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Bone mineral density reductions after tenofovir disoproxil fumarate initiation and changes in phosphaturia: a secondary analysis of ACTG A5224s

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Background: It is unknown if the greater reductions in bone mineral density (BMD) associated with initiation of tenofovir disoproxil fumarate compared with abacavir in previously untreated HIV-infected participants in the ACTG A5224s clinical trial were associated with potentially worsening tenofovir-related phosphaturia.

Methods: We correlated changes in BMD at the hip and spine with changes in phosphaturia [transtubular reabsorption of phosphorus (TRP) and tubular maximum phosphate reabsorption per glomerular filtration rate (TmP/GFR)] from entry through week 96 in those initiating tenofovir ($n = 134$) versus abacavir ($n = 135$) with efavirenz or atazanavir/ritonavir in A5224s. We also correlated changes in BMD with tenofovir AUC measured between weeks 4 and 24.

Results: Changes in TRP and TmP/GFR through week 96 between the tenofovir and abacavir arms were not significantly different (both $P \geq 0.70$) and did not differ with use of efavirenz versus atazanavir/ritonavir. There were no significant correlations between changes in either TRP or TmP/GFR and with either hip or spine BMD in the tenofovir arms. Tenofovir AUC was significantly correlated with changes in hip BMD, but not spine BMD, at week 24 ($r = -0.22$, $P = 0.028$) and week 48 ($r = -0.26$, $P = 0.010$), but not at week 96 ($r = -0.14$, $P = 0.18$).

Conclusions: Changes in phosphaturia were not different between the tenofovir and abacavir arms in A5224s. Changes in hip and spine BMD with tenofovir were not related to changes in phosphaturia. However, tenofovir exposure was weakly associated with changes in hip BMD through week 48.

Introduction

Compared with the HIV-uninfected population, fracture rates are higher in those who are HIV infected.[1,2] In addition, osteopenia, assessed as reduced bone mineral density (BMD), is more frequent and of greater severity in HIV-infected patients, particularly in those receiving ART incorporating tenofovir disoproxil fumarate.[3–6] The mechanism for tenofovir-induced reductions in BMD is widely believed to be due to urine phosphate wasting as a consequence of renal proximal tubulopathy that frequently accompanies tenofovir use. A recent study suggested that patients receiving tenofovir chronically had continued BMD loss due to, in part, urinary phosphate losses.[7] However, it is not known if phosphaturia explains the BMD reductions seen with initial tenofovir use.[8]

An alternative hypothesis for why tenofovir may lead to osteopenia is that circulating tenofovir concentrations (and by correlation bone levels of tenofovir) may directly increase bone turnover.[9,10] Indeed, with regimens containing tenofovir alafenamide fumarate, lower plasma levels of tenofovir and lower BMD losses were seen when compared with therapies containing tenofovir disoproxil fumarate.[11,12] This suggests that plasma levels of tenofovir are associated with bone loss directly, although this has not yet been proven.

Therefore, we sought to test these competing hypotheses in a secondary analysis of ACTG A5224s, which found that BMD losses were more pronounced with initiation of regimens containing

tenofovir disoproxil fumarate compared with those containing abacavir.[8]

Methods

Study design and procedures

AIDS Clinical Trials Group A5224s was a metabolic substudy of A5202 (ClinicalTrials.gov NCT00118898) in which ART-naive study participants from AIDS Clinical Trials Group sites in the United States and Puerto Rico aged ≥16 years and with an HIV-1 RNA level >1000 copies/mL were randomized to a blinded NRTI fixed-dose component, abacavir/lamivudine (600 mg/300 mg) or tenofovir disoproxil fumarate/emtricitabine (300 mg/200 mg), with either the open-label PI atazanavir/ritonavir (300 mg/100 mg) or the NNRTI efavirenz (600 mg).

