Title

Association of Higher Plasma Vitamin D Binding Protein and Lower Free Calcitriol Levels with Tenofovir Disoproxil Fumarate Use and Plasma and Intracellular Tenofovir Pharmacokinetics: Cause of a Functional Vitamin D Deficiency?

Journal

Antimicrobial Agents and Chemotherapy, 57(11)

ISSN

0066-4804

Authors

Havens, Peter L
Kiser, Jennifer J
Stephensen, Charles B
et al.

Publication Date

2013-11-01

DOI

10.1128/aac.01096-13

Peer reviewed

AAC Journals.ASM.org

Association of Higher Plasma Vitamin D Binding Protein and Lower Free Calcitriol Levels with Tenofovir Disoproxil Fumarate Use and Plasma and Intracellular Tenofovir Pharmacokinetics: Cause of a Functional Vitamin D Deficiency?

Peter L. Havens,[a] Jennifer J. Kiser,[b] Charles B. Stephensen,[c] Rohan Hazra,[d] Patricia M. Flynn,[e] Craig M. Wilson,[f] Brandy Rutledge,[g] James Bethel,[g] Cynthia G. Pan,[a] Leslie R. Woodhouse,[c] Marta D. Van Loan,[c] Nancy Liu,[g] Jorge Lujan-Zilbermann,[h] Alyne Baker,[i] Bill G. Kapogiannis,[d] Catherine M. Gordon,[j] Kathleen Mulligan,[k] the Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) 063 Study Team

Children's Research Institute, Medical College of Wisconsin, Children's Hospital of Wisconsin, Milwaukee, Wisconsin, USA[a]; University of Colorado, Skaggs School of Pharmacy and Pharmaceutical Sciences, Department of Pharmaceutical Sciences, Aurora, Colorado, USA[b]; United States Department of Agriculture–Agricultural Research Service Western Human Nutrition Research Center, Davis, California, USA[c]; Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, Maryland, USA[d]; St. Jude Children's Research Hospital, Memphis, Tennessee, USA[e]; University of Alabama at Birmingham, Birmingham, Alabama, USA[f]; Westat, Rockville, Maryland, USA[g]; University of South Florida College of Medicine, Tampa, Florida, USA[h]; Tulane University, New Orleans, Louisiana, USA[i]; Hasbro Children's Hospital and Brown University, Providence, Rhode Island, USA[j]; University of California at San Francisco, San Francisco, California, USA[k]

Tenofovir disoproxil fumarate (TDF) causes bone, endocrine, and renal changes by an unknown mechanism(s). Data are limited on tenofovir pharmacokinetics and these effects. Using baseline data from a multicenter study of HIV-infected youth on stable treatment with regimens containing TDF ($n = 118$) or lacking TDF ($n = 85$), we measured cross-sectional associations of TDF use with markers of renal function, vitamin D-calcium-parathyroid hormone balance, phosphate metabolism (tubular reabsorption of phosphate and fibroblast growth factor 23 [FGF23]), and bone turnover. Pharmacokinetic-pharmacodynamic associations with plasma tenofovir and intracellular tenofovir diphosphate concentrations were explored among those receiving TDF. The mean age was 20.9 (standard deviation [SD], 2.0) years; 63% were male; and 52% were African American. Compared to the no-TDF group, the TDF group showed lower mean estimated glomerular filtration rates and tubular reabsorption of phosphate, as well as higher parathyroid hormone and 1,25-dihydroxy vitamin D [1,25-OH(2)D] levels. The highest quintile of plasma tenofovir concentrations was associated with higher vitamin D binding protein, lower free 1,25-OH(2)D, higher 25-OH vitamin D, and higher serum calcium. The highest quintile of intracellular tenofovir diphosphate concentration was associated with lower FGF23. Higher plasma tenofovir concentrations were associated with higher vitamin D binding protein and lower free 1,25-OH(2)D, suggesting a functional vitamin D deficiency explaining TDF-associated increased parathyroid hormone. The finding of lower FGF23 accompanying higher intracellular tenofovir diphosphate suggests that different mechanisms mediate TDF-associated changes in phosphate handling. Separate pharmacokinetic properties may be associated with distinct TDF toxicities: tenofovir with parathyroid hormone and altered calcium balance and tenofovir diphosphate with hypophosphatemia and FGF23 regulation.

(The clinical trial registration number for this study is NCT00490412 and is available online at http://clinicaltrials.gov/ct2/show/NCT00490412.)

The prodrug tenofovir disoproxil fumarate (TDF) is commonly used in combination antiretroviral therapy (cART) for persons infected with HIV, and, when coformulated with emtricitabine, it is also used for preexposure prophylaxis in HIV-seronegative adults (1). Following absorption, TDF is converted to free tenofovir and then phosphorylated intracellularly by host enzymes to tenofovir diphosphate. TDF is highly effective, but its use is associated with bone, endocrine, and renal toxicity.

The mechanism(s) by which TDF causes these organ-specific toxicities is unclear. There is a complex interplay between bone, endocrine, and renal physiology (Table 1). TDF use is associated with decreased bone mineral density (2–6), possibly caused by TDF-induced phosphaturia (7–14) with accompanying hypophosphatemia and osteomalacia (15–17). The phosphaturic hormone fibroblast growth factor 23 (FGF23) is not altered by TDF use (18, 19). TDF-associated bone changes may occur through a TDF effect on parathyroid hormone (16, 20), as parathyroid hormone secretion increases soon after initiating TDF (21). Vitamin D deficiency (22) may exacerbate this increase in parathyroid hormone (20, 23), but TDF-associated increased parathyroid hormone is found even in persons with sufficient vitamin D (24). Short-term vitamin D supplementation reduces TDF-associated increased parathyroid hormone but does not ameliorate TDF-associated phosphate loss (24, 25). The magnitude of phosphaturia is generally small and only rarely clinically important, while significant declines in bone density may occur in 10 (6) to 28% (Viread product label, available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021356s046,022577s003lbl.pdf) of TDF-treated adults.

Received 22 May 2013 Returned for modification 29 June 2013
Accepted 24 August 2013
Published ahead of print 3 September 2013
Address correspondence to Peter L. Havens, phavens@mcw.edu, or Kathleen Mulligan, kathleen.mulligan@ucsf.edu.

doi:10.1128/AAC.01096-13

TABLE 1 Relationships between vitamin D, parathyroid hormone, fibroblast growth hormone 23, calcium, and phosphate

Stimulus	Response[g]						
	1,25-OH(2)D	PTH	FGF23	Calcium absorption[f]	Phosphate absorption[f]	Serum calcium	Serum phosphate
Increased 1,25-OH(2)D[a]		↓	↑	↑	↑	↑	↑
Increased PTH[b]	↑		↑	↑	↓	↑	↓
Increased FGF23[c]	↓	↑ ↓			↓		↓
Decreased serum calcium[d]	↑	↑					
Increased serum phosphate[e]	↓	↑	↑				

[a] For 1,25-OH(2)D, the main action is to enhance calcium and phosphate absorption in the gastrointestinal tract, which leads to increases in serum calcium and serum phosphate. 1,25-OH(2)D directly decreases parathyroid hormone synthesis. In vitamin D deficiency there is low 1,25-OH(2)D, low serum calcium, and low serum phosphate; low calcium and phosphate absorption in the GI tract; and low reabsorption in kidney, high parathyroid hormone, and low FGF23. With high vitamin D binding protein and high albumin, free 1,25-OH(2)D is low, which mimics vitamin D deficiency.

[b] For parathyroid hormone, the main action is to increase calcium and phosphate resorption from bone and increase calcium reabsorption from distal renal tubules. Elevated parathyroid hormone causes phosphaturia (low phosphate reabsorption in the proximal renal tubule). Parathyroid hormone increases 1,25-OH(2)D levels (which then decreases parathyroid hormone synthesis).

[c] For FGF23, the main action is to cause phosphaturia in response to increased serum phosphate. FGF23 increases in response to increased 1,25-OH(2)D and decreases 1,25-OH(2)D concentration by decreasing synthesis and increasing clearance. The effect of FGF23 on parathyroid hormone depends on the context.

[d] Low serum calcium is a strong trigger to increase parathyroid hormone, which then causes increased 1,25-OH(2)D, calcium reabsorption from bone, and increased calcium absorption from the gastrointestinal tract.

[e] High serum phosphate stimulates FGF23, which causes phosphaturia and decreases phosphate absorption from the gastrointestinal tract.

[f] Calcium and phosphate absorption refers to either absorption in the gastrointestinal tract or reabsorption in the renal tubule, as identified in the footnote for each stimulus category.

