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ORIGINAL CONTRIBUTION

Vitamin D supplementation does not prevent the testosterone decline in males with advanced heart failure: the EVITA trial

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Abstract

Purpose Observational studies indicate a positive association between circulating 25-hydroxyvitamin D (25OHD) and testosterone (T) concentrations. Because low 25OHD concentrations and T deficiency are considered to be a generalized phenomenon in patients with advanced heart failure (HF), we aimed to investigate whether vitamin D supplementation has beneficial effects on T indices in these patients.

Methods In a pre-specified secondary analysis of the EVITA (effect of vitamin D on mortality in heart failure) randomized controlled trial, we analyzed in male subjects with 25OHD concentrations < 75 nmol/L the effect of a daily vitamin D_3 supplement of 4000 IU for 3 years ($n = 71$) vs. placebo ($n = 62$) on total T (TT), sex hormone-binding globulin (SHBG), free T (fT), and bioactive T (BAT). We assessed changes from baseline until study termination and between-group differences at study termination.

Results 25OHD increased in the placebo group from 36.6 nmol/L by 9.2 nmol/L (95% CI 3.2–15.1 nmol/L; $P = 0.003$) and in the vitamin D group from 36.5 nmol/L by 63.9 nmol/L (95% CI 52.6–75.3 nmol/L; $P < 0.001$), with a significant between-group difference at study termination ($P < 0.001$). TT and SHBG concentrations did not change significantly, neither in the placebo group nor in the vitamin D group ($P = 0.845$–0.082), but concentrations of fT and BAT declined significantly in both groups ($P = 0.025$–0.008). At study termination, there were no between-group differences in TT ($P = 0.612$), SHBG ($P = 0.393$), fT ($P = 0.861$), or BAT ($P = 0.960$).

Conclusions In male patients with advanced HF and low 25OHD concentrations, a daily vitamin D_3 supplement of 4000 IU for 3 years did not prevent the decline in testosterone indices.

Keywords Vitamin D · Testosterone · Free testosterone · Bioactive testosterone · Sex hormone · Heart failure · Randomized controlled trial

Introduction

Testosterone (T) is the principal male sex hormone. As men age, T concentrations typically fall [1]. T deficiency is considered to be a generalized phenomenon in patients

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with advanced heart failure (HF) that may also be involved in the pathophysiology of the disease [2]. Briefly, typical symptoms of advanced HF such as reduced muscle mass, abnormal energy handling, fatigue, dyspnea, and cachexia are attributed, at least in part, to T deficiency [2].

Observational studies indicate a positive relation between circulating 25-hydroxyvitamin D (25OHD) concentrations, the generally accepted indicator of vitamin D status, and T concentrations [3–7]. In line with these findings, a prospective, non-randomized, non-controlled trial in middle-aged men showed that vitamin D_2 bolus administration was associated with an improvement in T concentrations [8]. Moreover, in a post hoc analysis of a randomized controlled trial (RCT) in overweight individuals [9], male subjects assigned to daily vitamin D_3 supplementation with 3332 IU showed a significant increase in concentrations of total T (TT), free T (fT) and bioactive T (BAT), whereas the aforementioned parameters did not change significantly in male patients assigned to placebo. However, no beneficial vitamin D effect on T indices was reported by two RCTs in healthy men [10, 11].

Low vitamin D status is a frequent finding in patients with HF [12]. The vast majority of patients with HF have 25OHD concentrations < 75 nmol/L [13–16] and the prevalence of deficient concentrations (i.e. < 30 nmol/L) varies between 28 and 66.7% [15, 16].

We, therefore, aimed to investigate in a pre-specified secondary analysis of the EVITA (Effect of vitamin D on mortality in heart failure) trial whether a daily vitamin D_3 supplement of 4000 IU for 3 years is able to improve male sex hormone concentrations in patients with advanced HF and 25OHD concentrations < 75 nmol/L.

