severe motion artifacts (Fig. 2).

Serum markers

Median serum 25OHD 12 weeks postfracture was 61 nmol/L (IQR, 42–72) for the control group, 70 nmol/L (IQR, 66 to 81) and 81 nmol/L (IQR, 70 to 95) for the low- and high-dose vitamin D_3 intervention groups, respectively. Compared with the control group, both intervention groups had a higher 25OHD level at visit 4 (low dose: *p* value = 0.016; high dose: *p* value <0.001).

Longitudinal analyses showed an increased level of CTX during the first 3–6 weeks post-fracture in the high-dose vitamin D group (Fig. 4) compared with the control group (β coefficient = 0.062; 95% CI, 0.0004–0.12; *p* value = 0.048), whereas no difference between the control and low-dose group was observed. No statistically significant differences were detected for the bone resorption marker PINP (*p* values >0.05).

Functional outcome

No differences in PRWE score were found between the control group and the low- or high-dose intervention groups during the study period (*p* value = 0.4 and 0.2 respectively).

Discussion

In this study, early bolus supplementation of vitamin D_3 after a distal radius fracture did not result in enhanced fracture healing as assessed using HR-pQCT nor in improved patient reported outcomes. Remarkably, a decreased trabecular number and compression stiffness and an increase of the serum marker of bone resorption CTX was observed in the high vitamin D_3 dose group compared with the control group.

The longitudinal changes of HR-pQCT and μFEA parameters during fracture healing seen in this study match the pattern we

