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ORIGINAL ARTICLE

A prospective cohort study of renal function and bone turnover in adults with hepatitis B virus (HBV)-HIV co-infection with high prevalence of tenofovir-based antiretroviral therapy use

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Abstract

Objective: Tenofovir disoproxil fumarate (TDF) is a common component of antiretroviral therapy in hepatitis B virus (HBV)-HIV co-infected adults but few studies have evaluated worsening renal function and bone turnover, known effects of TDF.

Methods: Adults from eight North American sites were enrolled in this cohort study. Research assessments were conducted at entry and every 24 weeks for ≤192 weeks. Bone markers were tested at baseline, week 96 and week 192 from stored serum. We evaluated changes in markers of renal function and bone turnover over time and potential contributing factors.

Results: A total of 115 patients were prospectively followed; median age 49 years, 91% male and 52% non-Hispanic Black. Duration of HIV was 20.5 years. TDF use ranged from 80% to 92% throughout follow-up. Estimated glomerular filtration rate (eGFR) (ml/min/1.73m^2) decreased from 87.1 to 79.9 over 192 weeks ($p < 0.001$); however, the prevalence of eGFR <60 ml/min/1.73m^2 did not appear to differ over time (always <16%; $p = 0.43$). From baseline to week 192, procollagen type I N-terminal propeptide (P1NP) (146.7 to 130.5 ng/ml; $p = 0.001$), osteocalcin (14.4 to 10.2 ng/ml; $p < 0.001$) and C-terminal telopeptides of type I collagen (CTX-1) (373 to 273 pg/ml; $p < 0.001$) decreased. Younger age, male sex and overweight/obesity versus normal weight predicted a decrease in eGRF. Black race, healthy weight versus underweight, advanced fibrosis, undetectable HBV DNA, and lower parathyroid hormone level predicted worsening bone turnover.

Conclusion: In this HBV-HIV cohort with high prevalence of TDF use, several biomarkers of renal function and bone turnover indicated worsening status over approximately 4 years, highlighting the importance of clinical awareness in co-infected adults.

KEYWORDS

AIDS, bone turnover, hepatitis B virus, human immunodeficiency virus, renal function, tenofovir

INTRODUCTION

Due to shared routes of transmission, co-infection with hepatitis B virus (HBV) and human immunodeficiency virus (HIV) is common [1–7]. Use of combination antiretroviral therapy (cART) has greatly reduced acquired immunodeficiency syndrome (AIDS)-related mortality. Current HIV treatment guidelines from the Department of Health and Human Services recommend that all persons living with HBV-HIV co-infection be treated with cART containing a tenofovir backbone, a nucleotide reverse transcriptase inhibitor (NRTI) [1–3, 8, 9] that suppresses both HIV and HBV replication. However, long-term use of tenofovir disoproxil fumarate (TDF) has been associated with adverse renal function and bone turnover in persons with HIV [10–15] and HBV [16–18]. There is evidence to suggest such effects are stronger when TDF is boosted with ritonavir or cobicistat [19, 20], as they increase plasma concentrations of TDF by one-quarter to one-third [19].

The mechanism of renal impairment may be related to the effect of TDF on the proximal tubule and has been associated with markers of tubular dysfunction including increased risk of proteinuria, phosphaturia and glucosuria [15, 21]. Risk factors for renal toxicity may also include older age, underweight, diabetes mellitus (DM), hypertension and first-generation protease inhibitors (PI) use [10, 22].

The mechanisms underlying the effect of TDF on bone turnover (indicative of acceleration of bone remodelling associated with bone loss) are not well defined. One hypothesis is that subclinical phosphate wasting leads to impaired bone mineralization and lower bone mineral density (BMD) [15]. TDF can also affect parathyroid hormone (PTH), a regulator of calcium and phosphate metabolism, and 1,25-dihydroxy vitamin D levels (25(OH)D), the major circulating form of vitamin D and precursor to the active form. However, despite its widespread use in HIV, the long-term renal and bone effects of TDF in HBV-HIV in the context of hepatic fibrosis and HBV suppression are unclear [14]. While a newer formulation, tenofovir alafenamide fumarate (TAF), has similar efficacy as TDF with lower nephrotoxicity and effects on bone turnover, TDF use remains part of the cART regimen in many settings.

To address these gaps in knowledge, our primary aim was to assess changes in renal function and bone turnover over time in an adult cohort with HBV-HIV co-infection and high (>80%) TDF use. Our secondary aim was to identify contributing clinical factors, including boosted TDF use, to changes in renal function and bone turnover.

