Title

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Vitamin D3 supplementation increases fibroblast growth factor-23 in HIV-infected youth treated with tenofovir disoproxil fumarate

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Abstract

Background—Tenofovir (TDF) is associated with phosphaturia and elevated 1,25 dihydroxy vitamin D (1,25-OH(2)D). Fibroblast growth factor 23 (FGF23) causes phosphaturia and increases

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The clinical trials registration number for this study is NCT00490412, available online at http://clinicaltrials.gov/ct2/show/NCT00490412

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None of the authors has a significant conflict of interest to report.

in response to elevated 1,25-OH(2)D. Vitamin D binding protein (VDBP) binds to 1,25-OH(2)D, decreasing its biologic activity, and is elevated in persons with higher plasma tenofovir concentrations. We compared FGF23 and VDBP before and after vitamin D3 (VITD) supplementation in youth treated with combination antiretroviral therapy (cART) containing or not containing TDF.

Methods—A randomized controlled trial in HIV+ youth ages 18–25 years enrolled participants based on cART treatment with TDF (TDF, N=118) or without TDF (no-TDF, N=85) and randomized within those groups to VITD (50,000 IU every four weeks) or placebo (PL). We measured FGF23 and VDBP and calculated free 1,25-OH(2)D at baseline and week 12, and compared changes by TDF treatment and VITD randomized group.

Results—At baseline, serum FGF23 concentration showed a quadratic relationship with 1,25-OH(2)D most pronounced in the TDF group. At week 12, total and free 1,25-OH(2)D increased in the VITD but not PL groups, independent of TDF use. FGF23 increased in the TDF group receiving VITD, but there was no FGF23 change in the no-TDF group receiving VITD or the PL groups. The adjusted mean change in FGF23 from baseline to week 12 was +7.7 pg/mL in the TDF/VITD group, compared to −1.7 (no-TDF/VITD, p=0.010); −1.3 (TDF/PL, p=0.006); and +1.1 (no-TDF/PL, p=0.035).

Conclusions—These results suggest that TDF-containing cART may alter the FGF23 response to vitamin D supplementation in HIV-infected youth.

Introduction

Tenofovir disoproxil fumarate (TDF) is associated with increased parathyroid hormone (PTH)[1], and phosphaturia[2, 3], as is vitamin D deficiency (as indicated by low serum 25 hydroxyvitamin D, 25-OHD) [4]. However, TDF therapy is associated with increased 1,25-OH(2)D [5], while vitamin D deficiency may be associated with decreased 1,25-OH(2)D [4]. Higher concentrations of tenofovir (the TDF plasma metabolite) are associated with higher vitamin D binding protein (VDBP) and albumin[5], which both bind 1,25-OH(2)D and decrease free 1,25-OH(2)D concentration[6]. Free 1,25-OH(2)D is the physiologically active form of 1,25-OH(2)D [7], and since TDF treatment is associated with low free 1,25-OH(2)D, TDF treatment approximates a state of “functional vitamin D deficiency” [5]. It may be that the high 1,25-OH(2)D associated with TDF use is in response to the low “functional” (free) 1,25-OH(2)D.

Vitamin D deficiency accentuates TDF-associated increased PTH [8, 9]. In adults [10] and youth [11] with HIV infection treated with TDF, vitamin D supplementation decreased serum PTH concentration but did not improve TDF-associated phosphaturia.

In non-HIV infected populations, fibroblast growth factor 23 (FGF23), a phosphaturic hormone produced in osteocytes, increases in response to elevations in 1,25-OH(2)D [12]. Paradoxically, although FGF23 levels are lower in persons with vitamin D deficiency [13], vitamin D supplementation leads to a further decline in FGF23 [13].

FGF23 concentrations are not different in HIV-infected persons treated with TDF compared to those treated with other antiretrovirals ([5, 14, 15]). In vitamin D-deficient persons with

HIV treated with TDF, open-label vitamin D supplementation was associated with a trend toward increased FGF23 [10], in contrast to what was observed in non-HIV infected persons with vitamin D deficiency [13].

