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Authors

Havens, Peter L
Stephensen, Charles B
Van Loan, Marta D
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MAJOR ARTICLE

Decline in Bone Mass With Tenofovir Disoproxil Fumarate/Emtricitabine Is Associated With Hormonal Changes in the Absence of Renal Impairment When Used by HIV-Uninfected Adolescent Boys and Young Men for HIV Preexposure Prophylaxis

Peter L. Havens,[1] Charles B. Stephensen,[2] Marta D. Van Loan,[2] Gertrud U. Schuster,[3] Leslie R. Woodhouse,[2] Patricia M. Flynn,[4] Catherine M. Gordon,[5] Cynthia G. Pan,[1] Brandy Rutledge,[6] Nancy Liu,[6] Craig M. Wilson,[7] Rohan Hazra,[8] Sybil G. Hosek,[9] Peter L. Anderson,[10] Sharon M. Seifert,[10] Bill G. Kapogiannis,[8] Kathleen Mulligan[11]; the Adolescent Medicine Trials Network for HIV/AIDS Interventions 117 study team

[1]Department of Pediatrics, Medical College of Wisconsin/Children's Hospital of Wisconsin, Milwaukee; [2]US Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, and [3]Department of Nutrition, University of California, Davis; [4]Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee; [5]Department of Pediatrics, University of Cincinnati College of Medicine/Cincinnati Children's Hospital Medical Center, Ohio; [6]Westat, Rockville, Maryland; [7]Department of Epidemiology, University of Alabama at Birmingham; [8]Maternal and Pediatric Infectious Disease Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, Maryland; [9]Department of Psychiatry, Stroger Hospital of Cook County, Chicago, Illinois; [10]Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora; and [11]Department of Medicine, Division of Endocrinology, Zuckerberg San Francisco General Hospital, University of California, San Francisco.

Background. We aimed to define the relative importance of renal and endocrine changes in tenofovir disoproxil fumarate (TDF)–related bone toxicity.

Methods. In a study of daily TDF/emtricitabine (FTC) preexposure prophylaxis (PrEP) in human immunodeficiency virus (HIV)–uninfected young men who have sex with men, we measured changes from baseline in blood and urine markers of the parathyroid hormone (PTH)–vitamin D–fibroblast growth factor 23 (FGF23) axis, creatinine, and renal tubular reabsorption of phosphate (TRP). We explored the relationship of those variables to changes in bone mineral density (BMD). Tenofovir-diphosphate (TFV-DP) in red blood cells was used to categorize participants into high and low drug exposure groups.

Results. There were 101 participants, median age 20 years (range 15 to 22). Compared with low drug exposure, high-exposure participants showed increase from baseline in PTH and decline in FGF23 by study week 4, with no differences in creatinine, phosphate, or TRP. At 48 weeks, the median (interquartile range) percent decline in total hip BMD was greater in those with high- compared to low- exposure (−1.59 [2.77] vs +1.54 [3.34] %, respectively; P = .001); in high-exposure participants, this correlated with week 4 TFV-DP (inversely; r = −0.60, P = .002) and FGF23 (directly; r = 0.42; P = .039) but not other variables.

Conclusions. These findings support the short-term renal safety of TDF/FTC PrEP in HIV-seronegative young men and suggest that endocrine disruption (PTH-FGF23) is a primary contributor to TDF-associated BMD decline in this age group.

Clinical Trials Registration. NCT01769469.

Keywords. tenofovir disoproxil fumarate; bone mineral density; parathyroid hormone; fibroblast growth factor 23; HIV preexposure prophylaxis.

Tenofovir disoproxil fumarate (TDF) is widely used in human immunodeficiency virus (HIV) treatment as part of combination antiretroviral therapy (cART) and in HIV pre-exposure prophylaxis (PrEP) combined with emtricitabine (FTC) [1]. Although generally safe, TDF has been associated with renal, endocrine, and bone toxicity, including decreased glomerular filtration rate (GFR) [2, 3] and renal tubular dysfunction with renal protein, glucose, and phosphate wasting [4–6]. Elevation of parathyroid hormone (PTH) occurs early after TDF initiation [7] in both the presence [8] and absence [9] of vitamin D deficiency. TDF increases bone turnover markers, including C-terminal telopeptides (CTX) and bone alkaline phosphatase [10], and decreases dual-energy X-ray absorptiometry (DXA)–measured bone mineral density (BMD) in individuals with HIV [10, 11] and those without HIV receiving TDF/FTC for PrEP [12].

The relative importance of renal or endocrine change as the mechanism of TDF-associated bone toxicity is unclear. Renal tubular damage [13] with phosphate wasting [14] may be a key factor. A primary role for disruption of vitamin D and calcium signaling is suggested because vitamin D supplementation improves TDF-associated PTH elevations [9, 15], and vitamin

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Correspondence: P. L. Havens, Suite C450, P.O. Box 1997, Milwaukee, WI 53201-1997 (phavens@mcw.edu).

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D plus calcium supplementation lessens the BMD decline associated with initiation of TDF-containing cART [16]. TDF use in cART [17], specifically higher plasma concentrations of tenofovir (TFV) [18], are associated with increased vitamin D binding protein (VDBP), which may lead to decreased calculated free 1,25 dihydroxyvitamin D (1,25-OH(2)D) [19], the metabolically active form of vitamin D, supporting a metabolic, not renal, mechanism for TDF-associated bone toxicity.

Because HIV infection [20] and cART initiation in persons with HIV infection [21] can cause BMD losses, study of the mechanism of TDF-associated bone toxicity is best undertaken in HIV-uninfected persons using TDF/FTC for PrEP, with drug effects measured in the absence of HIV or other ART.