METHODS

Study design

Adult patients in the HBV-HIV Cohort ($N = 139$) were recruited from eight sites to participate in this prospective

observational cohort study, regardless of type of ART used for HBV or HIV [23]. While the current analysis was part of the pre-planned objectives of the study, the target sample size was based on the primary aim to evaluate liver fibrosis progression. The study was not designed or powered to examine the impact of TDF on renal function and bone turnover. The study protocol specified study participants be at least 18 years old, chronically infected with HIV (anti-HIV-positive), hepatitis B surface antigen (HBsAg)-positive for at least 6 months, on cART including an anti-HBV nucleoside or nucleotide analogue, and agreeable to a liver biopsy within 1 year of study entry and ~3–4 years later [24] (Appendix S1). Those with decompensated cirrhosis, hepatitis C RNA and hepatocellular carcinoma were excluded. ART could be stopped, initiated or changed at any time per standard of care at the discretion of a treating physician. The institutional review board at each centre approved the protocol, and participants gave written informed consent. Study staff followed detailed manuals of operations to ensure consistency between sites. Data were entered by study coordinators or central laboratories and transmitted the Data Coordinating Center at the University of Pittsburgh, where they were was centrally managed and analysed. The study is registered at ClinicalTrials.gov (NCT01924455).

Participants underwent evaluation every 24 weeks. However, some laboratory measures were tested less often (details specified later). In addition to routine laboratory tests at each site, serum was sent to the Hepatitis B Research Network central laboratory (University of Washington) as previously reported [23, 24]. Follow-up ended with liver transplant or death. Otherwise, participants were followed up to ~4 years (192 weeks) or 31 January 2020, whichever came first. This report is limited to participants who were confirmed to be HBsAg-positive at study entry via central laboratory testing and had at least one bone or renal measure at baseline ($N = 134$). Longitudinal analysis also required participants to have at least one bone or renal measure at follow-up. While 115 participants had at least one outcome at baseline and follow-up, the number meeting this requirement ranged from 49 to 110 for renal and from 73 to 78 for bone turnover outcomes.

Assessments

The baseline and follow-up evaluations included assessment of demographics, medical history and current health status, with self-report and interviewer-administered questionnaires, a physical examination, and blood and urine tests, previously described [23, 24]. Relevant clinical, laboratory and radiological data were extracted from medical records, including standard of care test from local laboratories (e.g., liver enzymes, HIV-related parameters).

Research blood samples were collected at each assessment. Whole blood was processed and serum was stored at −70°C at each site, and shipped in batches to a central repository for subsequent transfer to central testing laboratories.

Renal function outcomes

Renal function studies included urinalysis for protein, creatinine and glucose, and fasting urine and serum phosphate (uPh, sPh) and creatinine (uCr, sCr) tested locally (tested every 48 weeks). Estimated glomerular filtration rate (eGFR) (tested every 24 weeks), was calculated with the CKD-EPI equation using serum creatinine (mg/dl) and demographic factors [25]. eGFR <90 ml/min/1.73m^2 is below normal and < 60 ml/min/1.73m^2 is indicative of chronic kidney disease [26, 27].

Renal threshold phosphate concentration was calculated as the ratio maximal tubular reabsorption capacity ($TmPO_4$)/eGFR, with $TmPO_4$ calculated as: sPh–([uPh*sCr]/uCr) [28]. A $TmPO_4$/eGFR ratio <2.5 was considered significant for urinary phosphate wasting [28, 29].

Bone turnover outcomes

Bone studies from stored serum samples collected in the fasting state at baseline, week 96 and week 192 were performed and included procollagen type I N-terminal propeptide (P1NP) (ng/ml) (enzyme-linked immunosorbent assay [ELISA], Abcam, ab210966) and osteocalcin (ng/ml) (ELISA, ImmunoDiagnostics, AC-11F1), both markers of bone formation, and C-terminal telopeptide of type I collagen (CTX-1) (pg/ml) (serum CrossLaps® ELISA, ImmunoDiagnostics, AC-02F1), which quantifies the degradation products of CTX-1, an established marker of bone resorption [30]. Intact parathyroid hormone (PTH) (pg/ml) (ELISA, ALPCO, cat# 21-IPTHU-E01) and 25 hydroxyvitamin D (25(OH)D) (ng/ml) (ELISA, Abcam, ab213966), which detects both 25(OH) vitamin D3 and 25(OH) vitamin D2, were also assessed [31]. All samples were run in duplicate, and analyte concentrations for each ELISA were determined using a four-parameter logistic (4PL) curve fitting approach, except CTX-1 for which a quadratic curve was applied. PTH >65 pg/ml was considered hyperparathyroidism [32]. 25(OH)D <20 ng/ml was considered vitamin D insufficiency and <12 ng/ml as vitamin D deficiency [33].