From baseline analyses in our randomized placebo-controlled trial of vitamin D supplementation in youth with HIV infection. we have reported previously that youth treated with TDF had higher PTH, higher total 1,25-OH(2)D, and lower tubular reabsorption of phosphate (TRP) but similar VDBP, free 1,25-OH(2)D, and FGF23 compared to youth not receiving TDF[5]. Secondary analyses using specimens available from that study allowed us to explore further the baseline relationships between TDF use, 1,25-OH(2)D, and FGF23; and changes in FGF23 from baseline to study week 12 in study groups randomized to vitamin D supplementation or placebo, which we report in this manuscript.

Methods

Design

The design and bioanalytic methods of Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) study 063 (NCT00490412) were previously reported [5, 11, 16]. HIV-infected youth 18–25 years were enrolled based on stable treatment with combination antiretroviral therapy (cART) containing TDF (N=118) or not containing TDF (no-TDF; N=85), with randomization within those groups to Vitamin D3 50,000 IU (VITD; N=102) or placebo (PL; N=101) (Bio-tech Pharmacal, Fayetteville, AR), administered as directly observed therapy at 0, 4, and 8 weeks. Serum samples collected at weeks 0 and 12 were stored for batch analysis. The study was approved by the Institutional Review Board of each participating center and required participants' written informed consent prior to enrollment.

Methods for measurement of PTH, tubular reabsorption of phosphate (TRP), 25-OHD, 1,25-OH(2)D, VDBP, albumin and FGF23 have been described previously[5][11, 16]. Albumin and VDBP were used to calculate free 1,25-OH(2)D) [6]. The current analysis reports the baseline relationships between TDF use, 1,25-OH(2)D, and FGF2; and the change from baseline to week 12 in FGF23, VDBP, total and free 1,25-OH(2)D in participants in whom stored samples were available for these secondary analyses.

Statistics

Data are presented as mean (standard deviation). Analyses of differences between antiretroviral therapy (TDF or no-TDF) and randomized (VITD or PL) groups used an intent-to-treat cohort (N=203) for baseline data and a per-protocol cohort for data on change from baseline to week 12.

Statistical significance of differences was identified using Pearson chi-square test for categorical variables and Wilcoxon rank sum test for continuous variables. Wilcoxon signed-rank test was used to test within-group differences. Spearman and Pearson correlations were considered significant only if the associated P value was < 0.010, and the correlation (Pearson or Spearman) with the lower P value is presented here.

Univariate analyses followed by multivariable models were used to measure the main effects of VITD and TDF as well as the interaction between those two exposures and to measure the effect of confounding variables. Generalized linear models with normal errors and identity link were used for the univariate and multivariable models. A rank-based analysis was also performed due to the presence of outliers. Covariates for baseline and change from baseline to week 12 analyses were chosen based on clinical significance as described previously [11] using the GLMSELECT procedure in SAS with stepwise selection (version 9.2, SAS Institute, Cary NC).

Results

In the TDF group, a significant (P<0.004) quadratic relationship was found between baseline total 1,25-OH(2)D and FGF23 concentrations, with an inverse association in the lower range of concentrations of total 1,25-OH(2)D and a positive association at higher concentrations (Figure 1). For example, with an increase in total 1,25-OH(2)D from 50 to 51 pmol/L, FGF23 would *decrease* by 0.171 pg/mL, whereas an increase in total 1,25-OH(2)D from 200 to 201 pmol/L would be accompanied by an *increas*e in FGF23 of 0.108 pg/mL. The magnitude of this association in the no-TDF group was less prominent (P=0.053).

From baseline to week 12, FGF23 increased in the TDF group receiving VITD, but not in the no-TDF group receiving VITD or in either of the PL groups (Table 1). VDBP showed no change from baseline in any group. Total and free 1,25-OH(2)D increased in the VITD but not PL groups, independent of TDF use (Table 1).

Multivariable analysis of FGF23 change from baseline to week 12 used a model that included TDF/no-TDF, VITD/PL, and week 12 total 1,25-OH(2)D (as a continuous variable). The model interaction term for TDF/VITD was statistically significant (p = 0.042), with no statistically significant covariates. In this model the adjusted mean FGF23 concentration change from baseline to week 12 was +7.7 pg/mL in the TDF/VITD group, compared to −1.7 (no-TDF/VITD, p=0.010); −1.3 (TDF/PL, p=0.006); and +1.1 (no-TDF/PL, p=0.035).

