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ORIGINAL ARTICLE

Effect of vitamin D supplementation on circulating fibroblast growth factor-23 concentration in adults with prediabetes

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Abstract

Background Recent meta-analyses report that vitamin D supplementation increases blood fibroblast growth factor-23 (FGF23) level.

Objectives To determine the effect of 4000 IU/day of vitamin D_3 for 12 months on circulating FGF23 levels. We also examined the association of the achieved 25-hydroxyvitamin D level [25(OH)D] with the FGF23 level at 12 months and with 12-month changes in FGF23.

Methods An ancillary analysis among adults 70 years and older with prediabetes who participated in a trial comparing vitamin D_3 4000 IU/day with placebo. Plasma intact FGF23 and serum 25(OH)D were measured at baseline and month 12 (M12).

Results Characteristics of the 52 participants (vitamin D_3 $n=28$; placebo $n=24$) did not differ significantly aside from more women than men in the vitamin D_3 group. Mean ± SD age was 73.8 ± 3.7 years, BMI 31.3 ± 4.2 kg/m2, and glomerular filtration rate (GFR) 76.3 ± 11.8 mL/min/1.73m^2 Baseline serum 25(OH)D level was 33.4 ± 10.8 ng/mL and increased at M12 to 54.9 ± 14.8 ng/mL in the vitamin D_3 group versus 33.4 ± 14.9 in the placebo ($p<0.001$). At baseline, GFR was inversely associated with FGF23 ($r=-0.349$, $p=0.011$). Change in FGF23 level at M12 did not differ significantly between vitamin D_3 and placebo. In all participants combined, the achieved serum 25(OH)D level at M12 was not significantly associated with the M12 plasma FGF23 or the M12 change in FGF23.

Conclusion In obese older adults with sufficient vitamin D status and normal renal function, vitamin D_3 4000 IU/day for 12 months did not significantly alter plasma intact FGF23 levels. Clinicaltrials.gov NCT 01,942,694, registered 9/16/2013.

Keywords FGF23 · Vitamin D · 25-hydroxyvitamin D · Prediabetes

Introduction

Recent large clinical trials in older adults have tested doses of vitamin D that are considerably higher than the 800 IU/day intake recommended by the National Academy of Medicine (NAM) to enhance musculoskeletal health and other age-related conditions [1]. Some of these trials in older adults have raised concerns that high doses of vitamin D may increase the risk of falls and fractures [2–5]. One of the proposed mechanisms by which high dose vitamin D may negatively impact musculoskeletal health involves an increase in fibroblast growth factor-23 (FGF23), a phosphaturic factor chiefly produced in osteoblasts and osteocytes [6–8].

FGF23 promotes renal phosphate wasting by decreasing expression of renal tubular sodium phosphate (NaPi)-2a and 2c transporters which reabsorb phosphate, and decreasing 1α-hydroxylase activity which in turn results in reduced blood 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] concentration [9]. Several studies in mice have also reported a negative feedback loop wherein an increase in 1,25$(OH)_2$D can stimulate expression of FGF23 [6, 8, 10]. The clinical concern with increasing this phosphaturic factor is suggested by congenital and acquired diseases of increased FGF23 production that cause muscle weakness and osteomalacia [11]. Observational studies of aging adults without frank hypophosphatemia or renal dysfunction have reported associations

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between higher blood levels of FGF23 (measured by different assays) and poor physical performance, falls, frailty and increased mortality [12–14]. In patients with type 2 diabetes, higher circulating FGF23 levels (some measured by the intact and others by C-terminal assays) have been associated with an increased risk of cardiovascular morbidity and mortality even among those with normal kidney function [15].

Notably two [16, 17] out of three [16–18] recent meta-analyses report that vitamin D supplementation leads to a significant increase in the circulating level of FGF23 using a mix of assays. A subgroup analysis in one of the meta-analyses suggested that a dose of ≥ 3000 IU/day could result in a more substantial increase in circulating FGF23 level compared to doses of 3000 IU or less [17, 19]. The aim of this study was to determine the effect of daily oral supplementation with 4000 IU of vitamin D_3 for 12 months vs. placebo on circulating intact FGF23 levels in adults aged 70 years and older with prediabetes. An additional aim was to examine the association of serum 25-hydroxyvitamin D level [25(OH)D] achieved at 12 months with FGF23 level at 12 months and with the 12-month change in FGF23 level.

