Published in final edited form as:

AIDS. 2016 July 31; 30(12): 1935–1942. doi:10.1097/QAD.0000000000001131.

Longitudinal Increase in Vitamin D Binding Protein Levels after Initiation of Tenofovir/Lamivudine/Efavirenz among Individuals with HIV

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Abstract

Objective—To examine longitudinal change in vitamin D binding protein (DBP) levels during the first year after initiation of tenofovir (TDF)/lamivudine (3TC)/efavirenz (EFV), and compare these findings to concurrent changes in markers of skeletal metabolism.

Design—Secondary analysis of plasma samples collected from an ongoing multi-center clinical trial.

Methods—Plasma samples collected at 0, 24, and 48 weeks after initiation of TDF+3TC+EFV from 134 adult participants enrolled in a multi-center randomized trial were analyzed. Data regarding socio-demographic and clinical characteristics were obtained as part of the parent study. Laboratory analyses included plasma DBP, intact parathyroid hormone (iPTH), total 25-hydroxyvitamin D (25OHD), phosphorus, the bone resorption marker collagen type 1 cross-linked

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Conflicts of Interest The authors state that they have no conflicts of interest.

Prior Presentations: Data presented previously at the annual meetings of the Conference on Retroviruses and Opportunistic Infections (February 24, 2015, Seattle, WA), the American College of Rheumatology (November 10, 2015, San Francisco, CA), and the European Calcified Tissue Society (May 16, 2016).

C-telopeptide, and the bone formation marker total procollagen type 1 N-terminal propeptide. Repeated measures ANOVA was used to measure change in biomarkers over time.

Results—Our sample included 108 men and 26 women (mean age 33.6 ± 9.6 yrs). Median levels of DBP increased significantly from baseline to 48 weeks [154(91.8-257.4) v. 198.3(119.6-351.9) $\mu\text{g/mL}$, $p < 0.001$]. A concurrent rise in iPTH levels was observed over the same period [32.3(24.4-40.9) v. 45.2(35.1-60.4) pg/mL , $p < 0.001$], however 25OHD and phosphorus levels remained stable. Bone resorption and formation markers increased rapidly from 0 to 24 weeks, followed by a slight decline or plateau, but remained significantly elevated at 48 weeks ($p < 0.001$).

Conclusion—Our study provides longitudinal data supporting a potential role for DBP in bone loss associated with TDF-based therapy. Further research to elucidate the mechanistic pathways and clinical impact of these findings is warranted.

Keywords

Tenofovir; Bone Loss; Vitamin D Binding Protein; Skeletal Metabolism

Introduction

Tenofovir disoproxil fumarate (TDF) is a nucleotide analog reverse transcriptase inhibitor that forms a critical component of first-line antiretroviral therapy (ART) and pre-exposure prophylaxis regimens for HIV worldwide. In epidemiologic studies its use has been associated with disrupted bone metabolism, decreased bone mineral density, and increased rates of fracture among individuals with HIV [1,2]. The exact mechanisms underlying these associations remain unclear. Proposed mechanisms for these changes include toxicity of the proximal renal tubular cells causing urinary phosphate wasting, alterations of calcium and phosphate homeostasis, and osteomalacia [3,4]. However cases of Fanconi's syndrome are rare and osteomalacia from clinically significant hypophosphatemia is relatively uncommon among patients receiving TDF, and does not seem to sufficiently account for the degree of bone loss and fracture reported [5,6].

