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ORIGINAL ARTICLE

Vitamin D supplementation and bone turnover in advanced heart failure: the EVITA trial

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Abstract

Summary Low vitamin D status is common in patients with heart failure and may influence bone health. A daily vitamin D dose of 4000 IU (moderately high dose) for 3 years had however no effect on parameters of bone metabolism, even in patients with very low vitamin D status.

Introduction Low vitamin D status is common in patients with heart failure (HF) and has been related to disturbed bone turnover. The present study investigated the effect of a daily vitamin D_3 dose of 4000 IU on bone turnover markers (BTMs) in patients with advanced HF and 25-hydroxyvitamin D (25OHD) concentrations < 75 nmol/L.

Methods In this pre-specified secondary analysis of a randomized controlled trial, we assessed in 158 male HF patients (vitamin D group: $n = 80$; placebo group: $n = 78$) between-group differences in calciotropic hormones (25OHD, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], intact parathyroid hormone [iPTH]), and BTMs (cross-linked C-telopeptide of type I collagen, bone-specific alkaline phosphatase, undercarboxylated osteocalcin). Comparisons were performed at the end of a 3-year vitamin D supplementation period with adjustments for baseline values.

Results Compared with placebo, vitamin D increased 25OHD on average by 54.3 nmol/L. At study termination, 25OHD and $1,25(OH)_2D$ were significantly higher ($P < 0.001$ and $P = 0.007$, respectively), whereas iPTH tended to be lower in the vitamin D group than in the placebo group ($P = 0.083$). BTMs were initially within their reference ranges and did not differ significantly between groups at study termination, neither in the entire study cohort nor when data analysis was restricted to the subgroup of patients with initial 25OHD concentrations < 30 nmol/L ($n = 54$) or to patients with initial hyperparathyroidism ($n = 65$) (all P values > 0.05).

Conclusions A daily vitamin D_3 dose of 4000 IU did not influence BTMs. Data indicate that vitamin D supplementation will not lower bone turnover in male patients with heart failure.

Keywords Bone formation · Bone resorption · Bone turnover · Heart failure · Parathyroid hormone · Vitamin D

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Introduction

The development of osteoporosis is a frequent complication in heart transplant recipients, with a prevalence of vertebral fractures between 18 and 50% [1–4]. Although the immunosuppression that is required for graft survival (glucocorticosteroids, calcineurin-inhibitors) is considered to play an important role in the pathogenesis of osteoporosis in these patients [4–6], heart failure (HF) per se is already associated with an increased risk of vertebral and hip fractures before transplantation [7, 8]. Low bone mineral densities have been frequently reported in HF [9, 10], and several risk factors such as hypogonadism, specific drugs (vitamin K antagonists, diuretics), reduced mobility, cachexia, and vitamin D deficiency may contribute to impaired bone health in these patients [11].

Low plasma levels of the vitamin D metabolites 25-hydroxyvitamin D (25OHD, indicator of vitamin D status) and 1,25-dihydroxyitamin D ($1,25(OH)_2D$; active vitamin D hormone) are frequent in HF [9, 12–14] and have been associated with elevated concentrations of the bone resorption markers hydroxyproline and deoxypyridinoline [9, 12]. Moreover, in a case-control study [15], levels of the bone formation marker intact osteocalcin (OC) were lower and the ratio of undercarboxylated OC (uOC; a biochemical marker of fracture risk, [16]) to intact OC was higher in HF patients than in controls. In a randomized controlled trial in advanced HF [17], a daily vitamin D_3 supplement of 2000 IU for 9 months had, however, no effect on uOC or the bone resorption marker cross-linked C-telopeptide of type I collagen (CTX).

At present, there still exist several knowledge gaps on the role of vitamin D on bone turnover as both, beneficial and detrimental effects of vitamin D on bone metabolism, have been described [18, 19]. The present study therefore aimed at investigating in a pre-specified secondary analysis of the EVITA (*e*ffect of *vi*tamin D on mor*ta*lity in heart failure) trial, whether a daily vitamin D_3 dose of 4000 IU (100 μg) and a treatment duration of 3 years are able to influence bone formation and resorption markers in patients with advanced HF and low vitamin D status.