Methods

Study participants/cohort

The Vitamin D and Type 2 Diabetes (D2d) study was a multicenter randomized controlled clinical trial comparing the effects of 4000 IU/day of vitamin D_3 vs. placebo on the development of type 2 diabetes in US adults with prediabetes (clinicaltrials.gov NCT 01,942,694, registered 9/16/2013) [20]. The eligibility criteria, design, and methods of the D2d study have been described in detail elsewhere [20, 21]. Briefly, inclusion criteria were age ≥ 30 years (> 25 years for American Indians, Alaska Natives, Native Hawaiians, or other Pacific Islanders) and body mass index (BMI) of 24–42 kg/m2 (22.5–42 kg/m2 for Asians). A low serum 25(OH)D level was not an eligibility criterion. The primary exclusion criteria were any glycemic criterion for diabetes; use of diabetes or weight-loss medications; history of hyperparathyroidism, nephrolithiasis, hypercalcemia, or bariatric surgery; or an estimated GFR [22] of < 50 ml/min per 1.73 m2 of body-surface area. The institutional review board at each clinical site approved the protocol, and all participants provided written, informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice.

The current analysis was conducted among a subset of 54 participants (vitamin D_3 $n = 28$; placebo n = 26) selected based on age 70 years and over, fasting blood drawn between 7 and 9 am, and available serum 25(OH)D levels at baseline and 12 months. Older participants were selected because they have a higher risk of falls, declining physical performance, and increased mortality at higher concentrations of FGF23 independent of their renal function [12, 13]. Plasma samples were collected and stored at − 80° C between January 2014 and December 2017. Samples underwent 1 freeze–thaw cycle in 2021 and were again stored at − 80° C until this analysis. Two placebo participants were excluded from the analyses due to grossly hemolyzed plasma resulting in a final sample of 52 participants (vitamin D_3 $n = 28$; placebo $n = 24$).

Biochemical measurements

Plasma intact FGF23 was measured using the MSD U-Plex FGF23 singleplex assay using the MSD Gold Small Spot Streptavidin plate with U-Plex Antibody set binding to complete the sandwich immunoassay (Meso Scale Diagnostics, Rockville, MD) with CVs 3–13.2%. Serum 25(OH)D was assayed by liquid chromatography-tandem mass spectrometry as described previously [20]. Serum creatinine was analyzed locally at each study site and the estimated GFR was calculated based on [22].

Statistical analysis

Mean baseline values of clinical characteristics, biochemical measurements, and the mean changes in these values from baseline to the month 12 visit were compared across groups with t tests for 2 independent samples. Pearson correlation coefficients were used to describe linear associations. Two-sided P-values < 0.05 were considered to indicate statistical significance. Statistical analyses were conducted using IBM SPSS Statistics 28.0.

Results

The clinical characteristics of the 52 participants are shown in Table 1. There were more women in the vitamin D_3 group vs. placebo, but otherwise baseline characteristics were balanced in the two groups (Table 1). More than 80% of the participants were White. Baseline mean plasma intact FGF23 levels did not differ significantly in the 2 groups (Table 2). At baseline, GFR was inversely associated with baseline FGF23 level ($r = -0.349$, $p = 0.011$).

Estimated GFR did not change significantly in either group at month 12 (Table 2). The mean serum 25(OH)D level at month 12 was significantly greater in the vitamin D_3 supplemented group vs. placebo ($p < 0.001$; Table 2). The difference in 12-month change in plasma FGF23 between the vitamin D_3 supplementation and placebo groups was 10.3 pg/mL (95% confidence interval (CI), − 44.8 to 24.3) (Table 2). Three participants in the vitamin D_3 group (all male, White, mean

Table 1 Baseline characteristics of participants

	Vitamin D_3 ($N=28$)	Placebo ($N=24$)
Characteristic		
Age, years	73.6 ± 3.5	74.1 ± 4.0
Women, no. (%)	5 (17.9)	9 (37.5)
Caucasian, no. (%) [2]	20 (82.1)	23 (83.3)
Vitamin D supplement intake, IU/day	495 ± 499	485 ± 395
Weight, kg	94.0 ± 14.8	92.4 ± 16.0
Body-mass index, kg/m^2	30.5 ± 3.7	32.4 ± 4.6

