Accepted Manuscript

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PII: S0735-1097(16)01694-6

DOI: 10.1016/j.jacc.2016.03.508

Reference: JAC 22425

To appear in: *Journal of the American College of Cardiology*

Received Date: 17 March 2016

Accepted Date: 21 March 2016

Please cite this article as: Witte KK, Byrom R, Gierula J, Paton MF, Jamil HA, Lowry JE, Gillott RG, Barnes SA, Chumun H, Kearney LC, Greenwood JP, Plein S, Law GR, Pavitt S, Barth JH, Cubbon RM, Kearney MT, Effects of Vitamin D on Cardiac Function in Patients With Chronic HF: The VINDICATE Study, *Journal of the American College of Cardiology* (2016), doi: 10.1016/j.jacc.2016.03.508.

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ACCEPTED MANUSCRIPT

Effects of Vitamin D on Cardiac Function in Patients With Chronic HF: The VINDICATE Study

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Running title: Vitamin D in chronic heart failure

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Conflict of Interest: There are no conflicts of interest for any of the authors relevant to the present manuscript.

Acknowledgements
KKW holds an NIHR Clinician Scientist Award
JG holds an NIHR-HCS Fellowship Award
MP is funded by a Leeds Charitable Foundation Fellowship
RMC holds an Intermediate Fellowship from the British Heart Foundation
MTK is a British Heart Foundation Professor of Cardiology

The authors acknowledge the consistent administrative support provided by Mrs Andrea Marchant and Miss Lisa Trueman that made VINDICATE possible. They also acknowledge methodological and analytical advice offered by Dr David A Cairns of the Leeds Clinical Trials Research Unit, University of Leeds.

This research took place in the National Institute for Health Research Leeds Cardiovascular Clinical Research Facility at Leeds Teaching Hospitals NHS Trust.

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Abstract

Background: Patients with chronic heart failure (CHF) secondary to left ventricular (LV) systolic dysfunction (LVSD) are frequently deficient in vitamin D. Low vitamin D levels are associated with a worse prognosis. It is unclear whether vitamin D deficiency is a marker of disease severity or plays a pathophysiological role.

Objectives: The VitamIN D treatIng patients with Chronic heArT failurE (VINDICATE) study was designed to establish the safety and efficacy of high-dose vitamin D supplementation in patients with CHF due to LVSD.

Methods: We enrolled 229 patients (179 men) with CHF due to LVSD and vitamin D deficiency ((25(OH) vitamin D_3 <50nmol/L (<20ng/mL)) into a randomised, placebo-controlled double-blind trial of vitamin D supplementation. Participants were either allocated to one year of vitamin D_3 supplementation (4000IU (100μg) 25(OH)D_3 daily) or matching non-calcium-based placebo. The primary endpoint was change in six-minute walk distance from baseline to 12 months. Pre-specified secondary endpoints included change in left ventricular ejection fraction at one year, and safety measures of renal function and serum calcium concentration assessed every three months.

Results: One year of high-dose vitamin D supplementation did not improve 6-minute walk distance at one year, but was associated with a significant improvement in cardiac function on echocardiography (left ventricular ejection fraction +6.07% (95% CI 3.20, 8.95; p<0.0001); and a reversal of left ventricular remodeling (left ventricular end diastolic diameter -2.49mm (95% CI -4.09, -0.90; p=0.002) and left ventricular end systolic diameter -2.09mm (95% CI -4.11; -0.06 p=0.043). There were no clinically significant effects on calcium levels or renal function.

Conclusions: One year of 100μg daily 25-OH vitamin D_3 supplementation does not improve 6-minute walk distance but has beneficial effects on LV structure and function in patients on contemporary optimal medical therapy. Further studies are necessary to determine whether these translate to improvements in outcomes.

Key words: Heart failure, vitamin D, left ventricular function, remodeling

Clinical Trial: VINDICATE was approved by the regional ethics committee (12/YH/0206), funded by the Medical Research Council-UK and is registered on ClinicalTrials.gov (NCT01619891).

Abbreviations

CHF	Chronic heart failure
LVSD	Left ventricular systolic dysfunction
SR	Sinus rhythm
AF	Atrial fibrillation
CMR	Cardiac magnetic resonance
LVEF	Left ventricular ejection fraction
LVEDD	Left ventricular end-diastolic diameter
LVEDV	Left ventricular end-diastolic volume
LVESD	Left ventricular end-systolic diameter
LVESV	Left ventricular end-systolic volume
25 (OH) vitamin D3	Cholecalciferol
1,25 (OH)2 vitamin D3	Calcitriol

Chronic heart failure (CHF) secondary to left ventricular (LV) systolic dysfunction is a common condition affecting 5 million individuals in the United States (1) and a similar number in Western Europe (2). While the prognosis of CHF has improved substantially over the last 2 decades (3) mortality remains high with 50% of patients dying within 5 years of diagnosis (4,5).

Patients suffering from cardiovascular disease are frequently deficient in the steroid hormone vitamin D and vitamin D deficiency has been shown to be associated with the development of CHF in a number of studies (6-10). Around 90% of CHF patients have hypovitaminosis D (11), even in sunny climates (12). The agent has a range of pleiotropic effects that in the setting of CHF may impact on disease severity (13,14), but despite this, clinical trials examining vitamin D supplementation in CHF patients have to date been inconclusive (15,16).

The aims of the VitamIN D treatIng patients with Chronic heArT failurE (VINDICATE) study were to describe the safety and efficacy of long-term, high-dose 25 (OH) vitamin D_3 supplementation on submaximal exercise capacity and cardiac function in patients with CHF due to LVSD.

Methods

Study population

VINDICATE was a randomised placebo-controlled double-blind trial of vitamin D supplementation in vitamin D-deficient CHF patients on optimal medical therapy. Patients were eligible if they had stable (>3 months) NYHA class II or III symptoms, a left ventricular ejection fraction (LVEF) ≤45% on maximally tolerated medical therapy (>3 months) and a 25(OH) vitamin D level of <50nmol/L (<20ng/mL).

Patients were ineligible if they were taking or had taken calcium or other vitamin supplements in the last three months, if their CHF was due to untreated valvular heart disease,

anaemia or thyrotoxicosis, if they had existing indications for vitamin D supplementation (e.g. previous osteoporotic fracture or symptoms of osteomalacia), a history of primary hyperparathyroidism, sarcoidosis, tuberculosis or lymphoma, a vitamin D concentration at the time of screening >50nmol/L (20ng/mL) or significant renal dysfunction (eGFR<30 mL/min).

Allocation and intervention

Patients enrolled into VINDICATE were allocated in blocks of 20 using minimisation balancing for aetiology of CHF (ischaemic/non-ischaemic), diabetes mellitus, sex, chronic obstructive pulmonary disease (COPD)(use of regular bronchodilators), ethnic origin (Caucasian/non-Caucasian). Each participant was asked to take two tablets per day providing either a total of 100µg 25(OH) vitamin D_3 (4000iu daily) or placebo (Cultech, Port Talbot, Wales, UK).