Methods

Study design and participants

EVITA is a randomized, placebo-controlled, single-center trial performed at the Heart and Diabetes Center North Rhine-Westphalia, Germany (geographic latitude 52° N). Major study results have already been published elsewhere [20]. Briefly, 400 heart failure patients aged 18 to 79 years (332 men and 68 women) who attended the heart failure unit of our clinic were enrolled. Major exclusion criteria were hypercalcemia, sarcoidosis, daily vitamin D intake > 800 IU, and baseline 25-hydroxyvitamin D levels > 75 nmol/L. Eligible participants were randomly allocated to receive either 4000 IU vitamin D_3 daily as eight drops of an oily vitamin D preparation (Vigantol oil, Merck, Darmstadt, Germany) or eight drops of a vitamin D-free oil (Migliol oil; Merck) in a 1:1 ratio for 3 years. For the present analysis, only male patients were considered because female bone turnover is significantly influenced by monthly fluctuations in sex hormone concentrations in premenopausal women [21], bone turnover differs between premenopausal and postmenopausal women, and the number of female participants finishing our trial was insufficient for adequately powered statistical analyses. Therefore, 68 female patients, of whom 40% were premenopausal, were not included in the data analysis. Of the 332 male study participants, 166 were assigned to the vitamin D group and 166 to the control group. During the study, all patients remained on guideline-recommended medications. Notably, our male patients do not receive medical treatment for osteoporosis prevention. Patient adherence was assessed by measuring in-study levels of circulating 25OHD. The study was registered at EudraCT as 010-020793-42 and clinicaltrials.gov as NCT01326650. All study participants gave written informed consent to the study procedures before study randomization. The study protocol was approved by the ethics committee of the Medical Council Westphalia-Lippe, Germany (No. 2010-052-f-A).

Biochemical analyses

Blood samples were taken in the morning between 7 and 11 am after an overnight fast. All blood samples were either measured at least 4 h after blood collection or stored at − 80 °C until analysis. The following parameters were measured at baseline and study termination (36-month visit): creatinine, bone-specific alkaline phosphatase (BSAP, bone formation marker), uOC, CTX, 25OHD, $1,25(OH)_2D$, and intact parathyroid hormone (iPTH). Moreover, calcium and inorganic phosphate values are presented at baseline. The DiaSorin auto-analyzer (DiaSorin, Stillwater, MN, USA) was used to measure 25OHD levels (sum of $25OHD_2$ and $25OHD_3$), $1,25(OH)_2D$ levels (sum of $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$), and iPTH. The latter parameter was measured using a new two-step, two-site sandwich assay that uses polyclonal antibodies to capture and detect iPTH. Calcium, inorganic phosphate, and creatinine were routinely assessed using the Architect auto-analyzer (Abbott, Wiesbaden, Germany). BSAP, uOC, and CTX were analyzed by ELISA test kit provided by ElabScience (BSAP, uOC: ElabScience Biotechnology Co., Ltd) and IDS (CTX: IDS Immunodiagnostic Systems GmbH, Frankfurt/M, Germany), respectively. We used the following cut-off values for classifying 25OHD [22]: < 30 nmol/L as deficient, 30–49.9 nmol/L as insufficient, and 50–74.9 nmol/L as borderline. With respect to other biochemical parameters, the following reference ranges were provided by the respective supplier: $1,25(OH)_2D$ 47.8–190.3 pmol/mL; iPTH 14.5–87.1 pg/mL; CTX 115–748 pg/mL; BSAP 80–2000 pg/mL, and uOC 13–65 ng/mL (males) or 18–72 ng/mL (females). According to the manufacturers, Intra- and inter-assay coefficients of variation were for the bone markers CTX, BSAP, and uOC as follows: 1.7 and 9.7%, 4.1 and 5.9%, and 5.6 and 5.6%, respectively. The MDRD formula was used to calculate estimated glomerular filtration rate (eGFR) [23]. The measurements of calciotropic hormones and BTMs were performed in batch analyses, with the exception of the 25OHD measurements, which were performed on the day of blood sampling.

Outcome measure

We assessed between-group differences in bone turnover markers (BTMs) such as CTX, BSAP, and uOC at study termination with adjustments for baseline values.

Statistics

Categorical variables are reported as a percentage of observations. Normally distributed continuous data (as checked by the Kolmogorov-Smirnov test) are shown as mean with standard deviation. Variables with a skewed distribution are presented as median with interquartile ranges (IQR), unless otherwise stated. Change from baseline data is shown as mean and 95% confidence interval (CI). Fisher's exact test, the unpaired *t* test, and the Mann-Whitney *U* test, respectively, were used for group comparison at baseline, when appropriate. The McNemar test was used to assess differences in hyperparathyroidism status within groups. ANCOVA with adjustments for baseline values was used to test for differences in calciotropic hormones and BTMs between the vitamin D and placebo groups at the 36-month follow-up visit. Skewed variables were normalized by log(e) transformation before use in ANCOVA, but all results are shown in the original units. The Wilcoxon test was used to test for differences within groups between the baseline and 36-month follow-up visit. Moreover, we used Spearman's rank correlation coefficient (r_s) to assess the interrelationship between biochemical variables. In addition, we performed subgroup analyses to assess the vitamin D effect on BTMs in patients with deficient initial 25OHD levels and hyperparathyroidism. Since 67 male patients died during the study, and an additional 107 patients dropped out or were lost to follow-up, 158 male patients (vitamin D group: $n = 80$; placebo group: $n = 78$) with at least one available calciotropic hormone parameter or bone turnover marker at study termination were included (Fig. 1). In detail, available values for iPTH, $1,25(OH)_2D$, BSAP, uOC, and CTX were 145, 145, 145, 145, and 136 at follow-up, respectively. No data imputation for missing values was performed. P values < 0.05 (two-sided) were considered as statistically significant. Given a total number of 136 patients in this two-treatment parallel-design study, there is a 90% probability that the study will detect a treatment difference in the bone resorption marker CTX at a two-sided 0.05 significance level if the true difference between treatments is 40 pg/mL. This is based on the assumption that the standard deviation of CTX concentrations is 70 pg/mL. We performed all analyses using IBM SPSS Statistics version 23 (IBM Corporation, Armonk, NY, USA).

