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Effect of Vitamin D Supplementation on Bone Turnover Markers During HIV Pre-Exposure Prophylaxis Using Tenofovir Disoproxil Fumarate-Emtricitabine in Men Who Have Sex with Men

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Abstract

Pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate-emtricitabine (TDF-FTC) reduces bone mineral density in HIV-uninfected men who have sex with men (MSM). We hypothesized that PrEP with TDF-FTC would increase bone turnover markers (BTMs) at week 24 and that vitamin D supplementation from weeks 24 to 48 would blunt this increase. Participants were from a cohort of 398 MSM and transgender women who received daily TDF-FTC for PrEP. At week 24, a prospective intervention group initiated vitamin D3 4,000 IU daily. Concurrent controls were selected from the cohort who took ≤400 IU/day of vitamin D3 matched by age, race, and body mass index. The primary endpoint was the change in procollagen-I N-terminal propeptide (P1NP) from weeks 24 to 48. Paired *t*-tests were used to compare changes in BTMs between intervention and controls. Among 48 intervention–control pairs, median age was 33 years. At baseline, 68.9% of the intervention group and 77.3% of controls were vitamin D sufficient (≥20 ng/mL, $p = .94$). P1NP, C-telopeptide, parathyroid hormone (PTH), and 25-OH vitamin D3 did not increase significantly at week 24. P1NP fell by a mean ± SD of −27.6 ± 49.9 pg/mL from weeks 24 to 48 with vitamin D and −2.5 ± 40.2 pg/mL in controls ($p = .01$). There were no significant between-group differences in the weeks 24–48 change in C-telopeptide, PTH, or 25-OH vitamin D3. Vitamin D3 supplementation with 4,000 IU/day resulted in a significant reduction in the BTM P1NP compared with controls, suggesting that this intervention has potential to improve bone health during PrEP.

Introduction

Tenofovir Disoproxil Fumarate-emtricitabine (TDF-FTC; Truvada®; Gilead Sciences) as a daily fixed dose tablet

is approved by the Food and Drug Administration for pre-exposure prophylaxis (PrEP) to prevent HIV acquisition for persons with increased risk for HIV infection. TDF has well-described toxicities, including greater loss of bone mineral density (BMD) after antiretroviral therapy (ART) initiation and increased risk of fragility fracture.[1–5] Daily use of TDF-FTC as PrEP is associated with significant reduction in BMD, although to a lesser degree than that seen during initial treatment for HIV.[6–8] Hence, long-term PrEP use is potentially associated with increased fracture risk. Glidden and colleagues showed that mean BMD returns to baseline levels within 12–18 months after discontinuation of TDF-based PrEP.[9] However, the effects of continued long-term exposure to TDF during PrEP on bone are not known.

The mechanism by which TDF induces BMD loss is unclear, but it appears to be associated with an increase in parathyroid hormone (PTH) levels and PTH-controlled calcium and phosphate metabolism.[10–12] Some data suggest that TDF induces a state of functional vitamin D deficiency.[13,14] Strategies to attenuate or prevent BMD loss when TDF-containing PrEP is given for prolonged periods of time are needed. Overton *et al.* reported a marked reduction in the degree of BMD loss during initial therapy of HIV infection with vitamin D3 4,000 IU/day and calcium supplementation.[15] When vitamin D–calcium was coadministered with efavirenz plus TDF-FTC-based ART for 48 weeks, the amount of BMD loss at the hip measured by dual-energy X-ray absorptiometry (DEXA) was approximately half that seen with placebo (−1.5% vs. −3.2%, respectively; $p < .001$).

The objective of our study was to evaluate if supplementation with vitamin D will favorably affect the adverse changes in bone turnover markers (BTM) that occur after initiation of PrEP with TDF-FTC. Our primary hypothesis was that supplementation with vitamin D 4,000 IU/day will significantly blunt the increase in the BTM procollagen-I N-terminal propeptide (P1NP) that occurs after initiation of PrEP with TDF-FTC. Secondary hypotheses included that supplementation with vitamin D 4,000 IU/day will significantly blunt the increases in BTM C-telopeptide (CTX) and PTH that occur after initiation of PrEP with TDF-FTC and increase levels of 25-OH vitamin D3 compared with control participants not receiving supplementation.