The supplement and dose were chosen based upon guidelines for studies of vitamin D supplementation (17). These guidelines suggest that studies should 1) aim to replace physiological requirements, supplementing between 75-250µg/day; 2) last at least 9 months; 3) supplement with vitamin D_3 (not D_2); 4) assay supplements for potency; 5) include a regular serum measurement of 25[OH] D levels; 6) aim to achieve serum levels in patients on active therapy between 100-160nmol/L (40-64 ng/mL). Also, on the basis of recent data demonstrating the adverse effect of hyperparathyroidism in CHF (18), we chose a dose likely to suppress parathyroid hormone release. Our proof of concept study, using the same inclusion and exclusion criteria and protocol as VINDICATE, had previously demonstrated the efficacy of 4000IU daily to achieve positive remodelling with significant reductions in left ventricular end-diastolic and end-systolic volumes and left ventricular end-diastolic dimension. The consort diagram and results from this study are presented in online supplementary datasets (**Figure 1 and Tables 1**

and 2). A simple linear model-based trend test from this study demonstrated a significant decrease in PTH over the year (p= 0.0095) in those allocated vitamin D, with no such trend in patients allocated to the placebo arm (p=0.977) (Online Figure 2) (19).

Outcome variables

The prespecified primary endpoint in VINDICATE was the difference in change in 6-minute walk test distance (6MWT) (baseline to 12 months) between the two groups. Key prespecified secondary endpoints included cardiac structure and function, and safety endpoints of serum calcium concentration, renal function and vitamin D levels. Hypervitaminosis D was defined as >200nmol/L (80ng/mL), hypercalcaemia as >2.6nmol/L (10.4 mg/dL).

Study procedures

At baseline each patient performed a 6MWT according to standard criteria (20).. Each patient also underwent echocardiography and blood sampling for serum calcium, serum creatinine, and vitamin D and PTH levels. Patients were also invited to undergo cardiac magnetic resonance imaging to measure left ventricular volumes. Subsequent visits took place at 3, 6, 9 and 12 months and blood draws were repeated at each visit for safety data.

Serum biochemistry

Serum 25(OH)D_2 and 25(OH)D_3 were analysed by tandem mass spectrometry. Samples were prepared using a protein precipitation reagent containing deuterated 25(OH)D_3. The supernatant was analysed on an API5000 LC-MS/MS (AB SCIEX, Warrington, UK) in APCI mode. The inter-assay CV was <10% at all concentrations ranging from 12-159 nmol/L (4.8-63.7 ng/mL). 25(OH)D_2 and vitamin D_3 concentrations were summed and reported as total 25(OH)D. We defined deficiency and insufficiency of vitamin D concentrations as <50nmol/L (20 ng/mL) and <75nmol/L (30 ng/mL) respectively (21,22). We also measured serum calcium, creatinine

and parathyroid hormone (Siemens Advia and Centaur, Siemens Healthcare Diagnostics, Camberley, UK). To confirm effective conversion of the supplement we also measured 1,25(OH)vitamin D_3 by radio-immuno-assay (IDS, Boldon, UK) at baseline and at 12 months.

Echocardiography

Echocardiography was performed on all patients at baseline and LV function was assessed according to European Society of Cardiology criteria using Simpson's biplane measure to determine left ventricular (LV) ejection fraction (LVEF) (23). At the 12 months visit echocardiography was repeated. Echocardiograms at both time points were analysed offline at the end of the study by two senior echocardiographers blinded to patient treatment.

Cardiac magnetic resonance imaging

CMR studies were performed on dedicated 1.5 Tesla or 3 Tesla CMR systems (Philips Healthcare, Best, Netherlands). The same system was used for baseline and follow-up studies (at 12 months) of individual patients. A multi-slice multiphase data set covering the entire left ventricle in 10-12 short axis slices was acquired using a validated 2D balanced steady state free precession (SSFP) pulse sequence (TR 2.8 ms, TE 1.4 ms, flip angle 55°, spatial resolution 2.0 mm x 2.0 mm x 10mm, no interslice gap, 30 phases/cardiac cycle, 1 slice per breath-hold). Off line analysis by an experienced CMR observer using QMASS V7.0 software (Medis, Leiden, Netherlands) blinded to study allocation derived end-diastolic and end-systolic LV volumes and ejection fraction.

Sample size

VINDICATE was powered to provide information on the patient-oriented outcome of 6MWT. A trial of iron supplementation in a similar patient group had demonstrated that improvements of 30-40metres could be expected with this type of intervention (24). We assumed, based upon our preliminary data from a pilot study (19), that there would be a change

between the two groups at 12 months of 30m. The SD of change in 6MWT was estimated from these data; the upper limit of the 80% confidence interval (estimated using bootstrapping) was used in these calculations to allow for the small sample size in the proof of concept. This determined that 210 patients were required to have 90% power to show a difference in change in 6MWT of 28m or more with 5% significance (SD=62). We aimed to recruit 230 patients (115 per group) to allow for ~10% dropout.

Statistical analysis

Differences in baseline variables between allocations were tested using t-tests (continuous data) or the chi-squared test (categorical data). The analysis of primacy for the main efficacy endpoints was based on analysis of covariance linear models relating differences in the final walk distance and imaging variables by treatment allocation, adjusting for baselines values and reported with 95% confidence intervals (CI) (25). All significance tests were two-sided and called significant at the 5% level. All analyses were conducted in Stata (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP)

Funding, ethical and safety considerations

VINDICATE was approved by the regional ethics committee (12/YH/0206), funded by the Medical Research Council-UK and is registered on ClinicalTrials.gov (NCT01619891).

A single unblinded observer with no involvement in the patients' care or study follow-up (JHB) reviewed each vitamin D result at each time point for safety. An agreed operating procedure for any subject develop a serum vitamin D concentration >200nmol/L (80ng/mL) involved reducing the dose of treatment from two to one tablets per day to maintain patient blinding.

Results

We enrolled 229 patients into VINDICATE. Six patients were found to be ineligible at the baseline visit, leaving 223 patients randomised to treatment. Figure 1 describes patient recruitment and loss to follow-up. A total of 163 patients completed the study. Baseline characteristics divided by treatment allocation are shown in **Table 1**. There were no important clinical differences at baseline between patients completing the study and those who dropped out. The two groups of completing participants were balanced for baseline clinical variables (**Table 1**).

The vitamin D_3 supplement was well-tolerated and achieved sustained normal serum 25(OH)D_3 concentrations by 3 months post-randomisation indicating excellent adherence to treatment (**Figure 2**). Patients in the placebo arm had lower median concentrations of 25(OH) vitamin D at 12-months post-randomisation, (24.5; range 10.0, 81.8 nmol/L (9.8; range 4, 32.7 ng/mL) than patients in the active supplement arm (115; range 17.8, 193 nmol/L (46 ng/mL (range 7.1, 77.2); $p<0.0001$) confirming the effectiveness of the vitamin D supplementation in restoring normal levels of 25-OH vitamin D_3. The supplement effectively normalised 1,25 $(OH)_2$ vitamin D_3 levels to 121pmol/L (range 40, 331), (46.5; range 15.4, 127.3pg/mL)) at 12 months and also suppressed parathyroid hormone levels leading to lower PTH levels in subjects allocated vitamin D (8.70; range 1.28, 22.2 pmol/L (82, range 12, 209 ng/mL)) than those allocated placebo (10.80; range 2.80, 53.10pmol/L (102, range 26, 499 ng/mL)); ANCOVA difference in mean change of -3.63 (95%CI -5.24, -2.03)pmol/L (-34, 95% CI -49, -19 ng/mL); $p<0.0001$.