Covariates

Current and past cART use were collected from medication reconciliation with participants throughout the

study. However, cART use prior to study entry could not be verified in many participants due to the fragmented care received from different health providers at various sites between the time of HIV diagnosis and study enrollment. cART including an anti-HBV nucleoside or nucleotide analogue was categorized as including TDF, TAF or Other. In addition, TDF use was categorized as boosted (with ritonavir or cobicistat) or unboosted. HBV therapies were also categorized (no/yes) as nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) and integrase inhibitors. Current use of calcium, vitamin D, multivitamin, anticoagulant and immunosuppressant (e.g., prednisone), which may affect bone metabolism [33–37], were also recorded.

Participants self-reported estimated duration of HIV. CD4 cell count (cells/mm^3) was measured and current HIV stage (1–4) was defined by CD4 count at entry according to 2005 World Health Organization Guidelines [38]. HIV RNA suppression was defined as <400 copies/ml.

Quantitative HBV DNA and hepatitis B e-antigen (HBeAg) (tested every 24 weeks) and quantitative HBsAg (tested every 48 weeks) were performed centrally at University of Washington, Seattle, WA, USA as previously described [22, 24]. HBV DNA was categorized as <10 (undetectable), 10–<1000 (suppressed) and ≥1000 IU/ml (not suppressed). HBV/HIV status was categorized as suppressed (HBV DNA <1000 IU/ml, HIV RNA <400 copies/ml), incompletely suppressed (HBV DNA ≥1000 IU/ml, HIV RNA <400 copies/ml) and not suppressed (HBV DNA ≥1000 IU/mL, HIV RNA ≥400 copies/ml); no participants had HBV DNA <1000 IU/ml and HIV RNA ≥400 copies/ml. Upper limit of normal (ULN) for alanine aminotransferase (ALT) was defined as 30 U/L for men and 19 U/L for women [39].

Age, sex, race, current smoking status and alcohol consumption were self-reported. Alcohol consumption in the past 12 months was categorized as none or minimal (<1 drink per month), low-risk (more than none or minimal but ≤4 drinks/day or 14 drinks/week in men, ≤3 drinks/day or 7 drinks/week in women) or at-risk (more than moderate or ≥5 drinks on ≥1 day in past month) [40]. Height and weight were measured and used to calculate body mass index (BMI) and determine weight status. A liver biopsy taken at study entry was scored blindly (Ishak fibrosis score) as previously described [41].

Statistical analysis

Cross-sectional

Descriptive statistics were used to report characteristics of the baseline ($N = 134$) and longitudinal ($N = 115$) analysis samples. Spearman's correlation was used to evaluate an association between PTH (pg/ml) and $TmPO_4$/eGFR. Fisher's exact test was used to test the association between vitamin D supplementation and vitamin D insufficiency and deficiency, respectively.

Associations between demographics (age, sex, race/ethnicity, smoking, alcohol use), weight-related (BMI and weight status), HIV-related (TDF use, integrase inhibitor use, CD4 cell count, duration of known HIV infection, HIV ≥20 years) and HBV-related (HBV DNA status, advanced fibrosis at baseline biopsy) variables, respectively, with renal markers (eGFR and renal threshold phosphate concentration, respectively) were tested with a series of simple linear regression models. Simple log-binomial models were used to evaluate the same independent variables with respect to eGFR <60 ml/min/1.73m^2 and significant urinary phosphate wasting, respectively.

The same modelling strategies were used to test associations with markers of bone turnover (P1NP, osteocalcin and CTX-1). The same independent variables were considered with the following exceptions: integrase inhibitor use was not considered, but eGFR, eGFR <60 ml/min/1.73m^2, renal threshold phosphate concentration, 25-(OH)D, vitamin D status, PTH, secondary hyperparathyroidism, and anticoagulant, vitamin D and multivitamin use were.

For each renal and bone outcome, variables with $p < 0.20$ in simple models were entered into a single multivariable model and retained via backward elimination if $p < 0.10$. Results are presented as relative risks or regression coefficients with 95% confidence intervals (CI) and p values.