Materials and Methods

Study design

This was a nonrandomized prospective interventional study with retrospectively matched concurrent controls as a nested substudy of an open-label clinical trial of the effect of a text messaging intervention versus standard of care on adherence to daily TDF-FTC as PrEP in men who have sex with men (MSM) and transgender male to female at increased risk for HIV acquisition (CCTG 595; ClinicalTrials.gov Identifier: NCT01761643). Both the vitamin D intervention group and the matched controls were part of the CCTG 595 clinical trial and were enrolled concurrently into the parent trial. Participation in the substudy was not required for continued participation in the CCTG 595 trial. The intervention included 48 men matched 1:1 with concurrent controls based on age (±5 years), race (black vs. nonblack), and body mass index (BMI; ±3 kg/m^2). The intervention group and controls were also matched based on season of enrollment to minimize the effect of sun exposure on baseline vitamin D levels.

Participants

Study participants were drawn from the men or transgender women at high risk of acquiring HIV infection who

received daily PrEP with TDF-FTC aged 18 years or older. All study participants were naive to TDF-FTC at the baseline visit. Participant enrollment to the substudy occurred at the 24-week visit of the CCTG 595 trial. Eligible participants had substantial ongoing risk of acquisition of HIV and tested negative for HIV infection by rapid HIV test and confirmed negative by another sensitive method. Laboratory values in the past 30 days included a calculated creatinine clearance of at least 60 mL/min, liver transaminase levels less than three times the upper limit of normal, hemoglobin >9 g/dL, absolute neutrophil count >750/mm^3, and platelet count >75,000/mm^3. Exclusion criteria for both groups included current or previous use of bisphosphonate therapy, current use of vitamin D supplements >400 IU/day, current use of androgenic or growth hormones, history of nephrolithiasis or fragility fracture, and no previous use of TDF.

Primary and secondary objectives

The primary objective was to compare the change in P1NP levels from week 24 to week 48 among participants in the intervention group who initiated vitamin D 4,000 IU/day to the change in levels seen in matched controls taking 400 IU/day or less of vitamin D. The secondary objectives were to (1) compare the change in CTX and PTH levels from week 24 through week 48 among participants who received vitamin D 4,000 IU/day to the change in levels seen in matched unsupplemented controls, (2) compare 25-OH vitamin D levels at week 48 among participants who received vitamin D 4,000 IU/day and matched controls, and (3) demonstrate the change in P1NP, CTX, PTH, and 25-OH vitamin D3 levels from baseline to week 24 in both the intervention group and controls.

Treatment

Vitamin D3 was supplied as 2,000 IU capsules (NatureMade, Mission Hills, CA) taken as two capsules once daily, taken from week 24 to week 48. In this study, supplemental calcium was not used because in ACTG 5280 dietary calcium intake among untreated HIV-infected participants was generally adequate.[15] We thus anticipated that the largely healthy HIV-uninfected participants in this study would also have adequate dietary calcium intake.

Clinical and laboratory evaluations

Plasma from participants was collected and stored at entry, 24, and 48 weeks. BTMs were measured after completion of the study on stored samples. PTH, CTX-1, and P1NP were measured using ELISA-based kits (Reddot Biotech, Inc., BC, Canada and MyBiosource, Inc., San Diego, CA), respectively. All samples were analyzed according to manufacturer's recommended procedures, where the replicates were all within acceptable percentage of coefficient of variation criteria set *a priori* at <15%. The concentration of 25-OH vitamin D3 was determined using a validated liquid chromatography–mass spectrometry method. Bone density measurements were not performed.