No patient was observed to suffer hypervitaminosis D according to our pre-specified safety concentration of 200nmol/L (80 ng/mL) 25(OH)D_3 and no subject required a down-titration of dose. One patient with borderline hypercalcaemia at baseline (2.66mmol/L

(10.64mg/dL)) had persistent hypercalcaemia throughout the study, and one other patient with hypercalcaemia at three months (2.73mmol/L (10.9mg/dL)) had a normal calcium by six months and throughout the remainder of the study (**Figure 2**). There was no concerning change in renal function (**Figure 2**) and there were no study drug-related admissions or adverse events. Twelve months of 4000IU of 25-OH did not improve or preserve 6MWT distance in CHF patients (**Figure 3**).

At 12 months, compared with patients randomised to placebo, patients in the vitamin D arm had a greater improvement in echocardiographic measures of left ventricular (LV) function (LVEF: +7.65 (5.21, 10.09) v +1.36 (-0.38, 3.11)%; p<0.0001), LV dimensions (LVEDD; -2.45 (-3.70, -1.21) v -0.08 (-1.25, 1.10)mm; p=0.002, LVESD; -2.72 (-4.52, -0.92) v -0.99 (-2.31, 0.33)mm; p=0.043) and volumes (LVEDV: -16.47 (-25.71, -7.22) v -3.83 (-13.36, 5.70)mL; p=0.04, LVESV -18.77 (-25.96, 9.59) v -8.49 (-17.98, 1.01)mL; p=0.041) (**Table 2 and Figure 3**). There was a dose-response relationship between increase in vitamin D levels and increase in LVEF (coefficient 0.04; p=0.023) and decrease in LVEDD (coefficient -0.02; p=0.035).

Enrolment into VINDICATE did not mandate CMR imaging and one third of patients in VINDICATE had cardiac devices incompatible with CMR imaging. Only 69 patients volunteered to undergo baseline CMR scanning. The CMR data are further limited as a result of withdrawal or death during follow-up (n=8), device implantation between baseline and follow-up (n=2 implantable cardioverter defibrillators), patient refusal to undergo a second scan (n=17) and technical problems with the second scan, such that we only had 34 patients with serial CMR images. Baseline characteristics of these patients are shown in Online Table 1. Patients agreeing to serial CMR scans were younger (61.5 (36.7, 84.8) v 71.3 (28.1, 92.3)years; p<0.0001) had better renal function (creatinine: 86 (43, 114) v 102 (48, 245)μmol/L; p=0.007) and were non-

significantly less deficient in 25-(OH) vitamin D at baseline (43.9 (10.0, 90.4) v 35.94 (10.0, 111.0)nmol/L (17.6 (4.0, 36.2) v 14.4 (4.0, 14.5) ng/mL); p=0.07), but were otherwise similar to patients who declined CMR scanning including the change in vitamin D from baseline to completion (p=0.64). The data from serial CMR scans showed improvements in cardiac function with vitamin D, but were not statistically significant possibly due to insufficient statistical power (LVEF: 4.12 (-0.11, 8.35) v 1.19 (-3.20, 5.59)%; p=0.317), LVEDV: --26.12 (-63.27, 11.04) v –0.10 (-12.88, 13.07)mls; p=0.168 and LVESV: -29.61 (-72.40, 13.18) v –1.36 (-19.19, 16.48)mls; p=0.206). There was however, a dose response relationship in our CMR data with a relationship between increases in vitamin D and reductions in left ventricular end-diastolic (coefficient -0.19; p=0.050) and end-systolic (coefficient -0.20; p=0.083) volumes.

Discussion

VINDICATE aimed to examine the effect of high-dose vitamin D supplementation in patients with CHF secondary to LVSD taking optimal medical therapy. The results demonstrate that 4000IU vitamin D_3 given for twelve months is safe, well tolerated, and not associated with concerning adverse biochemical effects.

There was no effect of vitamin D supplementation on the primary endpoint of 6MWT distance but there were statistically significant, and prognostically and clinically relevant improvements in the secondary outcomes of left ventricular ejection fraction, dimensions and volumes suggesting that vitamin D is leading to beneficial reverse remodelling.

New therapies for serious chronic conditions including CHF are often expensive, increasingly technical and frequently fail to meet the rigorous demands of large phase 3 clinical trials. Vitamin D might be a cheap and safe additional option for CHF patients and may have beneficial effects on multiple features of the syndrome (21).

Patients with CHF are frequently deficient in vitamin D, low vitamin D levels increase the risk of incident CHF (26), and are associated with more severe disease and worse outcomes in patients with CHF (6-9,12). Supplementation to treat or prevent osteoporotic fractures might be associated with a lower incidence of CHF (10).

However, despite the publication of studies exploring various doses and forms of vitamin D supplementation in patients with CHF there remains considerable uncertainty regarding the benefits of this therapeutic approach. In the first study by Schleithoff et al, 93 subjects received 50µg vitamin D_3+calcium (Ca^{2+}) per day for 9 months or placebo+Ca^{2+} (15). There was a trend to improvement of LV function measured by echocardiography and a smaller increase in pro-inflammatory cytokines during follow-up in those randomised to vitamin D. Both groups were given Ca^{2+} and both groups had some improvement in LV function with no differences between them. Witham et al examined vitamin D_2 supplementation in 105 elderly patients (20). Subjects were randomized to two doses of 100,000IU of vitamin D_2 or placebo at baseline and ten weeks and assessed at 20 weeks. There was no effect on walk distance, or immune function, and a slight deterioration in quality of life. The population in that study was heterogeneous including patients with and without LVSD, mean N-terminal B-type natriuretic peptide levels and daily furosemide doses were lower than those seen in a usual HF population, medical therapy was not optimised, the duration of treatment was short, patients randomised to vitamin D remained deficient (at 43.4 nmol/L (17.4ng/mL)) and PTH was not suppressed (27). Although Boxer et al did not demonstrate improvements in cardiac function or objective measures of muscle strength and exercise capacity in 64 CHF patients (of whom 34 underwent echocardiography) randomised to weekly doses of 50,000IU of vitamin D3 for six months, there was an improvement in serum aldosterone and quality of life in those allocated the supplement (28,29). In an open-label study,

Schroten et al demonstrated a reduction in plasma renin concentration after 6 weeks of 2000IU vitamin D3 daily in 101 patients with CHF (30). Finally, although Dalbeni et al (31) noted an increase in LVEF of almost 7% after only 25 weeks in 13 patients randomised to 600,000 IU vitamin D_3 at baseline and two further doses of 100,000IU at 10 and 20 weeks while the 10 patients randomised to placebo had a reduction in LVEF of more than 4%, the authors did not comment on cardiac dimensions and there was an increase in natriuretic peptide levels in both groups. In contrast to these studies, VINDICATE is a double-blind, placebo-controlled study of an oral non-calcium based daily supplement of 4000IU of vitamin D_3 given for 12 months in patients with CHF due to LVSD on otherwise optimal medical therapy. The supplement led to consistent biochemical evidence of replenishment and an effective suppression of parathyroid hormone levels.