We performed a *post hoc* analysis of available data from both A5224s and A5202. The bone DXA and estimated glomerular filtration rate (eGFR) data collected at entry, weeks 24, 48 and 96 have been previously published.[8,13]

Fasting serum and urine phosphate levels were also obtained at these study visits. Urine creatinine was measured centrally at Quest Diagnostics using a Jaffe colorimetric assay on a Beckman Coulter/Olympus platform. Urine phosphate was also measured centrally at Quest Diagnostics using a spectrophotometric assay using a molybdate reaction. Serum creatinine and serum phosphate were measured locally. We estimated urinary phosphate wasting as both transtubular reabsorption of phosphorus (TRP) and tubular maximum phosphate reabsorption per GFR (TmP/GFR). TRP was calculated as 1 – [serum creatinine × urine phosphate]/[urine creatinine × serum phosphate] and is reported here as a percentage. TmP/GFR was calculated as TRP × serum phosphate (if TRP ≤ 0.86) or as 0.3 × {TRP/[1 – (0.8 × TRP)]} × serum phosphate (if TRP >0.86).[14,15] GFR was estimated using the 2009 CKD-EPI creatinine equation.[16]

Sparse tenofovir plasma concentrations were collected between weeks 4 and 24. Individual Bayesian estimates of plasma oral clearance values of tenofovir were estimated from a two-compartment population pharmacokinetic model using the tenofovir concentrations.[17] AUC values were calculated based on individual clearance values where AUC = dose/clearance; the actual dose of tenofovir in 300 mg of tenofovir disoproxil fumarate was 136 mg. Tenofovir concentrations were not measured beyond week 24.

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Study participants

To be included in the main A5202 trial participants were required to have a screening CL_{CR} by Cockcroft–Gault >60 mL/min. To be included in the substudy A5224s, participants also could not have uncontrolled thyroid disease, hypogonadism, Cushing's syndrome or American Diabetes Association-defined diabetes mellitus. The participants also were excluded if using or had planned use during the study period of the growth hormone, anabolic steroids, glucocorticoids or osteoporosis medications.

Ethics

The human subjects ethics committee at each participating centre approved the study protocol and written informed consent was provided by all participants in compliance with the human experimentation guidelines of the US Department of Health and Human Services.

Statistical analysis

This analysis had three main objectives. First, we assessed the effects of NRTI backbone on phosphaturia changes. Second, we sought to determine the associations between changes in phosphaturia and changes in lumbar spine and hip BMD at 24, 48 and 96 weeks in the overall study population and by treatment arm in A5224s. We also determined if use of atazanavir/ritonavir versus efavirenz or if changes in eGFR modified the relationships between phosphaturia and tenofovir exposure with BMD. Third, we wished to correlate circulating tenofovir exposure (AUC) with lumbar spine and hip BMD at 24, 48 and 96 weeks and with phosphaturia at week 24 in the two tenofovir-containing regimens in A5224s. The first objective was assessed using both simple and multivariable linear regressions whereas Spearman correlations were used to address the second and third objectives.

The sample size estimate was based on the primary A5224s objective of week 96 lipoatrophy prevalence.[18] Complete details of the randomization procedures are described elsewhere.[19]

$P < 0.05$ was considered statistically significant and nominal values are reported without adjustment for multiple comparisons. Analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

A total of 269 participants in A5224s were included in this analysis. Overall, 85% were men and 47% were non-Hispanic white. The median age, CD4 cell count and HIV-1 RNA levels were, respectively, 38 years, 233 cells/μL and 4.62 $\log_{10}$ copies/mL.[8]

BMD changes

The changes in lumbar spine and hip BMD from entry to week 96 with use of tenofovir versus abacavir have previously been published.[8] The prevalence of osteopenia (defined as having a *t* score ≤ −1 at either the spine or the hip) overall at entry was 39%. In brief, the mean lumbar spine BMD was reduced from entry to week 96 by 3.35% versus 1.35% in the tenofovir- and abacavir-containing study arms, respectively, whereas the mean hip BMD was reduced by 3.96% versus 2.61% through week 96 in these same treatment arms.

Phosphaturia changes

As shown in Table 1, the TRP and TmP/GFR values at entry were well-balanced amongst the four study groups. The changes in TRP and TmP/GFR in each of the four study arms are also shown in Table 1. Reductions in both phosphaturia measures indicate less phosphate reabsorption (or more urinary phosphate wasting). Mean (SD) absolute changes in TRP and TmP/GFR in the entire study group were modest at −1.78% (6.59%) and −0.28 (0.92) mg/dL, respectively, from entry to week 96 and were similar amongst the four arms.