To calculate the average daily dietary intake of vitamin D and calcium from food, participants were asked to recall food items eaten over the last 3 days at the week 36 visit. A calculator adapted from the USDA database was used to quantify vitamin D and calcium intake.[15] Tenofovir diphosphate in dried blood spots (DBS) served as an objective measure of adherence to PrEP.[16,17]

Statistical analysis

Forty-four pairs of cases and controls provides 80% power to detect a difference of 20% in the weeks 24–48 change in P1NP between the intervention group and controls, assuming the standard deviation of change is 40% using a paired *t*-test with a two-sided alpha = 0.05.

Mean changes in BTMs from week 24 to week 48 were compared within groups using a paired *t*-test. The Wilcoxon signed-rank test was used for comparison of BTMs at each visit between groups because of the skewness of the data. Paired *t*-test was used to compare the change in BTMs from baseline to week 24 for both groups. Linear regression model assessed the association between change in P1NP and change in vitamin D levels, adjusting for the week 24 P1NP levels. All analyses were conducted in R (http://cran.r-project.org), version 3.3.2. Values of $p < .05$ were considered statistically significant.

Results

Forty-eight participants were enrolled to the vitamin D supplementation (intervention) group and compared with 48 matched controls. Baseline characteristics of the groups were similar with regard to age, race, and BMI (Table 1). All participants were MSM. The number of Hispanic participants was higher in the vitamin D group than in the control group (33% vs. 17%). Both at baseline and week 24, most study participants had sufficient vitamin D levels (≥ 20 ng/mL; Table 2).[18] Both the intervention group and controls demonstrated satisfactory adherence to PrEP, with 87.5% of the intervention group and 85.4% of controls having a DBS tenofovir diphosphate level of >719 fmol/punch at week 48, indicating at least four weekly doses.[16] Sixty-five percent of the intervention group at week 36 and 64% at week 48 self-reported >90% adherence to the vitamin D supplement.

Table 1. Patient Characteristics and Adherence to Pre-Exposure Prophylaxis

	Vitamin D supplementation (n = 48)	*Controls (n = 48)*	p *value*
Age at entry, median (Q1, Q3)	**33.5 (28, 37.5)**	**33.0 (29, 38.5)**	*
Race, *n* (%)			
White	**26 (55)**	**25 (53)**	*
Black	**13 (28)**	**14 (30)**	
Asian	**2 (4)**	**2 (4)**	

Multiracial	**6 (13)**	**6 (13)**	
Ethnicity, *n* (%)			
Hispanic	**16 (33)**	**8 (17)**	
BMI at week 24, kg/m^2, median (Q1, Q3)	**25.6 (24.1, 27.5)**	**26.0 (23.3, 28.4)**	*
Treatment assignment in parent trial, *n* (%)			
Standard of care arm	**25 (52)**	**22 (46)**	**.68**
Text messaging arm	**23 (48)**	**26 (54)**	
Dried blood spot tenofovir diphosphate fmol/punch, median (Q1, Q3)			
Week 12	**1,165 (909, 1,393)**	**1,148 (905, 1,408)**	**.39**
Week 48	**1,068 (935, 1,388)**	**1,110 (859, 1,475)**	**.87**
Percent with tenofovir diphosphate >719 fmol/punch, %			
Week 12	**91.7**	**87.5**	**.74**
Week 48	**87.5**	**85.4**	**>.99**

p value by paired *t*-test. **p* values not reported for age, race, and BMI because there were matching criteria.

BMI, body mass index; Q1, first quartile; Q3, third quartile.