Mean ± SD unless otherwise specified

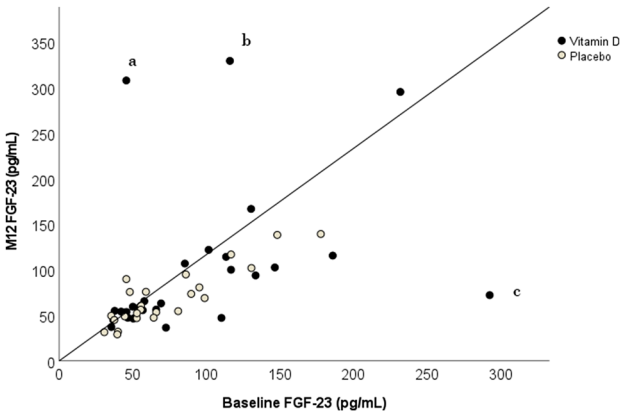

Fig. 1 Scatterplot of the baseline FGF23 (pg/mL) and M12 FGF23 (pg/mL) with the line of identity. *Clear circles* placebo, *Black circles* vitamin D

Table 2 Baseline, month 12, and changes in laboratory values (mean ± SD) for 25(OH)D, FGF23, and GFR

	Vitamin D_3 ($N=28$)	Placebo ($N=24$)	Between-group P value
Laboratory			
Serum 25-hydroxyvitamin D, ng/mL			
Baseline	34.0 ± 9.5	32.8 ± 12.3	0.711
Month 12	54.9 ± 14.8	33.4 ± 14.9	< 0.001
Change	21.0 ± 13.7	0.6 ± 6.9	< 0.001
Plasma FGF23, pg/mL			
Baseline	92.0 ± 62.8	72.9 ± 38.6	0.203
Month 12	98.6 ± 81.3	69.3 ± 31.0	0.103
Change	6.6 ± 82.4	− 3.7 ± 18.9	0.554
Estimated GFR, mL/min/1.73m^2			
Baseline	75.9 ± 13.4	76.7 ± 9.8	0.799
Month 12	72.0 ± 13.5	75.3 ± 10.5	0.338
Change	− 3.9 ± 8.0	− 1.3 ± 6.2	0.205

age 74.4 years, mean BMI 29.9 kg/m^2, GFR > 60 ml/min, and 25(OH)D level 23–40 ng/mL) had aberrantly large discrepancies between the baseline and 12-month FGF23 levels (Fig. 1, dots labeled a, b, and c). Re-evaluation without these three participants revealed a non-statistically significant group difference in 12-month change in FGF23 of − 5.5 pg/mL (95% CI − 17.1 to 28.1). Additional adjustments for potential covariates such as sex and/or baseline GFR, FGF23, and 25(OH)D levels did not significantly alter the results (data not shown).

In all participants combined, the achieved serum 25(OH)D level at month 12 was not significantly associated with month 12 plasma FGF23 level ($r=0.119$, $p=0.401$) or with 12-month change in FGF23 level ($r=0.039$, $p=0.782$).

Discussion

In this clinical trial of obese, vitamin D-sufficient adults aged 70 years and older with prediabetes, a daily dose of 4000 IU of vitamin D_3 for 12 months had no significant effect on plasma intact FGF23 levels compared to placebo, whether or not FGF23 outliers were included in the analysis. There also was no association between the achieved 25(OH)D level at month 12 and plasma FGF23 level at month 12 or with 12-month change in FGF23 level.

Our findings contrast with a recent meta-analysis by Zittermann et al. in which vitamin D doses of ≥ 3000 IU/day increased circulating levels of FGF23 by 18 pg/mL with a narrower 95% CI of 6–30 pg/ml in a group of studies that employed a variety of intact or C-terminal assays [17, 19]. Our study is specific to a vitamin D-replete population of older adults with prediabetes. This population is at risk from elevated FGF23 levels, which have been linked to increased cardiovascular morbidity and mortality even in patients with normal kidney function [15].