Methods

Study design and participants

The present investigation is a pre-specified secondary analysis of the EVITA trial. EVITA is a single-center, randomized, placebo-controlled, clinical trial performed at the Clinic for Thoracic and Cardiovascular Surgery of the Heart and Diabetes Center North Rhine Westphalia, Bad Oeynhausen, Germany. Main study results have already been published elsewhere [17, 18]. Briefly, between November 2010 and July 2013, 400 patients with HF (332 men and 68 women) were recruited. All patients were ambulatory and regularly seen at our outpatient clinic. Eligible study participants were adults aged ≥ 18 to 79 years with congestive HF, New York Heart Association functional class ≥ II, and circulating 25OHD concentrations < 75 nmol/L. Participants were randomly allocated to receive 4000 IU (100 µg) cholecalciferol per day as oily drops (Vigantol® Oel, provider: Merck KGaA, Darmstadt, Germany) or a matching placebo (Miglyol Oel, provider: Merck KGaA, Darmstadt, Germany) for 3 years. The daily vitamin D dose was identical with the upper tolerable intake level of the Institute of Medicine [19] and the European Food Safety Authority [20]. During the study, participants remained on guideline-recommended medications. Patient adherence was assessed by measuring in-study concentrations of circulating 25OHD and comparing results with expected values as calculated by a formula, based on vitamin D dose, body weight, age, and initial 25OHD concentration [21]. For the present analysis, only male patients were considered. Of the 332 male participants, 158 completed the study (Fig. 1), while 174 patients died, dropped out, or were lost to follow-up. Additional 25 patients had to be excluded because no sex hormone measurements were possible, due to insufficient sample volume. Finally, data on relevant parameters for this secondary analysis were available in 133 patients (vitamin D group, $n = 71$; placebo group, $n = 62$). 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 randomization. The study protocol was approved by the ethics committee of the Medical Council Westphalia-Lippe, Germany (No. 2010-052-fA).

Data assessment

The electronic records of the patients were used to assess baseline characteristics, such as anthropometric data, clinical parameters, and medication use. Fasting venous blood samples were collected on study visits between 8 and 11 a.m. under standardized conditions. Blood samples were either measured directly within 4 h of blood collection or stored at − 80 °C until analysis. Circulating total 25OHD, total $1,25(OH)_2D$, and intact parathyroid hormone (iPTH) concentrations were measured by the autoanalyzer Liaison (DiaSorin, Stillwater, MN, USA). The 25OHD test has equimolar cross-reactivity with $25OHD_2$ (104.5%) and $25OHD_3$ (100.7%). The measuring range for 25OHD lies between 10 and 375 nmol/L. Values < 10 nmol/L were considered 9.9 nmol/L. The $1,25(OH)_2D$ test has equimolar cross-reactivity with $1,25(OH)_2D_2$ (104.0%) and $1,25(OH)_2D_3$ (100.0%). The limit of $1,25(OH)_2D$ quantitation is 12 pmol/L and we considered values below this limit as 11 pmol/L. Albumin, brain natriuretic peptide (BNP), calcium, creatinine, TT, and sex hormone-binding globulin (SHBG) values were analyzed by the Architect Autoanalyzer (Abbott, Wiesbaden, Germany). Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula [22]. Measurements of calcium and 25OHD were performed on the day of blood sampling. Inter- and intra-assay coefficients of variation were 6.8 and 7.0%, respectively. Determinations of the calciotropic