We then examined the changes in TRP and TmP/GFR by NRTI and NNRTI/PI treatment components (Figure 1). Changes in TRP were similar in the tenofovir/emtricitabine and abacavir/lamivudine groups from entry through week 96. Changes in TmP/GFR between the tenofovir/emtricitabine and abacavir/lamivudine groups as well as between the atazanavir/ritonavir and efavirenz groups from entry to week 96 were also similar.

We then estimated the NRTI effects on phosphaturia changes and assessed treatment effects by the NNRTI/PI components as well as by change in eGFR from entry to week 96. The mean (95% CI) change in TRP (%) was −1.90 (−3.25 to −0.55) for

Table 1. Mean (SD; *n*) changes in phosphaturia for all four A5224s study treatment arms

	EFV + TDF/FTC, *N* = 69	EFV + ABC/3TC, *N* = 70	ATV/RTV + TDF/FTC, *N* = 65	ATV/RTV + ABC/3TC, *N* = 65	Total, *N* = 269
TRP (%)					
entry	89.17 (6.10; 66)	90.34 (4.58; 66)	88.90 (5.27; 57)	89.54 (5.38; 61)	89.51 (5.36; 250)
change from entry to week 24	0.26 (5.93; 57)	−1.50 (6.22; 60)	−0.71 (6.80; 50)	0.40 (6.32; 54)	−0.40 (6.31; 221)
change from entry to week 48	−0.65 (5.99; 54)	−2.20 (6.77; 55)	−0.66 (5.82; 45)	0.51 (5.99; 55)	−0.75 (6.21; 209)
change from entry to week 96	−1.44 (7.67; 49)	−1.86 (6.20; 50)	−2.41 (6.47; 44)	−1.43 (5.98; 45)	−1.78 (6.59; 188)
TmP/GFR (mg/dL)					
entry	3.43 (0.85; 66)	3.56 (0.83; 66)	3.40 (0.72; 57)	3.39 (0.68; 61)	3.45 (0.77; 250)
change from entry to week 24	−0.19 (0.83; 57)	−0.14 (0.90; 60)	−0.13 (0.58; 50)	0.27 (0.89; 54)	0.00 (0.83; 221)
change from entry to week 48	−0.21 (0.82; 54)	−0.30 (0.89; 55)	−0.08 (0.64; 45)	0.05 (0.75; 55)	−0.14 (0.79; 209)
change from entry to week 96	−0.32 (1.06; 49)	−0.30 (0.84; 50)	−0.29 (0.73; 44)	−0.20 (1.04; 45)	−0.28 (0.92; 188)

EFV, efavirenz; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; ABC, abacavir; 3TC, lamivudine; ATV, atazanavir; RTV, ritonavir.