Table 2. Vitamin D Status

	Vitamin D supplementation	*Controls*	*Total*	*p value*
Baseline				
Sufficient	**31 (68.89)**	**33 (73.33)**	**64 (71.11)**	**.94**
Insufficient	**10 (22.22)**	**8 (17.78)**	**18 (20)**	
Deficient	**4 (8.89)**	**4 (8.89)**	**8 (8.89)**	
Week 24				
Sufficient	**29 (60.42)**	**39 (81.25)**	**78 (70.83)**	**.093**
Insufficient	**15 (31.25)**	**8 (16.67)**	**23 (23.96)**	
Deficient	**4 (8.33)**	**1 (2.08)**	**5 (5.21)**	
Week 48				
Sufficient	**37 (77.08)**	**32 (66.67)**	**69 (71.88)**	**.388**
Insufficient	**10 (20.83)**	**12 (25)**	**22 (22.92)**	
Deficient	**1 (2.08)**	**4 (8.33)**	**5 (5.21)**	

Vitamin D sufficient was considered 25-OH vitamin D3 level ≥20 ng/mL, insufficient 12 to <20 ng/mL, and deficient <12 ng/mL. *p* value by paired *t*-test.

Values are given as *n* (%).

P1NP level was unexpectedly higher among the intervention group than controls at week 24 (Table 3). P1NP decreased by a mean of −27.6 ± 49.9 pg/mL from week 24 to 48 with vitamin D supplementation. The P1NP level decreased only −2.5 ± 40.2 pg/mL in controls (Table 3 and Fig. 1). The mean change in P1NP from week 24 to week 48 between groups was significantly different ($p = .011$). The percent reduction in P1NP level from week 24 to 48 was also greater with vitamin D supplementation (−14.6% ± 52.5%) than in controls (20.7% ± 67.3%, $p = .006$). In a multivariable linear regression model controlling for week 24 P1NP levels, greater increase in 25-OH vitamin D3 between weeks 24 and 48 was associated with greater decrease in P1NP between weeks

24 and 48 (coefficient estimate = −0.59, p = .006). *Post hoc* mixed model analysis of the absolute change in P1NP from week 24 to 48 showed no statistically significant difference between the groups after adjusting for the week 24 P1NP levels (p = .23).