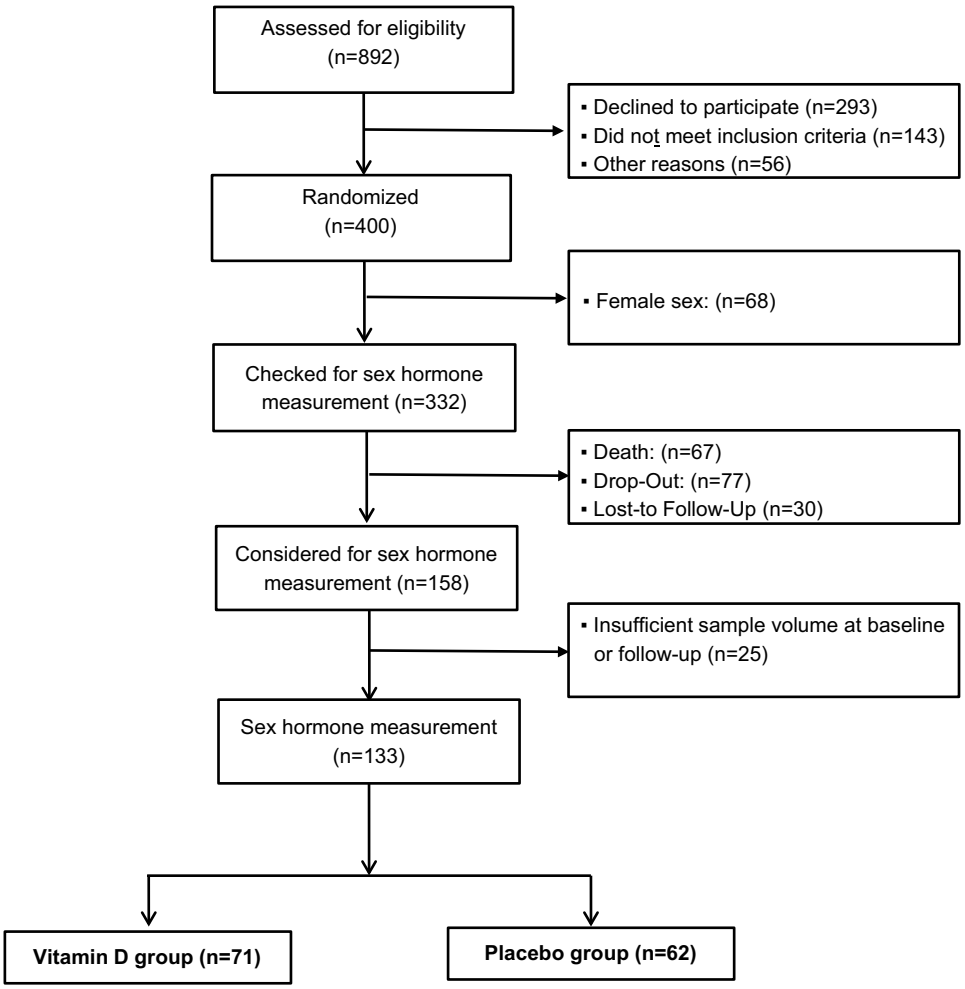

Fig. 1 Flow chart of study participants

hormones iPTH and 1,25[OH]$_2$D, and of TT and SHBG were performed in batch analyses.

According to earlier classifications [9], we considered the reference range for TT as 9.09–55.28 nmol/L for males aged 20–49 years and 6.28–26.30 nmol/L for males aged ≥ 50 years, and the SHBG reference range for males as 13–71 nmol/L. BAT (reference range 2.14–13.60 nmol/L) and fT (reference range 0.090–0.580 nmol/L) were calculated from TT and SHBG concentrations according to Vermeulen et al. [23]. For classifying 25OHD, we used the following cut-off values [19, 24, 25]: < 30 nmol/L as deficient, 30–49.9 nmol/L as insufficient, and 50–74.99 nmol/L as borderline.

Outcome measures

In the present analysis of the EVITA trial, we assessed changes from baseline until study termination in TT, fT, and BAT concentrations. Moreover, we assessed between-group differences of the aforementioned indices at study termination, with adjustment for baseline values.

Statistics

Categorical data are presented as numbers and percentages of observations. Continuous data are shown as mean and standard deviation. Fisher's exact test, the unpaired *t* test, and the Mann–Whitney *U* test were used for group comparisons at baseline, when appropriate. Change from baseline data is shown as mean and 95% confidence interval (CI). The paired *t* test and the Wilcoxon test were used to test for differences within groups between the baseline and 3-year follow-up visit, when appropriate. ANCOVA with adjustments for baseline values was used to test for differences in calcium, calciotropic hormones, and sex hormone parameters between the patients assigned to vitamin D or placebo at the 3-year follow-up visit. We also adjusted for those anthropometric/clinical/biochemical parameters that at least tended to differ ($P < 0.1$) between study groups at baseline. Skewed variables were normalized by log(e) transformation before use in ANCOVA, but all results are shown in the original units. We used Spearman's rank correlation coefficient (r_s) to assess the interrelationship

between biochemical variables. P values < 0.05 were considered statistically significant.