Results

Blood samples were collected in winter (January to March, $n = 52$), spring (April to June, $n = 45$), summer (July to September, $n = 28$), and fall (October to December, $n = 33$). Baseline characteristics of the patients are listed in Table 1 by study group. One fifth of the patients were already listed

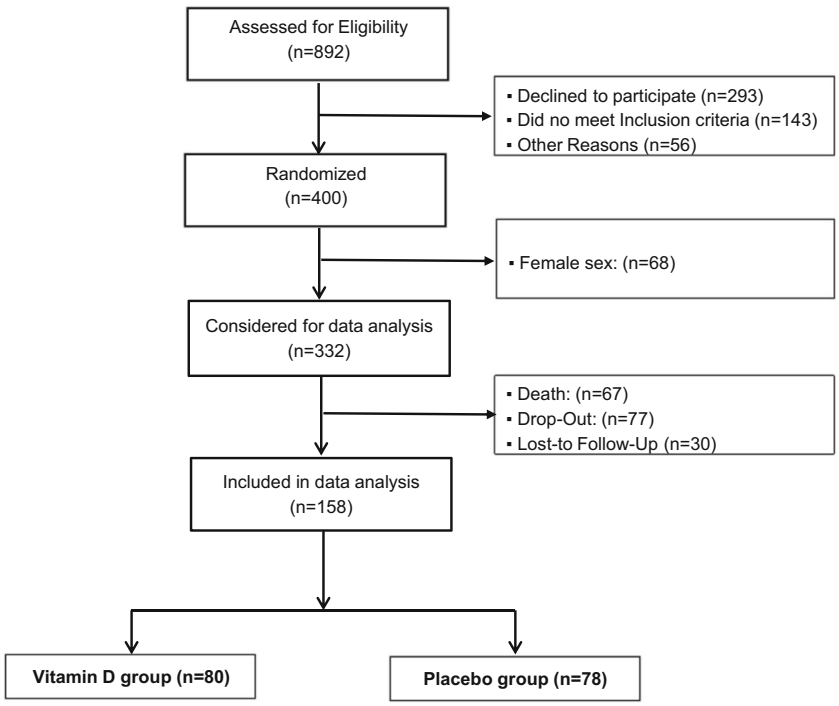

Fig. 1 Study flow chart

Table 1 Baseline characteristics of the study groups

Parameter	Vitamin D group ($n = 80$)	Placebo group ($n = 78$)	P value
Age (years)	56 (49–62)	54 (47–59)	0.093
Body mass index (kg/m^2)	28.7 (25.3–31.6)	28.0 (25.3–31.1)	0.618
Diagnosis			
Dilated cardiomyopathy, number (%)	34 (42.5)	38 (48.7)	0.327
Ischemic cardiomyopathy, number (%)	43 (53.8)	34 (43.8)	0.327
Others, number (%)	3 (3.8)	6 (7.7)	0.325
New York Heart Association functional class			
II, number (%)	64 (80.0)	58 (74.4)	0.451
III, number (%)	16 (20.0)	20 (25.6)	0.451
Arterial hypertension, number (%)	23 (28.7)	25 (32.1)	0.730
Listed for heart transplantation, number (%)	14 (17.5)	20 (25.6)	0.248
Diabetes mellitus, number (%)	22 (27.5)	14 (17.7)	0.185
Estimated GFR (mL/min/1.73 m^2)	70.6 ± 21.8	75.1 ± 24.8	0.233
Medications			
Aldosterone-antagonists, number (%)	64 (80.0)	65 (83.3)	0.682
Loop diuretics, number (%)	65 (81.3)	65 (83.3)	0.836
Thiazid-diuretics, number (%)	25 (31.3)	30 (38.5)	0.404
Beta-blockers, number (%)	76 (95.0)	77 (98.7)	0.367
ACE-inhibitors/ARB-Blockers, number (%)	77 (96.3)	77 (98.7)	0.620
Calcium-antagonists, number (%)	3 (3.8)	1 (1.3)	0.620
Antiarrhythmics, number (%)	22 (27.5)	22 (28.2)	>0.999
Digoxin, number (%)	26 (32.5)	35 (44.9)	0.141
Calcium supplement use, number (%)	1 (1.9)	2 (2.6)	0.618
Vitamin D supplement use, number (%)	0 (0.0)	0 (0.0)	>0.999
Biochemical parameters			
Calcium (mmol/L)	2.39 ± 0.11	2.39 ± 0.11	0.870
Phosphate (mmol/L)	0.84 (0.74–1.00)	0.94 (0.81–1.07)	0.070
25-hydroxyvitamin D (nmol/L)	34.8 (24.2–51.8)	34.3 (26.5–48.4)	0.873