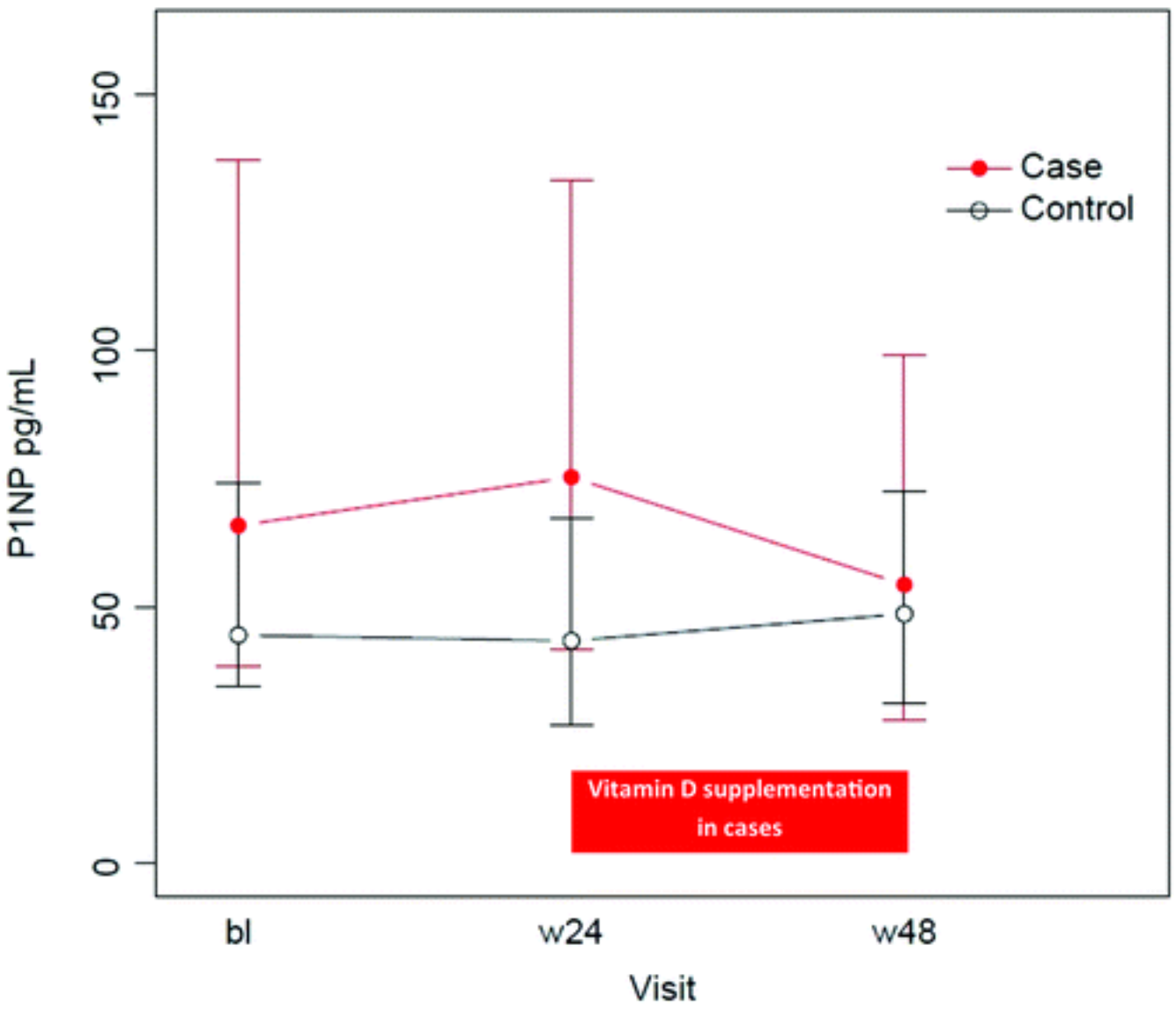

FIG. 1. Change in P1NP level from week 24 to 48. P1NP levels were higher among the vitamin D supplementation group (cases) compared with controls at week 24. P1NP fell by a mean of −27.6 ± 49.9 pg/mL from week 24 to 48 with vitamin D 4,000 IU/day supplementation and by −2.5 ± 40.2 in controls (p = .011, paired t-test). The paired Wilcoxon signed-rank test was used for comparison at each time point because of the skewness of the data. p = .14 at baseline; p = .003 at week 24; p = .35 at week 48. P1NP, procollagen-I N-terminal propeptide. Color images are available online.

Table 3. Bone Turnover Markers

	P1NP pg/mL	p *value*	*CTX ng/mL*	p *value*
Baseline, median (IQR)				
Vitamin D	**68.1 (37.6–137)**	**.137**	**1.3 (0.6–2.5)**	**.258[a]**

Controls	**44.5 (34.6–74.1)**		**1.4 (0.7–2.8)**	
Week 24, median (IQR)				
Vitamin D	**75.3 (41.6–133)**	**.003**	**1.1 (0.6–2.1)**	**.023[a]**
Controls	**43.4 (26.8–67.2)**		**1.5 (0.6–3.1)**	
Week 48, median (IQR)				
Vitamin D	**54.3 (28.0–99.0)**	**.349**	**0.8 (0.6–1.5)**	**.003[a]**
Controls	**48.6 (31.2–72.5)**		**1.35 (0.7–4.2)**	
Mean (±SD) change from week 24 to 48				
Vitamin D	**−27.6 ± 49.9**	**.011**	**0.03 ± 1.12**	**.398[b]**
Controls	**−2.5 ± 40.2**		**0.29 ± 2.10**	

[a]*p* value by paired Wilcoxon signed-rank test.

[b]*p* value by paired *t*-test.

P1NP, procollagen-I N-terminal propeptide; CTX, C-telopeptide; IQR, interquartile range.