Given a total number of 133 patients in this two-treatment parallel design study, there is a 90% probability that the study will detect a treatment difference in fT at a two-sided 0.05 significance level if the true difference between treatments is 0.051 nmol/L. This is based on the assumption that the standard deviation of fT is 0.090 nmol/L [9]. The calculated treatment difference of 0.051 nmol/L would be similar to the vitamin D effect in the aforementioned study in overweight men [9], indicating an increase in fT of 0.045 nmol/L by a daily vitamin D dose of 3332 IU. We performed all analyses using IBM SPSS Statistics, version 23 (IBM Corporation, Armonk, NY, USA).

Results

Baseline data

Baseline characteristics of the study participants are presented in Table 1 by study group. The presence of advanced HF in both study groups is confirmed by their low values of left ventricular ejection fraction, and their high values of left ventricular end-diastolic diameter and BNP. In the vitamin D and placebo groups, 42.3 and 40.3% of patients, respectively, had initial circulating 25OHD concentrations < 30 nmol/L. Body mass index and medication use were similar in the two study groups. However, compared with patients assigned to placebo, age was significantly higher in patients assigned to vitamin D. Other parameters did not differ significantly between groups.

Vitamin D effects on calciotropic hormones and sex hormones

Plasma calcium concentrations, calciotropic hormones, and sex hormone indices are presented in Table 2. In detail, mean 25OHD increased slightly by 9.2 nmol/L in the placebo group ($P = 0.003$) and markedly by 63.9 nmol/L in the vitamin D group ($P < 0.001$). Likewise, in the vitamin D group plasma calcium and $1,25(OH)_2D$ increased significantly ($P < 0.001$ and $P = 0.004$, respectively) and iPTH decreased significantly ($P = 0.005$), whereas these parameters remained constant in the placebo group ($P = 0.574$, $P = 0.397$, and $P = 0.638$, respectively). Overall, the adjusted between-group differences at study termination were significant for plasma calcium ($P = 0.003$), 25OHD ($P < 0.001$), and $1,25(OH)_2D$ ($P = 0.003$), but not for iPTH ($P = 0.182$). The mean vitamin D-induced increase in circulating 25OHD of 54.8 nmol/L was in line with the expected increase of 57.6 nmol/L, as calculated by a recently provided formula [21].

Initially, mean concentrations of TT, SHBG, fT, and BAT were in the vitamin D and placebo groups in their respective reference range. In the vitamin D and placebo groups, 19.7

Table 1 Baseline characteristics of the study groups

Parameter	Placebo group ($n = 62$)	Vitamin D group ($n = 71$)	P value
Age (years)	51.1 ± 10.5	55.0 ± 9.9	0.026
Body mass index (kg/m^2)	29.5 ± 5.3	29.2 ± 4.6	0.788
Left ventricular ejection fraction (n, %)	29.9 ± 10.5	29.9 ± 8.8	0.957
Left ventricular end-diastolic diameter (mm)	68.3 ± 13.9	67.4 ± 9.2	0.670
Diabetes mellitus (n, %)	21.9 (44)	20.1 (40)	0.099
Estimated GFR (ml/min/1.73 m^2)	77.8 ± 25.0	70.4 ± 22.3	0.075
Medications			
Aldosterone-antagonists (n, %)	53 (86)	57 (80)	0.495
Loop diuretics (n, %)	52 (84)	57 (80)	0.656
Thiazide diuretics (n, %)	21 (34)	20 (28)	0.573
Beta-blockers (n ,%)	61 (98)	69 (97)	> 0.999
ACE-inhibitors/ARB-blockers (n, %)	62 (100)	68 (96)	0.248
Digoxin (n, %)	26 (42)	22 (31)	0.209
Biochemical parameters			
Calcium (mmol/L)	2.38 ± 0.11	2.39 ± 0.13	0.885
25-Hydroxyvitamin D (nmol/L)	36.6 ± 17.6	36.5 ± 17.9	0.976
Albumin (mg/dL)	3824 ± 421	3810 ± 434	0.855
BNP (pg/mL)	511 ± 550	467 ± 940	0.801