We report metabolic data collected from a subset of participants in 2 Adolescent Medicine Trials Network for HIV/AIDS Intervention (ATN) demonstration and safety studies of open-label TDF/FTC PrEP in high-risk young men who have sex with men (YMSM) [22, 23]. We focused the analysis on the magnitude and timing of change in renal, endocrine, and bone turnover markers and their relationship with cumulative TDF exposure and BMD decline. Our aim in this study was to characterize the relative roles of renal (glomerular and tubular) vs endocrine (calcium–phosphate–vitamin D metabolism) changes in TDF-associated bone toxicity.

METHODS

Overview

ATN 117 was a substudy of ATN 113 [22] and ATN 110 [23], which enrolled participants between December 2012 and October 2014 at 12 ATN clinical trials sites in the United States. HIV-uninfected YMSM, born male, enrolled in the parent studies. There were no exclusions for substudy participation. The older age cohort (ATN 110; ages 18–22 years) enrolled faster than ATN 113 (ages 15–17 years), so we limited enrollment from ATN 110 to ensure inclusion of younger participants in this study. The study was approved by participating centers' local institutional review board and required participants' written consent prior to enrollment.

Parent Studies: Visits and Data

All participants were provided with coformulated TDF/FTC (Truvada) and advised to take 1 tablet by mouth daily. Study visits occurred at baseline and weeks 4, 8, 12, 24, 36, and 48. Dried blood spot specimens to quantify red blood cell tenofovir diphosphate (TFV-DP) concentrations (T1/2 = 17 days) [24] were collected at each follow-up visit. Spine, hip, and whole body DXA scans were performed at baseline and weeks 24 and 48. With few exceptions, participants were scanned on the same instrument throughout the study. Four sites had GE/Lunar scanners (Madison, Wisconsin), and 8 sites had Hologic devices (Waltham, Massachusetts). A standard phantom was scanned on each DXA instrument. Machine-generated *z* scores were used. All DXA scans were analyzed centrally at Tufts University.

Substudy Data

Urine and blood samples were collected at each study visit after a minimum 8-hour fast. We attempted to have all study visits in the morning. When this was not possible, we scheduled repeat visits for the same time of day. Serum samples were collected for measurement of PTH, fibroblast growth factor 23 (FGF23), VDBP, 25-hydroxyvitamin D (25-OHD), 1,25-OH(2)D), C-terminal telopeptides (CTX), osteocalcin, calcium, creatinine, phosphate, and albumin. Spot urine samples for creatinine, calcium, phosphate, glucose, protein, and retinol binding protein were collected upon arrival for the study visit. Spot urine samples for urine beta-2 microglobulin were collected approximately 1 hour after the first urine collection, after participants drank 8–12 ounces of water. All samples were initially processed at the study sites, then sent frozen for batch analysis of stored samples at the US Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, Davis, California.

Dietary or supplemental intake of calcium, vitamin D, phosphate, and total energy were assessed at baseline and week 48 (Block Dietary Systems Food Frequency Questionnaire [25], Nutritionquest, Berkeley, California). Information on exercise frequency, tobacco and alcohol use, and attempts to lose weight was collected by questionnaire at baseline and week 48.

Laboratory Variables

See Supplemental Material for specific laboratory methods.

Drug Exposure Categorization

Using the trapezoidal rule [26] for participants with at least 12 weeks of data, we calculated the area under the curve of TFV-DP concentration for each participant for the duration of the study and then divided that by the number of days to the last measurement to generate a measure of mean overall drug exposure. We divided participants into tertiles based on mean overall drug exposure. Once participants were assigned into these tertiles of mean overall drug exposure, their individual TFV-DP values for each study visit were identified as the visit-specific drug exposure.

Statistical Analyses

For each renal, metabolic, bone turnover, and DXA-measured variable, the change from baseline to each study visit was calculated. A Wilcoxon signed rank test was used to test for statistical significance of the changes from baseline. A Wilcoxon rank sum test was used to test for differences between high and low categories of visit-specific drug exposure and overall drug exposure. Spearman and Pearson correlations were used to identify the association of renal, metabolic, and bone turnover variables

Table 1. Baseline Characteristics of the Study Population

Characteristic[a]	Value
Number	101
ATN 110 (ages 18–22 y)	88
ATN 113 (ages 15–17 y)	13
Age, y	
Mean (SD)	19.6 (1.8)
Median (range)	20 (15–22)
Sexual maturity rating (Tanner stage),[b] N (%)	
3	2 (2)
4	10 (10)
5	89 (88)
Race, N (%)	
Black/African American	52 (51.5)
Non-black/African American	49 (48.5)
Ethnicity, N (%)	
Hispanic	34 (33.7)
BMI, kg/m^2	
Mean (SD)	24.6 (5.6)
Median (range)	22.7 (17.4–49.1)
BMI, N (%)	
Underweight (<18.5 kg/m^2)	5 (5.0)
Normal (18–25 kg/m^2)	59 (58.4)
Overweight (25–<30 kg/m^2)	23 (22.8)
Obese (≥30 kg/m^2)	14 (13.9)
Vitamin D serum concentration (25-OHD), ng/mL	
Mean (SD)	18.2 (7.5)
Median (range)	18.4 (3.2–40.3)
Serum vitamin D concentration category N, %[c]	
Deficient (<12 ng/mL)	24 (23.8)
Inadequate (12–<20 ng/mL)	35 (34.7)
Adequate (≥20–50 ng/mL)	42 (41.6)
Lifestyle, N (%)	
Exercise ≥ once weekly	76 (75.3)
Trying to lose weight	39 (39.0)
Smoke cigarettes	33 (32.7)
Drink alcohol	38 (37.6)
Calcium intake, mg/day[d]	
Mean (SD)	1438 (1131)
Median (range)	1250 (278–8607)
Inadequate daily calcium intake for age, N (%)[d]	52 (51.5)
Vitamin D intake, IU/day	
Mean (SD)	262 (298)
Median (range)	161 (11–1884)
Daily vitamin D intake, N (%)[e]	
<300 IU	72 (71.3)
300–600 IU	19 (18.8)
>600 IU	10 (9.9)
Phosphorus intake, mg/day[f]	
Mean (SD)	2205 (2015)
Median (range)	1693 (354–16 710)
Season enrolled, N (%)	
Winter	18 (18)
Spring	60 (59)
Summer	13 (13)
Fall	10 (10)