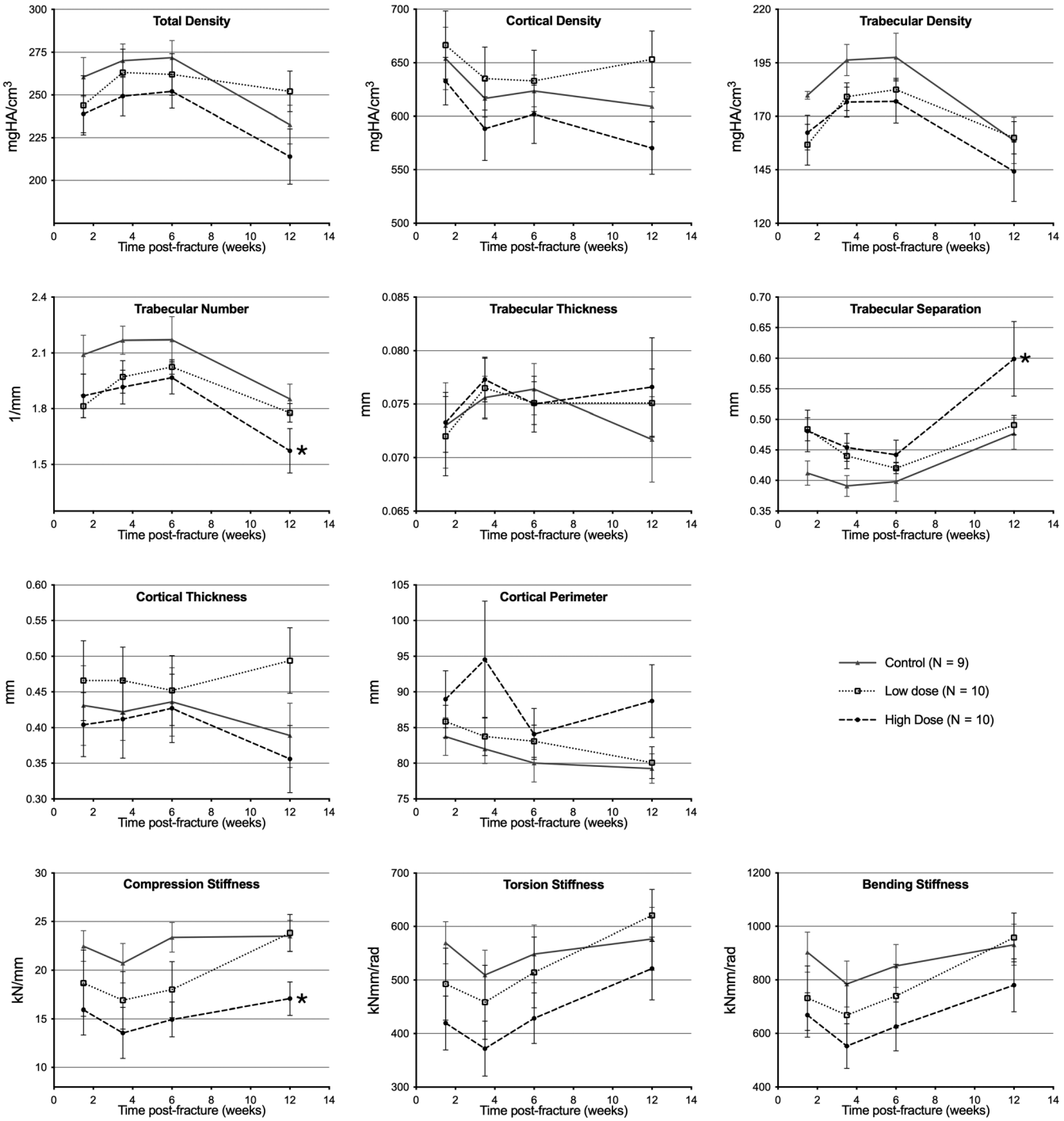

Fig 3. Longitudinal HR-pQCT–derived bone density, microarchitectural, geometric, and biomechanical parameters. Data presented as mean ± SEM. SEM, standard error of the mean. *Statistically significant different from control group.

described earlier in our observational studies: First an increase in (trabecular) bone density is seen, followed by a decrease corresponding to the formation and remodeling of the fracture callus, with increasing bone stiffness from 3–4 weeks postfracture onward.[17,26]

An interesting observation is that compression stiffness differs between the high dose and the control group, but torsion and bending stiffness do not. The latter two are predominantly determined by the cortical perimeter, whereas the trabecular compartment contributes primarily to compression stiffness.[35] Because the trabecular compartment features a large surface area to bone ratio as compared with the cortex, it is possible that metabolic changes (such as fracture healing influenced by 25OHD) can have more impact in the trabecular compartment.

TABLE 2. Results of the GEE Analyses of All Outcome Measures

	Low dose vs. control			High dose vs. control		
	β coefficient (95% CI)		*p* value	β coefficient (95% CI)		*p* value
Density parameters						
Total (mg/HA/cm^3)	6.48	(−4.8 to 17.77)	0.260	−8.39	(−27.44 to 10.67)	0.388
Cortical (mg/HA/cm^3)	0.98	(−29.91 to 31.87)	0.951	−19.4	(−50.25 to 11.46)	0.218
Trabecular (mg/HA/cm^3)	−1.05	(−16.28 to 14.18)	0.893	−11.95	(−32.29 to 8.39)	0.250
Microarchitectural parameters						
Trabecular number (1/mm)	−0.10	(−0.24 to 0.05)	0.180	**−0.22**	**(−0.36 to − 0.08)**	**0.002**
Trabecular thickness (mm)	0.003	(−0.002 to 0.008)	0.288	0.003	(−0.003 to 0.010)	0.295
Trabecular separation (mm)	0.00	(−0.046 to 0.046)	0.990	**0.05**	**(0.009 to 0.096)**	**0.018**
Geometric parameters						
Cortical thickness (mm)	−0.012	(−0.053 to 0.028)	0.799	0.004	(−0.030 to 0.038)	0.547
Cortical perimeter (mm)	−0.37	(−2.92 to 2.18)	0.777	3.0	(−2.54 to 8.53)	0.289
Biomechanical parameters						
Compression stiffness (kN/mm)	−0.95	(−3.61 to 1.7)	0.481	**−3.63**	**(−6.76 to − 0.50)**	**0.023**
Torsional stiffness (kNmm/rad)	6.89	(−68.04 to 81.81)	0.857	−43.57	(−147.22 to 60.08)	0.410
Bending stiffness (kNmm/rad)	15.43	(−89.3 to 120.16)	0.773	−84.47	(−245.83 to 76.9)	0.305
Serum markers						
PINP (ng/mL)	13.4	(−7.0 to 33.9)	0.199	8.1	(−3.4 to 19.6)	0.168
CTX (ng/mL)	0.048	(−0.033 to 0.13)	0.244	**0.062**	**(0.00 to 0.12)**	**0.048**
Functional outcome						
PRWE score	5.71	(−7.74 to 19.2)	0.405	9.72	(−6.79 to 26.2)	0.249

Note. The model was adjusted for baseline values and baseline 25OHD levels. Statistically significant results ($p < 0.05$) are marked in bold.
Abbreviations: GEE, generalized estimation equations; PRWE, patient-rated wrist evaluation.