There was no significant change in P1NP, CTX, PTH, or 25-OH vitamin D3 levels from baseline to week 24 (Tables 3 and 4). Levels of 25-OH vitamin D3 increased from week 24 to 48 among the intervention group receiving 4,000 IU of vitamin D3 daily, but this was not statistically significant (within-group $p = .065$). The change in P1NP levels observed from baseline to week 24 did not differ by adherence to PrEP assessed at week 12 (data not shown).

Table 4. Parathyroid Hormone and 25-OH Vitamin D_3 Levels

	PTH pg/mL	p *value*	*25-OH vitamin D_3 ng/mL*	p *value*
Baseline, median (IQR)				

Vitamin D	**165.2 (120.9–238)**	**.524**	**25.7 (17.6–31.9)**	**.769**
Controls	**143.9 (95.3–224.4)**		**29 (19.7–37)**	
Week 24, median (IQR)				
Vitamin D	**165.2 (126.9–214.9)**	**.354**	**23.6 (17.2–29.8)**	**.621**
Controls	**164.5 (92.4–216.7)**		**26.2 (21.7–30.4)**	
Week 48, median (IQR)				
Vitamin D	**183.5 (149.7–299.1)**	**.358**	**31.1 (20.8–38.7)**	**.055**
Controls	**190.5 (114.9–347.8)**		**35.4 (26.1–49.7)**	
Mean change from week 24 to 48				
Vitamin D	**57.3 ± 128.9**	**.206**	**4.4 ± 16.2**	**.098**
Controls	**108.4 ± 210.5**		**−0.9 ± 13.7**	

Paired *t*-test was used.

PTH, parathyroid hormone.

Discussion

Contrary to our expectations, there were no changes in BTMs or levels of PTH over the initial 24 weeks of PrEP with daily TDF-FTC and a high degree of adherence assessed by DBS TDF-DP levels. However, supporting our primary hypothesis, the addition of 4,000 IU/day of vitamin D3 at week 24 to week 48 of PrEP resulted in a significant decrease in P1NP levels compared with unsupplemented controls taking ≤400 IU/day. This suggests that vitamin D supplementation has the potential to blunt the decreases in BMD associated with daily TDF-FTC PrEP.

The fixed-dose combination of TDF-FTC has proven to be a highly effective PrEP agent.[19–21] Because PrEP involves long-term administration of drugs to generally healthy individuals, it is particularly important to monitor drug safety. The mechanism by which TDF induces BMD loss is unclear. Data suggest that TDF induces a state of functional vitamin D deficiency.[13,14] Havens *et al.* observed increased vitamin D binding protein and lower

free 1,25-OH(2)D levels in individuals with higher plasma tenofovir concentrations, which may explain TDF-associated increased PTH levels. A recent study reported that PTH-vitamin D-fibroblast growth factor 23 (FGF23), which causes phosphate wasting, is a primary contributor to TDF-associated decrease in BMD.[22,23] Moreover, the level of vitamin D binding protein has been shown to increase upon initiation of TDF-based therapy, which may be associated with bone loss.[24] Thus, when TDF-containing PrEP is used for prolonged periods of time, approaches to attenuate or prevent BMD loss are needed.

The baseline vitamin D status influences the effect of TDF on BMD, with vitamin D sufficiency showing a protective outcome.[11,25,26] The use of TDF and the level of 25-OH vitamin D level have both been shown to independently associate with PTH levels. In a study that examined the effect of vitamin D status and TDF use on PTH levels among HIV-positive patients receiving combination ART, higher PTH values were more commonly seen among TDF users with suboptimal vitamin D status compared with nonusers.[27] The underlying etiology is not completely understood; however. Bech *et al.* reported that 1-year treatment for vitamin and/or calcium deficiency improved vitamin D levels, decreased serum PTH, and improved calcium balance and BMD.[28] A recent randomized double-blind trial evaluated BTMs and BMD with supplementation of three different vitamin D3 doses in HIV-infected youth with baseline serum 25-OH vitamin D concentrations <30 ng/mL.[29] They found that high-dose vitamin D supplementation with 120,000 IU/month given over 12 months decreases BTMs. Furthermore, Havens *et al.* reported that vitamin D supplementation with 50,000 IU/month in youth aged 16–24 years with HIV taking TDF had increased lumbar spine BMD through 48 weeks, independent of baseline vitamin D status.[30]