The primary endpoint of VINDICATE was change in 6MWT distance. The study was based upon pilot data and powered to detect a 28m difference between the two groups at twelve months (24). The variability in the walk distance measure at baseline was much greater than predicted from our pilot study such that our sample size only had 7% *post hoc* power to detect a difference between the groups. VINDICATE was therefore underpowered to detect a clinically relevant change in walk distance. Six-minute walk distance is an increasingly frequently used patient-oriented outcome measure, but has greater variability than objective surrogate endpoints (19). The findings from VINDICATE have implications for future studies using 6-minute walk distance as an outcome measure.

Our secondary endpoints of cardiac function and structure measured by echocardiography were however highly statistically and clinically significant with improvements in both LV ejection fraction, dimensions and volumes. Similar changes were seen in a subgroup of patients

agreeing to serial cardiac magnetic resonance imaging, although did not reach conventional levels of statistical significance due to lack of power.

A pathophysiological hallmark of CHF secondary to LV systolic dysfunction is a progressive increase in LV cavity dimensions and impaired contractility, a process known as LV remodelling (32). Current accepted therapies for CHF which afford CHF patients improvements in survival such as angiotensin converting enzyme inhibitors (33), beta-adrenoceptor antagonists (34,35), and cardiac resynchronization therapy (36) have also been shown to have a favourable effect on LV remodelling by delaying progression of, or reversing LV dilatation. The degree of favourable remodelling induced by these treatments is related to long term outcomes (37). It is therefore plausible that the improvements in cardiac function demonstrated in VINDICATE have the potential to improve outcomes.

How does vitamin D contribute to beneficial remodelling?

Vitamin D deficiency could contribute to adverse remodelling through two major pathways. Vitamin D deficiency could lead to cardiomyocyte dysfunction by interfering with Ca^{2+} transport (38) at a cellular concentration. HF is a condition of intracellular calcium overload, which adversely affects both contraction and relaxation. Furthermore, vitamin D deficiency might contribute to cardiomyocyte hypertrophy, interstitial inflammation and fibrosis (39). Hence vitamin D deficiency could contribute to a more rapid progression to heart failure following myocardial damage due to more aggressive adverse remodelling (40).

However, adverse remodelling is also the result of persistent neurohormonal activation, particularly that of the renin angiotensin aldosterone system (RAAS) which strongly contributes to deteriorating cardiac function, cardiomyocyte loss and interstitial fibrosis (41). Inhibition of the RAAS leads to attenuated or reverse LV remodelling in patients with heart failure (42).

Vitamin D deficiency heightens RAAS activity (30,43), whereas vitamin D supplementation seems to reduce renin synthesis (44) and plasma renin activity (43).

Study Limitations

VINDICATE was performed at a single centre. However, the study was based upon results from a randomised, placebo-controlled pilot study in 53 patients using the same dose for 12 months that also showed a favorable effect of vitamin D on cardiac structure and function (24). We did not examine the effect of vitamin D supplementation in patients with CHF and preserved ejection fraction, a group of patients who may warrant such investigation.

Conclusions

VINDICATE has demonstrated that high-dose vitamin D supplementation is safe, well tolerated and associated with a clinically relevant improvement in cardiac function in CHF patients already taking current optimal therapies.

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FIGURE LEGENDS

Figure 1: Consort diagram demonstrating patient enrollment and disposition for VINDICATE.

Figure 2: Median and interquartile ranges for vitamin D (A), creatinine (B), calcium (C), and parathyroid (PTH) (D) concentrations at three monthly time points in VINDICATE by treatment allocation. Vitamin D concentrations are described in relation to deficiency (green line), sufficiency (yellow) and the accepted upper limit for hypervitaminosis D (red line). Serum calcium levels described in relation to upper limit of normal range (red line), and serum PTH concentrations in relation to the normal range (between red lines). Conversion factors: vitamin D nmol/L * 0.4 = ng/mL; creatinine mmol/L * 0.11 = mg/dL; calcium mmol/L * 4 = mg/dL; parathyroid hormone pmol/L * 9.4 = pg/mL.

Figure 3: Median and interquartile ranges for 6-minute walk test distance (A), and left ventricular ejection fraction (LVEF) (B), left ventricular end-diastolic dimension (LVEDD) (C), and left ventricular end-diastolic volume (LVEDV) measured by echocardiography at baseline and final visit in VINDICATE by treatment allocation.

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Table 1: Patient demographics (VINDICATE) at randomization - intention-to-treat population

	Total (n=163)	Placebo (n=83)	Vitamin D (n=80)
Male sex (n)[%]	129 [79.1]	62 [74.7]	67 [83.8]
Age	68.7 (13.10)	69.0 (13.78)	68.5 (12.45)
Caucasian (n)[%]	146 [90]	74 [89]	72 [90]
Aetiology (n)[%]			
Ischaemic heart disease	94 [57.7]	50 [60.2]	44 [55.0]
Non-ischaemic cardiomyopathy	61 [37.4]	29 [34.9]	32 [40.0]
Valvular heart disease	8 [4.9]	4 [4.8]	4 [5.0]
Diabetes mellitus (n)[%]	37 [22.7]	20 [24.1]	17 [21.3]
BMI (Kg/m^2)	30.0 (11.41)	30.3 (14.36)	29.8 (7.26)
NYHA (n)[%]			
II	145 [89]	71 [85.5]	74 [92.5]
III	18 [11.0]	12 [14.5]	6 [7.5]
Beta blockers (n)[%]	155 [95.1]	79 [95.2]	76 [95.0]
ACEi/ARB (n)[%]	150 [92.0]	76 [91.6]	74 [92.5]
Furosemide dose (mg/day)	61.4 (46.38)	64.4 (52.07)	58.6 (41.00)
Digoxin (n)[%]	29 [18.0]	15 [18.3]	14 [17.7]
Spironolactone (n)[%]	83 [51.2]	41 [50.0]	42 [52.5]
Device (ICD or CRT) (n)[%]	48 [29.5]	27 [32.5]	21 [26.3]
Atrial fibrillation (n)[%]	68 [45.0]	33 [42.9]	35 [47.3]
Baseline heart rate	70.5 (13.10)	72.7 (14.72)	68.2 (10.86)
Systolic BP (mmHg)	120.3 (20.81)	122.9 (22.44)	117.6 (18.74)
Diastolic BP (mmHg)	71.2 (13.21)	72.8 (14.96)	70.0 (10.99)
6 Minute walk test	292.9 (120.35)	283.7 (116.84)	302.2 (123.81)
LVEF (%)	26.1 (10.68)	26.5 (10.62)	25.6 (10.80)
LVEDD (mm)	57.8 (7.58)	58.0 (6.49)	57.6 (8.62)
LVESD (mm)	50.3 (8.50)	50.7 (7.58)	49.8 (9.42)
LVEDV (mls)	163.0 (66.60)	164.1 (60.07)	161.8 (73.58)
LVESV (mls)	115.4 (59.39)	119.4 (53.30)	111.0 (63.58)
25(OH) Vitamin D (nmol/L)	37.3 (22.56)	36.4 (20.24)	38.2 (24.81)
Parathyroid hormone (pmol/L)	11.4 (8.09)	11.7 (7.50)	11.0 (8.75)
Creatinine (µmol/L)	96 (29.3)	94.4 (29.42)	96.6 (29.26)

Continuous variables are mean (SD), categorical variables are n (%) as indicated.
BMI; body mass index, NYHA; New York Heart Association class, ACEi; angiotensin converting enzyme inhibitor, ARB; aldosterone receptor blocker, BP; blood pressure, LVEF; left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume. Conversion factors: vitamin D nmol/L * 0.4 = ng/mL; creatinine mmol/L * 0.11 = mg/dL; calcium mmol/L * 4 = mg/dL; parathyroid hormone pmol/L * 9.4 = pg/mL.