Abbreviations: *ACE* angiotensin converting enzyme, *ARB* angiotensin II receptor blocker

for heart transplantation and a substantial proportion of the patients were diagnosed as having diabetes mellitus. In both study groups, median 25OHD levels were only slightly above the deficiency range. Overall, 36.7% of patients had deficient and 38.6% insufficient initial 25OHD levels. None of the study participants were initially taking vitamin D supplements and only three patients were calcium supplement users. Compared with patients who were not included in the data analysis, the baseline body mass index of the included patients was slightly higher (29.2 ± 5.0 kg/m^2 vs. 27.9 ± 5.1 kg/m^2, $P = 0.021$). All other parameters listed in Table 1 did not differ significantly between included and excluded patients. Of the included 158 patients, clinically relevant heart failure progression, as indicated by the need of mechanical circulatory support, "high urgent" listing for heart transplantation and/or heart transplantation, occurred in seven patients assigned to vitamin D and in eight patients assigned to placebo.

Initial 1,25(OH)$_2$D and BTMs were, on average, within the respective reference range, whereas average iPTH concentrations exceeded the reference range, especially in the group assigned to vitamin D (Table 2). Briefly, the prevalence of hyperparathyroidism was in the vitamin D and placebo groups 56 and 41%, respectively. Vitamin D metabolites increased and iPTH levels decreased significantly in the group assigned to vitamin D. Moreover, the prevalence of hyperparathyroidism decreased from 56.0 to 33.0% in the group assigned to vitamin D ($P = 0.001$). There was also a slight, but significant increase in 25OHD levels in the group assigned to placebo. However, the prevalence of hyperparathyroidism remained constant in this group (41.4 vs 38.3%, $P = 0.824$). BTMs did not change, neither in the vitamin D nor in the placebo groups.

Compared with placebo, vitamin D increased circulating 25OHD on average by 54.3 nmol/L and 1,25(OH)$_2$D by 17.1 pmol/L (Table 2). In the placebo and vitamin D groups, 64.6 and 14.3% of 25OHD values, respectively, were <

Table 2 Results of vitamin D treatment on calciotropic hormones and bone turnover markers in patients with advanced chronic heart failure

	Vitamin D group ($n = 80$)			Placebo group ($n = 78$)			Treatment effect	P value[2]
Characteristics	Baseline	Follow-up (36 months)	Mean change from baseline[1]	Baseline	Follow-up (36 months)	Mean change from baseline[1]	Between-group differences	
Calciotropic hormones								
25OHD (nmol/L)	38.4 (34.4–42.4)	100.8 (88.4–113.1)	63.9 (52.6 to 75.2)***	37.5 (33.6–41.4)	46.3 (40.9–41.4)	9.9 (3.7 to 15.5)**	54.3 (41.3 to 67.3)	< 0.001
$1,25(OH)_2D$ (pmol/L)	83.7 (76.5–90.9)	96.6 (86.9–106.3)	13.0 (4.2 to 21.7)**	86.9 (79.7–94.1)	82.0 (72.8–94.1)	− 4.1 (− 12.6 to 4.2)	17.1 (5.1 to 29.2)	0.007
iPTH (pg/mL)	130 (103–156)	92 (71–113)	− 37.2 (− 62.6 to −11.7)**	106 (82–131)	97 (76–119)	− 8 (− 30 to 15)	− 30 (− 64 to 5)	0.083
Bone turnover markers								
BSAP (pg/mL)	990 (798–1182)	1103 (860–1348)	125 (− 161 to 411)	1412 (1123–1701)	1357 (1051–1663)	− 51 (− 427 to 325)	176 (− 288 to 640)	0.448
uOC (ng/mL)	22.1 (10.5–33.7)	19.6 (10.4–28.8)	− 2.3 (− 16.5 to 11.9)	18.9 (8.9–28.9)	16.8 (8.3–25.2)	−2.0 (− 12.8 to 8.7)	−0.3 (− 18.2 to 17.5)	0.707
CTX (pg/mL)	226 (192–260)	246 (201–292)	21 (−22 to 64)	291 (226–356)	295 (235–352)	29 (−45 to 103)	−8 (−93 to 78)	0.351

Abbreviations: *1,25(OH)$_2$D* 1,25-dihydroxyvitamin D, *25OHD* 25-hydroxyvitamin D, *iPTH* intact parathyroid hormone, *BSAP* bone-specific alkaline phosphatase, *uOC* undercarboxylated osteocalcin, *CTX*: cross-linked C-telopeptide of type I collagen

** $P < 0.01$ vs. baseline; *** $P < 0.001$ vs baseline (Wilcoxon test)

[1] Change from baseline data is shown as means and 95% confidence interval of the mean

[2] Probability of between group differences at study termination, with adjustments for baseline values (ANCOVA)

