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Effects of six months of vitamin D supplementation in patients with heart failure: A randomized double-blind controlled trial

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KEYWORDS
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Abstract *Background and aim:* Low plasma vitamin D levels have been associated with heart failure (HF). This research attempts to explain the role of vitamin D supplementation on myocardial function in elderly patients with HF.
Methods and results: Twenty-three chronic HF patients were randomized in a small parallel group, double-blind, placebo-controlled trial. All patients, with a mean age of 74 years and vitamin D levels <30 ng/mL, received 800,000 IU (4000 IU/daily) of cholecalciferol or placebo for 6 months. The outcomes measured at baseline and after 6 months were ejection fraction (EF) and other echocardiography parameters, carboxyterminal propeptide of procollagen type I (PIP), natriuretic peptides, lipid profile, renin, parathyroid hormone, blood pressure, and body mass index (BMI).

In 13 patients under active treatment for 6 months, mean plasma 25-hydroxy vitamin D concentrations (15.51 vs. −1.40 ng/mL, $p < 0.001$) and plasma calcium (from 9.3 to 9.6 mmol/L, $p < 0.05$) increased significantly. However, other biomarkers of bone metabolism did not differ between the treatment and placebo groups. EF increased significantly in the intervention group (6.71 vs. −4.3%; $p < 0.001$), and the serum concentration of PIP increased only in the placebo group after 6 months (1140.98 vs. −145 mcg/L; $p < 0.05$). Systolic blood pressure was lower after 6 months of cholecalciferol treatment (from 129.6 to 122.7 mm Hg, $p < 0.05$).

No significant variations were observed for other parameters.
Conclusions: Six months of vitamin D supplementation significantly improves EF in elderly patients with HF and vitamin D deficiency.

Introduction

Heart failure (HF), characterized by a reduced cardiac ejection fraction (EF), is one of the major medical problems in the Western world, and the incidence and prevalence are increasing. In fact, recent data show that approximately 1–2% of the adult population in developed countries has HF, with the prevalence rising to ≥10% among those over 70 years of age [1] and in the obese [2].

Low vitamin D levels also (<30 ng/mL) are more common in elderly patients in developed countries or in patients with an increased body mass index (BMI kg/m^2).

In fact, epidemiological data show that vitamin D levels are substantially decreased in patients with HF, compared with controls [3,4]. In different cohorts, it was confirmed that higher vitamin D levels are associated with more favorable outcomes in patients with HF [5].

These epidemiological data are supported by experimental data showing that nuclear vitamin D receptor (nVDR) is expressed in vascular smooth muscle cells, renal juxtaglomerular cells, and cardiac myocytes [6].

In laboratory experiments, VDR$^{-/-}$ mice (with genetic disruption of the VDR) showed the development of

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hypertension, cardiac fibrosis, and remodeling [7]. Moreover, vitamin D may also act rapidly through non-nuclear receptors via a putative membrane vitamin D receptor (mVDR) that modulate a complex signaling system involving the rapid opening of Ca2+ channels, which can increase cardiac contractility.

In mice models, the correction of vitamin D deficiency is associated with a reduction in ventricular hypertrophy, decreases in atrial natriuretic peptide, and the attenuation of hypertension [8,9].

These data, together with a few controlled randomized trials, prompted us to evaluate the role of vitamin D in elderly patients with HF.

Thus far, limited data exist with regard to vitamin D levels in HF patients and its association with clinical outcomes, especially functional parameters. Indeed, few studies [15,16], only two of which are randomized, double-blind trials, have tested vitamin D supplementation in humans, showing mixed results. In particular, no study has been designed to demonstrate a direct improvement in cardiac performance by echocardiography.

Therefore, we designed a randomized, double-blind trial to demonstrate *in vivo* that vitamin D supplements can produce benefits on heart functionality in elderly patients with HF. Additionally, we sought to study the main pathways involved in the mechanisms linking vitamin D to heart health.

Methods

Study design and population

The study was a double-blind, randomized, placebo-controlled trial. Thirty-six HF patients were recruited at Verona University Hospital between July 2011 and June 2012.