GFR glomerular filtration rate, *ACE* angiotensin converting enzyme, *ARB* angiotensin II receptor blocker, *BNP* brain natriuretic peptide

Table 2 Results of vitamin D treatment on calcium concentrations, calciotropic hormones, and sex hormone indices in male patients with advanced heart failure

Characteristics	Vitamin D group ($n=71$)			Placebo group ($n=62$)			Treatment effect	P value[b]
	Baseline	Follow-up (36 months)	Mean change from baseline[a]	Baseline	Follow-up (36 months)	Mean change from baseline[a]	Between-group differences	
Calcium (mmol/L)	2.39 (2.35 to 2.41)	2.47 (2.44 to 2.49)	0.08 (0.04 to 0.08)***	2.39 (2.36 to 2.41)	2.40 (2.36 to 2.43)	0.01 (−0.03 to 0.05)	0.07 (0.01 to 0.01)	0.003
25OHD (nmol/L)	36.5 (32.5 to 41.4)	99.8 (87.0 to 112.5)	63.9 (52.6 to 75.3)***	36.6 (32.2 to 41.9)	54.3 (39.6 to 51.0)	9.2 (3.2 to 15.1)**	54.8 (41.6 to 68.0)	<0.001
$1,25(OH)_2D$ (pmol/L)	82.8 (75.3 to 90.3)	95.7 (85.6 to 105.7)	13.0 (4.2 to 21.8)**	86.2 (78.2 94.1)	82.8 (73.0 to 92.6)	3.5 (−11.6 to 13.0)	16.5 (4.5 to 28.4)	0.003
iPTH (pg/mL)	132 (104 to 159)	93 (71 115)	−37.2 (11.7 to −62.6)**	101 (76 to 126)	95 (72 to 118)	−5.2 (−27.7 to 18.1)	−32 (−66 to 2)	0.182
TT (nmol/L)	11.2 (9.9 to12.5)	10.0 (8.8 to 11.3)	0.29 (−2.65 to 3.22)	12.4 (10.6 to 14.1)	11.1 (9.3 to 12.8)	−1.37 (−2.94 to 0.19)	0.48 (−0.51 to 1.47)	0.612
SHBG (nmol/L)	37.6 (32.3 to 43.0)	49.0 (35.9 62.0)	11.4 (−1.5 to 24.2)	35.5 (28.9 to 42.1)	38.4 (31.8 to 45.0)	3.0 (−0.6 to 6.5)	8.4 (−5.5 to 22.4)	0.393
FT(nmol/L)	0.217 (0.190 to 0.244)	0.186 (0.165 to 0.207)	−0.032 (−0.056 to −0.007)*	0.246 (0.216 to 0.275)	0.211 (0.180 to 0.243)	−0.038 (−0.066 to −0.011)**	0.019 (−0.008 to 0.013)	0.861
BAT (nmol/L)	5.08 (4.45 to 5.71)	4.39 (3.82 to 4.95)	−0.70 (−1.32 to −0.09)*	5.75 (5.06 to 6.44)	4.94 (4.17 to 5.70)	−0.90 (−1.56 to −0.025)**	0.06 (−0.20 to 0.31)	0.960

25OHD 25-hydroxyvitamin D, *1,25(OH)$_2$D* 1,25-dihydroxyvitamin D, *iPTH* intact parathyroid hormone, *TT* total testosterone, *SHBG* sex hormone-binding globulin, *FT* free testosterone, *BAT* bioactive testosterone