With respect to the HR-pQCT results, the baseline total BMD in the high-dose group is lower compared with the control group (Fig. 3). This same absolute difference can be observed to persist during follow-up. Part of the explanation of a lower trabecular number in the high-dose group could be that more tissue fell below the threshold that defines mineralized bone because of the lower baseline density. However, correcting the statistical model for the total density as measured with HR-pQCT does not change the results for the trabecular number (data not shown). Together with no statistically significant difference detected in total density, this suggests that the baseline total density does not fully explain the difference in trabecular number that emerges during follow-up. Nevertheless, statistical correction is not the same as adjusting image segmentation thresholds. Furthermore, it is important to state that HR-pQCT image analyses and μFEA have not yet been validated in fractured bone. Conclusions about the meaning of the different parameters should thus be drawn cautiously.

To the best of our knowledge, this work represents the only published RCT to date that investigates the effect of sole vitamin D_3 supplementation in human fracture healing. The single other published RCT concerning vitamin D and fracture healing looked at the effect of the combination of calcium and vitamin D_3 supplementation on the healing of proximal humerus fractures (PHF).[16] In that study, Doetsch and colleagues used DXA scans of the shoulder following a conservatively treated PHF and found a higher BMD of the fracture callus in the proximal humerus after 6 weeks of healing in the intervention group compared with the placebo group. This stands in contrast with our findings, which indicated no differences in volumetric BMD change at the fractured distal radius between the groups during 12 weeks of fracture healing as evaluated using HR-pQCT. A notable difference between the two studies is the daily calcium supplementation of 1 g in the RCT by Doetsch and colleagues, whereas in our study the dietary daily calcium intake was on average <750 mg and no supplementation was provided. Nevertheless, the patients in our study did not show a significant negative calcium balance as indicated by the normal PTH levels (Table 1). Another explanation is the baseline 25OHD level: In a vitamin D-deficient population such as the study by Doetsch and colleagues (with a baseline 25OHD of 40 nmol/L), calcium and vitamin D_3 supplementation could increase BMD in fracture callus, whereas the relatively normal 25OHD levels (∼60 nmol/L) in our population could preclude such an effect. The baseline levels of 25OHD could also be related to our observation that a higher dose of vitamin D_3 supplementation could be detrimental to fracture healing. Although unanticipated, this finding is in accordance with a recently published RCT by Burt and colleagues.[36] In that study, a (nonfracture) population with 75–80 nmol/L serum 25OHD at baseline received 400, 4000, or 10,000 IU vitamin D_3 supplementation a day. Their findings included a dose-dependent negative effect on BMD, with a higher dose of vitamin D_3 resulting in a more pronounced decrease in volumetric BMD as assessed with HR-pQCT.

The mechanism underlying the possible detrimental effect of high-dose vitamin D_3 supplementation on bone remains unclear, but several hypotheses have been suggested.[25] For instance, animal studies have shown that a high dose of vitamin D_3 can result in upregulation of 25-hydroxyvitamin D-24-hydroxylase (also known as CYP24), the enzyme responsible for catabolizing the biologically active form of vitamin D, 1,25-dihydroxyvitamin D_3.[37] This mechanism could protect the organism from vitamin D toxicity. However, a study where different dosing regimens of vitamin D_3 were compared, a monthly dose of 45,000 IU showed a steady increase of serum 25OHD when assessed at 1, 7, and 28 days after administration.[38] Although the higher dose used in our study could theoretically result in a transient drop of serum 25OHD between the measurements at baseline