The inclusion criteria were the following: patients older than 40 years with a recorded clinical diagnosis of chronic HF (according to Framingham criteria) in the last 5 years, documentation of left ventricular (LV) systolic dysfunction by echocardiography (EF $<$ 55%) and a New York Heart Association (NYHA) class $>$ II. The heart disease etiology was hypertensive, ischemic hypertensive, and valvular hypertensive. All patients were required to have a 25(OH)D level screening $<$30 ng/mL to be eligible for inclusion. Exclusion criteria were hypercalcemia, osteomalacia or fracture history, recent intake of Vitamin D supplements, serum creatinine $>$2 mg/dL, acute heart insufficiency, and no active smoking. All drugs for HF were permitted, but no addiction to drugs or dose changes were permitted during the follow-up period. All patients provided written informed consent for the study, which was approved by the Ethics Committee of the Verona University Hospital according to the principles of the Declaration of Helsinki. The study was registered with the EU Clinical Trials Register (AIFA-EudraCT 2011-002823-16), and the outcomes were prespecified.

At the time of study enrollment, all patients had clinically compensated HF and were receiving a stable dose of a medical regimen that included diuretics, an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, aldosterone receptor, and beta-adrenergic receptor antagonists (Tables 1a and 1b). According to the protocol, the patients were examined at a baseline visit and after 25 weeks. No change in therapy was made during follow-up, and no revascularization events or infarction appeared during follow-up.

Randomization was performed using a computer-generated random number table. An oral dose of 600,000 IU cholecalciferol or placebo was administered after baseline measurements were performed, and another oral dose of 100,000 IU cholecalciferol or placebo was administered at weeks 10 and 20. All the doses of placebo or active treatment (identic in size, color, and markings) were dispensed by physicians, who monitored the intake.

Thirty-six patients were randomized using 1:1 randomization; however, a total of 13 patients (eight placebo/five vitamin D group) were excluded by the analysis (eight refused to complete the study, two were lost during follow-up, and three violated the protocol) (Fig. 1). No patients died within the 25 weeks of the study.

Twenty-three patients completed the study; 10 subjects consumed a placebo (control group) and 13 consumed the vitamin D supplements (case or treated group).

The outcomes described in the per-protocol principle were measured at baseline and 25 weeks. The primary outcome measure was the EF% according to Simpson's method. All the echocardiographic measurements were performed by the same blinded operator.

Secondary outcomes were other echocardiographic parameters, including M Mode left ventricular end diastolic diameter (LVEDD) and end systolic diameter (LVESD), septal (IVS), and posterior wall (PW) thickness. Doppler studies on the mitral valve were used to determine the peak early (E) and atrial (A) velocities. The ratio of E and A waves (E/A) is a commonly used index of diastolic function. LV mass and LV mass indexed to body surface area estimated by LV cavity dimension and end-diastole wall thickness by applying the formula LV mass $= 0.8\ \{1.04\ [([\text{LVEDD} + \text{IVSd} + \text{PWd}]^3 - \text{LVEDD}\ [3])]\} + 0.6$ [10]. Other laboratory outcomes were cardiovascular and inflammatory markers and the measure of carboxyterminal propeptide of procollagen type I (PIP) as a marker of collagen type I-dependent myocardial fibrosis [11]. Serum vitamin D was measured using a radioimmunoassay test kit supplied by DiaSorin (Stillwater, MN, USA). Intact parathyroid hormone (PTH) was examined using the DSL-8000 ACTIVE Intact PTH immunoradiometric Kit (Diagnostic Systems Laboratories, Webster, TX, USA). Calcium and phosphate were analyzed using flame atomic absorption spectrometry (ca; AAS 3030, Perkin–Elmer, Ueberlingen, Germany) and colorimetric test kits (phosphate, Wako Chemicals, Neuss, Germany, and BioMerieux). Brain natriuretic peptide (BNP) was measured in batches using a radioimmunoassay (Becham, St Helens, UK); the intra-assay coefficient of variation was 13.2%. Aldosterone was measured using a radioimmunoassay (Dia-Sorin Ltd, Bracknell, UK); the intra-assay coefficient of variation was

Table 1a Baseline characteristics of 36 patients enrolled in the study.