In the TDF group at week 12, the FGF23 concentration showed a weak positive correlation with serum phosphate (r=0.299, p=0.003, Pearson) and a weak negative correlation with serum total 1,25-OH(2)D (r= −0.268, p=0.008, Spearman). These correlations were not seen in the no-TDF group at week 12, nor were they seen in either group at baseline.

Baseline FGF23 was similar in participants with serum 25-OHD<20 ng/mL or serum 25-OHD>=20 ng/mL [17] (Figure 2, Panel A). The baseline to week 12 increase in FGF23 in the TDF/VITD group was statistically significant overall, and in participants with baseline serum 25-OHD<20 ng/mL (15% increase; P=0.009). The FGF23 change did not achieve statistical significance in those with baseline serum 25-OHD>=20 ng/mL (18% increase; P=0.095; Figure 2, panel B). There were no statistically significant changes in the no-TDF group randomized to vitamin D (Figure 2, panel B), or in either group (TDF or no-TDF) randomized to placebo independent of baseline vitamin D status (data not shown).

Discussion

This study shows that in youth with HIV infection treated with TDF-containing cART, short-term vitamin D supplementation increases FGF23 compared to youth treated with cART not containing TDF. Since FGF23 causes phosphaturia, this may partially explain the continued renal phosphate loss seen in studies that have otherwise shown a positive response to vitamin D supplementation, with decreased PTH [10, 11] and improved bone density [10].

The FGF23 response to vitamin D supplementation may be context-sensitive, as suggested by our finding of differing results in those receiving TDF vs. no-TDF, and a quadratic relationship between baseline FGF23 and total 1,25-OH(2)D concentrations. In studies of children [18] and adults [19] with chronic kidney disease requiring dialysis, treatment with intravenous 1,25-OH(2)D led to a decrease in PTH and an increase in FGF23. Conversely, in a study of vitamin D-deficient adult women, treatment with oral vitamin D supplements was associated with declines in both PTH and FGF23, and persistent hypophosphatemia over the 6 weeks of study [13]. It is possible that at high concentrations of 1,25-OH(2)D such as those attained in the dialysis patients, the counter-regulatory effect of FGF23 becomes of prime importance, and FGF23 increases to decrease 1,25-OH(2)D production and also increase 1,25-OH(2)D clearance [12]. However, in vitamin D deficiency, with relatively low 1,25-OH(2)D and persistently low serum phosphate, low FGF23 would be an appropriate response to conserve phosphate further and improve bone health [13].

Strengths of this study include the prospective, placebo-controlled design and randomized assignment to vitamin D or placebo. This secondary analysis, however, was performed on a subset of participants for whom frozen samples were available, thus limiting the sample size. The findings need to be confirmed in other populations.

The reported change in FGF23 concentration is small, but may be clinically significant during prolonged treatment or in a subgroup of patients. Increased FGF23 has been associated with an increased mortality risk in patients with chronic kidney disease [20], but its causative role remains unclear. More work is needed to confirm the associations suggested by these findings and to clarify the role of FGF23 in TDF-associated phosphaturia, as well as the effects of long-term vitamin D supplementation in individuals treated with TDF.

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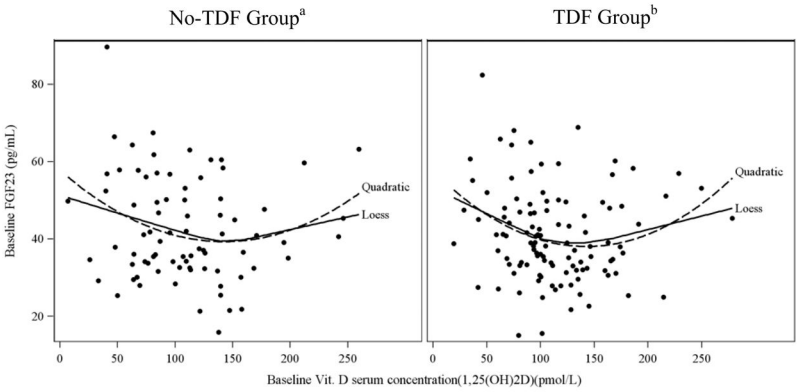