* $P<0.05$ vs. baseline; ** $P<0.01$ vs. baseline; *** $P<0.001$ vs. baseline

[a]Change from baseline data is shown as means and 95% confidence interval

[b]Between-group differences at study termination, with adjustments for baseline values (ANCOVA)

and 16.1%, respectively, had baseline TT values below the age-specific reference range. TT declined non-significantly between baseline and study termination in the placebo group ($P=0.084$) and remained constant in the vitamin D group ($P=0.845$). SHBG concentrations increased non-significantly in both groups ($P=0.098$ and $P=0.082$). There was a significant decline in fT concentrations between baseline and study termination in the placebo group ($P=0.008$) and in the vitamin D group ($P=0.012$). Likewise, BAT concentrations declined significantly between baseline and study termination in the placebo group ($P=0.008$) and in the vitamin D group ($P=0.025$). At study termination, there were no between-group differences in adjusted sex hormone indices (TT: $P=0.612$; SHBG: $P=0.393$; fT: $P=0.861$; BAT: $P=0.960$).

Subgroup analyses

Supplemental Table 1 shows the effect of vitamin D supplementation on sex hormone indices in patients with initial 25OHD concentrations < 30 nmol/L by study group. Even in patients with very low vitamin D status at baseline, vitamin D supplementation did not influence male sex hormone indices. Moreover, no significant vitamin D effect was observed in the small group of patients with initial testosterone concentrations below the age-dependent reference range (Supplemental Table 2).

Correlations

In correlation analyses, we included all samples where measurements of calciotropic hormones and sex hormone indices were available at baseline and study termination ($n=266$) (Table 3). Briefly, there were weak inverse relationships of 25OHD with fT and BAT, and of $1,25(OH)_2D$ with SHBG, whereas 25OHD was positively associated with SHBG. iPTH was not correlated with any sex hormone parameter. Notably, of the parameters included in the correlation analysis, age showed the strongest (inverse) association with fT and BAT. Body mass index was not significantly related to fT or BAT.

Discussion

The present investigation indicates a significant decline in male sex hormone indices in patients with advanced HF over a period of 3 years. This decline is not influenced by a vitamin D_3 supplement of 4000 IU daily, neither in the entire study group nor in the subgroup of patients with circulating 25OHD concentrations < 30 nmol/L.

Several observational studies indicate a positive association of circulating 25OHD concentrations with male sex

Table 3 Spearman's rank correlation coefficient between male sex hormone indices and calciotropic hormones in plasma samples of the EVITA trial ($n = 266$)

	Total testosterone	Sex hormone-binding globulin	Free testosterone	Bioactive testosterone
Age	−0.180*	0.204**	−0.326**	−0.327**
BMI	−0.248**	−0.271**	−0.138	−0.138
Calcium	−0.036	−0.038	−0.010	−0.017
25-Hydroxyvitamin D	−0.103	0.169**	−0.171**	−0.175**
Intact parathyroid hormone	0.061	0.121	−0.031	−0.034
1,25-Dihydroxyvitamin D	0.003	−0.139*	0.077	0.088

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

hormone concentrations [3–7]. However, observational studies are subject to reverse causation bias and unexplained confounding. Therefore, RCTs are considered to be the most appropriate way to demonstrate the role of vitamin D in health [26]. Data from RCTs regarding the effect of vitamin D supplementation on male sex hormone concentrations are scarce [9–11, 27] and mainly focus on apparently healthy men [10, 11, 27]. To the best of our knowledge, this is the first RCT investigating the vitamin D effect on male sex hormone indices in patients with advanced HF. Study participants were on average in their sixth decade of life and several male sex hormone indices such as TT, fT, and BAT were initially on average within the respective reference range. It is unclear at present whether the decline in these indices during the study period was a consequence of the disease or simply of advancing age, but age showed an inverse association with fT and BAT. Independent of the cause, our data indicate that this decline in male sex hormone indices cannot be prevented by vitamin D supplementation. There was also no treatment effect on testosterone status in vitamin D-deficient study participants, as indicated by the subgroup analysis in patients with initial circulating 25OHD concentrations < 30 nmol/L. This null finding is further underlined by no vitamin D effect on testosterone status in subgroup analysis of participants with low-baseline testosterone concentrations. The finding that circulating 25OHD was only weakly and inversely correlated with some sex hormone indices is in line with our null finding on vitamin D treatment effects. Moreover, iPTH was not correlated with any sex hormone parameter. The increase in plasma calcium and $1,25(OH)_2D$ indicates inadequate initial vitamin D status in our study cohort. The fact that compared with placebo vitamin D supplementation did not significantly decrease iPTH concentrations indicates that inadequate substrate availability rather than impaired hormonal regulation was the cause of low circulating $1,25(OH)_2D$ concentrations. Notably, in the entire study cohort of the EVITA trial [18] six cases of hypercalcemia have been reported in the vitamin D group and three in the placebo group ($P = 0.192$).