with each other and with DXA-measured variables. To identify early biomarkers of BMD change, we measured correlation of week 4 variables to BMD change from baseline to week 48. In the text, the correlation (Pearson or Spearman) with the more strongly significant *P* value is presented. Data are reported as mean (standard deviation [SD]) or median (interquartile range [IQR]) except as noted.

RESULTS

There were 101 participants, mean (SD) age 19.6 (1.8) years; 52 (51.5%) were African Americans and 34 (33.7%) Hispanic (Table 1). At baseline 36.7% were overweight or obese (body mass index >25 kg/m^2); 88% were sexual maturity rating (Tanner stage) 5. The majority had deficient or inadequate vitamin D status and inadequate intake of both calcium and vitamin D by Institute of Medicine standards [27] (Table 1). More participants enrolled in winter (18%) or spring (59%; $P < .001$). Mean change in height from baseline to week 48 was 0.14 (0.90) cm, with no difference between the younger ATN 113 (0.31 [2.01] cm) and the older ATN 110 cohort (0.12 [0.64] cm; $P = .085$). During the study 2 participants were diagnosed with HIV infection and were discontinued from this substudy.

Drug Exposure

The overall TFV-DP mean concentration was 646 (465) fmol/punch, which in adult MSM at steady state (approximately week 8 and beyond [28]) is consistent with use of 2–3 tablets of TDF/FTC per week on average [1] (Table 2). In the low-exposure tertile, TFV-DP was below the limit of quantitation in 50% of participants at week 24 and 89% at week 48, suggesting minimal usage of PrEP. Race was the only baseline variable to differ by drug exposure tertile: black/African Americans were 74%, 62%, and 33% and non-black/African Americans were 26%, 38%, and 67% of the low, moderate, and high drug exposure groups, respectively ($P = .009$). The categorization into high (>861 fmol/punch), moderate (≤861 to ≥345 fmol/punch), and

Abbreviations: ATN, Adolescent Medicine Trials Network for HIV/AIDS Intervention; BMI, body mass index; SD, standard deviation.

[a]These characteristics, which might affect vitamin D status, concentration of the other variables measured, and bone mass, had similar values in the high and low drug exposure groups, except for race, as discussed in the text. While median age did not differ across all 3 drug exposure tertiles, the median (range) age in the low drug exposure group was lower than in the high drug exposure group (19 [15–22] vs 21 [16–22] years), respectively ($P = .045$).

[b]Sexual maturity rating (Tanner stage) was determined by physical exam or self-assessment. Only 2 of the 101 participants were in Tanner stage 3, and Tanner stage did not differ by low vs high drug exposure tertile. Tanner stages 3, 4, and 5 were 0%, 8%, and 92% and 4%, 13%, and 83% of the low and high drug exposure groups, respectively ($P = .494$).

[c]Vitamin D status was categorized as deficient (25-OHD <12 ng/mL [<30 nmol/L]), insufficient (25-OHD 12–20 ng/mL [30–50 nmol/L]), sufficient (25-OHD >20-50 ng/mL [50-125 nmol/L], and excess (25-OHD >50 ng/mL [>125 nmol/L]) [27].

[d]Total calcium intake was categorized as inadequate if <1300 mg (age ≤18 years) or <1000 mg (age ≥19 years) [27].

[e]The recommended daily intake of vitamin D is 600 IU [27].

[f]The recommended daily intake of phosphorus for boys age 14–18 years is 1250 mg/day and for men 19–30 years is 700 mg/day (https://www.ncbi.nlm.nih.gov/books/NBK109813/; accessed 9/30/2016).

Table 2. Drug Exposure Categories During 48 Weeks of Study

Overall Drug Exposure Tertile[a]	Variable	Study Week					
		4	8	12	24	36	48
High (TFV-DP >861 fmol/punch), N = 27	TFV-DP median (fmol/punch)[b]	878	1121	1318	1329	1231	1102
	TFV-DP range	328–1588	539–2147	381–2294	807–2621	213–1930	163–2771
	Total number	26	26	27	27	24	22
	Visit-specific drug exposure category (N)[c]						
	High	15	23	25	26	21	17
	Moderate	10	3	2	1	2	4
	Low	1	0	0	0	1	1
Moderate (TFV-DP 345–861 fmol/punch), N = 26	TFV-DP median (fmol/punch)	653	742	841	530	436	368
	TFV-DP range	261–1096	250–1181	92–1281	21–1133	BLQ–917	BLQ–1224
	Total number	26	26	26	26	24	22
	Visit-specific drug exposure category (N)						
	High	5	7	12	5	1	2
	Moderate	20	17	13	15	13	10
	Low	1	2	1	6	10	10
Low (TFV-DP <345 fmol/punch), N = 27	TFV-DP median (fmol/punch)	385	436	208	25	13	13
	TFV-DP range	BLQ–865	BLQ–1076	BLQ–1312	BLQ–304	BLQ–429	BLQ–173
	Total number	26	26	26	26	21	18
	Visit specific drug exposure category (N)						
	High	2	1	2	0	0	0
	Moderate	12	15	9	0	1	0
	Low	12	10	15	26	20	18

There were 81 participants with at least 12 weeks of drug concentration data, but 1 was excluded from further analysis because of lack of adequate follow-up data.