Table 2: Change in primary and secondary outcome variables in VINDICATE at 12 months post-randomization - intention-to-treat population

Endpoint	Randomised treatment	Mean change after 12 months	Ancova Difference in mean change	p-value
Primary outcome				
Six minute walk distance (m)	Placebo Vitamin D	10.10 [-20.77, 40.96] -12.56 [-40.80, 15.68]	-24.11 [-65.81, 17.60]	0.255
Secondary outcomes				
LVEF (%)	Placebo Vitamin D	1.36 [-0.38, 3.11] 7.65 [5.21, 10.09]	**6.07 [3.20, 8.94]**	**<0.001**
LVEDD (mm)	Placebo Vitamin D	-0.08 [-1.25, 1.10] -2.45 [-3.70, -1.21]	**-2.49 [-4.09, -0.90]**	**0.002**
LVESD (mm)	Placebo Vitamin D	-0.99 [-2.31, 0.33] -2.72 [-4.52, -0.92]	**-2.09 [-4.11, -0.06]**	**0.043**
LVEDV (mls)	Placebo Vitamin D	-3.83 [-13.36, 5.70] -16.47 [-25.71, -7.22]	**-13.11 [-25.63, -0.60]**	**0.040**
LVESV (mls)	Placebo Vitamin D	-8.49 [-17.98, 1.01] -18.77 [-25.96, -9.59]	**-12.65 [-24.76, -0.54]**	**0.041**

Values are mean change [95% confidence intervals]; 95% significance shown in bold
LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume.

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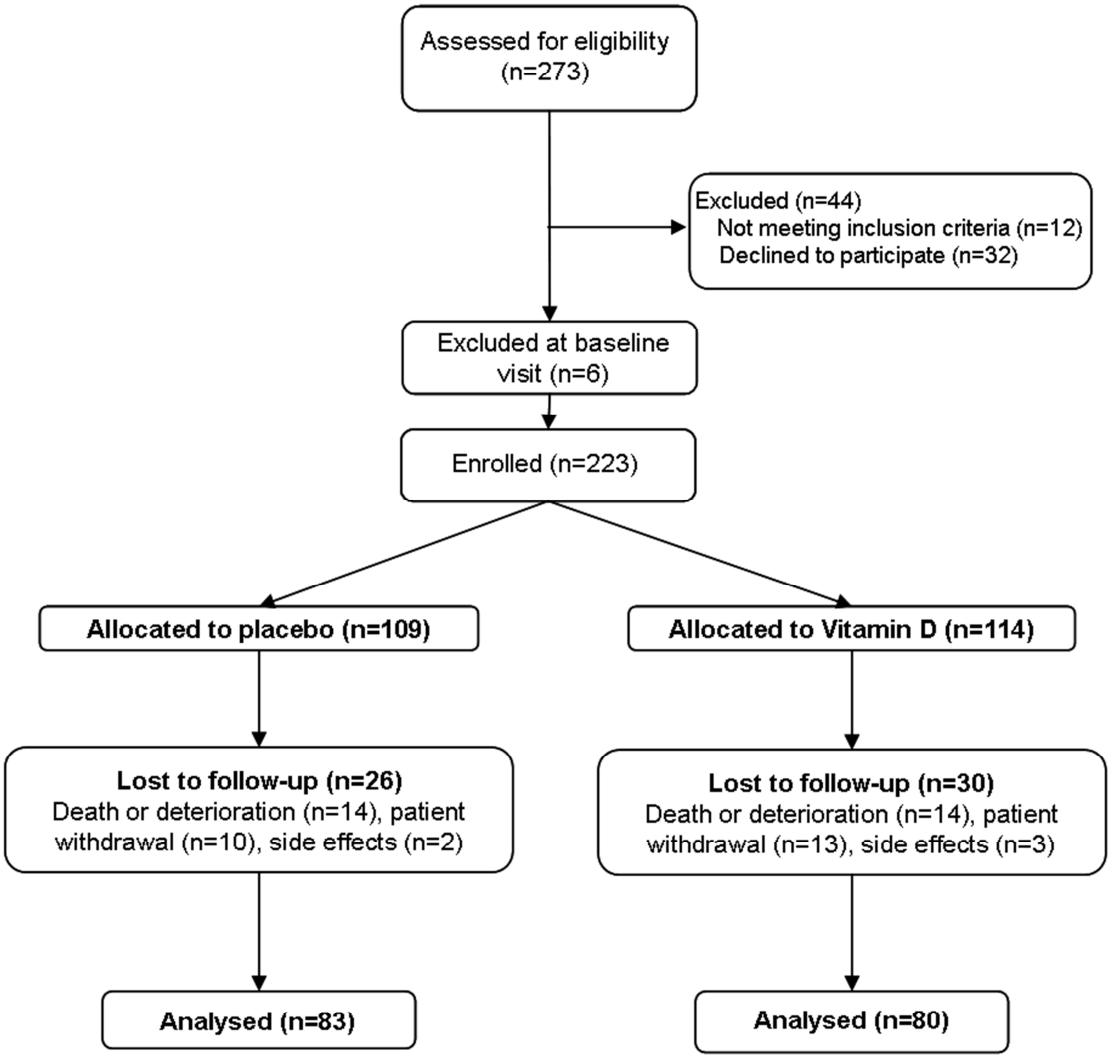