Group parameter	Vitamin D group (18)	Placebo group (18)	*p*
Mean age (CI 95%)	74.2 (66.6–81.9)	74.3 (62.7–85.0)	n.s
Gender (M/F)	12/6	10/8	n.s.
Heart failure causes			
Ischemic heart disease	16	17	n.s
Hypertension	17	18	n.s
Current medication (*n*/%)			
On ACE inhibitors/angiotensin blocker	15/85	16/90	n.s.
On B-blocker	15/85	14/75	n.s.
On spironolactone	12/69	11/60	n.s.
On digoxin	3/16	1/9	n.s.
On Ivabradine	0/0	0/0	n.s.
On statin	15/85	11/60	0.04
On aspirin	9/50	11/60	n.s.
Loop diuretic	17/94	16/89	n.s.
Mean body mass index Kg/m^2 (CI 95%)	29.0 (27.4–32.8)	28.2 (27.0–34.5)	n.s.
Mean SBP mmHg (CI 95%)	130.0 (119.6–140.2)	136.1 (122.5–152.8)	n.s
Mean DBP mmHg (CI 95%)	72.3 (67.6–77.0)	77.9 (69.9–86.0)	n.s
NYHA class (%)			
I and II	14 (77.8)[a]	12 (28.2)[a]	n.s
III and IV	4 (22.2)[a]	6 (77.8)[a]	
Mean plasma vitamin D ng/mL (CI 95%)	17.2 (10.1–26.3)	17.6 (11.2–27.0)	n.s
Mean plasma PTH pg/mL (CI 95%)	46.7 (17.6–82.6)	48.2 (26.2–59.6)	n.s
Mean plasma Calcium mmol/L (CI 95%)	9.3 (9.0–9.6)	9.3 (9.0–9.8)	n.s
Mean plasma Cholesterol mg/dL (CI 95%)	146.0 (123.0–172.4)	154.6 (120.5–180.3)	n.s
Mean plasma proBNP ng/L (CI 95%)	5242 (1799–5998)	2623 (2320–3980)	n.s
Mean plasma Renin pg/mL (C 95%)	41.9 (13.4–81.7)	60.1 (30.8–192.6)	n.s
Mean plasma Aldosterone pg/mL (CI 95%)	123.0 (80.8–170.5)	174.2 (98.7–249.8)	n.s.
Mean IVS cm (CI 95%)	1.24 (1.09–1.38)	1.16 (0.99–1.35)	n.s
Mean PW cm (CI 95%)	1.20 (1.00–1.36)	1.15 (0.96–1.37)	n.s
Mean EF% (CI 95%)	42.3 (35.0–50.1)	44.3 (36.7–51.0)	n.s

SBP, systolic blood pressure; DBP, diastolic blood pressure; NYHA, New York Heart Association class; PIP, carboxyterminal propeptide of procollagen type I; IVS, interventricular septum; PW, posterior wall; LVD, left ventricular diameter.
[a] Percentage by row.

5.5%. Renin was measured using a radioimmunoassay (DiaSorin Ltd, Bracknell, UK) and the intra-assay coefficient of variation was 4.8%. Serum PIP was determined using the enzyme-linked immunosorbent assay (ELISA) kit (Merck Millipore, Germany) and the inter-assay and intra-assay variations were 4.8–3.4.

We also collected baseline data on age, medical history, current drug use, BMI, and metabolic profile using an ELISA kit (Mercordia, Sweden). Blood pressure was measured with the oscillometric method (median of two measures).

Statistical analysis

Based on our pilot study (data not published) of 18 HF patients (eight treated patients versus eight placebo), we planned a study of a continuous response variable from independent control and experimental subjects with one control per experimental subject. In our previous study, the response within each subject group was normally distributed with a standard deviation (SD) of 5.5%. Thus, if the true difference in the experimental and control means is 8%, we would need to examine eight experimental subjects and eight control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with a probability (power) of 0.8. The type I error probability associated with this null hypothesis test is 0.05. Accordingly, we aimed to recruit 36 participants in anticipation of a 50% dropout rate at 25 weeks to yield a final valuable sample of 18 participants.

Because of the sample's low number, the statistical analysis was performed using a non-parametric test. Categorical variables were compared by a bivariate statistical analysis with Fisher's exact test. All continuous variables were expressed as the mean ± SD or 95% confidence interval (CI 95%). The Mann–Whitney *U* test was used to compare continuous variables between cases and controls. The Wilcoxon signed-rank test was used in both the vitamin D and placebo groups to compare the continuous variable at 0 and 25 weeks.

The Spearman non-parametric test was performed to explore correlations between the variables studied. Alpha was set at 0.05 for all the statistical analyses, which were performed using the SPSS 11.5 statistical software package (SPSS 11.5, SPSS Inc., Chicago, IL, USA).

Results

Analysis at baseline (enrollment time or time 0)

The characteristics of the 36 enrolled patients and the 23 study patients who completed the study are shown in

Table 1b 23 Patients' baseline characteristics.