Abbreviations: BLQ, below the limit of quantitation of the assay used; TFV-DP, dried blood spot red blood cell tenofovir diphosphate concentration.

[a]Overall drug exposure tertiles were assigned based on the mean red blood cell TFV-DP for the 48 weeks of study.

[b]Values shown are median and range TFV-DP concentration at each study week (the visit-specific drug exposure) for each category of overall drug exposure. For comparison, in adult men who have sex with men at steady-state (approximately week 8 and beyond), Grant et al [1] estimated median steady-state TFV-DP ≥1250 fmol/punch for persons taking 7 doses/week; 700–1249 fmol/punch (4–6 doses/week); 350–699 fmol/punch (2–3 doses/week), and <349 fmol/punch (<2 doses/week). Drug concentrations were closely associated with adherence in ATN 110 [23] and other PrEP studies [1]. Dried blood spots were used because of their proven comparability to venous samples [24] and their ease of use regarding storage and shipping.

[c]Visit-specific drug exposure category was assigned using the visit-specific TFV-DP concentration and the overall drug exposure categories. Highlighted cells show, for each study week, the number of participants with TFV-DP concentration measured at that visit for whom the visit-specific concentration category was the same as their overall drug exposure category.

low (<345 fmol/punch) categories based on overall drug exposure did not fully capture the variability in adherence over time. For example, at study week 12, of the 26 participants categorized as having "moderate" overall drug exposure, 12 had "high" visit-specific drug exposure at that visit (TFV-DP >861 fmol/punch; Table 2).

Bone Mass, Renal Function, and Endocrine Changes

BMD and/or BMD *z* scores declined during the study, with statistically significant changes from baseline in total hip BMD and in lumbar spine (L1-L4), total hip, and total body BMD *z* scores at both 24 and 48 weeks (Table 3). Two participants sustained fractures, both after trauma.

While serum creatinine rose slightly during the first 12 weeks, Estimated glomerular filtration rate (eGFR) did not decline significantly at any time point. There were no significant changes in any other measure of renal toxicity (Table 3).

FGF23 decreased at most measured time points (Table 3). This decrease was not accompanied by a statistically significant change in serum phosphate or renal phosphate excretion. Serum 25-OHD and free 1,25-OH(2)D increased significantly from week 4 through week 12. There were no statistically significant changes in intake of vitamin D, calcium, or energy or in exercise or tobacco or alcohol use (data not shown). Serum CTX, a marker of bone resorption, increased from baseline at weeks 8 and 12, but osteocalcin, a marker of bone turnover, did not change (Table 3).

Effects of Drug Exposure

Compared to the low drug exposure group, the high drug exposure group exhibited a PTH increase from baseline and an FGF23 decline from baseline, present by week 4 and statistically significant by week 8 (Table 3). These endocrine changes occurred in the absence of change in serum creatinine, eGFR, or markers of renal tubular damage. Changes in 25-OHD and free 1,25-OH(2)D did not differ by drug exposure. Osteocalcin increased in the high drug exposure but not the low exposure group, while CTX showed no change by drug exposure category.

Compared to the low drug exposure group, those with high drug exposure had progressive declines in BMD (% difference from the baseline value) and BMD *z* score in the total hip and femoral neck. At week 48 the median (IQR) decline was

Table 3. Baseline and Changes in Metabolic and Dual-Energy X-Ray Absorptiometry Measurements in the High Drug Exposure Group Compared to the Low Drug Exposure Group During 48 Weeks of Study