[g] ↑, increase; ↓, decrease.

TDF may induce a state of functional vitamin D deficiency (24), or a parathyroid hormone-like factor may cause these TDF-associated metabolic changes (18). The active form of vitamin D, 1,25 dihydroxy vitamin D [1,25-OH(2)D], is increased by TDF administration (Viread product label [http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021356s046,022577s003lbl.pdf]), in contrast to the trend expected with vitamin D deficiency but consistent with the changes expected with increased parathyroid hormone secretion (Table 1). The association between TDF use and vitamin D binding protein (VDBP), which could alter free 1,25-OH(2)D and affect circulating parathyroid hormone concentration, total 1,25-OH(2)D, and bone density, has not been assessed.

Different TDF-associated toxicities (bone, phosphate, and renal) may be associated with different pharmacokinetic properties of TDF, either plasma tenofovir or intracellular tenofovir diphosphate. This distinction may be important given the development of tenofovir alafenamide fumarate (TAF), a tenofovir prodrug with low concentrations of plasma tenofovir but high concentrations of intracellular tenofovir diphosphate (26). A study of TAF use showed equivalent virologic suppression and less bone toxicity compared to TDF, suggesting that bone toxicity is more closely related to tenofovir, while virologic efficacy is most closely related to intracellular tenofovir diphosphate concentrations (27).

This report uses baseline data from a trial of vitamin D supplementation in HIV-infected youth treated with cART containing or not containing TDF (24) to explore the relationship of TDF use with vitamin D-calcium balance and phosphate metabolism and to evaluate the relationship of plasma tenofovir and intracellular tenofovir diphosphate concentrations with those metabolic and endocrinologic variables.

(Presented in part at the 18th Conference on Retroviruses and Opportunistic Infections [CROI], Boston, MA, February 2011.)

MATERIALS AND METHODS

Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) study 063 (NCT00490412) was a 12-week randomized, double-blind, placebo-controlled, multicenter trial performed between November 2007 and April 2010 at 16 ATN and 19 International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) sites in the United States and Puerto Rico. The study was approved by the Institutional Review Board of each participating site and required participants' written informed consent prior to enrollment.

Persons aged 18 to 25 years with HIV-1 infection who were treated with unchanged cART consisting of ≥3 antiretrovirals for ≥90 days and with HIV-1 plasma RNA (viral load) of <5,000 copies/ml within 60 days before entry were enrolled. Subjects were excluded if they had renal disease or current or recent pregnancy; used medicines that affect bone mineral density, interfere with vitamin D absorption or tenofovir excretion, or cause nephrotoxicity; or had an estimated glomerular filtration rate (eGFR) of <70 ml/min/1.73 m^2, hypercalcemia, or hypercalciuria. Subjects taking standard multivitamins or calcium supplements coformulated with vitamin D, up to 400 IU/day, could enroll. There was no exclusion based on pretreatment vitamin D status.

Enrollment into two groups was based on current antiretroviral treatment: cART containing TDF (TDF) or cART not containing TDF (no TDF); the baseline (cross-sectional) differences between these two groups are the focus of this report. Partial results of unadjusted baseline comparisons (Table 2) and the prospective portion of the study have been reported (24, 28). The study was powered based on the primary outcome: anticipated decline in tubular reabsorption of phosphate with vitamin D treatment at 12 weeks (24).

Samples were obtained after a minimum 4-h fast, and participants in the TDF group were asked to take their regular dose of TDF approximately 24 h prior to the study visit in an attempt to obtain trough concentrations of tenofovir and tenofovir diphosphate. Spot urine samples for creatinine, calcium, phosphate, glucose, and retinol binding protein were collected upon arrival for the study visit. Spot urine samples for β2 microglobulin were collected ≤1 h after the first urine collection and after drinking 8 to 12 oz of water. Whole blood was obtained in EDTA and sodium heparin cell preparation tubes for quantification of tenofovir in plasma and tenofovir diphosphate in peripheral blood mononuclear cells (PBMC), respectively. EDTA tubes were centrifuged at 1,200 × *g* for 10 min at 4°C, and plasma was isolated within 30 min of collection. PBMCs were isolated, counted, and lysed from sodium heparin cell preparation tubes using a published protocol (29). Serum and urine samples were obtained

TABLE 2 Characteristics of the study population[a]

Characteristic	Overall no. (%; $n = 203$)	Tenofovir (%; $n = 118$)	No tenofovir (%; $n = 85$)	P value[b]
Age (yr)	20.9 (2.0)	21.1 (2.0)	20.6 (2.0)	0.058
Male sex	127 (63)	86 (73)	41 (48)	<0.001
African American race	106 (52)	59 (50)	47 (55)	0.48
BMI (kg/m^2)	25.6 (7.0)	25.5 (7.0)	25.8 (7.0)	0.72
Currently smokes cigarettes	57 (28)	44 (37)	13 (15)	<0.001
Currently drinks alcohol	114 (56)	76 (64)	38 (45)	0.005
Exercises regularly	116 (57)	64 (54)	52 (61)	0.32
ATN study site (versus IMPAACT site)	151 (74)	108 (92)	43 (51)	<0.001
Season enrolled: winter/spring	106 (52)	70 (59)	36 (42)	0.023
Geographic latitude >40° N	86 (42)	47 (40)	39 (46)	0.47
Serum 25-OH vitamin D (ng/ml)	21.2 (12.3)	20.8 (12.7)	21.7 (11.8)	0.39
Calcium intake (mg/day)	796 (523)	795 (536)	798 (507)	0.82
Vitamin D intake (IU/day)	211 (199)	205 (195)	220 (204)	0.56
Age at HIV acquisition <9 years (presumed perinatally acquired)	51 (25)	14 (12)	37 (44)	<0.001
HIV duration (years)	6.6 (6.4)	4.0 (4.8)	10.2 (6.6)	<0.001
CDC disease stage C	49 (24)	22 (19)	27 (32)	0.045
Current CD4 cell count (cells/μl)	587 (246)	534 (204)	660 (279)	<0.001
Current viral load below quantitation limit	139 (68)	96 (81)	43 (51)	<0.001
Maximum cART exposure of >24 months	111 (55)	40 (34)	71 (84)	<0.001
Efavirenz included in current cART	84 (41)	63 (53)	21 (25)	<0.001
Ritonavir included in current cART	101 (50)	57 (48)	44 (52)	0.67

[a] Participants were enrolled based on inclusion of TDF in their cART. Continuous measures are shown as means (SD) with unadjusted P values determined by the Wilcoxon rank-sum test. Categorical measures are shown as numbers and percentages, with P values determined by the Pearson chi-square test. BMI, body mass index.
[b] Variables with $P < 0.10$ were included as covariates for multivariable modeling of physiologic variables shown in Table 3 (see the text).

at study sites, frozen, and shipped for central analysis. Information on calcium and vitamin D intake from diet and supplements was collected (Block calcium/vitamin D screener [30], Nutritionquest, Berkeley, CA).

Bioanalyses. Tenofovir and tenofovir diphosphate were measured at the University of Nebraska Antiviral Pharmacology Laboratory (director, C. V. Fletcher) using previously published methods (29, 31). The range of concentrations in this age group was previously described (32). Briefly, tenofovir in plasma was determined using a validated liquid chromatography-mass spectrometry (LC-MS) assay. Linearity was in the range of 10 to 1,500 ng/ml. The assay had a minimum quantifiable limit of 10 ng/ml when 0.25 ml of plasma was analyzed. Intracellular tenofovir diphosphate was determined using a validated LC-MS assay. The assay was linear in the range of 50 to 10,000 fmol. The minimum quantifiable limit was 10 fmol/million cells when 5 million cells were analyzed. Accuracy and precision for both methods was ±15%.

Measurements of laboratory variables were performed by batch analysis of stored samples at the United States Department of Agriculture–Agricultural Research Service–Western Human Nutrition Research Center, Davis, CA, unless otherwise indicated. Assays for 25-hydroxy vitamin D (25-OHD), 1,25-OH(2)D, parathyroid hormone, urine β-2 microglobulin, bone alkaline phosphatase (BAP), and C-telopeptide (CTX) were previously described (24). Assays performed for this secondary analysis and not previously described are included below.

Serum FGF23 was measured by enzyme-linked immunosorbent assay (ELISA) (FGF23 ELISA kit; Kainos Laboratories, Inc., Tokyo, Japan). The assay is sensitive to 3 pg/ml; no normal ranges are available.

Serum vitamin D binding protein (VDBP) was measured by ELISA (Quantikine VDBP ELISA kit [DVDBP0]; R&D Systems, Inc., Minneapolis, MN) with a normal range of 56 to 473 μg/ml.