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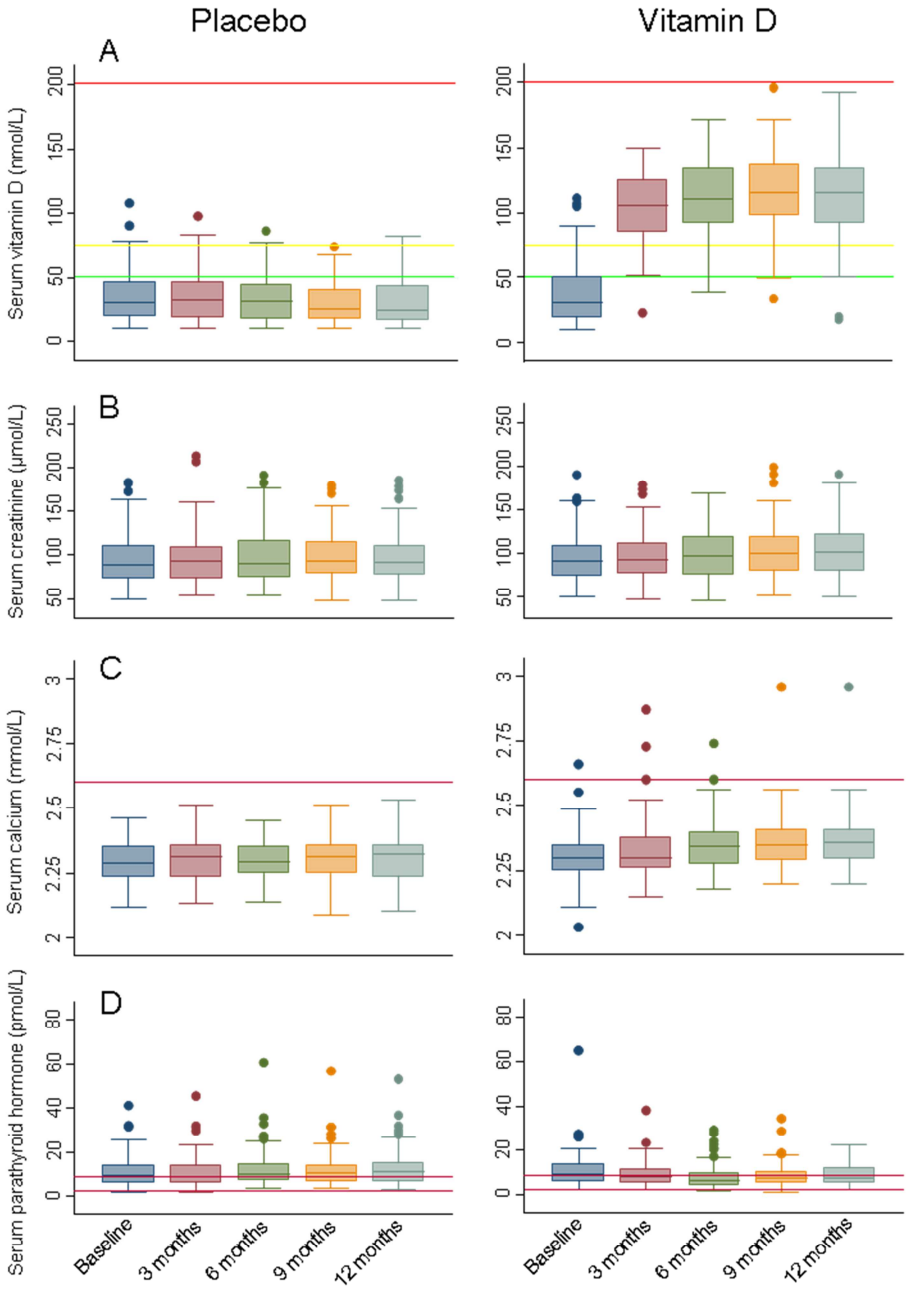
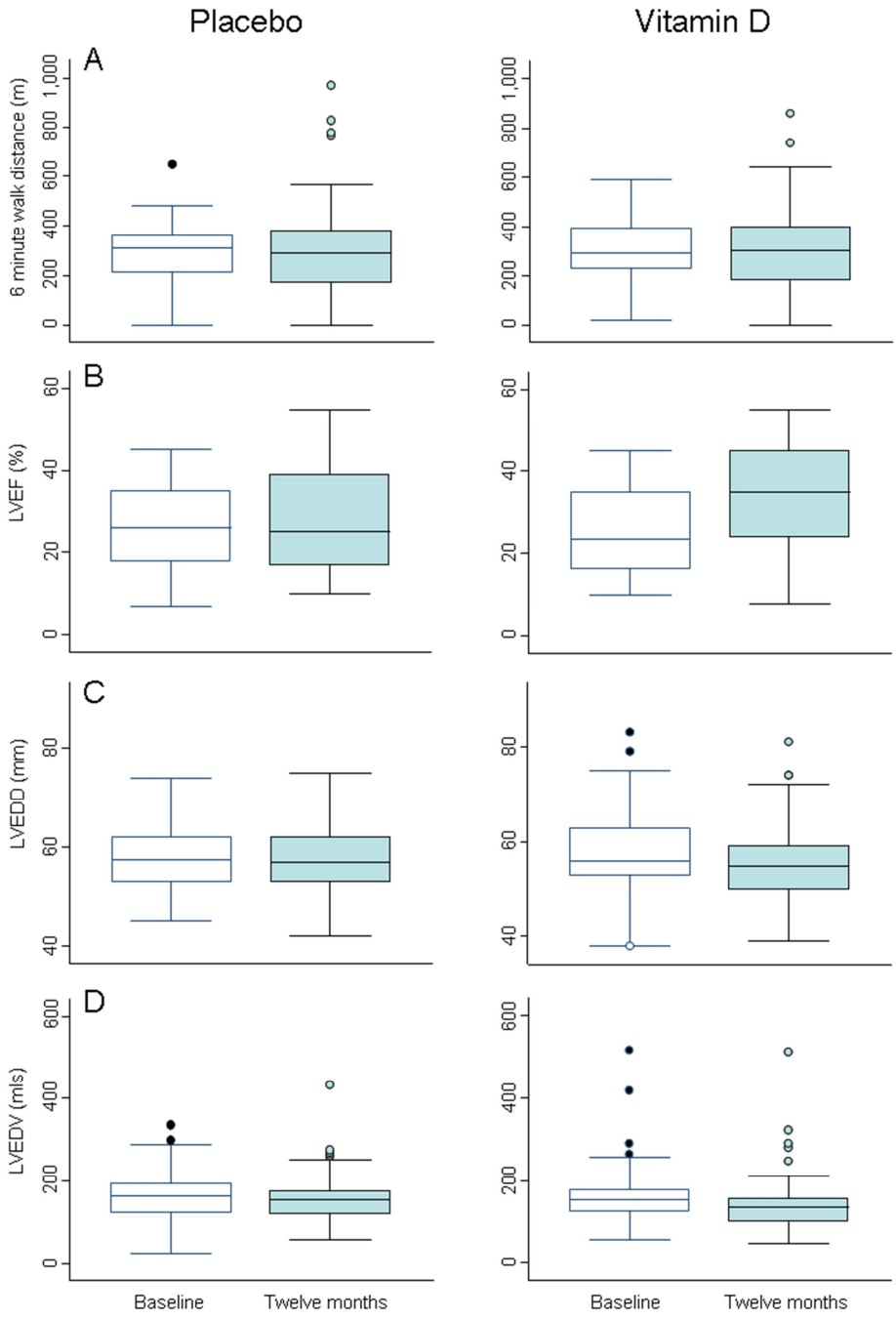

Placebo
Vitamin D
A
B
C
D
Serum vitamin D (nmol/L)
Serum creatinine (μmol/L)
Serum calcium (mmol/L)
Serum parathyroid hormone (pmol/L)
Baseline
3 months
6 months
9 months
12 months


Placebo
Vitamin D
A
6 minute walk distance (m)
0
200
400
600
800
1,000
B
LVEF (%)
0
20
40
60
C
LVEDD (mm)
40
60
80
D
LVEDV (mls)
0
200
400
600
Baseline
Twelve months
Baseline
Twelve months

Supplementary table 1: Patient demographics at randomisation in proof of concept study - intention-to-treat population