Variable[a]	Drug Exposure Category	Value at Baseline[b]	Change from Baseline at Study Week[c]					
			4	8	12	24	36	48
Serum parathyroid hormone (pg/mL)	Overall	27.2 (12.1)	0.9 (13.2)[c]	1.0 (9.4)	1.2 (8.9)	1.3 (13.3)	2.7 (15.0)†d	2.1 (11.4)
	High	**25.7 (6.7)**	**4.4 (11.0)**	**2.2 (9.8)**	**3.3 (5.4)**^**	**0.5 (15.5)**	**2.6 (19.3)**	**–0.1 (12.5)**
	Low	**29.4 (15.1)**	**–0.8 (12.4)**	**–0.6 (9.6)**	**–1.0 (12.2)**	**0.5 (13.3)**	**2.9 (9.5)**	**3.6 (11.3)**
Serum fibroblast growth factor 23 (pg/mL)	**Overall**	**38.2 (12.1)**	**–.3 (14.5)**	**0.6 (16.7)**	**–3.4 (13.5) †**	**–3.1 (12.2) †**	**–3.1 (14.8) † †**	**–1.0 (14.6)**
	High	**41.9 (10.3)**	**–1.4 (14.6)**	**–5.6 (15.3)***	**–4.2 (13.9)**	**–6.9 (11.0)^**	**–7.1 (12.6)***	**1.7 (15.7)**
	Low	**35.8 (11.9)**	**1.8 (11.9)**	**3.4 (13.2)**	**0.2 (16.5)**	**0.8 (10.3)**	**–0.9 (16.0)**	**0.6 (10.5)**
Serum vitamin D binding protein (μmol/L)	Overall	2.97 (3.14)	–0.03 (0.27)	–0.05 (0.27)	–0.00 (0.36)	0.03 (0.32)	–0.01 (0.35)	–0.00 (0.28)
	High	3.46 (3.22)	0.05 (0.37)	–0.05 (0.41)	–0.04 (0.38)	–0.02 (0.33)	–0.02 (0.30)	–0.01 (0.46)
	Low	1.98 (2.74)	–0.09 (0.26)	–0.03 (0.26)	0.02 (0.30)	0.01 (0.38)	0.01 (0.39)	0.08 (0.30)
Serum 25-OHD (ng/mL)	**Overall**	**17.7 (11.0)**	**1.5 (5.6) ††**	**2.7 (5.7) ††**	**2.9 (8.5) ††**	**1.9 (8.1) †**	**–0.1 (8.1)**	**0.3 (5.9)**
	High	19.1 (10.06)	2.0 (5.1)	1.6 (8.3)	3.8 (7.4)	3.2 (10.2)	0.0 (10.1)	0.7 (5.9)
	Low	16.7 (12.2)	1.3 (5.6)	3.2 (3.7)	2.4 (8.5)	0.5 (6.8)	–1.6 (5.7)	1.2 (4.7)
Serum 1,25-OH(2)D (pmol/L)	Overall	179.2 (81.5)	14.2 (75.8)	11.2 (76.1)	15.3 (82.9) †	7.3 (72.1)	–3.6 (72.6)	–13.1 (54.8)
	High	170.9 (78.4)	4.8 (94.9)	3.6 (81.5)	19.8 (94.5)	10.8 (94.1)	1.9 (50.0)	–12.6 (32.6)
	Low	182.6 (105.6)	16.4 (76.1)	14.0 (73.3)	19.3 (98.1)	7.1 (70.7)	–9.4 (77.9)	–8.6 (79.5)
Serum free 1,25-OH(2)D (fmol/L)[d]	**Overall**	**1102 (1182)**	**85 (546)**	**92 (405) †**	**80 (413) †**	**38 (475)**	**–53 (461)**	**–73 (456)**
	High	1101 (1263)	10 (704)	110 (571)	188 (815)	51 (471)	–20 (299)	–86 (304)
	Low	1285 (1530)	116 (669)	74 (373)	55 (460)	48 (531)	–80 (460)	–73 (630)
Urine glucose (mg/dL)	Overall	0.07 (0.05)	–0.00 (0.06)	–0.00 (0.05)	–0.01 (0.05)	0.01 (0.07)	0.00 (0.06)	–0.01 (0.05)
	High	0.07 (0.06)	–0.01 (0.07)	–0.01 (0.05)	–0.01 (0.07)	–0.02 (0.06)	0.01 (0.07)	–0.01 (0.04)
	Low	0.07 (0.04)	0.01 (0.06)	0.01 (0.05)	0.00 (0.05)	0.01 (0.08)	0.02 (0.08)	0.02 (0.04)
Urine retinol binding protein to creatinine ratio (μg/g)	Overall	7312 (7818)	–1029 (8247)	–845 (6630)	–672 (8200)	153 (8594)	–1242 (7130)	–678 (7267)
	High	6740 (10426)	–1137 (7460)	–1898 (8239)	–2727 (12280)	2282 (12301)	–680 (5934)	–711 (6189)
	Low	6385 (7388)	327 (4877)	266 (5542)	–417 (4873)	809 (10171)	1273 (6989)	1606 (7267)
Urine beta-2 microglobulin (ng/mL)	Overall	165.0 (290.0)	17.0 (271.3)	–2.4 (242.6)	4.9 (280.4)	–14.6 (364.2)	–16.7 (263.6)	–40.1 (248.8)
	High	129.2 (204.0)	–11.7 (389.0)	1.84 (208.4)	4.9 (234.9)	–7.3 (281.2)	59.7 (268.0)	–0.2 (288.2)
	Low	207.0 (315.9)	42.0 (326.7)	–11.2 (241.8)	–40.6 (260.2)	–23.3 (379.0)	–35.3 (253.5)	–79.8 (221.0)
Urine protein to creatinine ratio (mg/mg)	Overall	0.05 (0.02)	–0.00 (0.02)	–0.00 (0.02)	0.00 (0.02)	0.00 (0.02)	0.00 (0.02)	0.00 (0.01)
	High	0.05 (0.03)	–0.00 (0.02)	–0.00 (0.02)	0.00 (0.03)	–0.00 (0.03)	0.01 (0.02)	0.00 (0.02)
	Low	0.05 (0.02)	–0.00 (0.02)	0.00 (0.02)	–0.00 (0.01)	0.00 (0.01)	–0.00 (0.02)	–0.00 (0.01)
Serum creatinine (mg/dL)	**Overall**	**0.87 (0.18)**	**0.03 (0.08) ††**	**0.02 0.08) ††**	**0.02 (0.08) ††**	**0.01 (0.07)**	**–0.00 (0.11)**	**–0.01 (0.13)**
	High	0.84 (0.19)	0.03 (0.06)	0.02 (0.08)	0.02 (0.08)^	0.02 (0.08)	0.01 (0.12)	0.01 (0.15)
	Low	0.89 (0.17)	0.01 (0.10)	0.01 (0.09)	0.00 (0.06)	–0.00 (0.07)	–0.04 (0.08)	–0.02 (0.11)
Estimated glomerular filtration rate (mL/min)	Overall	129 (43)	0 (18)	–3 (21)	–1 (24)	–1 (26)	2 (27)	3 (34)
	High	127 (49)	1 (10)	0 (14)	–2 (15)	–4 (21)	–2 (22)	–0 (33)
	Low	134 (42)	1 (17)	–4 (21)	–0 (31)	5 (28)	10 (20)	–0 (40)
Urine calcium to creatinine ratio (mg/mg)	Overall	0.05 (0.05)	–0.00 (0.04)	0.00 (0.06)	–0.00 (0.05)	0.00 (0.05)	–0.00 (0.05)	0.00 (0.05)
	High	0.05 (0.05)	–0.01 (0.04)	–0.01 (0.04)^	–0.00 (0.05)	0.00 (0.06)	–0.02 (0.05)	–0.00 (0.05)
	Low	0.06 (0.07)	0.00 (0.05)	0.02 (0.09)	0.00 (0.06)	–0.01 (0.06)	–0.01 (0.06)	–0.00 (0.07)
Tubular reabsorption of phosphate (%)	Overall	91 (8)	–0 (6)	–0 (8)	1 (7)	1 (7)	1 (6)	–1 (6)
	High	93 (7)	–1 (7)	1 (5)	0 (6)	1 (7)	2 (6)	0 (6)
	Low	91 (9)	–1 (6)	–1 (8)	2 (6)	1 (10)	–0 (6)	–1 (6)
Serum calcium (mg/dL)	Overall	9.8 (0.3)	0.0 (0.5)	–0.0 (0.4)	–0.0 (0.4)	0.0 (0.5)	0.0 (0.6)	–0.1 (0.6)
	High	9.8 (0.5)	0.0 (0.5)*^	–0.0 (0.5)	0.0 (0.3)	0.1 (0.4)	0.2 (0.7)	–0.0 (0.6)
	Low	9.8 (0.3)	–0.1 (0.54)	–0.02 (0.6)	–0.0 (0.5)	–0.1 (0.6)	0.0 (0.6)	0.1 (0.8)
Serum phosphate (mmol/L)	Overall	1.15 (0.18)	0.00 (0.25)	0.03 (0.20)	–0.01 (0.22)	0.01 (0.22)	–0.01 (0.25)	–0.00 (0.19)
	High	1.11 (0.21)	–0.02 (0.27)	–0.00 (0.23)	0.01 (0.33)	0.02 (0.27)	0.04 (0.28)	–0.00 (0.24)
	Low	1.18 (0.16)	0.01 (0.17)	0.05 (0.20)	–0.03 (0.13)	0.01 (0.26)	–0.05 (0.25)	0.01 (0.24)
Serum albumin (g/dL)	Overall	4.6 (0.3)	–0.0 (0.4)	–0.0 (0.4)	0.0 (0.3)	–0.1 (0.3)	–0.0 (0.4)	–0.1 (0.4)
	High	4.7 (0.4)	–0.1 (0.4)^^	–0.1 (0.4)	–0.0 (0.3)	0.0 (0.3)	–0.1 (0.4)	–0.0 (0.4)
	Low	4.6 (0.1)	–0.1 (0.4)	–0.0 (0.4)	0.0 (0.4)	–0.1 (0.5)	0.1 (0.6)	0.1 (0.5)
Serum osteocalcin (μg/L)	Overall	9.38 (4.00)	0.01 (1.90)	0.13 (2.26)	–0.04 (2.56)	–0.17 (2.72)	0.13 (2.89)	0.03 (2.95)
	High	**9.80 (2.87)**	**0.29 (1.80)**	**0.44 (3.16)**	**0.40 (1.94)**	**0.87 (3.54)***	**0.62 (3.72)^**	**1.12 (2.95)**
	Low	**9.44 (3.92)**	**–0.20 (1.68)**	**0.22 (2.09)**	**–0.23 (2.32)**	**–1.03 (3.20)**	**0.03 (2.59)**	**–0.10 (3.15)**