Figure 1. Baseline FGF23 shows a quadratic relationship with baseline total 1,25-OH(2)D, more pronounced in the TDF than the no-TDF group

a. No tenofovir disoproxil fumarate in cART (No-TDF Group):

Regression Equation: 57.716 + [(−0.2583) * Baseline 1,25-OH(2)D(pmol/L)] + [(0.0009) * Baseline 1,25-OH(2)D (pmol/L) * Baseline 1,25-OH(2)D (pmol/L)]

P value = 0.053

Critical point = 143.50 pmol/L

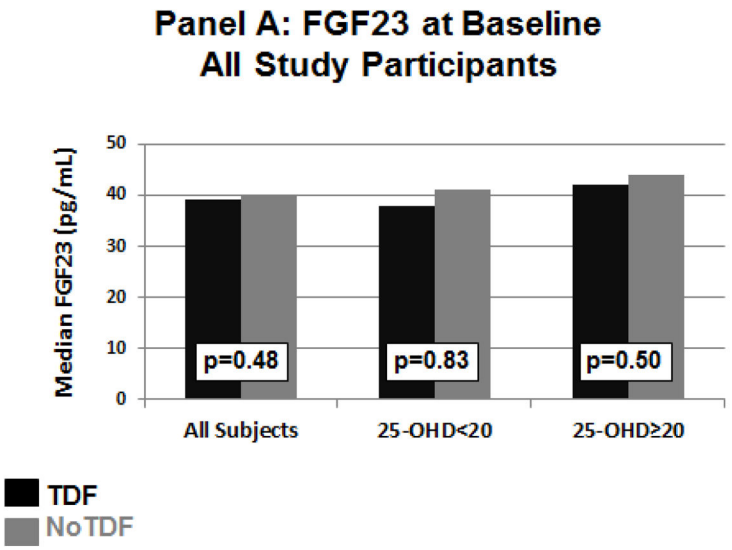
b. Tenofovir disoproxil fumarate in cART (TDF Group):

Regression Equation: 57.547 + [(−0.2733) * Baseline 1,25-OH(2)D (pmol/L)] + [(0.0010) * Baseline 1,25-OH(2)D (pmol/L)] * Baseline 1,25-OH(2)D (pmol/L)

P value = 0.004

Critical point = 136.65 pmol/L

The nonparametric Loess smoothed curve confirms the presence of a quadratic relationship.



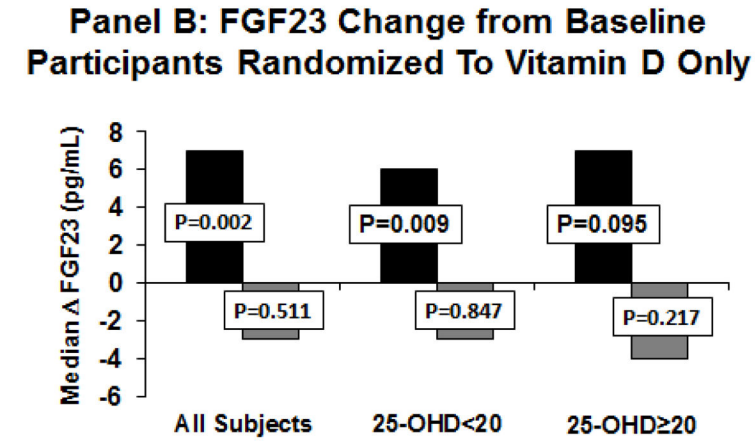


Figure 2. Serum FGF23 concentration at baseline, and change in FGF23 from baseline to week 12, by baseline vitamin D status and tenofovir disoproxil fumarate use

Panel A. Median Fibroblast Growth Factor 23 (FGF23) serum concentrations at baseline for all study participants, by presence of tenofovir disoproxil fumarate (TDF; black bars) or absence (no-TDF; grey bars) in combination antiretroviral (cART) regimen; and by baseline vitamin D nutritional status of serum 25-OHD < 20 ng/mL or serum 25-OHD>=20 ng/mL. P values for comparison between TDF and no-TDF groups.