50 nmol/L at study termination. At study termination, 25OHD and $1,25(OH)_2D$ were significantly higher ($P < 0.001$ and $P = 0.007$, respectively), whereas iPTH tended to be lower in the vitamin D group than in the placebo group ($P = 0.083$). BTMs did however not differ significantly between groups at study termination, neither in the entire study cohort (Table 2) nor when data analysis was restricted to the subgroup of patients with initial 25OHD concentrations < 30 nmol/L (vitamin D group: $n = 29$; placebo group: $n = 25$) or to the subgroup of patients with initial hyperparathyroidism (vitamin D group: $n = 40$; placebo group: $n = 25$) (Supplemental Tables 1 and 2). Moreover, results did not change substantially when patients with eGFR values < 30 mL/min/1.73 m^2 ($n = 15$) were excluded from data analysis (data not shown). At study termination, mean values and standard deviations of the entire vitamin D and placebo groups were for CTX, BSAP, and uOC as follows: CTX 228 ± 130 pg/mL vs 287 ± 250 pg/mL, BSAP 1004 ± 800 pg/mL vs. 1412 ± 1157 pg/mL, and uOC 22.0 ± 48.0 ng/mL vs. 19.0 ± 40.0 ng/mL.

In correlation analyses where all available samples at baseline and study termination were included, circulating 25OHD was significantly related to $1,25(OH)_2D$ and iPTH (Table 3). $1,25(OH)_2D$ was also correlated with BMI, BSAP, and eGFR. iPTH concentrations were interrelated with eGFR, uOC, and CTX concentrations. Regarding the relationship between iPTH and eGFR, a logarithmic equation was slightly superior over a linear equation in describing the association. Nevertheless, the significant association between iPTH and eGFR persisted in patients with eGFR values above 30 mL/min/1.73 m^2 (Supplemental Figure).

Discussion

In the present study in male patients with advanced HF, a daily vitamin D_3 supplement of 4000 IU for 3 years increased circulating 25OHD and $1,25(OH)_2D$ concentrations significantly, but did not influence BTMs, neither in the entire study group nor in the subgroup of patients with deficient initial vitamin D status. Moreover, there was no significant treatment effect on BTMs in patients with initial hyperparathyroidism.

The parameters we used to measure bone turnover (CTX, BSAP, and uOC) have been shown to be sensitive biomarkers for monitoring preventive and therapeutic effects on bone [24–27]. Moreover, they have been demonstrated to be predictive of future bone loss and osteoporosis [28, 29]. Therefore, our results indicate that vitamin D did not affect bone turnover. Data concur with earlier findings in patients with advanced HF [17] and cardiovascular disease [30]. In these RCTs, daily supplementation with 2000 or 2800 IU vitamin D_3 had no significant effect on BTMs such as BSAP, OC, CTX, uOC, or procollagen type 1 N-terminal propeptide.

Table 3 Interrelationships between study variables according to Spearman's rank correlation coefficient

	eGFR	25OHD	iPTH	$1,25(OH)_2D$	BSAP	uOC	CTX	BMI	Age
eGFR									
25OHD	− 0.113								
iPTH	− 0.212***	− 0.235**							
$1,25(OH)_2D$	0.248***	0.134*	− 0.019						
BSAP	0.118	− 0.031	− 0.115	0.208***			–		
uOC	− 0.132*	− 0.008	0.203***	− 0.019	0.133*				
CTX	− 0.185**	− 0.098	0.234***	− 0.082	− 0.059	− 0.014			
BMI	− 0.034	0.025	− 0.051	− 0.214***	− 0.019	0.452***	0.025		
Age	− 0.227***	0.140*	0.046	0.137	− 0.062	0.053	0.140*	− 0.077	

*$P < 0.05$;**$P < 0.01$;***$P < 0.001$

Abbreviations: *$1,25(OH)_2D$* 1,25-dihydroxyvitamin D, *25OHD* 25-hydroxyvitamin D, *NTACT-PTH II* second generation intact parathyroid hormone assay, *BSAP* bone-specific alkaline phosphatase, *uOC* undercarboxylated osteocalcin, *CTX* cross-linked C-telopeptide of type I collagen, *BMI* body mass index

Several factors may explain why there was only a tendency for a treatment effect on PTH levels and no significant effect on BTMs: It is a general problem with vitamin D supplementation studies that the study groups are usually also exposed to endogeneously synthesized vitamin D, and that the placebo group may have access to vitamin D supplements and vitamin D blood tests [31]. The slight in-study 25OHD increase in the placebo group, accompanied by a slight, non-significant decrease in PTH concentrations, is an indication of this problem. It may have prevented a significant treatment effect on PTH levels, despite the in-study suppression in PTH concentrations in the vitamin D group. Although fasting PTH and $1,25(OH)_2D$ concentrations were significantly related to both circulating 25OHD concentrations and BTMs, the associations were obviously too small to provide a significant treatment effect of vitamin D supplementation on BTMs. Notably, all BTMs were initially lying within the reference range of the respective assay. This may explain why no improvements in bone turnover markers were seen at study termination. In line with our data, season-related differences in circulating 25OHD and $1,25(OH)_2D$ concentrations did not influence BTMs in young healthy adults [32]. Even in trials where different vitamin D doses (up to 600 IU/day, 4000 IU/day, 20,000 IU/weekly) resulted in significantly lower PTH levels, BTMs remained unaffected [33–35]. Only a tendency towards decreased CTX levels were reported in obese subjects who were supplemented with 7000 IU vitamin D_3 daily, despite a significant treatment effect on PTH levels [36]. Thus, even a vitamin D-induced suppression of PTH levels does not necessarily result in reduced bone turnover.