Table 3. Continued

Variable[a]	Drug Exposure Category	Value at Baseline[b]	Change from Baseline at Study Week[c]					
			4	8	12	24	36	48
Serum C-teminal telopeptides (pM)	**Overall**	**937 (488)**	**37 (541)**	**123 (510) †**	**147 (503) †**	**51 (450)**	**5 (473)**	**18 (485)**
	High	1028 (589)	53 (410)	80 (313)	222 (420)	106 (443)	104 (456)^	51 (412)
	Low	937 (585)	38 (540)	113 (436)	57 (506)	–26 (439)	83 (610)	–21 (533)
Lumbar spine (L1-L4) BMD (g/cm^2)[e]	Overall	1.09 (0.18)				–0.09 (2.91)%		0.40 (3.26)%
	High	1.04 (0.15)				–0.33 (3.61)%		–0.32 (2.65)%
	Low	1.12 (0.19)				0.00 (2.63)%		0.88 (4.35)%
Lumbar spine (L1-L4) BMD z score[f]	**Overall**	**–0.4 (1.6)**				**–0.1 (0.3) ††**		**–0.1 (0.3) ††**
	High	–0.7 (1.3)				–0.1 (0.3)		–0.2 (0.3)
	Low	–0.2 (1.9)				0.0 (0.3)		–0.1 (0.5)
Femoral neck BMD (g/cm^2)	Overall	1.03 (0.23)				–0.42 (3.68)%		0.10 (4.98)%
	High	**0.97 (0.21)**				**–0.80 (3.91)%***		**–1.89 (5.09)%***
	Low	**1.03 (0.26)**				**0.62 (2.41)***		**1.14 (3.85)%**
Femoral neck BMD z score	**Overall**	**–0.4 (1.4)**				**0.0 (0.3) †**		**0.0 (0.4)**
	High	**–0.5 (1.1)**				**–0.1 (0.2)*^**		**–0.1 (0.3)**
	Low	**–0.3 (1.4)**				**0.0 (0.1)**		**0.1 (0.3)**
Total hip BMD (g/cm^2)	**Overall**	**1.07 (0.19)**				**–0.51 (2.48)% †**		**–1.00 (3.86)% ††**
	High	**1.04 (0.18)**				**–0.79 (2.81)%**		**–1.59 (2.77)%****
	Low	**1.09 (0.23)**				**0.18 (1.85)**		**1.54 (3.34)**
Total Hip BMD z score	**Overall**	**–0.5 (1.2)**				**0.0 (0.3) †**		**–0.1 (0.3) ††**
	High	**–0.3 (0.9)**				**–0.1 (0.2)**		**–0.1 (0.3)***
	Low	**–0.3 (1.4)**				**0.0 (0.2)**		**0.1 (0.4)**
Total body BMD (g/cm^2)	Overall	1.19 (0.16)				–0.35 (2.59)%		–0.39 (2.68)%
	High	1.15 (0.17)				–0.16 (3.19)%		–0.76 (2.68)%
	Low	1.20 (0.15)				–0.46 (1.67)%		–0.43 (2.70)%
Total body BMD z score	**Overall**	**–0.4 (1.3)**				**–0.1 (0.4) ††**		**–0.1 (0.3) ††**
	High	–0.4 (1.5)				0.0 (0.3)		–0.2 (0.25)
	Low	–0.1 (1.4)				–0.1 (0.3)		–0.2 (0.4)
Total body bone mineral content (g)	Overall	2688 (642)				–0.26 (2.47)%		–0.41 (3.01)%
	High	2611 (588)				–0.40 (2.41)%		–0.6 (3.23)%
	Low	2829 (803)				–0.24 (2.78)%		–1.24 (3.35)%
Body weight (kg)	Overall	69.4 (19.0)	0.0 (1.8)	–0.1 (2.3)	–0.4 (3.2)	–0.3 (3.0)	–0.4 (4.0)	0.1 (3.9)
	High	70.3 (27.0)	–0.6 (2.6)	–0.4 (2.0)	–0.9 (4.7)	–1.4 (4.5)*	–0.4 (4.0)	0.5 (3.4)
	Low	68.5 (21.7)	0.0 (1.3)	0.0 (2.3)	–0.4 (1.9)	–0.2 (2.7)	–0.4 (4.8)	–0.3 (7.3)