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	Placebo (n=26)	Vitamin D (n=27)	Total (n=53)
Male Sex (n)[%]	20 (76.9%)	23 (85.2%)	43 (81.1%)
Age	68.9 (10.50)	74.1 (7.98)	71.6 (9.59)
Aetiology (n)[%]			
Dilated cardiomyopathy	6 (23.1%)	8 (29.6%)	14 (26.4%)
Ischemic heart disease	19 (73.1%)	19 (70.4%)	38 (71.7%)
Valvular heart disease	1 (3.8%)	0 (0.0%)	1 (1.9%)
Diabetes mellitus (n)[%]	4 (15.4%)	5 (18.5%)	9 (17.0%)
NYHA (n)[%]			
II	21 (80.8%)	18 (65.7%)	39 (73.6%)
III	5 (19.2%)	9 (33.3%)	14 (26.4%)
Beta blockers (n)[%]	22 (84.6%)	27 (100.0%)	49 (92.5%)
ACEi/ARB (n)[%]	25 (96.2%)	24 (88.9%)	49 (92.5%)
Furosemide dose (mg/day)	53.1 (35.30)	48.9 (38.56)	50.9 (36.70)
Digoxin (n)[%]	7 (26.9%)	7 (25.9%)	14 (26.4%)
Spironolactone (n)[%]	11 (42.3%)	13 (48.1%)	24 (45.3%)
Atrial fibrillation (n)[%]	10 (38.5%)	13 (48.1%)	23 (43.4%)
Systolic BP (mmHg)	127.2 (22.03)	119.3 (19.93)	123.2 (21.16)
Diastolic BP (mmHg)	70.8 (10.80)	65.9 (14.76)	68.3 (13.09)
LVEF (%)	34.0 (7.36)	32.2 (6.95)	33.1 (7.14)
LVEDD (mm)	58 (11)	59 (8.6)	59 (9.8)
LVESD (mm)	48 (13.3)	49 (8.7)	49 (11.1)
LVEDV (mls)	156.0 (67.46)	166.3 (72.41)	161.3 (69.55)
LVESV (mls)	105.5 (53.05)	114.0 (57.39)	109.8 (54.94)
Vitamin D (nmol/L)	31.0 (17.37)	24.4 (9.63)	27.4 (13.99)
Parathyroid hormone (pmol/L)	9.6 (5.27)	10.6 (5.02)	10.1 (5.11)

Continuous variables are mean (SD), categorical variables are n (%) as indicated.
NYHA, New York Heart Association class; ACEi; angiotensin converting enzyme inhibitor, ARB; aldosterone receptor blocker, BP; blood pressure, LVEF; left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume.

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Supplementary table 2: Changes in echocardiographic variables with vitamin D supplementation adjusted for baseline for all patients in the intention-to-treat population of the proof of concept study

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Endpoint	Randomised treatment	Mean (adjusted for baseline) 95% CI	Mean difference in change (adjusted for baseline)	95% CI	p-value
LVEF (%)	Placebo	35.8 [33.16, 38.51]	2.7	[-1.01, 6.43]	0.1490
	Vitamin D	38.5 [35.98, 41.11]	.		.
LVEDD (mm)	Placebo	57 [55.4, 59.1]	-1.0	[-3.4, 1.7]	0.5212
	Vitamin D	56 [54.7, 58.2]	.		.
LVESD (mm)	Placebo	47 [44.6, 50.0]	**-4.0**	**[-7.6, -0.1]**	**0.0428**
	Vitamin D	43 [40.9, 46.0]	.		.
LVEDV (mls)	Placebo	177.0 [163.0, 191.1]	**-23.4**	**[-42.86, -3.94]**	**0.0196**
	Vitamin D	153.6 [140.2, 167.1]	.		.
LVESV (mls)	Placebo	116.1 [104.5, 127.6]	**-19.5**	**[-35.44, -3.49]**	**0.0181**
	Vitamin D	96.6 [85.56, 107.6]	.		.

LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume.

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Supplementary table 3: Baseline variables of patients undergoing serial CMR scans - intention-to-treat population

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	Placebo (n=17)	Vitamin D (n=17)	Total (n=34)
Male Sex (n)[%]	12 [71]	14 [82]	26 [76]
Age	60.6 (16.0)	62.3 (16.8)	61.4 (16.2)
Aetiology (n)[%]			
Ischemic heart disease	14 [41]	6 [35]	20 [59]
Non-ischaemic cardiomyopathy	3 [18]	11 [65]	13 [38]
Diabetes mellitus (n)[%]	4 [24]	1 [6]	5 [15]
BMI	27.6 (3.8)	26.8 (5.7)	27.2 (4.8)
NYHA (n)[%]			
II	17 [100]	17 [100]	34 [100]
Beta blockers (n)[%]	16 [94]	15 [88]	31 [91]
ACEi/ARB (n)[%]	17 [100]	17 [100]	34 [100]
Furosemide dose (mg/day)	33.5 (61.7)	34.1 (40.0)	33.8 (51.2)
Digoxin (n)[%]	0 [0]	2 [12]	2
Spironolactone (n)[%]	9 [53]	8 [47]	17 [50]
Atrial fibrillation (n)[%]	2 [12]	8 [47]	10 [29]
Heart rate (beats/min)	68.8 (11.1)	68.8 (9.6)	68.8 (10.2)
Systolic BP (mmHg)	119.5 (17.5)	107.0 (19.3)	113.3 (19.3)
Diastolic BP (mmHg)	70.7 (11.3)	67.8 (9.6)	69.3 (10.4)
CMR-LVEF (%)	33.6 (8.0)	38.2 (12.1)	36.0 (10.4)
CMR-LVEDV (mls)	214.2 (61.2)	211.8 (105.6)	213.0 (85.0)
CMR-LVESV (mls)	144.5 (55.0)	139.5 (106.1)	142.0 (83.1)
Creatinine (µmol/L)	88.1 (28.6)	83.3 (16.2)	85.7 (23.0)
Vitamin D (nmol/L)	43.0 (22.8)	44.6 (34.3)	43.9 (23.2)
Parathyroid hormone (pmol/L)	8.7 (4.3)	8.1 (3.9)	8.4 (4.0)

Continuous variables are mean (SD), categorical variables are n (%) as indicated.
NYHA, New York Heart Association class; ACEi; angiotensin converting enzyme inhibitor, ARB; aldosterone receptor blocker, BP; blood pressure, LVEF; left ventricular ejection fraction; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume.

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Supplementary Figure Legends

Supplementary figure 1: Consort diagram for the randomised, placebo-controlled, proof of concept study of vitamin D_3 supplementation.

Supplementary figure 2: Biochemical changes during the randomised, placebo-controlled, proof of concept study of vitamin D_3 supplementation by allocated group. Vitamin D concentrations are described in relation to deficiency (green line), sufficiency (yellow) and the accepted upper limit for hypervitaminosis D (red line). Serum calcium levels described in relation to upper limit of normal range (red line), and serum PTH concentrations in relation to the normal range (between red lines).