In addition to effects mediated through renal toxicity, multiple studies have also found TDF to be associated with elevations in parathyroid hormone (PTH) both in the presence of vitamin D deficiency, as measured by 25-hydroxy vitamin D (25OHD) levels, and among persons with sufficient 25OHD levels [7-10]. Vitamin D is a key hormonal regulator of bone mineral metabolism. It is initially obtained through dietary intake (D_2) or synthesized in the skin upon exposure to sunlight (D_3) then subsequently transported to the liver where it is converted to 25OHD by vitamin D-25-hydroxylase. In the kidney 25OHD undergoes further hydroxylation by the renal 1- α -hydroxylase to 1,25(OH) $_2$ D, the active form of vitamin D that acts upon target cells by binding to the vitamin D receptor [11]. During each step of this process, 85-90% of the body's vitamin D metabolites circulate tightly bound to the carrier protein, vitamin D binding protein (DBP). The non DBP-bound portion, known as bioavailable vitamin D, circulates less tightly bound to albumin, with <1% in the free form [12,13]. It is this free form which acts upon target cells in the intestine, kidney and bone to maintain calcium homeostasis. In the setting of vitamin D deficiency, decreased calcium

absorption leads to secondary elevations in PTH levels, which in turn leads to excessive bone resorption [14]. Clinical studies have shown that vitamin D deficiency is linked to decreased BMD, increased falls, and increased fracture rates [15-17].

In 2013, Havens and colleagues showed that in a cross-sectional cohort of HIV-infected youth receiving TDF-containing ART, higher plasma TDF concentration was associated with higher DBP levels, and lower levels of free 1,25(OH)₂D [18]. Furthermore, the authors found that PTH levels were elevated in this group compared with a cohort of HIV-infected youth on ART regimens without TDF, and that supplementation with Vitamin D₃ for 12 weeks led to subsequent decreases in PTH levels compared to placebo regardless of baseline 25OHD status [19]. These findings raise the possibility that treatment with TDF leads to a relative vitamin D deficiency that in part mediates the subsequent changes seen in bone metabolism, bone mineral density and fracture.

No studies have longitudinally examined the potential role of alterations in DBP in the pathogenesis of TDF-associated skeletal changes. We therefore performed a secondary analysis using plasma samples from an existing cohort of treatment-naïve individuals with HIV who were initiated on TDF + lamivudine (3TC) + efavirenz (EFV) and subsequently followed for one year. We hypothesized that plasma levels of DBP would increase during this period concurrently with iPTH and bone turnover markers (BTMs), and that this change would be accompanied by stable or decreased 25OHD and phosphorus levels.

Methods

Study Design and Study Population

We performed a secondary analysis of stored plasma samples collected from patients enrolled in an ongoing large multi-center open-label, clinical trial in eight cities across China including Beijing, Shanghai, Zhengzhou, Nanning, Liuzhou, Shenyang, Chengdu, and Changsha (ClinicalTrials.gov identifier: NCT01844297). The objective of the parent study was to evaluate the efficacy and safety of the first-line ART regimen in China, TDF+3TC+EFV, for ART-naïve Chinese Patients with HIV-1 Infection. Men and women were eligible for inclusion if they were aged 18-65 years with HIV-1 infection confirmed by Western Blot and documented CD4⁺ count < 500 cell/mm³ within the last 1 month, and ART-naïve. Exclusion criteria included: acute HIV infection, currently active AIDS-defining illness, pregnancy, breastfeeding, women of child-bearing age not on contraception, current injection drug use or alcohol abuse, acute or chronic pancreatitis, peripheral neuropathy, severe psychiatric or neurologic diseases, severe peptic ulcers, white blood cell count < 2000/μL, hemoglobin <9 g/dL, platelet count < 75 × 10⁹/L, aspartate transaminase, alanine transaminase or alkaline phosphatase three times the upper limit of normal (ULN), or serum creatinine one and a half times the ULN, creatinine clearance < 60mL/min, bilirubin and creatinine kinase two times the ULN.

All participants enrolled in the parent trial had a clinical and laboratory evaluation at baseline prior and were subsequently initiated on TDF+3TC+EFV. Follow up clinical and laboratory evaluations were performed at weeks 2, 4, 8, 12, and every 12 weeks thereafter. All plasma samples were collected fasting in the morning and stored at -80°C.

Of 409 participants enrolled in the parent study between 7/17/2012-7/24/2013, 390 (95%) individuals had completed one year of follow up as of 8/1/2014. From this group, we chose a random sample of 134 subjects from six cities (Beijing, Zhengzhou, Liuzhou, Nanning, Chengdu, and Changsha) in order to achieve a sample that maximized geographic diversity while minimizing variability in sample collection procedures. We excluded women who were over 45 years of age to avoid confounding by menopausal status. For each subject, we utilized plasma samples from the baseline, 24 week, and 48 week time points.