For the 80 participants with at least 12 weeks of drug concentration data and complete DXA data at baseline and week 48.

Abbreviations: 1,25-OH(2)D, 1,25 dihydroxyvitamin D; 25-OHD, 25-hydroxyvitamin D; BMD, bone mineral density.

[a]Rows in bold met the following criteria: for variables measured at every visit, either at least 2 *P* values ≤ .05 or at least 1 *P* value ≤ .01. Dual-energy X-ray absorptiometry (DXA) variables are in bold if they had *P* ≤ .05 at least once. For the overall group, this refers to the *P* value for the change from baseline to the study week identified by each column. For the high and low drug exposure groups, this refers to the *P* value for the difference between the high and low drug exposure groups, as defined in Table 2 and in the text. The variable names are in bold only if the *P* value criteria were met for the difference between high and low drug exposure groups.

[b]Cells labelled baseline show median (interquartile range [IQR]) of the value at the baseline visit. IQR is Q3-Q1.

[c]Cells in study week columns show change from baseline as median (IQR).

[d]Calculated variables included in the table are as follows: serum-free 1,25-OH(2)D was calculated from serum albumin, vitamin D binding protein (VDBP), and 1,25-OH(2)D by the method of Bikle [19]. Free 1,25-OH(2)D concentration decreases with increases in VDBP and albumin. Estimated glomerular filtration rate was calculated by the Cockcroft-Gault formula [29] for participants age >18 years and by the bedside Schwartz formula [30] for those age <18 years. The urine calcium/creatinine (UCa/UCr) ratio was used to estimate urinary calcium excretion, with normal being <0.21 mg/mg. Tubular reabsorption of phosphate was calculated as (1– [(UPhos × SCr)/(SPhos × UCr)]) × 100.

[e]BMD at baseline has the units gm/cm^2 and bone mineral content has the units gm. Changes in BMD and bone mineral content from baseline to weeks 24 and 48 are given in percent.

[f]BMD z score is reported as the number of standard deviations away from the mean BMD value in comparison to an age-, sex-, and race-matched population. Changes in BMD z score are given as standard deviation units, not percentages.

† *P* value ≤ 0.05, †† P value ≤ 0.01: Wilcoxon signed rank test for difference from baseline to study week identified by the column heading (overall group only).

* *P* value ≤ .05, ** *P* value ≤ .01: Wilcoxon rank sum test for differences between high and low overall drug exposure categories.

^ *P* value ≤ .05, ^^ *P* value ≤ .01: Wilcoxon rank sum test for differences between high and low visit specific drug exposure categories.

greatest in total hip BMD in the high drug exposure group compared to the low drug exposure group (−1.59 [2.77] vs +1.54% [3.34], respectively; *P* = .001; Table 3). Spine BMD changes followed a similar pattern but did not achieve statistical significance (*P* = .19 at week 48). None of the indices of renal function (urine glucose, RBP, B2MG, protein/creatinine or calcium/creatinine ratio; serum creatinine, calcium, or phosphate; or calculated eGFR or TRP) differed by drug exposure category.

Correlations

In the group as a whole, change in total hip BMD from baseline to week 48 negatively correlated with drug concentration at weeks 12, 24, and 48. Change in spine BMD at week 48 negatively correlated with drug concentration only at week 12. In the analysis of the high drug exposure group for correlations of week 4 variables with changes from baseline to week 48, change in total hip BMD at week 48 correlated inversely with week 4 TFV-DP (r = −0.61, P = .002, Spearman) and positively with FGF23 (r = 0.42, P = .039, Spearman) but not with any other renal or metabolic variables tested, including osteocalcin. For spine BMD change at week 48, there was no association with drug exposure but there was a negative correlation with week 4 urine RBP/creatinine (r = −0.43, P = .039, Pearson) and a positive correlation with week 4 urine calcium/creatinine (r = +0.49, P = .015, Spearman).