Urinary retinol binding protein was measured by ELISA (DetectX urinary RBP enzyme immunoassay kit [KUO4-H1]; Arbor Assays, Ann Arbor, MI). Retinol binding protein values are normalized to urine creatinine concentrations, with a normal range of <130 μg retinol binding protein/g creatinine for individuals under 50 years of age.

Serum albumin was determined using a clinical chemistry analyzer (Cobas Integra 400 Plus [04469658]; Roche Diagnostics Corp., Indianapolis, IN), with a reference range for adults of 35 to 52 g/liters.

Urine calcium (UCa), phosphorus (UPhos), glucose (UGluc), and creatinine (UCr) were measured centrally. Serum calcium (SCa), phosphorus (SPhos), and creatinine (SCr) were measured at the local site laboratories, which were CLIA (Clinical Laboratory Improvement Amendments)-certified laboratories (24). eGFR was calculated by the modification of diet in renal disease formula (33). 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 (TRP) was calculated as $\{1 - [(\text{UPhos} \times \text{SCr})/(\text{SPhos} \times \text{UCr})]\} \times 100$, with normal being >96%. Serum-free 1,25-OH(2)D was calculated from serum albumin, vitamin D binding protein, and 1,25-OH(2)D by the method of Bikle et al. (34). Free 1,25-OH(2)D concentration decreases with increases in vitamin D binding protein and albumin.

Statistics. Data are presented as means (standard deviations [SD]) or median values for continuous measures with frequency and percentage presented for categorical measures. Bivariate analyses compared the TDF and no-TDF groups for differences in demographic and participant characteristics, as well as for differences in markers of renal, endocrine, and bone physiology. Except as noted, statistical significance of differences was identified using the Pearson chi-square test for categorical variables and the Wilcoxon rank-sum test for continuous variables; a rank-based analysis was used for continuous measures due to the presence of outliers.

Generalized linear regression models were used to measure the main effect of TDF use (versus no TDF use), adjusting for the effect of potential confounding variables. Covariates for inclusion in the multivariable analyses were chosen based on their relationship with TDF use (statistical significance at $P < 0.10$ in Table 2).

Spearman and Pearson correlations were used to explore interrelationships among the physiologic variables, tenofovir, and tenofovir diphosphate. Correlations were considered statistically significant only if the associated P value was ≤0.010; in the text the correlation (Pearson or Spearman) with the lower P value is presented.

Prior studies suggest that clinically significant changes in bone density occur in a subset of 10% (6) to 33% (4) of TDF-treated patients. To

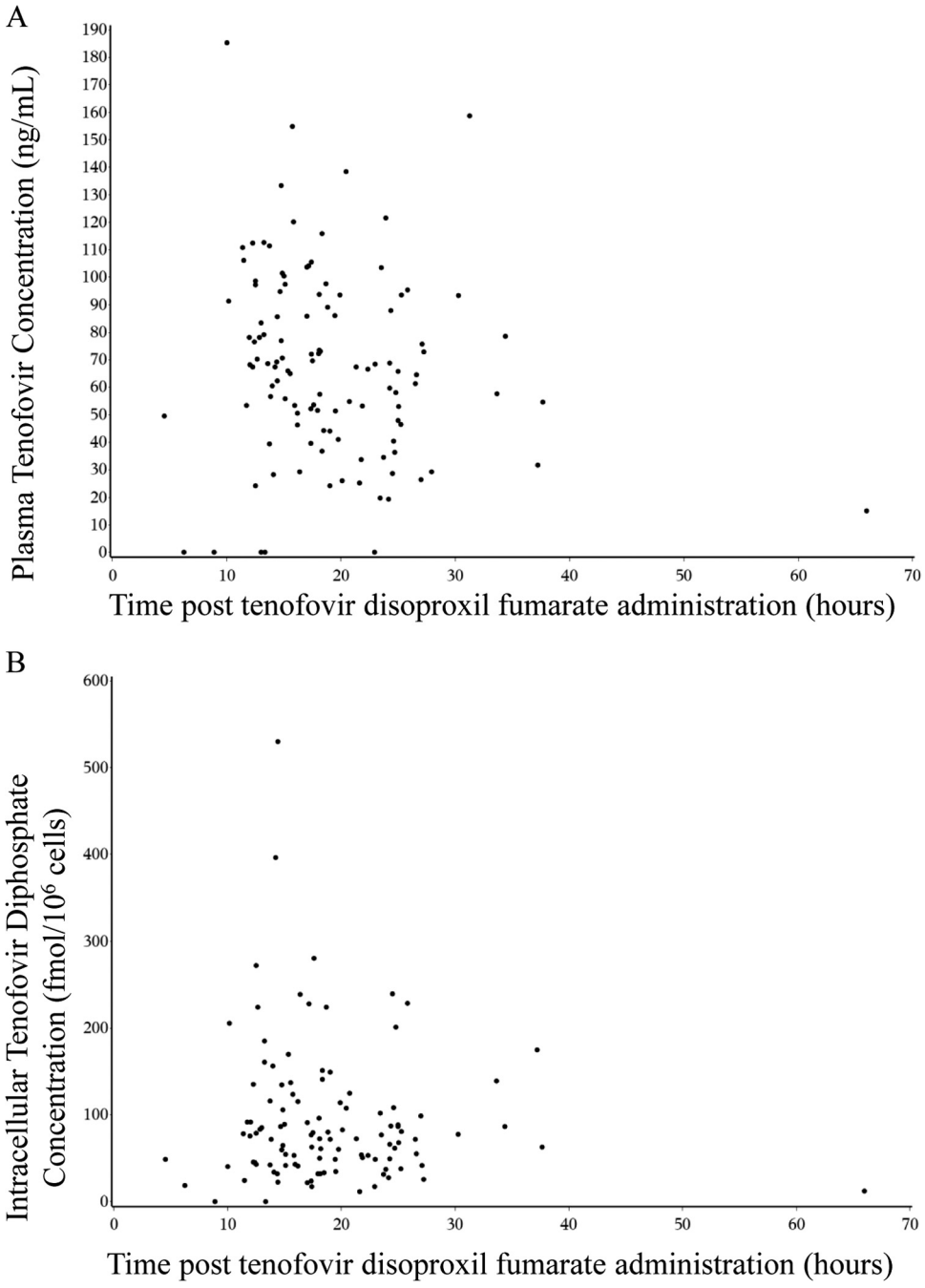

FIG 1 Plasma tenofovir and intracellular tenofovir diphosphate concentration by time following administration of tenofovir disoproxil fumarate. (A) Plasma tenofovir concentration by time postdose. (B) Intracellular tenofovir diphosphate concentration by time postdose.

further explore the interrelationships between TDF exposure and physiologic variables that might be occurring in small subgroups of participants, plasma tenofovir and intracellular tenofovir diphosphate were categorized into quintiles, with the median of each physiologic variable compared between the first (Q1) and fifth (Q5) quintile using the Wilcoxon rank-sum test to assess statistical significance of differences between the two quintiles. For those variables with $P < 0.05$ between the first and fifth quintiles of plasma tenofovir or intracellular tenofovir diphosphate, the Kruskal-Wallis test was used to test for overall differences between the quintiles. To confirm the relationship of plasma tenofovir quintiles with eGFR, 25-OHD, vitamin D binding protein, and free 1,25-OH(2)D, covariates shown to be associated with TDF use (described above) were included in an analysis of covariance (ANCOVA) performed on the subset of participants in the no-TDF group and in either the first or fifth quintile of plasma tenofovir concentration.