Baseline demographic and clinical data, including sex, age, body mass index (BMI), ethnicity, education level, alcohol use and smoking history, route of transmission, CD4⁺ cell counts, HIV-1 viral load, chronic hepatitis B infection (hepatitis B surface antigen positive, hepatitis B core antibody positive, hepatitis B surface antibody negative, and persistently detectable HBV DNA viral load over a 6 month period) and hepatitis C status (based upon HCV antibody), and creatinine were collected for all participants as part of the parent study.

This study was reviewed and exempt by the institutional review board of PUMCH and the human investigations committee of Yale School of Medicine prior to initiation, and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All samples and data used were previously collected and de-identified prior to analysis.

Laboratory Testing

Plasma DBP was measured using the Quantikine Human DBP enzyme-linked immunosorbent assay kit (R&D Systems, Inc., Minneapolis, MN). The bone resorption marker, C-terminal cross-linking telopeptide of type-1 collagen (CTX), and bone formation marker, total procollagen type 1 N-terminal propeptide (PINP), 25OHD, and iPTH were assayed by an electrochemiluminescence immunoassay (cobas e 601, Roche Diagnostics, Mannheim, Germany). Phosphorus was assessed using a molybdate-based method (Beckman Coulter AU5800 Chemistry Analyzer, Beckman Coulter Inc., Brea, CA, USA). Plasma HIV-1 RNA viral load was measured by the COBAS Ampliprep/TaqMan 48 (Roche Molecular Systems, Pleasanton, CA, USA). CD4⁺ T cell count was determined by 3-color flow cytometry (Epics XL flow cytometer, Beckman Coulter, USA).

Statistical Analysis

All statistical analyses were performed using Stata Intercooled 13 (StataCorp, College Station, TX). Descriptive statistics were used to report means, standard deviations, medians, interquartile ranges (IQR), and frequencies. Paired t-tests and the Wilcoxin signed-rank test were used to compare measures of central tendency at baseline versus 48 weeks for normally distributed (BMI and creatinine) and non-parametric (CD4⁺ cell count and viral load) continuous variables, respectively. A one-way repeated measures ANOVA was conducted to compare the effect of ART initiation on DBP and markers of vitamin D and bone metabolism (iPTH, 25OHD, Phosphorus, CTX, and PINP) at baseline, 24 weeks, and 48 weeks. Stratified analyses based upon age (<35 v. ≥35 years) BMI (<24 kg/m² v. ≥24 kg/m²), sex or baseline CD4 count (<250 cells/mm³ v. ≥250 cells/mm³) were performed to look for differences within subgroups. Correlation between CTX and PINP levels at each

time point were assessed using Pearson's correlation to confirm appropriate coupling of bone formation and resorption.

Results

Baseline Sociodemographic and Clinical Characteristics

Baseline characteristics of our cohort are shown in **Table 1**. Our sample included a total of 108 (80.6%) men and 26 (19.4%) women with a mean age of 33.6 ± 9.6 years and mean BMI of 22.3 ± 2.9 kg/m². Forty (30%) individuals had baseline CD4⁺ counts >350 cells/mm³, 61 (45.5%) had counts between 200-350 cells/mm³, and 33 (24.6%) had counts <200 cells/mm³. Mean creatinine levels were within normal limits at 71.4 ± 13.5 µmol/L. Twelve individuals (9.0%) had a concurrent diagnosis of chronic HBV, and four of 125 (3.2%) individuals had positive serology for HCV.

Change in Clinical and Laboratory Markers from Baseline to 48 Weeks

No significant change in mean BMI was observed over the study period (22.3 ± 2.9 v. 21.2 ± 3.1 kg/m², $p=0.47$). After initiation of ART, median CD4⁺ count increased significantly from baseline to 48 weeks [$290.5(201-362)$ v. $424(294-555)$ cells/mm³, $p<0.001$] with a concurrent decline in median viral load from 52,723 (IQR 19802 to 137798) to 0 (IQR 0 to 10) copies/mL over the same time period ($p<0.001$).