Longitudinal

Generalized linear mixed-effects models with the gamma distribution were used to test for change over time in select renal and bone biomarkers (eGFR, renal threshold phosphate concentration, P1NP, osteocalcin, CTX-1, 25(OH)D, PTH). Each model included a random intercept, site (related to missing follow-up data), the outcome as a repeated measure (every 24 or 48 weeks for renal measures; every 96 weeks for bone measures) and time (days from baseline) entered as fixed effects. TDF use and an interaction term between TDF and time were considered in each model to test whether values differed by TDF use and whether change over time differed by TDF use; these terms were retained in $p < 0.20$. eGFR <60 ml/min/1.73m^2, significant urinary phosphate wasting, vitamin D insufficiency and hyperparathyroidism were similarly evaluated over time with Poisson

TABLE 1 Demographics and clinical characteristics of an adult hepatitis B virus (HBV)-HIV co-infected cohort, by analysis sample

Variable	Baseline sample $n = 134$[a]	Longitudinal sample $n = 115$ [a]
Demographics		
Age, years		
Median (25th:75th)	49 (44: 54)	49 (45: 55)
Biological sex, *n* (%)		
Male	123 (91.8)	105 (91.3)
Race, *n* (%)	$n = 130$	$n = 111$
Non-Hispanic White	42 (32.3)	35 (31.5)
Non-Hispanic Black	68 (52.3)	58 (52.3)
Non-Hispanic Asian	5 (3.8)	5 (4.5)
Other	15 (11.5)	13 (11.7)
Current smoker, *n* (%)	30 (22.4)	24 (20.9)
Alcohol use, *n* (%)		
None	73 (54.5)	64 (55.7)
Moderate	43 (32.1)	36 (31.3)
At-risk	18 (13.4)	15 (13.0)
Weight-related		
BMI (kg/m^2)	$n = 128$	$n = 109$
Median (25th:75th)	25.9 (22.3: 30.4)	26.1 (22.6: 30.4)
Weight status, *n* (%)	$n = 128$	$n = 109$
Underweight	8 (6.3)	7 (6.4)
Healthy	45 (35.2)	36 (33.0)
Overweight	41 (32.0)	37 (33.9)
Obese	34 (26.6)	29 (26.6)
HIV-related		
Duration of HIV	$n = 122$	$n = 108$
Median (25th:75th)	20.0 (11.0: 25.0)	20.5 (14.0: 26.0)
CD4 cell count ($cells/mm^3$), per 100 units	$n = 116$	$n = 101$
Median (25th:75th)	564.5 (337: 702)	562 (366: 680)
HIV stage, *n* (%)	$n = 103$	$n = 86$
1–2 ($\geq$350 $cells/mm^3$)	86 (83.5%)	72 (83.7%)
3–4 (<350 $cells/mm^3$)	17 (16.5%)	14 (16.3%)
HIV RNA (copies/ml)	$n = 120$	$n = 105$
< 400	108 (90.0%)	97 (92.4%)
$\geq$400	12 (10.0%)	8 (7.6%)
HBV-related		
HBV DNA (IU/ml), *n* (%)		
Undetectable (<10 IU/ml)	45 (33.6)	39 (33.9)
Suppressed (10–<1000 IU/ml)	64 (47.8)	54 (47.0)
Not suppressed ($\geq$1000 IU/ml)	25 (18.7)	22 (19.1)
HBeAg-positive, *n* (%)	77 (57.5)	70 (60.9)
HBeAg (log_{10} IU/ml)	$n = 129$	$n = 112$
Median (25th:75th)	0.0 (−0.8: 1.3)	0.1 (−0.7: 1.6)

(Continues)

TABLE 1 (Continued)