RESULTS

ATN 063 had 203 participants at baseline, but not all participants had data available for all variables in this secondary analysis. There were 28 to 34 nonevaluable participants, depending on the variable. There were no statistically significant differences between the

TABLE 3 Association of tenofovir disoproxil fumarate with markers of renal, endocrine, and bone physiology

Markers of renal, endocrine, and bone physiology	Overall[a]	Tenofovir[a]	No tenofovir[a]	*P* value[b]	Adjusted *P* value[c]
Renal glomerular					
eGFR (MDRD[d]; ml/min/1.73 m^2)	123 (26)	119 (20)	129 (31)	0.008	**0.010**[e]
Renal tubular					
Urine beta-2 microglobulin (ng/ml)	169 (381)	197 (398)	130 (354)	0.008	0.065
Urine retinol binding protein/urine creatinine ratio (pmol/umol)	0.40 (0.59)	0.40 (0.47)	0.40 (0.73)	0.31	0.797
UGluc (mg/dl)	7.6 (5.5)	7.8 (5.0)	7.4 (6.1)	0.21	0.39
Vitamin D and calcium					
SCa (mg/dl)	9.4 (0.4)	9.4 (0.4)	9.5 (0.4)	0.60	0.19
UCa/UCr (mg/mg)	0.06 (0.04)	0.05 (0.04)	0.07 (0.05)	0.031	0.92
PTH (pg/ml)	40.8 (24.5)	47.7 (25.7)	31.2 (19.1)	<0.001	**<0.001**
Serum 1,25-OH(2)D (pmol/liter)	111.5 (47.7)	113.7 (46.3)	108.5 (49.8)	0.36	**0.016**
VDBP (μg/ml)	3.8 (2.0)	4.0 (2.1)	3.4 (1.8)	0.026	0.066
Albumin (micromoles/liter)	0.66 (0.05)	0.66 (0.05)	0.65 (0.05)	0.069	0.080
Free 1,25-OH(2)D (fmol/liter)	908 (721)	850 (602)	993 (863)	0.42	0.65
Phosphate					
SPhos (mEq/liter)	3.6 (0.5)	3.7 (0.5)	3.6 (0.6)	0.18	0.71
TRP (%)	92.5 (4.3)	92.0 (3.8)	93.3 (4.8)	0.003	**0.011**
FGF23 (pg/ml)	41.7 (12.6)	41.0 (12.0)	42.6 (13.4)	0.48	0.66

[a] Values are means (SD).
[b] *P* values were determined by the Wilcoxon rank-sum test.
[c] *P* values were obtained for each marker from a generalized linear regression model, which was adjusted for demographic variables identified in Table 2 that differed significantly ($P < 0.10$) among subjects in the TDF versus no-TDF group. These variables are age (years), sex, current cigarette smoking, current use of alcohol, study site (ATN versus IMPAACT), season enrolled (winter/spring versus summer/fall), age of HIV acquisition of <9 years (presumed perinatally acquired), HIV duration (years), CDC disease stage C, current CD4 cell count (cells/μl), current viral load below quantitation limit, maximum cART exposure for >24 months (versus ≤24 months), and efavirenz included in current cART. Boldface indicates *P* values < 0.05.
[d] MDRD, modification of diet in renal disease formula (33).
[e] The relationship of eGFR to TDF use was further analyzed using a model that adjusted for the covariates described in footnote *c* plus body surface area (m^2); body weight (kg); and duration of TDF use (≤1 month, >1 to ≤6 months, >6 to ≤24 months, or >24 months); in that model, $P = 0.069$.

evaluable and nonevaluable participants except for urine glucose (7.35 and 9.08 mg/dl, respectively; $P = 0.049$).

Tenofovir and tenofovir diphosphate were measured in all 118 youths in the TDF group, but 3 were excluded from analyses due to undetectable tenofovir and tenofovir diphosphate concentrations. The remaining 115 participants provided blood samples at a median of 18 h (interquartile range, 14.4 to 23.8 h) post-TDF dose (Fig. 1). Mean (SD) tenofovir and tenofovir diphosphate concentrations were 68.8 (33) ng/ml and 95.5 (79) fmol/10^6 cells, respectively. There was higher variability in intracellular tenofovir diphosphate than in plasma tenofovir, with coefficients of variation equal to 83 and 48%, respectively.

Differences in demographic and HIV variables by TDF treatment. The TDF group ($n = 118$) included a higher proportion of males, more current users of cigarettes and alcohol, more enrollees from ATN study sites, and subjects with shorter duration of HIV and cART treatment compared to the no-TDF group ($n = 85$). The no-TDF group included a higher proportion of participants from IMPAACT sites, more with presumed perinatally acquired HIV, more with CDC stage C disease, and fewer with viral load below assay quantitation limits (Table 2).

Differences in renal, endocrine, and bone physiology by TDF treatment. Multiple markers of kidney function (including eGFR and markers of renal tubular function), vitamin D, calcium, phosphate status, and bone turnover were measured in all subjects. Unadjusted analyses suggested there were differences in several of these measures between the TDF and no-TDF groups, but after adjustment for demographic and HIV-related variables (Table 2), the only significant differences found were lower eGFR and TRP and higher 1,25-OH(2)D and parathyroid hormone in the TDF than in the no-TDF group (Table 3).

Associations of tenofovir concentrations with renal, endocrine, and bone physiology. Plasma tenofovir concentrations were correlated positively with the variables that regulate free 1,25-OH(2)D, including vitamin D binding protein ($r = 0.26$; $P = 0.007$) and albumin ($r = 0.28$; $P = 0.004$); plasma tenofovir concentrations correlated negatively with free 1,25-OH(2)D ($r = -0.34$; $P < 0.001$) (data not shown). Plasma tenofovir concentrations correlated negatively with eGFR ($r = -0.29$; $P = 0.002$) but not with markers of tubular function (urine β2 microglobulin, urine retinol binding protein/UCr, and UGluc), calcium balance (SCa, UCa/UCr, and parathyroid hormone), phosphate handling (SPhos, TRP, and FGF23), or bone turnover (bone alkaline phosphatase [BAP] and C-telopeptide [CTX]). Correlation analysis did not identify any associations between intracellular tenofovir diphosphate concentrations and any of these variables (data not shown).

Quintile analysis showed that, compared to the lowest quintile (Q1) of tenofovir, in the highest quintile of tenofovir (Q5) there were lower eGFR, higher SCa, higher 25-OHD, higher vitamin D binding protein, and lower free 1,25-OH(2)D levels (Table 4). For all of these variables, an overall difference by quintiles (Q1 through Q5) was shown using the Kruskal-Wallis test (denoted by asterisks in Table 4). The gradual increase in vitamin D binding

TABLE 4 Relationship between quintiles of plasma tenofovir or intracellular tenofovir diphosphate and physiologic variables

Physiologic variable[a]	Plasma tenofovir (ng/ml)			Intracellular tenofovir diphosphate (fmol/10^6 cells)		
	Q1 (≤39.5)	Q5 (>95.3)	*P* value[b]	Q1 (≤40.7)	Q5 (>140.3)	*P* value[b]
eGFR[c]	128	108	**<0.001***	128	113	0.10
Urine beta-2 microglobulin	113	90	0.34	139	106	0.68
Urine retinol binding protein/urine creatinine ratio	0.25	0.29	0.66	0.24	0.34	0.46
UGluc	7.6	7.0	0.47	7.5	7.8	0.45
SCa	9.4	9.5	**0.033***	9.4	9.5	0.51
UCa/UCr	0.04	0.05	0.99	0.06	0.04	**0.035**
PTH	36.6	47.5	0.51	32.6	44.2	0.26
25-OHD[c]	15	21	**0.013***	23	17	0.35
1,25-OH(2)D	99	102	0.96	124	99	0.97
VDBP[c]	3.02	4.70	**0.018***	3.96	4.48	0.93
Albumin	0.64	0.66	0.11	0.64	0.67	0.15
Free 1,25-OH(2)D[c]	959	558	**0.008***	891	591	0.613
SPhos	3.7	3.7	0.79	3.7	3.6	0.40
TRP	93	93	0.80	93	91	0.895
FGF23	40.8	35.6	0.10	45.6	36.4	**0.045**
BAP	31.1	32.3	0.71	40.0	29.9	**0.020**
CTX	6061	6502	0.22	6774	5704	0.83
Tenofovir				51.5	70.2	**0.030**
Tenofovir diphosphate	67.0	91.1	0.076			

[a] The medians of the physiologic variables are reported for participants categorized in the first (Q1) and fifth (Q5) quintiles of plasma tenofovir and intracellular tenofovir diphosphate. BAP, bone-specific alkaline phosphatase; CTX, C-telopeptide.

[b] *P* values were derived using the Wilcoxon rank-sum test to assess the statistical significance of differences in the physiologic variables between these two quintiles. For the variables with $P < 0.05$ (boldface) for the difference between the first and fifth quintiles, an asterisk denotes that the overall difference in all quintiles was statistically significant at $P < 0.05$ by the Kruskal-Wallis test.