Figure 1 illustrates the change in DBP levels from baseline through 48 weeks after initiation of ART, and the changes observed in iPTH, 25OHD, phosphorus, CTX and P1NP. Median levels of both DBP and iPTH increased significantly over the 48 weeks of study [DBP: $F(2,265)=60.9$, $p<0.001$; iPTH: $F(2,266)=266.0$, $p<0.001$] yielding a +30.6% change in DBP levels (IQR 10.9-53.9) and +42.1% change in iPTH levels (IQR 12.5-89.9). By contrast median 25OHD and phosphorus levels remained essentially unchanged from baseline to 48 weeks [25OHD: $F(2,266)=2.1$, $p=0.14$; phosphorus: $F(2,265)=1.7$, $p=0.20$]. BTM levels rose rapidly between baseline and week 24, then plateaued or declined slightly between weeks 24 to 48, but remained significantly elevated compared to baseline at 48 weeks [CTX: $F(2,266)=107.3$, $p<0.001$; P1NP: $F(2,266)=163.0$, $p<0.001$] resulting in a +60.2% percent change in CTX (IQR 18.4-110.5) and a +59.7% change in P1NP (IQR 31.8-87.4).

The number of individuals meeting criteria for vitamin D deficiency (25OHD <20ng/mL) and insufficiency (25OHD 20-30ng/mL) did not change significantly over 48 weeks (**Table 2**), however the proportion of individuals with elevated iPTH levels (>65ng/mL) increased from 2.2% to 20.1% from baseline to week 48 ($p<0.001$).

No significant differences were seen in the pattern of change in DBP, iPTH, 25OHD or BTMs when stratified by age, BMI, sex, or baseline CD4 count (data not shown). In addition, mean creatinine levels remained stable from baseline to 48 weeks (71.5 v. 71.4 µmol/L, $p=0.996$). Levels of CTX and P1NP were significantly correlated at all time points (baseline: $r=0.65$, $p<0.001$; Week 24: $r=0.66$, $p<0.001$; week 48: $r=0.63$, $p<0.001$).

Discussion

Our study is the first to examine longitudinal change in DBP levels during the first year after initiation of TDF-based ART, and compare these findings to concurrent changes in markers of skeletal metabolism including iPTH, 25OHD, phosphorus and BTMs. We found that median levels of DBP increased significantly over 48 weeks in our cohort with a concurrent rise in plasma iPTH levels. The latter change occurred in the setting of relatively stable levels of 25OHD indicating that a deficiency in total 25OHD levels did not drive the increase in iPTH levels. Furthermore, both creatinine and phosphorus levels remained constant making it unlikely that renal dysfunction and altered phosphorus homeostasis contributed to the elevated iPTH levels. These collective findings support our hypothesis that exposure to TDF-based ART—in this case TDF+3TC+EFV—may cause DBP levels to rise resulting in decreased bioavailability of 25OHD, with a compensatory rise in iPTH.

DBP is a highly polymorphic serum protein, possessing a host of biological functions including vitamin D binding, actin scavenging, fatty acid transport, macrophage activation and chemotaxis [14]. As a vitamin D transporter, DBP is postulated to stabilize circulating vitamin D concentrations by binding vitamin D and its hydroxylated metabolites, thereby slowing their action on target tissues, and reducing uptake by the liver [15]. Under normal physiologic states DBP circulates in high molar excess to its ligands [14]. However it is less clear how DBP levels and activity are affected in the setting of chronic illness.