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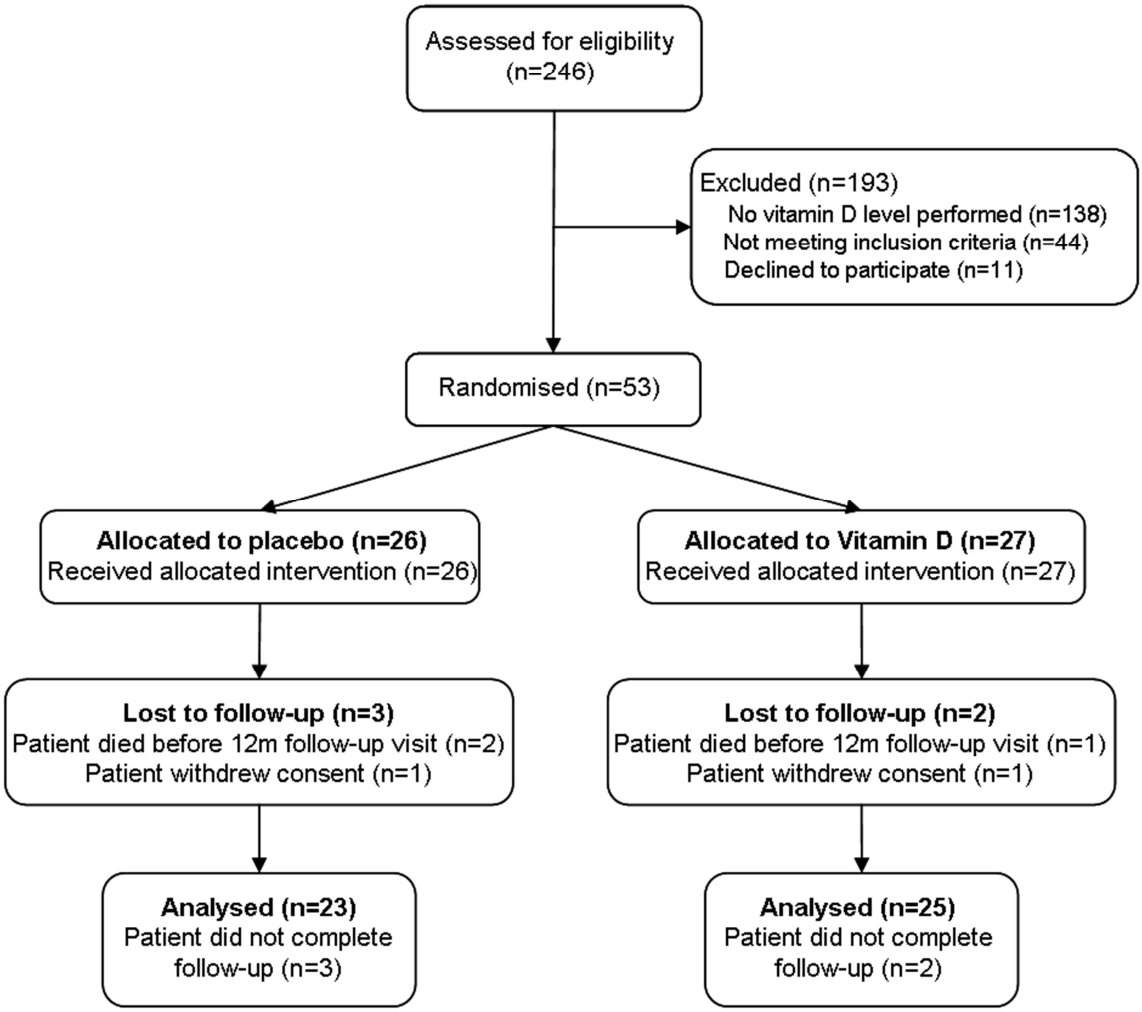

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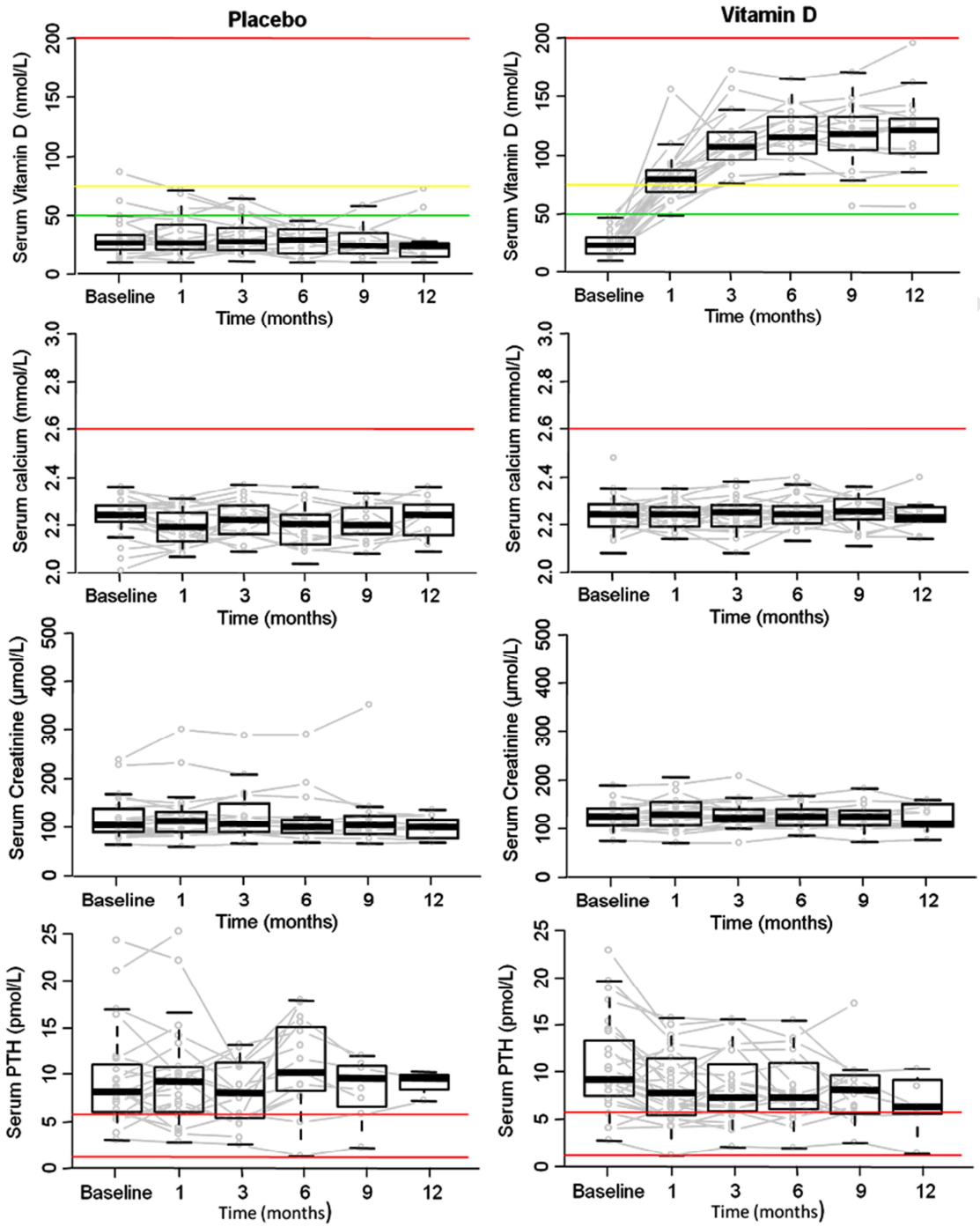
Placebo
Vitamin D
Serum Vitamin D (nmol/L)
Serum calcium (mmol/L)
Serum calcium mnmol/L)
Serum Creatinine (µmol/L)
Serum PTH (pmol/L)
Baseline
1
3
6
9
12
Time (months)