Group parameter	Vitamin D group (13)	Placebo group (10)	*p*
Mean age (CI 95%)	71.2 (67.0–75.4)	73.4 (64.1–82.7)	n.s
Gender (M/F)	11/2	6/4	n.s.
Heart failure causes			
Ischemic heart disease	10	9	n.s
Hypertension	13	10	n.s
Current medication (n/%)			
On ACE inhibitors/angiotensin blocker	11/85	9/90	n.s.
On B-blocker	11/85	7/70	n.s.
On spironolactone	9/69	6/60	n.s.
On digoxin	2/15	1/10	n.s.
On Ivabradine	0/0	0/0	n.s.
On statin	11/85	6/60	0.04
On aspirin	7/54	6/60	n.s.
Loop diuretic	12//92	8/80	n.s.
Mean body mass index Kg/m^2 (CI 95%)	30.1 (27.6–32.7)	30.9 (27.2–34.5)	n.s.
Mean SBP mmHg (CI 95%)	129.6 (119.4–139.8)	138.6 (122.5–154.7)	n.s
Mean DBP mmHg (CI 95%)	72.3 (67.6–77.0)	77.9 (69.9–86.0)	n.s
NYHA class (%)			
I and II	10 (58.8)[a]	7 (41.2)[a]	n.s
III and IV	3 (50.0)[a]	3 (50.0)[a]	
Mean plasma vitamin D ng/mL (CI 95%)	16.2 (11.8–20.7)	16.0 (11.9–20.2)	n.s
Mean plasma PTH pg/mL (CI 95%)	51.1 (19.6–82.6)	40.4 (26.2–54.6)	n.s
Mean plasma Calcium mmol/L (CI 95%)	9.3 (9.0–9.5)	9.4 (9.0–9.8)	n.s
Mean plasma Potassium mmol/L (CI 95%)	3.2 (2.9–3.5)	3.3 (2.8–3.7)	n.s
Mean plasma Glucose mg/dL (CI 95%)	112.6 (97.1–128.2)	111.9 (84.1–139.7)	n.s
Mean plasma Cholesterol mg/dL (CI 95%)	146.9 (124.3–169.6)	149.5 (121.9–177.1)	n.s
Mean plasma proBNP ng/L (CI 95%)	3867 (1773–5961)	1962 (229–4154)	n.s
Mean plasma Renin pg/mL (C 95%)	47.2 (13.4–81.1)	79.8 (−31.7 to −191.3)	n.s
Mean plasma Aldosterone pg/mL (CI 95%)	125.7 (80.8–170.5)	174.2 (98.7–249.8)	n.s.
Mean plasma PIP mcg/L (CI 95%)	3413.1 (1682.2–5144.1)	2373.02 (1833.4–2912.5)	n.s.
Mean IVS cm (CI 95%)	1.23 (1.09–1.38)	1.17 (1.00–1.34)	n.s
Mean PW cm (CI 95%)	1.19 (1.04–1.33)	1.18 (0.99–1.37)	n.s
Mean LVD cm (CI 95%)	5.39 (4.93–5.85)	5.06 (4.58–5.53)	n.s
Mean Max index g/m^2 (CI 95%)	137.1 (104.9–169.3)	125.7 (93.0–158.4)	n.s
Mean EF% (CI 95%)	39.08 (35.1–45.9)	43.6 (38.5–48.8)	n.s

SBP, systolic blood pressure; DBP, diastolic blood pressure; NYHA, New York Heart Association class; PIP, carboxyterminal propeptide of procollagen type I; IVS, interventricular septum; PW, posterior wall, LVD, left ventricular diameter.
[a] Percentage by row.

Tables 1a and 1b The treatment and placebo groups were not significantly different with regard to the distribution of gender, age, BMI, and other variables listed in Table 1.

In particular, the mean and distribution of low vitamin D plasma levels did not differ statistically between the two groups (mean plasma vitamin D ng/mL of 16.2 (CI 95% 11.8–20.7) versus 16.0 (CI 95% 11.9–20.2).

The echocardiographically determined EF (%) obtained at the time of enrollment was 39.08 ± 7.48 in the vitamin D group and 42.19 ± 6.74 in the placebo group and thus not significantly different. Additionally, the mean levels of PIP and systolic blood pressure (SBP) at baseline were not significantly different between the two groups.

Analysis at 25 weeks

After 6 months, the mean vitamin D levels increased in the treatment group to 31.7 ng/mL (CI 95% 27.4–36.1); as expected, the mean vitamin D plasma levels in the placebo group did not show any significant change (14.6 ng/mL (CI 95% 6.2–23.0)). This difference between the groups was statistically significant ($p < 0.001$) (Table 2 and Fig. 2).