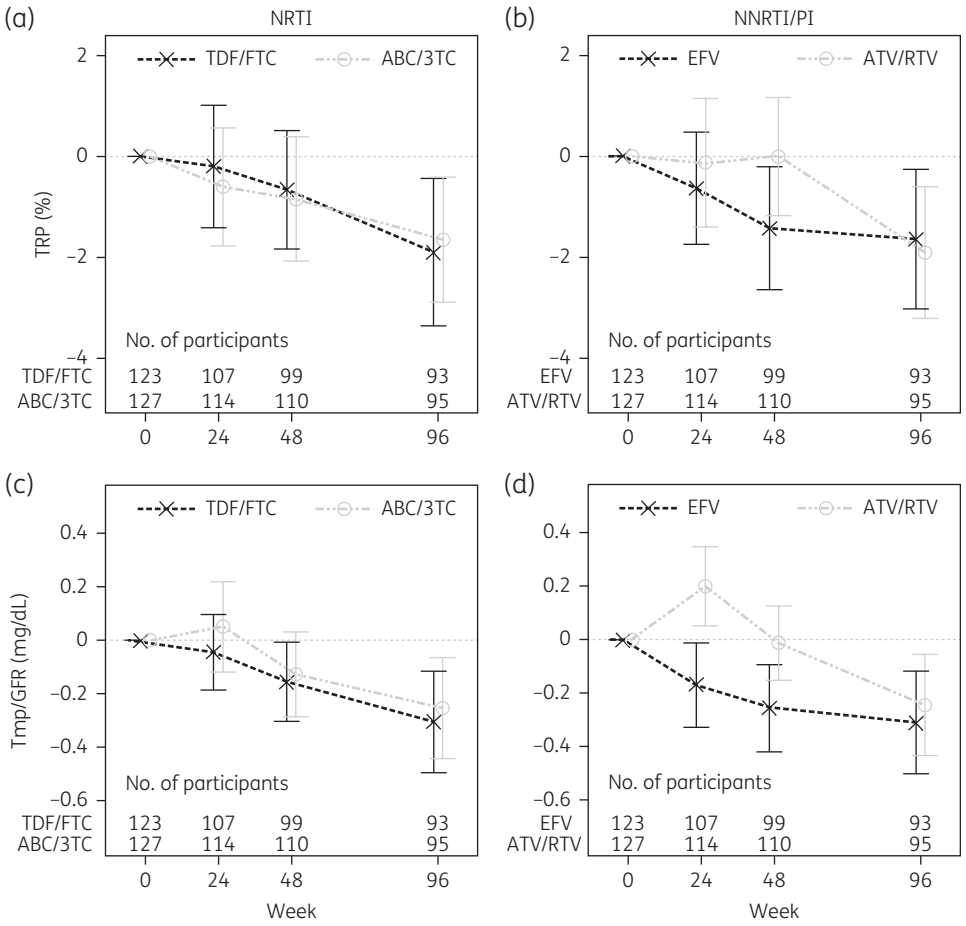

Figure 1. Changes in phosphaturia from entry to week 96 by NRTI and NNRTI/PI treatment components. (a) and (b), respectively, show absolute mean (95% CI) changes in TRP (%) in the NRTI treatment components (TDF/FTC and ABC/3TC) and in the NNRTI/PI treatment components (EFV and ATV/RTV). (c) and (d), respectively, show absolute mean (95% CI) changes in TmP/GFR (mg/dL) in the NRTI and NNRTI/PI treatment components. TDF/FTC, tenofovir/emtricitabine; ABC/3TC, abacavir/lamivudine; EFV, efavirenz; ATV/RTV, atazanavir/ritonavir.

Table 2. Correlations (*r*; *P*) between changes in TRP or TmP/GFR and changes in BMD from entry through week 96

		Overall		TDF		ABC	
Urine phosphate reabsorption	Study week	hip	spine	hip	spine	hip	spine
TRP	24	**−0.17; 0.013**	−0.09; 0.19	−0.10; 0.33	−0.03; 0.77	**−0.23; 0.018**	−0.11; 0.27
	48	**−0.17; 0.020**	−0.09; 0.22	−0.09; 0.40	0.05; 0.63	**−0.26; 0.012**	−0.20; 0.058
	96	−0.06; 0.47	0.05; 0.55	0.06; 0.60	0.04; 0.73	−0.18; 0.090	0.06; 0.60
TmP/GFR	24	−0.05; 0.46	−0.01; 0.92	0.00; 0.99	−0.09; 0.39	−0.12; 0.24	0.05; 0.62
	48	−0.08; 0.26	−0.07; 0.32	−0.17; 0.11	−0.10; 0.33	−0.04; 0.71	−0.05; 0.63
	96	−0.03; 0.70	0.13; 0.082	0.04; 0.73	0.03; 0.77	−0.12; 0.27	**0.23; 0.033**

TDF, tenofovir disoproxil fumarate; ABC, abacavir.
Items in bold are correlations that were statistically significant.

tenofovir/emtricitabine and −1.66 (−2.99 to −0.32) for abacavir/lamivudine, which produces a mean difference (95% CI) of 0.24 (−1.66 to 2.14) ($P = 0.80$). The mean (95% CI) absolute changes in TmP/GFR (mg/dL) were −0.31 (−0.49 to −0.12) for tenofovir/emtricitabine and −0.25 (−0.44 to −0.07) for abacavir/lamivudine, with a mean (95% CI) difference of 0.05 (−0.21 to 0.32) ($P = 0.70$). In exploratory analyses, we did not find significant interactions between the NRTI component and either the NNRTI/PI component or the change in eGFR through week 96 (all $P > 0.13$).