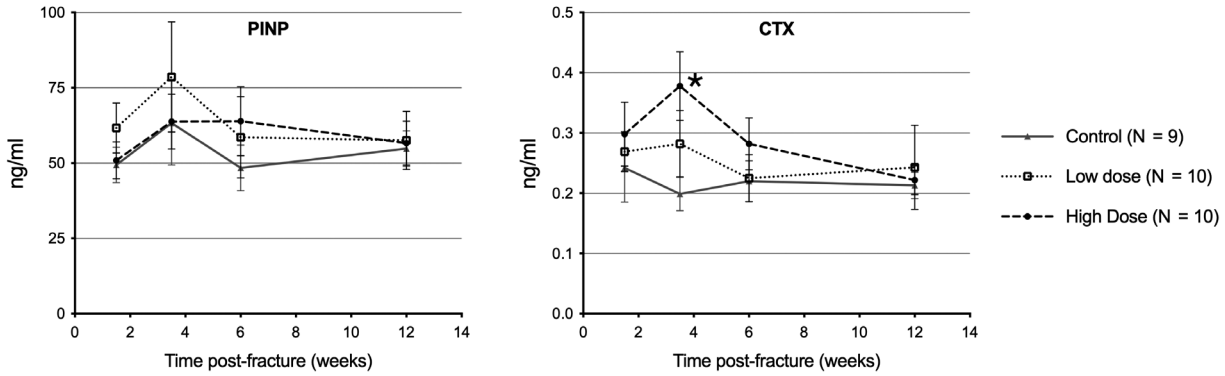

Fig 4. Longitudinal levels of serum markers of bone formation (PINP) and bone resorption (CTX). Data presented as mean ± SEM. SEM, standard error of the mean. *Statistically significant different from control group.

and 12 weeks, further evidence to support this hypothesis is warranted.

The supplementation regimen of a bolus dose once per 6 weeks is also an item of discussion. This method of supplementation was chosen to ensure full compliance and corresponds to 60,000 and 150,000 IU/d in the intervention groups, corresponding to a daily dose of 700 and 1800 in the low- and high-dose groups, respectively. This was based on the national guidelines,[21] although other guidelines recommend 1500–2000 IU/d.[39]

However, a bolus dose is not similar to the daily equivalent dose. Studies have shown detrimental effects of monthly bolus doses of cholecalciferol on falls.[40,41] Although the mechanism underlying this observation is still under investigation, reviews have described a positive effect of daily and weekly cholecalciferol supplementation, but not of monthly bolus dose administration.[42,43] In our study, although thebolus regimen is relatively short compared with Bischoff-Ferrari and colleagues (3 months vs 1 year), it is currently unclear whether the detrimental effect in the 1800 IU/d equivalent group is the result of the 6-week bolus dose or the higher supplementation itself is responsible. Future studies should address this issue, but there is increasing data in support of daily dosing regimens for cholecalciferol supplementation.

Studies on the effect of vitamin D supplementation in the treatment of osteoporosis focus on the increased intestinal calcium absorption as the mechanism of action. However, the direct effects of vitamin D on bone tissue[23] are probably of greater importance in the current study. The vitamin D receptor (VDR) on osteoblasts controls the expression of RANKL. This important osteoclastic factor binds on its receptor (RANK) on osteoclasts, stimulating proliferation and thus enhancing bone resorption.[44] A similar mechanism was also found in chondrocytes,[45] indicating that this pathway could be important in both the early as well as in the later stages of fracture healing. Indeed, the observed increase of the serum marker of bone resorption CTX in this study (Fig. 4) supports this hypothesis. Furthermore, no differences between the groups were detected in PINP levels, the marker for bone formation, supporting the hypothesis that the decrease in bone strength is caused by vitamin-D mediated increased resorption.

Nevertheless, the intricacies of VDR signaling in osteoblasts and the subsequent indirect effects on osteoclast activity remain to be elucidated.[46]

With respect to the bone turnover markers, it is known that CTX is influenced by a circadian rhythm, as well as by food intake. Therefore, guidelines recommend using morning overnight fasting samples for CTX monitoring.[47] However, considering the challenging logistics caused by the multiple procedures during study visits (regular clinical follow-up, blood sample collection, HR-pQCT scanning) combined with the average age of our population, we anticipated that adding the fasting requirement to the study protocol would result in a lower inclusion rate and higher loss to follow-up. Unfortunately, this introduces a preanalytical variability that invalidates the comparison of CTX levels to other studies.[47] Nevertheless, because the samples were taken under similar nonfasting conditions during the same time of the day in follow-up visits, the serial measurements of CTX are usable in our study design.[47]