A variety of physiologic and pathophysiologic conditions can alter levels of DBP [20]. Pregnancy and exposure to exogenous estrogen are associated with significant increases DBP levels [21]. DBP levels are lowered in the setting of chronic liver disease due to decreased synthesis of DBP, chronic kidney disease due to increased renal losses, and in type 1 diabetes mellitus [22-4]. A few studies have found that DBP levels are decreased among adults and children in acute inflammatory states such as critical illness, sepsis, or following surgery, possibly due to increased urinary losses of DBP or increased actin binding to protect against polymerization of actin released from damaged tissue [25-7]. Based upon these reports, it is possible that DBP levels at baseline were relatively low in our cohort due to the inflammation associated with untreated HIV infection, and that after ART initiation, DBP normalized in parallel with a return to health. However our stratified analysis did not find a difference in baseline DBP levels based upon baseline CD4⁺ cell counts, nor did change in DBP and iPTH over one year differ based upon baseline CD4⁺ cell counts. In the cross-sectional study by Havens et al., the authors found that levels of DBP increased with successive quintiles of plasma TDF concentration among youth were stably treated with TDF, suggesting a pharmacologic effect independent of disease status [18]. No studies to date have examined whether TDF exposure impacts hepatocyte production or renal excretion of DBP.

With regards to BTMs, the magnitude and pattern of change in bone resorption and formation observed in our cohort after initiation of TDF-based ART was notable and consistent with previous studies from various countries, including our own prior studies among individuals with HIV in China [28-30]. CTX and P1NP levels increased by approximately 60% over one year, and were correlated at all time points demonstrating

appropriate coupling of bone resorption and formation among our participants. Also consistent with previous studies in other ethnic groups, the majority of that change occurs during the first six months.

Our data as well as earlier cross sectional studies, suggest that vitamin D supplementation may be efficacious in ameliorating these changes. In fact, a few studies have begun to examine the role of vitamin D supplementation to mitigate bone loss associated with ART, and in particular TDF. Havens and colleagues examined the impact of monthly vitamin D₃ supplementation for 12 weeks on HIV-infected youth and found that vitamin D₃ supplementation decreased PTH levels among youth receiving ART with TDF regardless of baseline 25OHD status, whereas these changes were not observed among youth receiving regimens without TDF [19]. Recently, a randomized clinical trial conducted by the AIDS Clinical Trials Group showed that among patients on TDF/emtricitabine/EFV who received 4000IU vitamin D₃ plus 1000mg calcium carbonate daily, BMD loss in the hip and lumbar spine were attenuated compared with individuals receiving a placebo [30]. In the placebo group, patterns of 25OHD, iPTH, and BTM are very similar to that found in our study, including a rise in iPTH and BTM levels without significant change in 25OHD levels or evidence of phosphate wasting. By contrast, in the supplementation group, 25OHD levels rose steadily and iPTH remained stable over 48 weeks, regardless of baseline 25OHD status. These findings provide corroborating evidence regarding the relationships observed in our cohort between markers of vitamin D and bone metabolism. Of note, baseline levels of 25OHD were somewhat lower in our Chinese cohort, consistent with prior studies in China showing high rates of vitamin D deficiency [31,32]. Accordingly, baseline iPTH levels in our cohort were higher and a more dramatic increase in iPTH levels (+40%) was observed over the course of 48 weeks as compared with that reported in the U.S. cohort (+20%).

It is important to recognize that in these prior studies, as in ours, the median iPTH levels remain within the normal reference range (0-65ng/mL). Therefore the marked changes in bone turnover markers observed are likely multifactorial and not solely attributed to secondary hyperparathyroidism. However, in our study 20% of patients did have iPTH levels that surpassed 65pg/mL by 48 weeks. As described in the introduction, other proposed drivers for bone loss among individuals treated with TDF include renal impairment and proximal tubulopathy (as evidenced by elevations in retinol-binding protein and β 2-microglobulin) however in practice these alterations are subclinical in the vast majority of patients and do not explain the rapid and dramatic decline in BMD seen during the first year of ART initiation [3-6]. A recent study exploring the role of the immunoskeletal interface in ART-associated bone loss showed that among 20 individuals with HIV treated with Lopinavir/ritonavir + TDF/emtricitabine, CTX levels increased significantly after ART initiation and was correlated with the magnitude of CD4⁺ T cell recovery. The authors observed an increase in key osteoclast stimulating inflammatory cytokines [tumor necrosis factor-alpha (TNF- α) and receptor activator of nuclear factor kappa-B ligand (RANKL)] and hypothesized that inflammation driven largely by reconstituting T cells may be responsible for the observed increase in bone resorption [33]. However, the sample size of this study was limited and other studies have also reported decreases in TNF- α and RANKL levels after initiation of ART regimens with and without TDF [9,34]. Therefore further research is necessary to fully understand how these inter-related mechanisms of renal dysfunction,