Other biochemical variables, including PTH concentrations, calcium and phosphorus levels, and natriuretic peptides, were not significantly affected by vitamin D supplementation (Table 2).

At 25 weeks, the mean EF (%) in the treated group was 47.2 (CI 95% 41.6–52.9) and was 39.4 (CI 95% 33.5–45.2) in the placebo group, with a statistically significant difference ($p = 0.047$).

Figure 3 shows the EF (%) variation from baseline to 6 months (delta) in the treatment group versus the placebo group; a statistically significant increase of 6.7 (CI 95% 0.8–12.6) in the vitamin D group compared to the EF variation of −4.3 in the placebo group (CI 95%−9.5 to −0.9) ($p = 0.007$) was observed.

Furthermore, the Spearman test showed a statistically significant positive correlation between the increase in vitamin D plasma levels and EF increase (in total group: $r = 0.428$; $p = 0.041$). There were no any statistically significant variations in any of the other echocardiographic parameters.

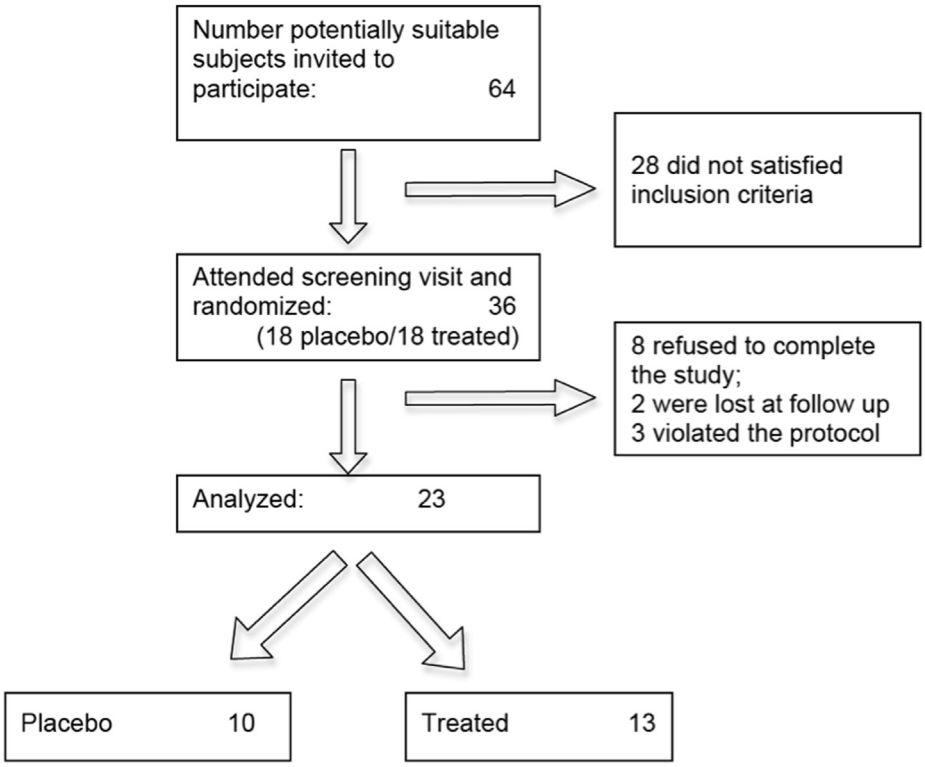

Figure 1 Flow chart showing the numbers of included, excluded, and analyzed patients.

Table 2 Characteristics at 25 weeks.