Correlations between changes in phosphaturia and BMD

We then correlated changes in both TRP and TmP/GFR with changes in BMD at the hip and spine in the overall study group and by NRTI treatment components (Table 2). In the overall study group, we found weak inverse correlations between changes in TRP and changes in hip BMD from entry to week 24 (−0.17, $P = 0.013$) and week 48 (−0.17, $P = 0.02$), thereby suggesting that increased urinary phosphate losses were correlated with less BMD loss at the hip at these time points. These correlations were driven primarily by significant inverse correlations in the abacavir/lamivudine-containing regimens. However, there was not a significant correlation between TRP and hip BMD at week 96 in the overall study group. We did not find any significant correlations between changes in phosphaturia, assessed as either TRP or TmP/GFR, and BMD at the hip or spine in the tenofovir/emtricitabine arms. We did find, however, a significant, albeit weak, positive correlation (0.23, $P = 0.033$) between increased urinary phosphate reabsorption and increased spine BMD in the abacavir-containing arms from entry to week 96.

In additional exploratory analyses, we then assessed the potential modifications of the effects of changes in phosphaturia on changes in hip and spine BMD by NNRTI/PI component and change in eGFR from entry to week 96. These analyses were performed to determine if these additional variables would affect renal clearance of phosphate and consequently unmask associations between phosphaturia and BMD change. No significant interactions were found in our models (data not shown). In additional models, we also did not find any significant interactions between the NRTI component and change in eGFR on the change in either hip or spine BMD.

Correlations between tenofovir exposure and changes in BMD and phosphaturia

The mean (SD) tenofovir AUC (mg·h/L) exposures estimated through sparse PK analysis of concentrations measured between weeks 4 and 24 in the overall group, in the efavirenz groups and in the atazanavir/ritonavir groups were 3.34 (1.19), 3.07 (1.30) and 3.61 (0.99), respectively. We found significant, but weak, correlations between tenofovir AUC and changes in hip BMD from entry to week 24 ($\rho = -0.22$, $P = 0.028$) and from entry to week 48 ($\rho = -0.26$, $P = 0.010$), but not through week 96 ($\rho = -0.14$, $P = 0.18$). There were no significant correlations between tenofovir AUC and spine BMD at any time point or between tenofovir AUC and either TRP or TmP/GFR from entry to week 24 (data not shown).

Discussion

The aetiology of BMD loss with initiation of ART is likely multifactorial, with growing evidence supporting immune reconstitution with increased net bone resorption as one possible explanation.[20] However, the reasons why regimens containing tenofovir disoproxil fumarate result in even greater BMD loss compared with other regimens remain unclear. The present study is, to our knowledge, the first systematic and comparative evaluation of changes in phosphaturia and correlation with changes in BMD in HIV-infected patients initiating ART with either tenofovir/emtricitabine or abacavir/lamivudine. We did not find evidence supporting a relationship between worsening urinary phosphate wasting and reductions in either the lumbar spine or the hip with use of tenofovir. However, instead we found significant, albeit weak, relationships between greater tenofovir exposures during the first 24 weeks of therapy and reduced hip BMD through week 48 of the trial, though not through week 96 of therapy. It should be noted that the reductions in BMD in A5224s occurred primarily during the first 48 weeks of therapy and plateaued or improved modestly afterwards to week 96, which may explain the lack of association with tenofovir exposure and changes in BMD at this latter time point.

These results corroborate those found by Hamzah *et al.*[21] in a cross-sectional study of 293 men receiving tenofovir disoproxil fumarate chronically. In that study, the investigators found no relationship between phosphaturia and BMD.[21] Moreover, in a trial

comparing continued emtricitabine/tenofovir disoproxil fumarate/efavirenz use with the switch to darunavir/ritonavir monotherapy, a significant improvement in BMD of the hip and spine in the nucleos(t)ide-sparing, PI monotherapy switch arm was observed compared with the tenofovir-containing arm after 48 weeks; however, no changes in phosphaturia were noted between study arms, thereby suggesting a lack of a relationship between phosphaturia and BMD change with tenofovir use.[22] However, our results stand in contrast to a recent study by Casado *et al.*[7] who examined 90 patients who had been receiving tenofovir for a median of 38 months. Greater phosphaturia was significantly related to lower BMD and predicted additional BMD loss over time;[7] however, this study did not measure tenofovir concentrations to examine the relationship between circulating exposure of tenofovir and bone loss. If we assume that the greater reductions in BMD with tenofovir are indeed due to circulating exposure with consequent direct bone toxicity and not due to urinary phosphate wasting, how can one explain the apparently disparate results between these two studies? It is plausible that greater tenofovir circulating concentrations, and by extension greater tenofovir renal proximal tubule concentrations, increase the risk and severity of proximal tubulopathy and worsening urinary phosphate wasting. If so, greater circulating concentrations of tenofovir may lead both to phosphaturia and to bone toxicity, but this does not necessarily mean that phosphaturia is causally related to bone loss; in other words, these two potentially pathological results of increased circulating tenofovir concentrations may be 'true, true and unrelated'.