alterations in DBP and vitamin D metabolism, and the changing inflammatory and immunologic milieu intersect in TDF-associated bone loss.

Our study has several limitations that warrant mention. First, for the purposes of this secondary analysis we were not able to directly measure ionized calcium, calcitriol, or free 25OHD levels, nor could we measure albumin levels to enable calculation of free vitamin D based upon previously published algorithms. Second, our sample was not powered for subgroup analysis by individual seasons. However, DBP levels were not associated with seasonality within any individual time point, and no statistically significant differences between seasons were found, consistent with prior studies suggesting that DBP levels do not vary significantly with season. Third, the ART regimen used included the non-nucleoside reverse transcriptase inhibitor, EFV, which has been shown to be independently associated with low 25OHD levels via induction of the cytochrome P450 hydroxylase enzymes involved in vitamin D metabolism [35,36]. However in our cohort, 25OHD levels did not decline significantly during this period of time suggesting that the impact of EFV is less pronounced or perhaps was offset by other competing factors. Fourth, we did not have access to a cohort of patients treated with a comparison regimen to confirm that our findings are specific to TDF. Finally, we did not have access to clinical outcomes such as bone mineral density or fracture data as part of the original study. Future prospective studies should aim to incorporate these data to provide a more comprehensive picture of the factors contributing to TDF-associated bone loss among individuals with HIV.

Conclusions

TDF remains a critical component of ART regimens and pre-exposure prophylaxis for individuals with HIV world-wide, particularly in low- and middle-income countries, which bear the overwhelming burden of this pandemic. In these regions, infrastructure to diagnosis and treat bone disease is scarce and access to alternative ART regimens, including the recently FDA-approved tenofovir alafenamide (which is largely converted to active tenofovir intracellularly and therefore has reduced systemic toxicity), is severely limited. Therefore elucidating the mechanisms behind TDF-associated bone loss remains necessary in order to identify strategies to mitigate this effect.

Our findings provide the first longitudinal evidence that DBP levels increase over time following initiation of TDF-3TC-EFV among individuals with HIV. Concurrently we found increases in iPTH and BTMs levels, despite stable 25OHD and phosphorus levels. Future research should focus on elucidating the interconnections between these drivers and clinical outcomes such as BMD and fracture. As well, given what is known about DBP's biology and function, further study is warranted regarding the potential mechanisms underlying our findings and the downstream effects on bone and immune cell function in this population. Finally, our findings point to a strong rationale for additional research evaluating vitamin D supplementation among individuals with HIV initiating TDF-based ART.

Acknowledgements

This study was designed by EH, LF, WX, KLI, MTY, and TL. Data collection was carried out by EH, YH, TZ, QC, and TL. Data analysis was performed by EH and LF. Data was then interpreted by EH, LF, WX, KLI, MTY, and TL. Manuscript organization and writing was undertaken by EH, LF, KLI, MTY. All authors participated in the

manuscript review and approved the final version of the text as submitted to AIDS (EH, LF, YH, WX, KLI, MTY, TZ, QC, and TL).

Funding for this study was provided by the China National Key Technologies R&D Program for the 12th Five-year Plan (2012ZX10001-003) and the Rheumatology Research Foundation Scientist Development Award. L.F. is supported by NIAMS K24 AR060231-01. Our deepest gratitude to all the study participants and to the participating centers of the original parent study: The Infectious Disease Hospital of Henan Province, 302 Military Hospital of China, The Fourth People's Hospital of Nanning, Guangxi Zhuang Autonomous Region Longtan Hospital, Changsha Public Health Salvation Center, and Public Health Clinical Center of Chengdu. Special thanks as well to Jing Xie, Ling Luo, Yijia Li, and Kunli Li for helping make this study possible.