Group parameter	Vitamin D group (13)	Placebo group (10)	*p*
Mean Body mass Index Kg/m^2 (CI 95%)	29.1 (26.1–32.1)	30.1 (25.9–34.4)	n.s
Mean SBP mmHg (CI 95%)	122.7 (113.2–132.1)	134.5 (118.5–150.5)	n.s
Mean DBP mmHg (CI 95%)	72.3 (67.3–77.3)	74.5 (66.4–82.6)	n.s
NYHA class (%)			
I and II	12 (63.2)[a]	7 (36.8)[a]	n.s
III and IV	1 (25.0)[a]	3 (75.0)[a]	
Mean plasma vitamin D ng/mL (CI 95%)	31.7 (27.4–36.0)	14.6 (6.2–23.0)	<0.001[b]
Mean plasma PTH ng/mL (CI 95%)	33.1 (24.4–41.8)	46.2 (29.8–62.6)	n.s
Mean plasma Calcium mmol/L (CI 95%)	9.6 (9.3–9.9)	9.5 (9.1–9.9)	n.s
Mean plasma Potassium mmol/L (CI 95%)	3.3 (2.9–3.7)	3.3 (2.9–3.7)	n.s
Mean plasma Glucose mg/dL (CI 95%)	130.8 (107.0–154.6)	139.6 (99.4–179.8)	n.s
Mean plasma Cholesterol mg/dL (CI 95%)	156.6 (132.6–180.6)	154.9 (124.1–185.7)	n.s
Mean plasma proBNP pg/L (CI 95%)	4096 (−1621 to −9812)	2376 (−820 to −5573)	n.s
Mean plasma PIP mcg/L (CI 95%)	3268.1 (2001.3–4534.8)	3514.9 (2648.4–4381.5)	0.003[b]
Mean plasma Renin pg/mL (CI 95%)	76.2 (21.8–130.6)	112.0 (−7.2 to −231.3)	n.s
Mean IVS cm (CI 95%)	1.16 (0.99–1.32)	1.19 (1.01–1.37)	n.s
Mean PW cm (CI 95%)	1.24 (1.12–1.37)	1.16 (0.95–1.37)	n.s
Mean LVD cm (CI 95%)	5.22 (4.70–5.75)	4.89 (4.39–5.39)	n.s
Mean Max index g/m^2 (CI 95%)	126.6 (109.2–144.0)	116.4 (90.3–142.5)	n.s
Mean EF% (CI 95%)	47.2 (41.6–52.9)	39.3 (33.5–45.2)	0.018[b]
Delta EF% (CI 95%)	6.7 (0.8–12.6)	−4.3 (−9.5 to −0.9)	0.007[b]

SBP, systolic blood pressure; DBP, diastolic blood pressure; NYHA, New York Heart Association class; PIP, carboxyterminal propeptide of procollagen type I; IVS, interventricular septum; PW, posterior wall; LVD, left ventricular diameter carboxyterminal propeptide of procollagen type I; IVS, interventricular septum; PW, posterior wall; LVD, left ventricular diameter.

[a] Percentage by row.

[b] Mann–Whitney *U* Test.

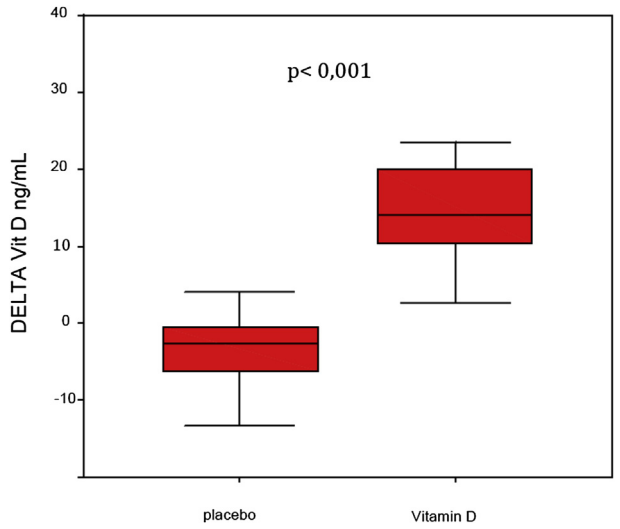

Figure 2 Differences in Vitamin D level after 25 weeks treatment in the two groups.

The serum PIP concentration was higher ($p < 0.05$) in the placebo group after 6 months, from 2373.0 μg/L (CI 95% 1833.4–2912.2) at baseline to 3514.9 μg/L (CI 95% 2648.4–4381.5), whereas there were no significant change in the vitamin D group after 6 months of therapy.

When we analyzed the only placebo subgroup, the ranks test for the continuous variable at time 0 and 25 weeks did not show any significant change. However, the plasma vitamin D level, calcium level, and EF significantly increased in the analysis of the vitamin D subgroup (as shown in Table 3).

Additionally, SBP exhibited a statistically significant decrease in the vitamin D subgroup after 6 months of supplementation, changing from 129.6 (CI 95% 119.4–139.8) to 122.7 mm Hg (CI 95% 113.2–132.1).