Several factors may explain the discord with the meta-analysis by Zittermann et al. [17]. The dose schedule (e.g., daily vs. bolus dosing), as opposed to the cumulative dose of vitamin D, may influence the FGF23 response to supplemental vitamin D differently [23–29]. Of the seven published trials testing weekly or monthly bolus dosing of parent vitamin D supplementation (vitamin D_2 or D_3) in adults with normal renal function [23–28, 30], all except one [30] found significant increases in circulating concentrations of intact FGF23 [23, 24, 26–28] or C-terminal FGF23 [25, 28]. Bolus dosing has been hypothesized to upregulate the expression of 24,25-hydroxylase, an important enzyme that hydroxylates 25(OH)D to the inactive metabolite $24,25(OH)_2D$ [29]. Production of this enzyme is promoted by FGF23 [31]. A recent trial in older

adults testing large weekly bolus dosing of vitamin D_3 (12,000 IU, 24,000 IU, or 48,000 IU/month) for 12 months found a dose-dependent increase serum $24,25(OH)_2D$ levels [28].

Another factor to consider in the effect of vitamin D on FGF23 is the assay type (intact vs. C-terminal) and the medium measured (serum vs. plasma. The methods used for the various intact or C-terminal assays have not been systematically compared [9]. There is no international standardization [9]. Additionally, there are matrix differences within and across assays [9]. The assay used in this study reported greater variability in the range of intact FGF23 levels when using EDTA plasma (difference from low to high of 139.4 pg/mL) versus serum (difference from low to high of 83 pg/mL). This wider range of values can reduce the ability to detect treatment group differences.

The FGF23 assay we use has been used for research purposes only and has a higher CV than commercially-available assays [9, 32]. The ranges for FGF23 levels using EDTA plasma were wider than serum with this assay, but serum samples were not available for this analysis. Our samples had been thawed and refrozen once and had been stored for 5 to 9 years. A recent review suggested no significant effects on the measured FGF23 levels following multiple freeze–thaw cycles [9]. One study found no significant change in FGF23 levels after multiple freeze–thaw cycles and no significant change over a 6-year storage period (37). Stability over a longer period of up to 9 years, as in this study, has not been established [33].

Our study findings are supported by two randomized controlled trials testing 4000 IU daily doses of vitamin D_3 on C-terminal FGF23 concentration [34, 35]. A randomized placebo-controlled trial in 165 overweight and obese adults with mean age of ~ 74 years and GFR ~ 70 ml/min/m^2 found no significant effect of vitamin D_3 4000 IU/day for 36 months on C-terminal FGF23 levels [34]. Another study of 54 overweight and obese adults with normal renal function randomized to receive a single oral dose of 100,000 IU of vitamin followed by 4000 IU/day or placebo for 4 months also found no significant change in C-terminal FGF23 levels [35]. Other trials testing doses of vitamin D_3 of 2800–3000 IU/day have also shown no effects on intact [36] and C-terminal FGF23 levels [37]. Our findings add support to the evidence that daily doses of vitamin D_3 of 4000 IU/day, the Tolerable Upper Intake Level (UL) as defined by the NAM [1], do not trigger increased intact FGF23 production.

There are very limited data [28] on whether a specific 25(OH)D level achieved by daily dosing results in higher FGF23. Our study did not find an association between the two concentrations at 12 months. It is possible that daily doses resulting in higher 25(OH)D levels than were achieved in our study ($> 54.9 \pm 14.8$ ng/mL) may influence FGF23 levels, but evidence for this is lacking.

Strengths of this study include the randomized controlled study design and balanced groups. Furthermore, in our analysis of associations with achieved 25(OH)D, our participants started with replete vitamin D status and thus were able to achieve high 25(OH)D levels on vitamin D supplementation. Limitations include the small sample size, limited generalizability of findings to older adults with prediabetes, and potential imbalances in dietary phosphate and iron intakes, both of which can influence FGF23 production but were not assessed in this study.

In conclusion, this study in obese vitamin D replete older adults with prediabetes and normal renal function found no significant effect of 4000 IU of vitamin D_3 daily for 12 months on plasma intact FGF23 levels. Currently there is no evidence that daily dosing of vitamin D at doses as high as the UL assigned by the NAM, 4000 IU per day, increases circulating levels of FGF23.

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Author contributions All authors contributed to the study conception and design. All authors performed material preparation and data collection. LC performed statistical analyses. The first draft of the manuscript was written by LC and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The data including individual records are not publicly available due to privacy or ethical restrictions. However, aggregated data will be shared on reasonable request to the corresponding author.

Declarations

Conflict of interest The authors have conflicts of interest.

Ethical approval This is a post-hoc analysis using existing archived de-identified plasma samples. The Tufts Health Sciences IRB has confirmed that no ethical approval is required.

Informed consent Informed consent was obtained from all individual participants included in the study.

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