Unfortunately, no data on habitual calcium intake were available in the present study. The effect of calcium supplementation on BTMs such as CTX has been demonstrated to be higher than the effect of improved vitamin D status [37]. However, due to the fact that all BTMs were lying within the reference range, it seems rather unlikely that calcium supplementation or combined vitamin D and calcium supplementation would have been more effective than vitamin D supplementation alone.

A beneficial vitamin D effect can only be expected in patients with impaired bone turnover. In our study, however, even the subgroups of patients with initial vitamin D deficiency or hyperparathyroidism had, on average, similar BTM concentrations than the entire study cohort, indicating that disturbed bone turnover is not a general problem in male HF patients. Others have reported elevated bone resorption markers only in HF patients with NYHA functional class III-IV, but not in patients with NYHA functional class I-III [38]. In line with these findings in patients with HF, a daily vitamin D_3 supplement of 4000 IU only decreased CTX concentrations in women > 49 years or postmenopausal—a group that is well-known for increased bone turnover—but not in women ≤ 49 years or premenopausal [39]. In our study, some patients had clinically relevant HF progression. However, the number of patients was too small to analyze the effect of vitamin D on BTMs in these patients with end-stage HF.

It also has to be addressed that in the present study, PTH and the bone markers uOC and CTX were inversely related to eGFR values, whereas $1,25(OH)_2D$ was directly related to eGFR values. Data suggest an association of renal insufficiency with secondary hyperparathyroidism and bone turnover. Wu et al. [38] reported similar associations of renal insufficiency with secondary hyperparathyroidism and bone turnover in patients with HF. An association between impaired renal function and increased bone resorption has also been reported in heart transplant candidates [40]. Thus, nutritional vitamin D status may be of minor importance regarding bone turnover in these patients. It is also noteworthy that in our study, a high percentage of patients had elevated PTH levels despite median eGFR values ≥ 70 mL/min/1.73 m^2, whereas in the general

population, PTH levels usually do not rise until chronic kidney disease stage III is achieved [41]. Since PTH can increase heart rate, myocardial blood flow, and cardiac output, it has been assumed that in patients with HF, high PTH levels may be an adaptation to the severity of the disease [42]. In this context, the small but significant association between eGFR values and PTH concentrations even above eGFR values of 30 mL/min/1.73 m^2 may be indication for the fact that HF is not only associated with elevated PTH levels but can cause renal impairment.

Importantly, our findings also underscore the safety of vitamin D supplementation with regard to bone metabolism, which is not trivial due to some reports on potential adverse vitamin D effects on the risk of osteoporotic fractures [19, 43].

The present study has some limitations: One limitation is a lack of bone mineral density measurements, as well as missing data on falls and fractures. Another limitation is the relatively low number of patients in the subgroups with vitamin D deficiency and hyperparathyroidism. This may have resulted in low statistical power to achieve significant results. A further limitation is the relatively high rate of patients who died, dropped out, or were lost-to-follow-up. In addition, the variance of the CTX measurements at study termination was higher than assumed for the statistical power calculation. This may at least in part be due to the relatively large time window of blood collection (7 am to 11 am), given the circadian rhythm of CTX concentrations [44]. The high variance may also be due to the broad reference range of CTX, indicating a high degree of biological variability. Likewise, we cannot definitively rule out that the large number of medications, which were prescribed to our patients, such as aldosterone antagonists, diuretics, and ß-blockers, have caused increased variance in BTMs. Together, the variance of CTX concentrations and other BTMs may have been too large and the between-group difference too small to obtain significant results. A main strength of our work is that, to the best of our knowledge, no other RCT has ever evaluated a higher cumulative vitamin D dose with regard to BTMs. Furthermore, the correlation analyses between vitamin D metabolites, PTH, and BTMs confirm established relationships thus underscoring the validity of our measurements.

In conclusion, the present study found no significant effects of a daily vitamin D_3 supplement at 4000 IU for 36 months on BTMs in patients with advanced HF. It seems to be that vitamin D supplementation will not lower bone turnover in male HF patients.

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Compliance with ethical standards

Conflicts of interest Armin Zittermann has received speaker honoraria from DiaSorin, Germany. Jana B. Ernst, Sylvana Prokop, Uwe Fuchs, Jens Dreier, Joachim Kuhn, Heiner K Berthold, Stefan Pilz, Ioanna Gouni-Berthold, and Jan F. Gummert declare that they have no conflict of interest.