[c] For these variables, the statistical significance of the difference between the first and fifth quintiles of plasma tenofovir was further tested using ANCOVA models with the covariates used for adjusted analyses listed in Table 3 (see the text). *P* values were the following: eGFR, $P = 0.001$; 25-OHD, $P = 0.032$; VDBP, $P = 0.008$; free 1,25-OH(2)D, $P = 0.011$. When the same analyses were repeated excluding participants with a tenofovir sampling time post-TDF administration of ≤10 h or >40 h, the results were similar: eGFR, $P = 0.003$; 25-OHD, $P = 0.032$; VDBP, $P = 0.021$; free 1,25-OH(2)D, $P = 0.021$.

protein by tenofovir quintile ($P = 0.023$ for the overall difference between quintiles) was matched by a gradual decline in free 1,25-OH(2)D from the lowest to highest tenofovir quintiles ($P = 0.001$ for the overall difference between quintiles) (Fig. 2). The changes in vitamin D binding protein and in free 1,25-OH(2)D by quintile of plasma tenofovir were still statistically significant when modeled with the covariates used for the adjusted analysis shown in Table 3 (Table 4, footnote *c*).

The highest quintile of tenofovir diphosphate concentrations (Q5) showed lower UCa/UCr ratios, lower FGF23 levels, and lower bone alkaline phosphatase levels than the lowest tenofovir diphosphate quintile (Q1) (Table 4). Higher tenofovir diphosphate concentrations (93.5 fmol/10^6 cells) were found in the lowest quintile of FGF23 than in the highest FGF23 quintile (41.9 fmol/10^6 cells) ($P = 0.012$) (data not shown).

The intracellular tenofovir diphosphate concentration trended toward higher values in the highest compared to the lowest quintile of plasma tenofovir ($P = 0.076$), and the level of plasma tenofovir was higher in the highest than the lowest quintile of intracellular tenofovir ($P = 0.030$) (Table 4).

DISCUSSION

Central conclusions. We found that a higher plasma tenofovir concentration was associated with higher vitamin D binding protein and lower free 1,25-OH(2)D, offering a possible mechanism to explain the higher parathyroid hormone and higher total 1,25-OH(2)D seen in participants taking TDF (Fig. 3A). The finding of higher intracellular tenofovir diphosphate concentration associated with lower FGF23 levels argues against a primary role of FGF23 in TDF-associated hypophosphatemia and suggests a compensatory drop in FGF23 in response to primary perturbations in other endocrine pathways (Table 1 and Fig. 3B).

TDF, tenofovir, vitamin D, and parathyroid hormone. The association of TDF use with elevations in parathyroid hormone and total 1,25-OH(2)D has been reported by others (Viread product label [http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/021356s046,022577s003lbl.pdf] and references 20, 21, 23, and 25). The finding of a 26% increase in vitamin D binding protein and a 42% decrease in free 1,25-OH(2)D between the lowest and highest quintiles of plasma tenofovir has not been previously reported. Vitamin D binding protein and albumin are proteins that bind to 1,25-OH(2)D, and when their levels are increased they lead to a decrease in free 1,25-OH(2)D (34). Vitamin D binding protein is synthesized constitutively in hepatocytes in conjunction with albumin and other liver-specific proteins, with some regulation resulting from sex hormones, malnutrition, and liver failure and during pregnancy (35). Vitamin D binding protein binds fatty acids, which may change its affinity for vitamin D metabolites. Vitamin D binding protein has roles in innate immunity (macrophage activation and neutrophil chemotaxis) and response to tissue damage (binding actin released from damaged cells), activities which may affect serum levels (36). Since free 1,25-OH(2)D is an important physiologic determinant of 1,25-OH(2)D signaling, decreased free 1,25-OH(2)D could lead to a secondary increase in parathyroid hormone caused by the deficiency in unbound 1,25-OH(2)D sensed by the parathyroid gland. Lower free 1,25-

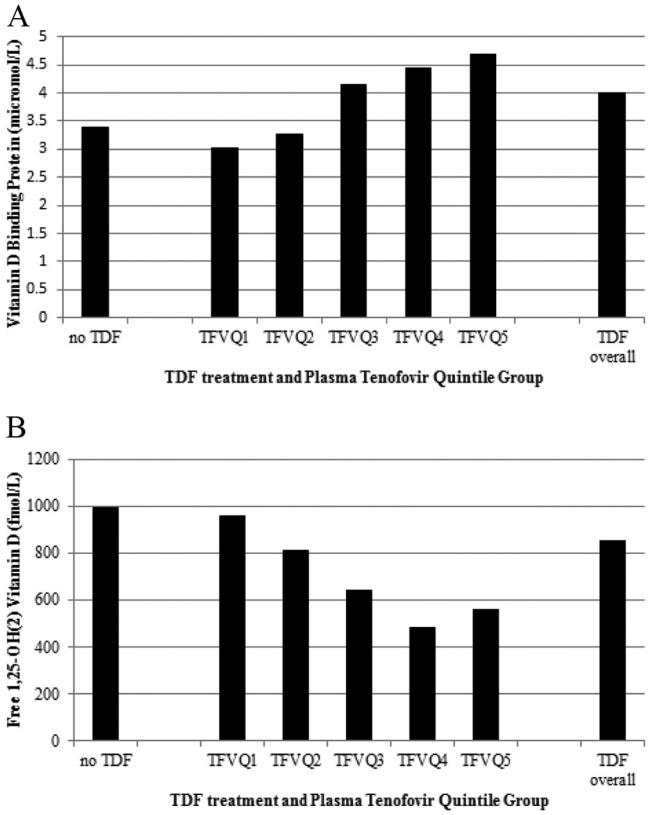

FIG 2 Changes in Vitamin D binding protein and free 1,25-OH(2) vitamin D by quintile of plasma tenofovir concentration. (A) Vitamin D binding protein by TDF use and plasma tenofovir concentration. Shown is the increase in vitamin D binding protein by quintile of plasma tenofovir concentration (TFVQ1 to TFVQ5). $P = 0.023$ for the overall difference in vitamin D binding protein by quintile of plasma tenofovir, and $P = 0.018$ for the difference between quintile 1 and quintile 5. (B) Free 1,25-OH(2) vitamin D by TDF use and plasma tenofovir concentration. Shown is the decrease in free 1,25-OH(2) vitamin D by quintile of plasma tenofovir concentration. $P = 0.001$ for the overall difference in vitamin D binding protein by quintile of plasma tenofovir, and $P = 0.008$ for the difference between quintile 1 and quintile 5. Values for participants treated with tenofovir disoproxil fumarate or left untreated (TDF and no TDF, respectively) overall are shown for comparison in both panels.

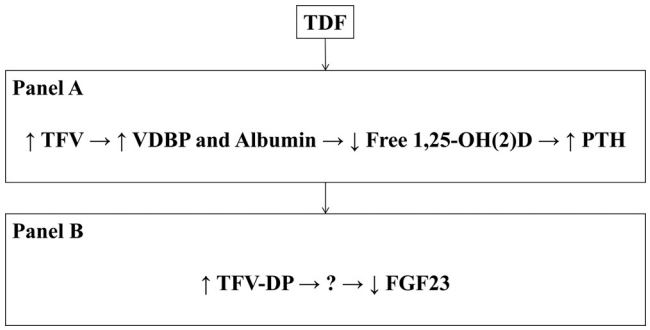

FIG 3 Potential differential endocrine effects of plasma tenofovir and intracellular tenofovir diphosphate. TDF, tenofovir disoproxil fumarate (prodrug); TFV, tenofovir (metabolite in plasma); TFV-DP, tenofovir diphosphate (active intracellular metabolite); VDBP, vitamin D binding protein; free 1,25-OH(2)D, free 1,25 dihydroxy vitamin D3; PTH, parathyroid hormone; FGF23, fibroblast growth hormone 23. (A) Plasma tenofovir may be linked to increased parathyroid hormone through the mechanism of increased vitamin D binding protein and albumin, which causes decreased free 1,25 OH(2)D. (B) The association of higher tenofovir diphosphate and lower fibroblast growth hormone 23 may occur through a mechanism that remains to be explained.

OH(2)D has been associated with lower calcium absorption, which would also cause an increase in parathyroid hormone (37). Chronic elevation of parathyroid hormone causes low bone density, which is observed in association with TDF use (2–5). Lower eGFR concentrations (also associated with higher tenofovir concentrations) have also been found to be associated with lower free 1,25-OH(2)D concentrations and with higher concentrations of parathyroid hormone (38). Since this is the first time an association of plasma tenofovir concentration with vitamin D binding protein and free 1,25-OH(2)D has been found, it merits confirmation in other cohorts.

TDF, tenofovir, renal phosphate loss, and FGF23. In the TDF group, we found mild phosphaturia in the absence of significant tubular proteinuria or glycosuria, consistent with other studies showing that TDF-associated increased renal excretion of phosphate occurs in the absence of proteinuria or more generalized renal tubular dysfunction (14, 39). Note that the low TRP was not associated with high parathyroid hormone levels. Even though high parathyroid hormone can directly cause renal tubular phosphate losses (40, 41), the data in this report support the findings of others (25) suggesting that TDF-associated phosphaturia is not due primarily to increased parathyroid hormone.