Generally, vitamin D supplementation is well-tolerated and safe. The dose of vitamin D (4,000 IU/day) in our study carries little risk. The present U.S. Dietary Reference Intake of 2,000 IU/day is considered to be below the actual physiologic requirements.[31,32] The Institutes of Medicine guideline considered 4,000 IU/day to be the safe upper limit for daily intake for adults.[18] Several studies have demonstrated the safety of chronic daily administration of 4,000 IU of vitamin D.[31,32]

Our study has several limitations. The P1NP level was unexpectedly higher among the intervention group compared with controls at week 24, before initiation of vitamin D supplementation. It is possible that the lower P1NP levels at week 24 among the controls were because of greater vitamin D intake by this group, as compared with the individuals who agreed to vitamin D supplementation starting at week 24. However, week 24 vitamin D use, as assessed by self-report, was not different between groups. The *post hoc* analyses that tried to explore the impact of the between-group difference in week 24 P1NP levels (multivariable linear regression examining change in P1NP and the vitamin D with adjustment of week 24 P1NP and mixed model adjusting for week 24 P1NP) provided conflicting results. Nonetheless, there was a positive result in our prespecified primary analysis comparing the week 24 to 48 change in P1NP between groups.

A placebo-controlled study of vitamin D supplementation would have been optimal, as compared with using a matched concurrent control group. However, findings of this study provide the rationale for a large-scale randomized, placebo-controlled study evaluating the effect of vitamin D supplementation on BMD among TDF-FTC PrEP users. Because of difficulties and expense of performing DEXA in this clinical trial, we used biochemical BTMs in this pilot study. P1NP is a marker that is specific to bone formation and CTX is a marker specific to bone resorption. In ACTG 5280, there was a good correlation between change in BMD by DEXA and changes in BTMs, making BTMs a plausible surrogate marker of BMD changes. The percentage change of

P1NP at week 48 correlated with the percentage change in total hip BMD ($r = -0.32$, $p = .01$) and the change in lumbar spine BMD ($r = -0.37$, $p = .002$) (Overton ET, unpublished data). Measurement of circulating BTMs can be confounded by within-patient and biologic variability (age, gender, BMI, and circadian variation). In this study however, we matched for age and BMI, and all were male at birth.

Adherence to vitamin D in this study was high by self report, and measured adherence to PrEP was also high and unaffected by the daily use of vitamin D. However, we observed only a nonsignificant trend toward increased 25-OH vitamin D3 levels from week 24 to 48 with 4,000 IU/day, suggesting a possible contribution of unreported suboptimal adherence. A majority of our study participants had sufficient vitamin D levels at baseline and at week 24, thus there may have been limited room for improvement with supplementation. Sunny southern California weather may have contributed to this phenomenon. However, additional vitamin D still has the potential to provide benefit, as was observed in patients with HIV initiating TDF-containing ART in ACTG 5280, where baseline vitamin D status did not influence the effect of vitamin D supplementation on BMD and this benefit occurred at all vitamin D levels.[15] We did not observe a differential effect on P1NP levels based on baseline 25-OH vitamin D3 levels in our study.

Conclusion

Our study demonstrated that daily PrEP with Truvada in MSM did not adversely affect the BTMs P1NP and CTX, nor did it affect levels of PTH and vitamin D over the initial 24 weeks. The addition of vitamin D3 4,000 IU/day beginning at week 24 resulted in a significant decrease in P1NP levels at week 48 compared with matched controls taking ≤400 IU/day. Hence, vitamin D3 4,000 IU/day in MSM taking Truvada as PrEP should be further studied as an intervention to improve bone health in this population.

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Author Disclosure Statement

No competing financial interests exist.