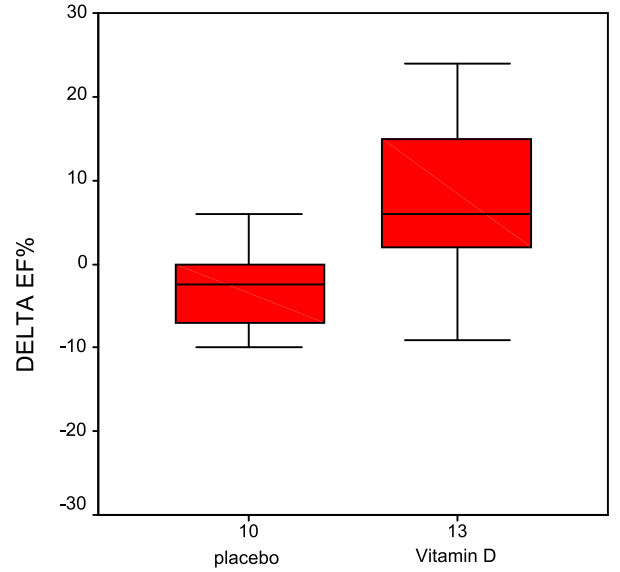

Figure 3 The EF (%) variation (delta) from baseline to 6 months in treated group versus placebo group.

Table 3 The subgroup vitamin D analysis: rank test for the continuous variable at time 0 and 25 weeks.

Group parameter	Baseline	25 weeks	*p*
Mean plasma vitamin D ng/mL (CI 95%)	16.2 (11.8–20.7)	31.7 (27.4–36.1)	0.001[a]
Mean EF% (CI 95%)	40.5 (35.1–45.9)	47.2 (41.6–52.9)	0.042[a]
Mean plasma Calcium mmol/L (CI 95%)	9.3 (9.0–9.5)	9.6 (9.3–9.9)	0.030[a]
Mean SBP mmHg (CI 95%)	129.6 (119.4–139.8)	122.7 (113–132.1)	0.043[a]

[a] Wilcoxon-signed rank test.

All the other bioumoral parameters, such as cholesterol, renin, aldosterone and glycemia, and clinical parameters, such as diastolic blood pressure (DBP) and BMI, remained unchanged in the two groups after intervention (Table 2).

Discussion

Our small group of HF patients was characterized by an inadequate vitamin D status. We showed that a supplement of 800,000 IU vitamin D over 25 weeks (repletion dose, 600,000; IU, plus 1600 IU/daily) is capable of achieving the normal range of plasma vitamin D level, as established by the WHO [12]. Although a consensus has not been reached regarding the concentration of vitamin D needed to define the normal range, recent committees have decided that it is approximately 30 ng/mL [12]. The Institute of Medicine (IOM) supports vitamin D concentrations above 20 ng/mL. These recommendations are based upon evidence related to bone health. Other experts (Endocrine Society, National Osteoporosis Foundation, International Osteoporosis Foundation, and American Geriatric Society) suggest that a minimum level of 30 ng/ml is necessary in older adults to minimize the risk from falls and fractures. A systematic review by the IOM also concluded that there are insufficient data to determine the safe upper limit of serum vitamin D [13]. The supplement dose can range from 50,000 UI of vitamin D orally per week for 8 weeks, followed by the dose needed to maintain the target vitamin D level, often at least 800 IU daily [14]. Due to an absence of safety data, the Endocrine Society guidelines suggest a maintenance dose of vitamin D (1500–2000 IU daily) to maintain a serum level concentration above 30 ng/mL.

However, this level was not achieved in most studies published to date [15,16], likely due to the low doses used. Nonetheless, the majority of treated patients in our study achieved the normal range, without collateral effects.

Furthermore, we are able to demonstrate for the first time that this normalization is paralleled by a significantly improved EF, as measured by echocardiography (%).

Our results confirm experimental data showing that the correction of vitamin D deficiency is able to reduce ventricular hypertrophy and most likely attenuates hypertension [8,9].

There is only one study in the literature that can be compared with ours. In a 2006 double-blind, randomized study by Schleithoff et al. [15], patients with HF (NYHA class I–IV) received either a daily dose of vitamin D, 2000 IU plus 500 mg Ca/d or a placebo plus 500 mg Ca/d for 9 months. In that study, the group of patients actively treated with vitamin D (540,000 IU in 9 months) showed increased plasma vitamin D levels but no significant change in echocardiographic parameters. However, it is important to note that the patients in that study were severely ill (high number of NYHA 3–4), and the researchers observed a high number of dropouts (37%).

Another double-blind, randomized study by Witte et al. [16] tested elderly patients with HF for an improvement in EF using multivitamin supplements, including vitamin D. Due to the study's design, the contribution of 400 IU vitamin D administered daily to the described improvements is not discernable from the potentially synergistic components of the benefits of the multivitamin preparation. Furthermore, vitamin D levels were not investigated.