FGF23 concentrations did not differ between TDF treatment groups, consistent with other studies (18, 42), but the highest quintile of tenofovir diphosphate was associated with lower FGF23 concentration. FGF23, produced in osteocytes and osteoblasts, is increased in response to elevations in dietary and serum phosphorus (43) and in response to increased 1,25-OH(2)D (44) (Table 1). Increased FGF23 directly causes increased renal phosphate excretion in the proximal renal tubule, thereby decreasing serum phosphate (45). Increased FGF23 also causes a decline in 1,25-OH(2)D (44), and that decline would further decrease phosphate absorption in the gastrointestinal (GI) tract and increase renal phosphate excretion. Low FGF23 is found with hypophosphatemia and in individuals with vitamin D deficiency (46).

The finding of low FGF23 in participants with the highest quintile of tenofovir diphosphate could result from a compensatory response to the decreased free 1,25-OH(2)D (similar to the situation in vitamin D deficiency [46]), but the free 1,25-OH(2)D was associated with plasma tenofovir and not with intracellular tenofovir diphosphate. Low FGF23 was most closely associated with the highest quintile of intracellular tenofovir diphosphate, which suggests that tenofovir diphosphate is the primary determinant of TDF-associated hypophosphatemia, and FGF23 decreases in response to that stimulus in order to maintain phosphate homeostasis. Other mechanisms could also be postulated. For example, higher tenofovir diphosphate levels could affect osteocyte function directly and cause low FGF23, a possibility supported by the association of higher tenofovir diphosphate levels with lower bone alkaline phosphatase levels. The possibility of separate endocrine effects of tenofovir (on vitamin D-parathyroid hormone) and tenofovir diphosphate (on phosphate-FGF23) is important to explore given the ongoing development of TAF, which has lower tenofovir and higher tenofovir diphosphate levels than TDF (26).

TDF, tenofovir, and renal function: association with parathyroid hormone? The association of lower eGFR with TDF use

(47–49) and higher plasma tenofovir concentrations (50) have been previously described. In this cross-sectional analysis, causality cannot be assessed; it may be that participants with the lowest pretreatment eGFR were the slowest to clear tenofovir.

It has been suggested that TDF-associated renal toxicity causes elevations in parathyroid hormone (17, 48). A lower eGFR is associated with parathyroid hormone elevations in both children and adults with early renal failure (38). While TDF use was associated with a decrease in eGFR and increase in parathyroid hormone, 1,25-OHD was higher in the TDF than in the no-TDF group. In patients with mild renal insufficiency, 1,25-OH(2)D decreases (38). This finding argues against the hypothesis that glomerular damage is causative in TDF-associated increases in parathyroid hormone.

We used analysis of variation in one variable by quintiles of another variable as a way to more fully evaluate changes that might occur in only a small subgroup of persons treated with TDF. This approach seemed justified given the relative infrequency of severe toxicity associated with TDF use. The TDF effect on bone density may be strongest in a subgroup of patients: in treatment studies, 5 of 15 (33%) children (3) and 15 of 143 (10%) (6) adults experienced a ≥6% decline in spine bone density after 48 weeks of TDF treatment. TDF-associated renal failure occurs extremely rarely (47). Quintile analysis offered the best demonstration of the relationship between tenofovir concentrations and vitamin D binding protein and free 1,25-OH(2)D, and it allowed us to identify the relationship between tenofovir diphosphate and FGF23, which has not been evaluated by others.

Major strengths of the study include the lack of comorbidities in the young population studied compared to studies in older populations where a drug effect may be confounded by aging-related diseases. Measurement of plasma tenofovir and intracellular tenofovir diphosphate concentrations allows a more nuanced understanding of exposure-effect relationships compared to studies that only allow analysis by TDF use. However, we measured tenofovir and tenofovir diphosphate concentrations at a single time point after an unobserved dose, a limitation that might have kept us from identifying other pharmacodynamic associations.

A cross-sectional analysis can only be seen as hypothesis generating. The findings of this study need to be confirmed with data obtained in longitudinal studies, including measurements of bone density in individuals initiating therapy with TDF. These *post hoc* analyses were performed to better understand the relationship between TDF use and increased parathyroid hormone. Further studies are needed to confirm these findings.

In summary, these data suggest that tenofovir-associated increased vitamin D binding protein is associated with decreased free 1,25-OH(2)D and functional vitamin D deficiency, which in turn leads to increased parathyroid hormone secretion. The data further suggest that tenofovir diphosphate induces a hypophosphatemic stress related more closely to intracellular tenofovir diphosphate concentrations than plasma tenofovir concentrations. In response, FGF23 production decreases to compensate and maintain phosphate homeostasis. It is also possible that tenofovir diphosphate directly inhibits FGF23 production within osteocytes. Further study is needed to test these hypotheses and to clarify whether the apparent plasma tenofovir-vitamin D-parathyroid hormone relationship is truly distinct from the intracellular tenofovir diphosphate relationship with FGF23.

ACKNOWLEDGMENTS

We greatly appreciate the careful manuscript review and advice of Robert Harris.

Vitamin D and placebo were supplied by Bio-tech Pharmacal, Fayetteville, AR. Laboratory assays were performed by Xiaowen Jiang, Melissa Zerofsky, Brian Piccolo, and Gertrud Schuster at the USDA-ARS-WHNRC, Davis, CA.

The following ATN sites participated in this study: University of South Florida, Tampa (P. Emmanuel, J. Lujan-Zilbermann, and P. Julian), Children's Hospital of Los Angeles (M. Belzer, N. Flores, and L. Tucker), University of Southern California at Los Angeles (A. Kovacs, J. Homans, and Y. Lozano), Children's National Medical Center (L. D'Angelo, R. Hagler, and C. Trexler), Children's Hospital of Philadelphia (S. Douglas, M. Tanney, and A. DiBenedetto), John H. Stroger, Jr., Hospital of Cook County and the Ruth M. Rothstein CORE Center (J. Martinez, K. Bojan, and R. Jackson), University of Puerto Rico (I. Febo, H. Ayala-Flores, and H. Fuentes-Gomez), Montefiore Medical Center (D. Futterman, E. Enriquez-Bruce, and M. Campos), Mount Sinai Medical Center (J. Steever and M. Geiger), University of California-San Francisco (B. Moscicki, C. Auerswald, and L. Irish), Tulane University Health Sciences Center (S. Abdalian, L. Kozina, and A. Baker), University of Maryland (L. Peralta and Gorle), University of Miami School of Medicine (L. Friedman, D. Maturo, and H. Major-Wilson), Children's Diagnostic and Treatment Center (Puga, Leonard, and Inman), St. Jude's Children's Research Hospital (P. M. Flynn and Dillard), and Children's Memorial (R. Garofalo, L. Brennan, and E. Flanagan).

The following IMPAACT sites participated in the study: Children's Memorial–Chicago (R. Yogev and M. Sanders), Duke University Medical Center (C. Cunningham, S. Patil, and J. Wilson), New York University School of Medicine (W. Borkowsky and S. Deygoo), University of Medicine and Dentistry of New Jersey (A. Dieudonne, L. Bettica, and D. Monti), Bronx-Lebanon Hospital (M. Purswani, M. Vachon, and S. Chittalae), Baylor College of Medicine (W. Shearer, N. Cooper, and C. McMullen-Jackson), Boston Medical Center (E. Cooper, D. McLaud, and L. Tucker), University of Colorado/The Children's Hospital of Denver (E. McFarland, C. Chambers, and A. Katai) San Juan Hospital (M. Acevedo, M. Gonzalez, and L. Angeli), Seattle Children's Hospital (L. Frenkel, C. Venema-Weiss, and G. Bowen), University of California at San Diego (S. Spector, R. Viani, and J. Manning), State University of New York–Stony Brook (S. Nachman, J. Puccio, and D. Ferraro), Howard University (S. Rana and P. Yu), Miller Childrens Hospital (T. Chen, D. Michalik, and J. Jackson-Alvarez), Metropolitan Hospital-New York (M. Bamji, S. Paul, and S. Riley), Jacobi Medical Center (A. Wiznia, K. Kassen, and M. Burey), Habor UCLA Medical Center (M. Keller, J. Hayes, and Y. Gonzalez), Children's Hospital of Los Angeles (C. Rodier and J. Rockwood), University of Miami (G. Scott, A. Falk, and C. Florenz), and Columbia University (P. LaRussa and A. Higgins).