In multivariable models incorporating the NNRTI/PI component and eGFR, we did not find differences in changes in phosphate wasting between tenofovir/emtricitabine and abacavir/lamivudine. This result corroborates the findings from smaller trials comparing tenofovir disoproxil fumarate with abacavir in previously ART-naive patients.[23,24] The relatively small changes in urinary phosphate wasting, measured as either TRP or as TmP/GFR, in our current study, both overall and specifically in the tenofovir-containing regimens, may be a result of offsetting factors. HIV infection itself leads to renal proximal tubulopathy,[25] and reductions in viraemia with ART initiation, even with tenofovir, may alleviate this phenomenon.[26] Thus, any potential increase in tenofovir disoproxil fumarate-induced urinary phosphate wasting may be blunted during initial therapy, but then may become more obvious with chronic therapy after viraemia is controlled, as was previously found in a trial of virologically suppressed patients switching to either tenofovir or abacavir.[27]

We acknowledge that the development of tenofovir alafenamide with resulting lower circulating tenofovir concentrations and tenofovir disoproxil fumarate-sparing ART regimens may minimize the concern for tenofovir-induced osteopenia and subsequent fracture risk in resource-replete settings.[11,28] However, the use of tenofovir disoproxil fumarate is becoming more widespread in resource-limited settings and thus presents continued concerns for bone safety in these populations. In addition, increasing tenofovir disoproxil fumarate use has been advocated in high-risk uninfected persons, both in resource-replete and constrained settings, as a means to prevent HIV infection. In fact, tenofovir use has been linked with modest reductions in BMD even in these HIV-negative pre-exposure prophylaxis populations.[29] Thus, understanding the mechanisms underlying tenofovir-related osteopenia remains clinically significant and relevant.

The effects of NNRTIs or PIs on phosphaturia have not been fully examined. Our results (Figure 1b and d) suggest that atazanavir/ritonavir-containing regimens did not appreciably impact either TRP or TmP/GFR during the first 48 weeks of treatment, but these parameters then fell from week 48 to week 96. Conversely, both phosphaturia measures fell steadily with the efavirenz regimens from entry to week 48, but then plateaued afterwards to week 96. The changes in TRP and TmP/GFR levels from entry to week 96 were then similar in each of the four study groups. It is possible that efavirenz initiation led to the induction of 24-hydroxylase activity and consequently reduction of vitamin D.[30] If so, then this could have led to an increase in parathyroid hormone[31] and increased phosphaturia during the first 48 weeks of treatment, although we cannot prove this as we did not measure vitamin D and parathyroid hormone levels in this study. The lack of changes in phosphaturia during the first 48 weeks of therapy with atazanavir/ritonavir, but then the increased phosphaturia during the next 48 weeks is not easily explained and, so, these results will require corroboration in future studies.

There are several limitations to these analyses that should be acknowledged. First, we did not measure tenofovir concentrations throughout the entire study period. Second, we did not measure bone turnover markers, vitamin D levels, parathyroid hormone levels or other bone or renal measures that might have suggested additional pathways by which tenofovir may have exacerbated bone loss in this population. This is particularly important given that the significant relationships we identified between tenofovir exposure and BMD reductions were weak and limited to the hip, thereby suggesting additional mechanisms of bone loss are likely. Third, we also did not correct for multiple testing, which may have led to false positive findings.

In conclusion, we did not find that the increased BMD loss with tenofovir disoproxil fumarate initiation in ACTG A5224s was associated with changes in urinary phosphate wasting. Instead, we found weak relationships between greater circulating tenofovir exposures and hip BMD reductions through the first 48 weeks of treatment. Additional studies are warranted to determine the aetiologies of bone loss with tenofovir.

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