Panel B. Median FGF23 change from baseline to week 12 for those participants randomized to receive vitamin D supplementation. P values are for statistical significance of the change from baseline to week 12, Wilcoxon signed rank test. There were no statistically significant changes in the no-TDF group randomized to vitamin D, or in either group (TDF or no-TDF) randomized to placebo. It is the interaction of TDF and vitamin D supplementation that is associated with increased FGF23 (see text).

Table 1

Change from baseline to week 12 by presence of TDF in cART and by randomized treatment with vitamin D or placebo

Variable	Timing	N	Study Group								
			Overall	**TDF[e]**	**No-TDF**	**VITD**	**Placebo**	**TDF-VITD**	**TDF-PL**	**No-TDF-VITD**	**No-TDF-PL**
Fibroblast Growth Factor 23 (FGF23; pg/mL)	Baseline	188[a]	41.7 (12.6)[c]	41.0 (12.0)	42.6 (13.4)	41.8 (12.7)	41.5 (12.5)	**40.3 (11.1)**	41.7 (13.0)	44.1 (14.8)	41.3 (12.1)
	Change	155[b]	+1.9 (16.1)	+3.2 (15.8)	−0.22 (16.5)	+3.9 (17.8)	−0.2 (14.0)	**+7.55 (17.9)**	−1.32 (11.8)	−1.92 (16.4)	+1.43 (16.7)
	P value		0.384[d]	0.093	0.60	0.077	0.508	**0.002**	0.414	0.512	0.944
Vitamin D Binding Protein (VDBP; μmol/L)	Baseline	184	3.77 (2.00)	4.04 (2.09)	3.37 (1.79)	3.72 (2.03)	3.83 (1.98)	4.16 (2.12)	3.93 (2.07)	3.05 (1.69)	3.67 (1.85)
	Change	144	−0.03 (0.68)	−0.03 (0.80)	−0.04 (0.45)	−0.06 (0.66)	+0.01 (0.71)	−0.08 (0.75)	−0.02 (0.84)	−0.05 (0.50)	−0.03 (0.39)
	P value		0.593	0.769	0.607	0.527	0.924	0.434	0.679	0.912	0.480
Total 1, 25 dihydroxy vitamin D (1,25-OH(2)D; pmol/L)	Baseline	203	**112 (48)**	**114 (46)**	109 (50)	**111 (48)**	112 (47)	**116 (47)**	112 (46)	**105 (49)**	112 (51)
	Change	168	**+12.7 (56)**	**+15.3 (58)**	+9.0 (53)	**+21.3 (59)**	+3.3 (53)	**+19.3 (62)**	+11.0 (54)	**+24.2 (53)**	−7.6 (49)
	P value		**0.005**	**0.001**	0.131	**<0.001**	0.438	**0.003**	0.118	**0.006**	0.500
Free 1,25-OH(2)D (fmol/L)	Baseline	177	**908 (721)**	**850 (602)**	993 (863)	**869 (527)**	945 (867)	**791 (447)**	909 (723)	**989 (617)**	995 (1044)
	Change	141	**+99 (655)**	**+146 (508)**	+18 (850)	**+198 (499)**	−1 (773)	**+144 (487)**	+149 (533)	**+290 (515)**	−253 (1027)
	P value		**0.008**	**0.012**	0.291	**0.001**	0.600	**0.023**	0.191	**0.020**	0.512

[a] N for “overall” group at baseline.

[b] N for “overall” group for the change from baseline to week 12.

[c] Mean (Standard deviation)

[d] P value by Wilcoxon signed rank test for change from baseline to week 12 within each group

[e] TDF, tenofovir disoproxil fumarate, used to identify the group treated with TDF in combination antiretroviral therapy (cART); no-TDF, group without TDF in cART; VITD; group randomized to vitamin D supplementation; Placebo (PL), group randomized to placebo.