Eight of these sites utilized their General Clinical Research Center (GCRC)/Pediatric Clinical Research Center (PCRC) for the study; the centers were supported by grants from the General Clinical Research Center Program of the National Center for Research Resources, National Institutes of Health, and Department of Health and Human Services under the following grant numbers: Children's National Medical Center, M01RR020359; University of Pennsylvania/Children's Hospital of Philadelphia, NCRRUL1-RR-024134; University of California at San Francisco, UL1-RR024131; Seattle Children's Hospital, UL1-RR025014; Texas Children's Hospital and Baylor College of Medicine, M01-RR00188; Boston University Medical Center, UL1-RR02517; and SUNY–Stony Brook, M01-RR10710. The Tulane University Health Sciences Center utilized its Clinical and Translational Research Center (CTRC) for the study; the center was supported in whole or in part by funds provided through the Louisiana Board of Regents RC/EEP (RC/EEP–06).

We declare no conflicts of interest.

This work was supported by the Adolescent Trials Network for HIV/AIDS Interventions (ATN) from the National Institutes of Health (U01

HD 040533 and U01 HD 040474] through the Eunice Kennedy Shriver National Institute of Child Health and Human Development [B. Kapogiannis]), with supplemental funding from the National Institutes on Drug Abuse (N. Borek) and Mental Health (P. Brouwers and S. Allison). The protocol was coendorsed by the International Maternal Pediatric Adolescent AIDS Clinical Trials Group. Support for the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) was provided by the National Institute of Allergy and Infectious Diseases (NIAID), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and the National Institute of Mental Health (NIMH) (U01 A1068632). The study was scientifically reviewed by the ATN′s Therapeutic Leadership Group. Network, scientific, and logistical support was provided by the ATN Coordinating Center (C. Wilson and C. Partlow) at The University of Alabama at Birmingham. Network operations and analytic support was provided by the ATN Data and Operations Center at Westat, Inc. (J. Korelitz and B. Driver).

We thank the ATN Community Advisory Board and the youth who participated in the study.

REFERENCES

1. **Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, Goicochea P, Casapia M, Guanira-Carranza JV, Ramirez-Cardich ME, Montoya-Herrera O, Fernandez T, Veloso VG, Buchbinder SP, Chariyalertsak S, Schechter M, Bekker L-G, Mayer KH, Kallas EG, Amico KR, Mulligan K, Bushman LR, Hance RJ, Ganoza C, Defechereux P, Postle B, Wang F, McConnell JJ, Zheng J-H, Lee J, Rooney JF, Jaffe HS, Martinez AI, Burns DN, Glidden DV.** 2010. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N. Engl. J. Med. **363:**2587–2599.
2. **Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, Coakley DF, Lu B, Toole JJ, Cheng AK, 903 Study Group.** 2004. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA **292:**191–201.
3. **Gafni RI, Hazra R, Reynolds JC, Maldarelli F, Tullio AN, DeCarlo E, Worrell CJ, Flaherty JF, Yale K, Kearney BP, Zeichner SL.** 2006. Tenofovir disoproxil fumarate and an optimized background regimen of antiretoviral agents as salvage therapy: impact on bone mineral density in HIV-infected children. Pediatrics **118:**e711–e718.
4. **Hazra R, Gafni RI, Maldarelli F, Balis FM, Tullio AN, DeCarlo E, Worrell CJ, Steinberg SM, Flaherty J, Yale K, Kearney BP, Zeichner SL.** 2005. Tenofovir disoproxil fumarate and an optimized background regimen of antiretroviral agents as salvage therapy for pediatric HIV infection. Pediatrics **116:**e846–e854.
5. **Martin A, Bloch M, Amin J, Baker D, Cooper DA, Emery S, Carr A.** 2009. Simplification of antiretroviral therapy with tenofovir-emtricitabine or abacavir-lamivudine: a randomized, 96-week trial. Clin. Infect. Dis. **49:**1591–1601.
6. **Stellbrink H-J, Orkin C, Arribas JR, Compston J, Gerstoft J, Van Wijngaerden E, Lazzarin A, Rizzardini G, Sprenger HG, Lambert J, Sture G, Leather D, Hughes S, Zucchi P, Pearce H, ASSERT Study Group.** 2010. Comparison of changes in bone density and turnover with abacavir-lamivudine versus tenofovir-emtricitabine in HIV-infected adults: 48-week results from the ASSERT study. Clin. Infect. Dis. **51:**963–972.
7. **Judd A, Boyd KL, Stohr W, Dunn D, Butler K, Lyall H, Sharland M, Shingadia D, Riordan A, Gibb DM.** 2010. Effect of tenofovir disoproxil fumarate on risk of renal abnormality in HIV-1-infected children on antiretroviral therapy: a nested case-control study. AIDS **24:**525–534.
8. **Kinai E, Hanabusa H.** 2005. Renal tubular toxicity associated with tenofovir assessed using urine-beta 2 microglobulin, percentage of tubular reabsorption of phosphate and alkaline phosphatase levels. AIDS **19:**2031–2033.
9. **Kinai E, Hanabusa H.** 2009. Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. AIDS Res. Hum. Retrovir. **25:**387–394.
10. **Rodriguez-Novoa S, Labarga P, Soriano V, Egan D, Albalater M, Morello J, Cuenca L, Gonzalez-Pardo G, Khoo S, Back D, Owen A.** 2009. Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study. Clin. Infect. Dis. **48:**e108–116.
11. **Buchacz K, Brooks JT, Tong T, Moorman AC, Baker RK, Holmberg SD, Greenberg A, HIV Outpatient Study (HOPS) Investigators.** 2006. Evaluation of hypophosphataemia in tenofovir disoproxil fumarate (TDF)-exposed and TDF-unexposed HIV-infected out-patients receiving highly active antiretroviral therapy. HIV Med. **7:**451–456.
12. **Essig M, Duval X, Kaied FA, Iordache L, Gervais A, Longuet P, Blanchet F, Peytavin G, Leport C.** 2007. Is phosphatemia the best tool to monitor renal tenofovir toxicity? J. Acquir. Immune Defic. Syndr. **46:**256–258.
13. **Labarga P, Barreiro P, Martin-Carbonero L, Rodriguez-Novoa S, Solera C, Medrano J, Rivas P, Albalater M, Blanco F, Moreno V, Vispo E, Soriano V.** 2009. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. AIDS **23:**689–696.
14. **Maggi P, Montinaro V, Bellacosa C, Pietanza S, Volpe A, Graziano G, Strippoli GFM, Angarano G.** 2012. Early markers of tubular dysfunction in antiretroviral-experienced HIV-infected patients treated with tenofovir versus abacavir. AIDS Patient Care Stds. **26:**5–11.
15. **Fux CA, Rauch A, Simcock M, Bucher HC, Hirschel B, Opravil M, Vernazza P, Cavassini M, Bernasconi E, Elzi L, Furrer H, Swiss HIV Cohort Study.** 2008. Tenofovir use is associated with an increase in serum alkaline phosphatase in the Swiss HIV cohort study. Antivir. Ther. **13:**1077–1082.
16. **Woodward CL, Hall AM, Williams IG, Madge S, Copas A, Nair D, Edwards SG, Johnson MA, Connolly JO.** 2009. Tenofovir-associated renal and bone toxicity. HIV Med. **10:**482–487.
17. **Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sanchez-Nino MD, Izquierdo MC, Poveda J, Sainz-Prestel V, Ortiz-Martin N, Parra-Rodriguez A, Selgas R, Ruiz-Ortega M, Egido J, Ortiz A.** 2011. Tenofovir nephrotoxicity: 2011 update. AIDS Res. Treatment **2011:**354908.
18. **Bech A, Van Bentum P, Nabbe K, Gisolf J, Richter C, De Boer H.** 2012. Fibroblast growth factor 23 in hypophosphataemic HIV-positive adults on tenofovir. HIV Med. **13:**558–563.
19. **Saidenberg-Kermanac'h N, Souabni L, Prendki V, Prie D, Boissier M-C.** 2011. Normal plasma FGF23 levels kinetic in tenofovir-related hypophosphatemic osteomalacia in an HIV-infected patient with von Recklinghausen disease. Joint Bone Spine **78:**306–308.
20. **Rosenvinge MM, Gedela K, Copas AJ, Wilkinson A, Sheehy CA, Bano G, Hay PE, Pakianathan MR, Sadiq ST.** 2010. Tenofovir-linked hyperparathyroidism is independently associated with the presence of vitamin D deficiency. J. Acquir. Immune Defic. Syndr. **54:**496–499.
21. **Masiá M, Padilla S, Robledano C, López N, Ramos JM, Gutierrez F.** 2012. Short communication: early changes in parathyroid hormone concentrations in patients initiating antiretroviral therapy with tenofovir. AIDS Res. Hum. Retrovir. **28:**242–246.
22. **Institute of Medicine.** 2011. Dietary reference intakes for calcium and vitamin D. National Academies Press, Washington, DC.
23. **Childs KE, Fishman SL, Constable C, Gutierrez JA, Wyatt CM, Dieterich DT, Mullen MP, Branch AD.** 2010. Short communication: inadequate vitamin D exacerbates parathyroid hormone elevations in tenofovir users. AIDS Res. Hum. Retrovir. **26:**855–859.
24. **Havens PL, Stephensen CB, Hazra R, Flynn PM, Wilson CM, Rutledge B, Bethel J, Pan CG, Woodhouse LR, Van Loan MD, Liu N, Lujan-Zilbermann J, Baker A, Kapogiannis BG, Mulligan K, Adolescent Medicine Trials Network (ATN) for HIV/AIDS Interventions 063 Study Team.** 2012. Vitamin D3 decreases parathyroid hormone in HIV-infected youth being treated with tenofovir: a randomized, placebo-controlled trial. Clin. Infect. Dis. **54:**1013–1025.
25. **Bech A, Van Bentum P, Telting D, Gisolf J, Richter C, De Boer H.** 2012. Treatment of calcium and vitamin D deficiency in HIV-positive men on tenofovir-containing antiretrovial therapy. HIV Clin. Trials **13:**350–356.
26. **Lee WA, He GX, Eisenberg E, Cihlar T, Swaminathan S, Mulato A, Cundy KC.** 2005. Selective intracellular activation of a novel prodrug of the human immunodeficiency virus reverse transcriptase inhibitor tenofovir leads to preferential distribution and accumulation in lymphatic tissue. Antimicrob. Agents Chemother. **49:**1898–1906.
27. **Zolopa A, Ortiz R, Sax P, Brar I, Elion R, Wang H, Callebaut C, Ramanathan S, Fordyce M, McCallister S.** 2013. Comparative study of tenofovir alafenamide versus tenofovir disoproxil fumarate, each with elvitegravir, cobicistat, and emtricitabine, for HIV treatment. Abstr. 20th Conf. Retrovir. Opportun. Infect., abstr 99LB.
28. **Havens PL, Mulligan K, Hazra R, Flynn P, Rutledge B, Van Loan MD, Lujan-Zilbermann J, Kapogiannis BG, Wilson CM, Stephensen CB,**