Another recent study by Zia et al. [17] involved a small group of 14 African-American patients with HF and vitamin D deficiency (<20 ng/mL). That open study, which was not randomized and had no control group, demonstrated an improvement in EF (24.3 ± 1.7% to 31.3 ± 4.3%) after 50,000 IU vitamin D given orally for 8 weeks and 2.000 IU given per day for 6 additional weeks.

In a double-blind controlled study in 2012, Shedeed [18] reported a significant improvement in HF score, LV end diastolic, systolic diameter, and EF in infants with chronic congestive HF after 12 weeks of vitamin D supplementation.

The present study is the first double-blind, small randomized study able to achieve a normal vitamin D range by analyzing echocardiographic parameters and able to demonstrate, as already demonstrated in animals models, that the normalized vitamin D plasma levels in the treatment group were linked to increased EF.

In patients with HF, characterized by LV systolic dysfunction, the maladaptive changes occurring in surviving myocytes and extracellular matrix after myocardial injury (e.g., myocardial infarction) lead to the pathological "remodeling" of the ventricle, with dilatation and impaired contractility, which is mirrored by a reduction in the ejected fraction (EF). This might be caused by at least two mechanisms: the occurrence of further events leading to additional myocyte death (e.g., recurrent myocardial infarction) and the systemic response induced by the decline in systolic function, particularly neurohumoral activation.

The two key neurohumoral systems activated in HF are the renin–angiotensin–aldosterone (RAA) system and the sympathetic nervous system [19,20]. The interruption of these two key processes is the basis of the most effective treatments for HF.

Through its genomic pathway (binding to VDR) and its non-genomic pathway, vitamin D can have anti-hypertrophic effects [6–21], modulating the differentiation and proliferation of cardiomyocytes. Vitamin D also can have an effect on neurohumoral systems.

Therefore, the main potential mechanism that could explain a direct protective effect of vitamin D in HF includes effects on myocardial contractile function, regulation of natriuretic hormone secretion [14], regulation of the RAA system [19], regulation of blood pressure effects [22], and heart remodeling and reduced left ventricular (LV) hypertrophy (17-18-22 3-24-30) [24,30]. Vitamin D can also indirectly affect cardiac function by altering PTH [25] and serum calcium levels [26].

Among the above-mentioned mechanisms, we may exclude the role of PTH, NT-pro-BNP, renin or aldosterone, as we did not measure any statistically significant variation in these variables in the treatment group. However, we did document variations in SBP though not in DBP.

We also measured the levels of PIP, which is considered a biomarker of myocardial type I collagen synthesis [10]. Previous studies have demonstrated that collagen synthesis is increased in hypertensive patients [28] and patients with HF compared to controls, and there is a direct correlation between PIP levels and myocardial collagen concentration, as measured by cardiac biopsies [11–29].

In our study, we found increased PIP levels in the placebo group after 6 months, yet no variations in the patients taking the vitamin D supplement. This may support the hypothesis that collagen synthesis increased only in the placebo group, which may represent a mirror of cardiac remodeling, though this detrimental effect on cardiac function appears to be prevented in patients with to vitamin D supplementation. We could hypothesize that, in the placebo group, the increase in PIP is linked to the natural history of HF disease, leading to cardiac fibrosis, whereas the absence of any increase in PIP levels demonstrates that the treatment group's outcome may be caused by vitamin D supplementation.

Procollagen type 1 N pro-peptide (P1NP), which is derived from PIP, is known as a marker of bone turnover and is linked with bone parameters, including vitamin D levels. However, no significant correlation has been reported between P1NP concentrations and vitamin D concentrations in the literature [27].

Even though we are far from uncovering the inner mechanisms of the protective effect of normalizing vitamin D plasma levels on the heart of HF patients, we here propose that an antifibrotic mechanism could be involved.

Our study has some limitations. First, the population recruited using a sufficient statistical power was rather limited in size. Second, follow up was limited to 6 months. Third, the results were analyzed according to the per-protocol principle, which can also create a limitation. Fourth, the number of dropout was high, probably due to the advanced age of enrolled patients. As far as this last point, we must emphasize that this is also a surprisingly short period of time for producing such an increase of EF.

Although decreases in SBP have been reported, it is necessary to conduct other studies to confirm these data.

In conclusion, 800,000 IU of vitamin D taken for 25 weeks improves EF in older patients with HF. If confirmed with larger trials, this finding could represent a safe and

cost-effective additional therapy for HF in elderly patients with vitamin D deficiency.

Conflict of interest

There are no financial issues or other relationships that could cause a conflict of interest.

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