DISCUSSION

In this study of adolescent boys and young men, biochemical markers of calcium (PTH), phosphate (FGF23), and bone turnover (osteocalcin) showed greater and more consistent change than markers of renal glomerular or tubular dysfunction over 48 weeks of TDF/FTC PrEP when comparing participants with high and low drug exposure measured by red blood cell TFV-DP concentrations. In those with high drug exposure, only drug concentration and FGF23, but none of the other measured renal or metabolic variables, were significantly correlated with change from baseline to week 48 in total hip BMD, the DXA measurement that showed the greatest effect of TDF exposure in this group.

There was no apparent effect of TDF/FTC exposure on any marker of glomerular or tubular function, even though there was a strong effect of drug exposure on BMD. This lack of renal effect could result from the high baseline eGFR in this young study group. In HIV-infected persons, older age, pre-existing renal dysfunction [6, 31, 32] and TFV concentration or exposure [18, 33] are most closely associated with eGFR decline during TDF treatment. The absence of renal effects may also be from the relatively short treatment period, as duration of exposure to TDF was associated with proteinuria in perinatally HIV-infected children and adolescents [5]. Longer exposure may be required to reveal a relationship between tubular impairment and bone loss [13].

We speculate that the relatively small, albeit significant, increase in average 25-OHD concentrations in the early phase of the study occurred because the majority of participants enrolled in winter and spring, when their vitamin D stores were low, and continued into the spring and summer, when vitamin D concentrations rose in response to sun exposure. This "background effect" of rising 25-OHD may be reflected in the increase in total hip and femoral neck BMD and BMD z scores seen in participants with low drug exposure. However, these increases in BMD are also consistent with bone growth, which would be expected in this age group [34].

The ability of TDF to decrease or impair accrual of BMD in this young study cohort is demonstrated by the greater than 3% difference in change in total hip BMD from baseline to week 48 between high and low drug exposure groups. There was a smaller effect on spine BMD. However, spine BMD z score declined, suggesting that the expected bone accrual was not occurring. This pattern of BMD change suggests a stronger effect of TDF on cortical bone (the hip is a predominantly cortical site) and a lesser effect on trabecular bone (predominant in the spine) in this group that included adolescent males as young as age 15 years. Use of TDF PrEP may be a particular risk for the younger men studied, since adolescence is a critical period for attainment of peak bone mass [35]. The density of cortical bone is lower among children and adolescents than adults and may even go through a transient period of increased porosity, particularly in boys [36]. Therefore, the striking cortical deficits in these young men raise concern and warrant follow-up to see if these deficits persist or reverse with age or discontinuation of PrEP.

There was a decrease in FGF23 in the high drug exposure group and an increase in FGF23 in the low drug exposure group. In the high drug exposure group, the change in total hip BMD was correlated with week 4 FGF23 but not with other endocrine or renal measurements. The TDF-associated fall in FGF23 occurred in the absence of a decline in serum phosphate or increase in renal phosphate excretion, which would be expected since the main function of FGF23 is to increase phosphate excretion in the presence of hyperphosphatemia [37]. Low FGF23 has been identified in persons with HIV treated with TDF [38], and low FGF23 has been specifically associated with high concentrations of intracellular TFV-DP [18]. However, the exact role that FGF23 might play independent of vitamin D deficiency in TDF-associated bone disease is unclear [15, 39].

PTH increased in the high drug exposure group, consistent with TDF use in other studies [7, 8]. It is possible that this change was not seen in the overall group because of the increase in 25-OHD, which would lead to a PTH decrease. The fact that increased PTH was still seen in the high compared to the low exposure group shows the potency of TDF to cause this endocrine change, in spite of an overall increase in 25-OHD. This increase could result from a TDF-induced decrease in FGF23, since FGF23 directly suppresses PTH production in an animal model [40], though this relationship seems more complex in clinically relevant situations in humans [37].

We did not find the VDBP increase that has been seen with TDF initiation in persons with HIV [17]. Increased VDBP, associated with decreased free 1,25-OHD, suggests that TDF use could lead to a "functional vitamin D deficiency" [18] with resultant PTH increase. In this study, the FGF23 decline remains consistent with the concept that TDF causes

"functional vitamin D deficiency," since a fall in effective 1,25-OHD would cause a fall in FGF23 (as well as an increase in PTH) via decreased absorption of calcium and phosphate from the intestine [40]. Since vitamin D and calcium supplementation lessens BMD decline at TDF-containing cART initiation [16], a study of the effect of vitamin D supplementation on BMD decline in TDF/FTC PrEP is warranted.

This study of TDF as PrEP allowed us to measure the effects of TDF in the absence of HIV, and the study of healthy youth allowed evaluation in the absence of other morbidities. Variability in adherence, measured by TFV-DP concentrations, allowed us to examine the effects of different levels of drug exposure on bone and renal outcomes. Since high and low drug exposure groups were not assigned by the study team, but rather "chosen" by the participants and categorized post hoc for analysis, there may be unidentified variables confounding our findings. Variability in adherence decreased the number of participants exposed to the highest concentrations of TDF, potentially decreasing the power of the study to show statistically significant associations. Within the age range of participants studied (15–22 years), differences in the rates of linear and skeletal growth may have contributed to variability in results. We also acknowledge that the limitations of DXA, which provides only 2-dimensional measurements of BMD, are particularly prominent in populations still undergoing linear growth.

These findings support the short-term renal safety of TDF-based PrEP in HIV-uninfected adolescent boys and young men. The early fall in FGF23 and increase in PTH in the high drug exposure group suggest that endocrine disruption, rather than renal toxicity, is the primary factor associated with early BMD decline with TDF/FTC PrEP in this age group. This suggests that endocrine-focused interventions might be reasonable to consider for young men taking TDF/FTC for PrEP.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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