Adolescent Medicine Trials Network for HIV/AIDS Interventions (ATN) 063 Study Team. 2012. Serum 25-hydroxyvitamin D response to vitamin D3 supplementation 50,000 IU monthly in youth with HIV-1 infection. J. Clin. Endocrinol. Metab. **97:**4004–4013.
29. **King T, Bushman L, Kiser J, Anderson PL, Ray M, Delahunty T, Fletcher CV.** 2006. Liquid chromatography-tandem mass spectrometric determination of tenofovir-diphosphate in human peripheral blood mononuclear cells. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **843:**147–156.
30. **Cummings SR, Block G, McHenry K, Baron RB.** 1987. Evaluation of two food frequency methods of measuring dietary calcium intake. Am. J. Epidemiol. **126:**796–802.
31. **Delahunty T, Bushman L, Fletcher CV.** 2006. Sensitive assay for determining plasma tenofovir concentrations by LC/MS/MS. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **830:**6–12.
32. **Kiser JJ, Fletcher CV, Flynn PM, Cunningham CK, Wilson CM, Kapogiannis BG, Major-Wilson H, Viani RM, Liu NX, Muenz LR, Harris DR, Havens PL, Fletcher CV, Flynn PM, Cunningham CK, Wilson CM, Kapogiannis BG, Major-Wilson H, Viani RM, Liu NX, Muenz LR, Harris DR, Havens PL, Adolescent Trials Network for HIV/AIDS Interventions.** 2008. Pharmacokinetics of antiretroviral regimens containing tenofovir disoproxil fumarate and atazanavir-ritonavir in adolescents and young adults with human immunodeficiency virus infection. Antimicrob. Agents Chemother. **52:**631–637.
33. **Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F, Chronic Kidney Disease Epidemiology Collaboration.** 2006. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann. Int. Med. **145:**247–254. [Erratum, 149:519, 2008.]
34. **Bikle DD, Siiteri PK, Ryzen E, Haddad JG.** 1985. Serum protein binding of 1,25-dihydroxyvitamin D: a reevaluation by direct measurement of free metabolite levels. J. Clin. Endocrinol. Metab. **61:**969–975.
35. **Laing CJ, Cooke NE.** 2005. Vitamin D-binding protein, p 117–134. *In* Feldman D, Glorieux FH, Pike JW (ed), Vitamin D, 2nd ed, vol 2. Elsevier Academic Press, Boston, MA.
36. **Speeckaert M, Huang G, Delanghe JR, Taes YE.** 2006. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin. Chim. Acta **372:**33–42.
37. **Pattanaungkul S, Riggs BL, Yergey AL, Vieira NE, O'Fallon WM, Khosla S.** 2000. Relationship of intestinal calcium absorption to 1,25-dihydroxyvitamin D [1,25(OH)2D] levels in young versus elderly women: evidence for age-related intestinal resistance to 1,25(OH)2D action. J. Clin. Endocrinol. Metab. **85:**4023–4027.
38. **St John A, Thomas MB, Davies CP, Mullan B, Dick I, Hutchison B, van der Schaff A, Prince RL.** 1992. Determinants of intact parathyroid hormone and free 1,25-dihydroxyvitamin D levels in mild and moderate renal failure. Nephron **61:**422–427.
39. **Fux CA, Christen A, Zgraggen S, Mohaupt MG, Furrer H.** 2007. Effect of tenofovir on renal glomerular and tubular function. AIDS **21:**1483–1485.
40. **Peacock M.** 2002. Primary hyperparathyroidism and the kidney: biochemical and clinical spectrum. J. Bone Miner. Res. **17**(Suppl 2)**:**N87–N94.
41. **Marks KH, Kilav R, Naveh-Many T, Silver J.** 1996. Calcium, phosphate, vitamin D, and the parathyroid. Pediatr. Nephrol. **10:**364–367.
42. **Gupta SK, Shen C, Moe SM, Kamendulis LM, Goldman M, Dubé MP.** 2012. Worsening endothelial function with efavirenz compared to protease inhibitors: a 12-month prospective study. PLoS One **7:**e45716. doi:10.1371/journal.pone.0045716.
43. **Vervloet MG, van Ittersum FJ, Buttler RM, Heijboer AC, Blankenstein MA, ter Wee PM.** 2011. Effects of dietary phosphate and calcium intake on fibroblast growth factor-23. Clin. J. Am. Soc. Nephrol. **6:**383–389.
44. **Martin A, David V, Quarles LD.** 2012. Regulation and function of the FGF23/klotho endocrine pathways. Physiol. Rev. **92:**131–155.
45. **Bergwitz C, Juppner H.** 2010. Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. Annu. Rev. Med. **61:**91–104.
46. **Uzum AK, Salman S, Telci A, Boztepe H, Tanakol R, Alagol F, Ozbey NC.** 2010. Effects of vitamin D replacement therapy on serum FGF23 concentrations in vitamin D-deficient women in short term. Eur. J. Endocrinol. **163:**825–831.
47. **Coca S, Perazella MA.** 2002. Rapid communication: acute renal failure associated with tenofovir: evidence of drug-induced nephrotoxicity. Am. J. Med. Sci. **324:**342–344.
48. **Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M.** 2010. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin. Infect. Dis. **51:**496–505.
49. **Nelson MR, Katlama C, Montaner JS, Cooper DA, Gazzard B, Clotet B, Lazzarin A, Schewe K, Lange J, Wyatt C, Curtis S, Chen SS, Smith S, Bischofberger N, Rooney JF.** 2007. The safety of tenofovir disoproxil fumarate for the treatment of HIV infection in adults: the first 4 years. AIDS **21:**1273–1281.
50. **Poizot-Martin I, Solas C, Allemand J, Obry-Roguet V, Pradel V, Bregigeon S, Faucher O, Lacarelle B.** 2012. Renal impairment in patients receiving a tenofovir-cART regimen: impact of tenofovir trough concentration. J. Acquir. Immune Defic. Syndr. [Epub ahead of print.] doi:10.1097/QAI.0b013e31827ce4ee.