References

1. Shane E, Rivas M, Staron RB, Silverberg SJ, Seibel MJ, Kuiper J, Mancini D, Addesso V, Michler RE, Factor-Litvak P (1996) Fracture after cardiac transplantation: a prospective longitudinal study. J Clin Endocrinol Metab 81:1740–1746
2. Meys E, Terreaux-Duvert F, Beaume-Six T, Dureau G, Meunier PJ (1993) Bone loss after cardiac transplantation: effects of calcium, calcidiol and monofluorophosphate. Osteoporos Int 3:322–329
3. Muchmore JS, Cooper DK, Ye Y, Schlegel V, Pribil A, Zuhdi N (1992) Prevention of loss of vertebral bone density in heart transplant patients. J Heart Lung Transplant 11:959–963
4. Rich GM, Mudge GH, Laffel GL, LeBoff MS (1992) Cyclosporine a and prednisone-associated osteoporosis in heart transplant recipients. J Heart Lung Transplant 11:950–958
5. Katz IA, Epstein S (1992) Posttransplantation bone disease. J Bone Miner Res 7:123–126
6. Epstein S (1996) Post-transplantation bone disease: the role of immunosuppressive agents and the skeleton. J Bone Miner Res 11:1–7
7. van Diepen S, Majumdar SR, Bakal JA, McAlister FA, Ezekowitz JA (2008) Heart failure is a risk factor for orthopedic fracture: a population-based analysis of 16,294 patients. Circulation 118:1946–1952
8. Lyons KJ, Majumdar SR, Ezekowitz JA (2011) The unrecognized burden of osteoporosis-related vertebral fractures in patients with heart failure. Circ Heart Fail 4:419–424
9. Shane E, Mancini D, Aaronson K, Silverberg SJ, Seibel MJ, Addesso V, McMahon DJ (1997) Bone mass, vitamin D deficiency, and hyperparathyroidism in congestive heart failure. Am J Med 103:197–207
10. Anker SD, Clark AL, Teixeira MM, Hellewell PG, Coats AJ (1999) Loss of bone mineral in patients with cachexia due to chronic heart failure. Am J Cardiol 83:612–615
11. Zittermann A, Schleithoff SS, Koerfer R (2006) Markers of bone metabolism in congestive heart failure. Clin Chim Acta 366:27–36
12. Christ E, Linka A, Junga G, Odermatt M, Steinert H, Kiowski W, Schmid C (1996) Bone density and laboratory parameters of bone metabolism in patients with terminal heart disease. Schweiz Med Wochenschr 126:1553–1559 **[In German]**
13. Zittermann A, Schleithoff SS, Tenderich G, Berthold HK, Körfer R, Stehle P (2003) Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure? J Am Coll Cardiol 41:105–112
14. Zittermann A, Schleithoff SS, Koerfer R (2006) Vitamin D insufficiency in congestive heart failure: why and what to do about it? Heart Fail Rev 11:25–33
15. Schleithoff SS, Zittermann A, Stüttgen B, Tenderich G, Berthold HK, Körfer R, Stehle P (2003) Low serum levels of intact osteocalcin in patients with congestive heart failure. J Bone Miner Metab 21:247–252