Our data do not support the results of two aforementioned earlier studies [8, 9], which indicated positive effects of vitamin D supplementation on male sex hormone indices: One of these studies [8] was not a placebo-controlled trial and results may thus have been biased by confounding. In the other study including obese individuals [9], all participants were also on a weight reduction program. Since weight loss seems to reverse obesity-related male hypogonadism [28], the increase in TT, fT, and BAT in that study in the patients assigned to vitamin D, which was not seen in the patients assigned to placebo, may have been influenced by the weight loss. However, results of the present investigation are in line with two other publications [10, 11]: Jorde et al. [10] combined three RCTs of patients with mean initial 25OHD concentrations of 48 nmol/L, who were supplemented with 20,000–40,000 IU vitamin D per week vs. placebo for 6–12 months. Their analysis showed no significant vitamin D effect on serum TT or fT concentrations. Results remained unchanged in sub-analyses of subjects with low-circulating 25OHD or TT concentrations. In a post hoc analysis by Heijboer et al. [11] of three small clinical trials of limited duration (6–16 weeks) in men with normal baseline TT concentrations, vitamin D supplementation (daily doses of 600, 1200 or 2000 IU) was not associated with an increase in circulating TT concentrations. A very recent RCT in 100 healthy eugonadal men with 25OHD below 75 nmol/L also failed to show an effect of vitamin D supplementation (20,000 IU weekly for 12 weeks) on T status [27]. Collectively, data indicate that there is currently no convincing evidence for an improvement in male sex hormone concentrations by vitamin D supplementation.

Our investigation has several strengths, but also some limitations. Strengths include the study design of an RCT, the high cumulative vitamin D_3 dose of 4000 IU daily for 3 years, the homogenous group of patients, and the fact that sex hormone indices declined over time, predestining the patients for testing a potential vitamin D effect on these parameters. One limitation is the relatively small number of patients in this secondary analysis of the EVITA trial.

Therefore, we cannot definitively preclude the possibility of a statistical type II error. Moreover, the present analysis was performed only in those patients who terminated the study as planned. A higher prevalence of TT deficiency in those patients who were not included in our data analysis cannot be ruled out. However, since vitamin D supplementation did not prevent the decline in fT and BAT, and others have already shown that vitamin D supplementation had no significant effect on TT and fT concentrations in patients with low TT concentrations [10], it is rather unlikely that the results would have changed substantially if all study participants had been included in the data analysis. It is also noteworthy that the restriction of a statistical analysis to the per-protocol group usually overestimates rather than underestimates effect sizes [29]. Another drawback of our investigation is that we did not use a "gold standard" mass spectrometry method for T measurements, but we do not consider this as a serious limitation in view of our clear negative result. Mass spectrometry is also the 'gold standard' for measuring vitamin D metabolites. Therefore, the methods we used for measuring vitamin D metabolites are no longer optimal, but as this study aimed to evaluate long-term consequences of placebo vs. vitamin D this handicap does not jeopardize our conclusion. Our study is, however, limited by the lack of measurements of luteinizing hormone and follicle-stimulating hormone.

In conclusion, a daily vitamin D_3 supplement of 4000 IU for 3 years did not prevent the decline in fT and BAT concentrations in male patients with advanced HF, even in those with baseline vitamin D deficiency or inadequate TT concentrations.

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Compliance with ethical standards

Conflict 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, Ioanna Gouni-Berthold, Jan F. Gummert, Jochen Börgermann, and Stefan Pilz declare that they have no conflict of interest.

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