Sources of Funding

Funding for this study was provided by the China National Key Technologies R&D Program for the 12th Five-year Plan (2012ZX10001-003) and the Rheumatology Research Foundation Scientist Development Award. L.F. is supported by NIAMS K24 AR060231-01.

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Figure 1.

Median levels and interquartile range of markers of vitamin D and bone metabolism at baseline, 24 and 48 Weeks. The asterisks (*) signify markers for which repeated measures analysis demonstrated a statistically significant change over time from baseline ($p < 0.001$). A. Levels of vitamin D binding protein (DBP); B. Levels of intact parathyroid hormone (iPTH); C. Levels of 25-hydroxy vitamin D (25OHD); D. Levels of plasma phosphorus; E. Levels of collagen type 1 cross-linked C-telopeptide (CTX); F. Levels of total procollagen type 1 N-terminal propeptide (P1NP).

Table 1

Baseline Sociodemographic and Clinical Characteristics (N=134)

Characteristic	Baseline
Age <i>mean years ±SD</i>	33.6 ± 9.6
BMI <i>kg/m²</i> [†]	
Normal (<24)	99 (73.9)
Overweight (24-27.9)	29 (21.6)
Obese (≥ 28)	6 (4.5)
Male <i>n(%)</i>	108 (80.6)
Study Site <i>n(%)</i>	
Beijing	21 (15.5)
Changsha	15 (11.2)
Chengdu	27 (20.2)
Liuzhou	33 (24.6)
Nanning	19 (14.2)
Zhengzhou	19 (14.2)
Ethnicity <i>n(%)</i>	
Han	115 (85.8)
Zhuang	17 (12.7)
Other (Hmong, Tujia)	2 (1.5)
Marital Status <i>n(%)</i>	
Single	62 (46.2)
Married	60 (44.8)
Divorced / Widowed	12 (8.9)
Education <i>n(%)</i>	
Primary/Junior High	49 (36.6)
High School/Vocational School	27 (20.1)
College and Beyond	57 (42.5)
Unspecified	1 (0.7)
Occupation <i>n(%)</i>	
Farmer/Laborer	24 (17.9)
Other *	108 (80.6)
Unemployed	2 (1.5)
Current Alcohol Use <i>n(%)</i>	45 (33.6)
Smoking Ever <i>n(%)</i>	35 (26.1)
Route of HIV Transmission <i>n(%)</i>	
Sexual	132 (98.5)
Homosexual	57 (42.5)
Heterosexual	70 (52.2)
Unknown	5 (2.7)
Blood-borne	2 (1.5)
CD4⁺ cell count <i>mean cells/mm³</i>	290.5 (201-362)

Characteristic	Baseline
Viral Load <i>median copies/mL(IQR)</i>	52723 (19802-137798)
HBV Coinfection <i>n (%)</i>	12 (9.0)
HCV Coinfection <i>n/N (%)</i>	4/125 (3.2)

* The category “Other” includes administrator, civil servant, commerce/service profession, other professional, technician, student.

* BMI categories shown here are based on the accepted classification system for Chinese populations.

Table 2

Change in proportion of individuals with vitamin D deficiency and hyperparathyroidism over 48 weeks following initiation of tenofovir/ lamivudine/efavirenz (N=134)

	0 weeks	24 weeks	48 weeks	<i>p</i>-value
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	(0-48 weeks)
25OHD				
20-30 ng/mL	47 (35.0)	41 (30.6)	52 (38.8)	0.53
< 20ng/mL	52 (38.8)	47 (35.1)	52 (38.8)	1.00
iPTH				
> 65pg/mL	3 (2.2)	11 (8.1)	27 (20.1)	<0.001

25OHD: 25-hydroxy vitamin D; iPTH: intact parathyroid hormone