The strengths of this study include the randomized design with two distinct dosing regimens, where patients in the intervention groups were administered vitamin D_3 supplementation in 6-week bolus doses during study visits, thus ensuring full patient compliance.[48] Furthermore, outcome measures were assessed with a blinded, detailed evaluation using HR-pQCT with μFEA. These outcome measures focus on the target of the intervention: bone density, structure, and strength, and are more precise than fracture healing quantification using conventional X-ray imaging.[49] Although in the clinical setting, patient-reported outcome measures such as the PRWE are important, in this study we were primarily interested in the direct action of vitamin D_3 supplementation on the healing bone and chose to power the trial on this outcome measurement.

An important limitation of this study is the lack of significant serum 25OHD deficiency at baseline in all groups (mean of approximately 60 nmol/L), although we expected our study to include a vitamin D deficient population based on our previous work showing that a substantial proportion of patients with a fracture has 25OHD levels below 50 nmol/L.[8] The healthy user bias may be a likely explanation for this finding. In combination with the required follow-up regimen of four visits in 12 weeks, the in- and exclusion criteria likely resulted in the selection of a relatively healthy subset of postmenopausal women presenting

with a distal radius fracture. As a result, the findings in our study are limited to a predominantly 25OHD nondeficient population.

Second, the control group had less severe fractures compared with the intervention groups, as indicated by the need for fracture reduction (one in the control group vs five in each intervention group) and the baseline bone stiffness of the fracture region (Fig. 3). Although a correction for baseline was used in the statistical model, it cannot be discounted that the difference in baseline has an effect on the range of observed changes during fracture healing. Future trials should consider more strict inclusion criteria regarding fracture type or facilitate stratification by baseline bone stiffness in the analyses. Furthermore, based on the osteoporosis screening after completion of this trial, a low BMD (*T* score <-2.5) was present in 8 out of 20 patients in the intervention groups and in none of the control group.

Finally, the number of patients in each group was low, although the high-resolution outcome measurements ensured adequate power to detect an effect in bone microarchitecture and estimated strength. Two currently active RCTs investigating vitamin D_3 supplementation in fracture healing will provide further evidence on this interesting topic.[50,51]

In conclusion, this RCT did not show a beneficial effect of early bolus vitamin D_3 supplementation during distal radius fracture healing on bone density, microarchitecture, or bone stiffness based on HR-pQCT scans in a nonvitamin D-deficient patient group. A possible detrimental effect of a high-dose vitamin D_3 bolus dose was observed, requiring further investigation. Because of the small sample size and limitations as discussed, these conclusions should be regarded as preliminary findings.

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Author Contributions

Frans Heyer: Data curation; formal analysis; investigation; project administration; resources; validation; visualization; writing-original draft; writing-review & editing. **Joost de Jong:** Conceptualization; investigation; methodology; project administration; resources; software; supervision; writing-review & editing. **Paul Willems:** Conceptualization; investigation; methodology; resources; supervision; writing-review & editing. **J. Arts:** Conceptualization; methodology; resources; supervision; writing-review & editing. **Sandrine Bours:** Conceptualization; investigation; methodology; project administration; writing-review & editing. **Sander van Kuijk:** Formal analysis; methodology; supervision; writing-review & editing. **Judith Bons:** Data curation; investigation; resources; writing-review & editing. **Martijn Poeze:** Funding acquisition; methodology; resources; supervision; writing-review & editing. **Piet Geusens:** Conceptualization; funding acquisition; methodology; resources; supervision; writing-review & editing. **Bert van Rietbergen:** Conceptualization; methodology; resources; software; supervision; writing-review & editing. **Joop Bergh:** Conceptualization; formal analysis; funding acquisition; methodology; project administration; resources; supervision; validation; writing-original draft; writing-review & editing.

Conflict of Interest

Bert van Rietbergen is a consultant for Scanco Medical AG. J J Arts is a board member of the workgroup Biotechnology of the Dutch Orthopedic Association (NOV). All other authors declare that they have no conflicts of interest.

Peer Review

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Data Availability Statement

Research data are not shared at this time, due to follow-up studies still ongoing.

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