16. Szulc P, Chapuy MC, Meunier PJ, Delmas PD (1993) Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. J Clin Invest 91:1769–1774
17. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R (2008) Combined calcium and vitamin D supplementation is not superior to calcium supplementation alone in improving disturbed bone metabolism in patients with congestive heart failure. Eur J Clin Nutr 62:1388–1394
18. Anderson PH, Lam NN, Turner AG, Davey RA, Kogawa M, Atkins GJ, Morris HA (2013) The pleiotropic effects of vitamin D in bone. J Steroid Biochem Mol Biol 136:190–194
19. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, Nicholson GC (2010) Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA 303:1815–1822
20. Zittermann A, Ernst JB, Prokop S et al (2017) Effect of vitamin D on all-cause mortality in heart failure (EVITA): a 3-year randomized clinical trial with 4,000 IU vitamin D daily. Eur Heart J 38: 2279–2286
21. Zittermann A, Schwarz I, Scheld K, Sudhop T, Berthold HK, von Bergmann K, van der Ven H, Stehle P (2000) Physiologic fluctuations of serum estradiol levels influence biochemical markers of bone resorption in young women. J Clin Endocrinol Metab 85: 95–101
22. Zittermann A, Kuhn J, Ernst JB et al (2015) 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and postoperative outcome in cardiac surgery. J Clin Endocrinol Metab 100:72–80
23. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group Ann Intern Med 130:461–470
24. Iwamoto J, Sato Y, Uzawa M, Takeda T, Matsumoto H (2009) Comparison of the effects of alendronate and alfacalcidol on hip bone mineral density and bone turnover in Japanese men having osteoporosis or osteopenia with clinical risk factors for fractures. Yonsei Med J 50:474–481
25. Binkley N, Bone H, Gilligan JP, Krause DS (2014) Efficacy and safety of oral recombinant calcitonin tablets in postmenopausal women with low bone mass and increased fracture risk: a randomized, placebo-controlled trial. Osteoporos Int 25:2649–2656
26. Purwosunu Y, Muharram RIA, Reksoprodjo S, Sekizawa A (2006) Vitamin K2 treatment for postmenopausal osteoporosis in Indonesia. J Obstet Gynaecol Res 32:230–234
27. Yasui T, Uemura H, Tomita J, Umino Y, Yamada M, Kuwahara A, Matsuzaki T, Maegawa M, Miura M, Irahara M (2006) Different effects of oral conjugated equine estrogens and transdermal estrogen on undercarboxylated osteocalcin concentration in postmenopausal women. Menopause 13:651–659
28. Pi YZ, Wu XP, Liu SP, Luo XH, Cao XZ, Xie H, Liao EY (2006) Age-related changes in bone biochemical markers and their relationship with bone mineral density in normal Chinese women. J Bone Miner Metab 24:380–385
29. Lenora J, Ivaska KK, Obrant KJ, Gerdhem P (2007) Prediction of bone loss using biochemical markers of bone turnover. Osteoporos Int 18:1297–1305
30. Schwetz V, Trummer C, Pandis M, Grübler MR, Verheyen N, Gaksch M, Zittermann A, März W, Aberer F, Lang A, Treiber G, Friedl C, Obermayer-Pietsch B, Pieber TR, Tomaschitz A, Pilz S (2017). Effects of vitamin D supplementation on bone turnover markers: a randomized controlled trial. Nutrients 9. https://doi.org/10.3390/nu9050432
31. Scragg R (2017) Limitations of vitamin D supplementation trials: why observational studies will continue to help determine the role of vitamin D in health. J Steroid Biochem Mol Biol. https://doi.org/10.1016/j.jsbmb.2017.06.006
32. Zittermann A, Scheld K, Stehle P (1998) Seasonal variations in vitamin D status and calcium absorption do not influence bone turnover in young women. Eur J Clin Nutr 52:501–506
33. Seamans KM, Hill TR, Wallace JM, Horigan G, Lucey AJ, Barnes MS, Taylor N, Bonham MP, Muldowney S, Duffy EM, Strain JJ, Kiely M, Cashman KD (2010) Cholecalciferol supplementation throughout winter does not affect markers of bone turnover in healthy young and elderly adults. J Nutr 140:454–460
34. Aloia JF, Dhaliwal R, Shieh A, Mikhail M, Islam S, Yeh JK (2013) Calcium and vitamin d supplementation in postmenopausal women. J Clin Endocrinol Metab 98:E1702–E1709
35. Holmøy T, Lindstrøm JC, Eriksen EF, Steffensen LH, Kampman MT (2017) High dose vitamin D supplementation does not affect biochemical bone markers in multiple sclerosis—a randomized controlled trial. BMC Neurol 17:67
36. Wamberg L, Pedersen SB, Richelsen B, Rejnmark L (2013) The effect of high-dose vitamin D supplementation on calciotropic hormones and bone mineral density in obese subjects with low levels of circulating 25-hydroxyvitamin d: results from a randomized controlled study. Calcif Tissue Int 93:69–77
37. Aloia J, Bojadzievski T, Yusupov E, Shahzad G, Pollack S, Mikhail M, Yeh J (2010) The relative influence of calcium intake and vitamin D status on serum parathyroid hormone and bone turnover biomarkers in a double-blind, placebo-controlled parallel group, longitudinal factorial design. J Clin Endocrinol Metab 95:3216–3224
38. Wu C, Kato TS, Pronschinske K, Qiu S, Naka Y, Takayama H, Schulze-Späte U, Cremers S, Shane E, Mancini D, Schulze PC (2012) Dynamics of bone turnover markers in patients with heart failure and following haemodynamic improvement through ventricular assist device implantation. Eur J Heart Fail 14:1356–1365
39. von Hurst PR, Stonehouse W, Kruger MC, Coad J (2010) Vitamin D supplementation suppresses age-induced bone turnover in older women who are vitamin D deficient. J Steroid Biochem Mol Biol 121:293–296
40. Kerschan-Schindl K, Strametz-Juranek J, Heinze G, Grampp S, Bieglmayer C, Pacher R, Maurer G, Fialka-Moser V, Pietschmann P (2003) Pathogenesis of bone loss in heart transplant candidates and recipients. J Heart Lung Transplant 22:843–850
41. Andress DL (2006) Vitamin D in chronic kidney disease: a systemic role for selective vitamin D receptor activation. Kidney Int 69:33–43
42. Zittermann A, Schleithoff SS, Götting C, Dronow O, Fuchs U, Kuhn J, Kleesiek K, Tenderich G, Koerfer R (2008) Poor outcome in end-stage heart failure patients with low circulating calcitriol levels. Eur J Heart Fail 10:321–327
43. Bleicher K, Cumming RG, Naganathan V, Blyth FM, Le Couteur DG, Handelsman DJ, Waite LM, Seibel MJ (2014) U-shaped association between serum 25-hydroxyvitamin D and fracture risk in older men: results from the prospective population-based CHAMP study. J Bone Miner Res 29:2024–2031
44. Bjarnason NH, Henriksen EE, Alexandersen P, Christgau S, Henriksen DB, Christiansen C (2002) Mechanism of circadian variation in bone resorption. Bone 30:307–313