Variable	Baseline sample $n = 134$[a]	Longitudinal sample $n = 115$ [a]
HBsAg ($\log_{10}$ IU/ml)	$n = 129$	$n = 112$
Median (25th:75th)	3.2 (2.6: 3.9)	3.3 (2.8: 3.9)
ALT (U/L)	$n = 129$	$n = 111$
Median (25th:75th)	28 (19: 42)	27 (19: 42)
AST (U/L)	$n = 129$	$n = 111$
Median (25th:75th)	29 (23: 41)	28 (22: 41)
Albumin (g/dl)	$n = 129$	$n = 111$
Median (25th:75th)	4.3 (4.1: 4.6)	4.3 (4.1: 4.6)
Platelets ($\times 10^3$/mm^3)	$n = 130$	$n = 112$
Median (25th:75th)	200 (174: 234)	200.5 (175: 238)
Fibrosis level, *n* (%)	$n = 116$	$n = 112$
None/minimal (0–1)	74 (63.8)	72 (64.3)
Significant (2)	15 (12.9)	14 (12.5)
Advanced (≥3)	27 (23.3)	26 (23.2)
HIV-HBV related		
HBV DNA (IU/ml) and HIV RNA (copies/ml) suppression status, *n* (%)	$n = 115$	$n = 104$
Suppressed (HBV DNA <1000 and HIV RNA <400)	92 (80.0%)	83 (79.8%)
Incomplete (HBV DNA ≥1000 and HIV RNA <400)	16 (13.9%)	14 (13.5%)
Not suppressed (HBV DNA ≥1000 and HIV RNA ≥400)	7 (6.1%)	7 (6.7%)
Medication use		
HBV therapy, *n* (%)		
None	4 (3.0)	3 (2.6)
TDF	112 (83.6)	97 (84.3)
Boosted	56 (41.8%)	49 (42.6%)
Unboosted	56 (41.8%)	48 (41.7%)
TAF	1 (0.7%)	1 (0.9%)
Other	17 (12.7%)	14 (12.2%)
NRTI, *n* (%)	127 (94.8)	110 (95.7)
NNRTI, *n* (%)	43 (32.1)	39 (33.9)
Protease inhibitors, *n* (%)	61 (45.5)	55 (47.8)
Integrase inhibitor, *n* (%)	61 (45.5)	54 (47.0)
Anticoagulant use, *n* (%)	5 (3.7)	5 (4.3)
Calcium use, *n* (%)	4 (3.0)	4 (3.5)
Multivitamin and vitamin D use, *n* (%)		
Neither	70 (52.2)	60 (52.2)
Multivitamin only	31 (23.1)	28 (24.3)
Vitamin D only	19 (14.2)	15 (13.0)
Multivitamin and vitamin D	14 (10.4)	12 (10.4)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CD4, cluster of differentiation 4; DNA, deoxyribonucleic acid; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis index based on four factors; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; RNA, ribonucleic acid; TAF, tenofovir alafenamide; TDF, tenofovir disoproxil fumarate.

[a]Data presented among the full samples unless a subset is indicated due to missing data.

TABLE 2 Cross-sectional associations between baseline factors and markers of renal health among adults with hepatitis B virus (HBV)-HIV co-infection

	eGFR (ml/min/1.73 m^2)				$TmPO_4$/eGFR				Significant urinary phosphate wasting (TmPO4/eGFR ratio <2.5)			
	N = 128 *Beta* (95% CI)	*p Value*	*N* = 111 Adj. *Beta* (95% CI)	*p Value*	*N* = 80 *Beta* (95% CI)	*p Value*	*N* = 77 Adj. *Beta* (95% CI)	*p*	*n/N* = 35/80 RR (95% CI)	*p Value*	*n/N* = 33/77 Adj. RR (95% CI)	*p*
Demographics												
Age, per 5 years	−6.23 (−8.31, −4.14)	<0.001	−6.09 (−8.23, −3.94)	<0.001	−0.08 (−0.17, 0.01)	0.09			1.01 (0.85, 1.19)	0.92		
Biological sex (ref = Male)		0.41				0.49				0.37		
Female	6.13 (−8.43, 20.69)				0.21 (−0.40, 0.82)				0.44 (0.07, 2.66)			
Race (ref = Non-Hispanic White)		0.02				0.01		0.002		0.02		0.006
Non-Hispanic Black	13.48 (4.34, 22.63)				0.47 (0.17, 0.77)		0.54 (0.24, 0.84)		0.41 (0.22, 0.77)		0.37 (0.20, 0.67)	
Other	6.79 (−5.63, 19.21)				0.22 (−0.17, 0.61)		0.27 (−0.11, 0.65)		0.68 (0.34, 1.33)		0.70 (0.37, 1.32)	
Current smoker (ref = No)		0.50				0.03				0.06		
Yes	−3.44 (−13.46, 6.57)				−0.39 (−0.75, −0.03)				1.60 (0.98, 2.63)			
Alcohol use (ref = None/minimal)		0.04		0.04		0.28				0.49		
Low-risk	−8.94 (−17.86, −0.03)		−9.93 (−18.21, −1.65)		0.25 (−0.06, 0.57)				0.71 (0.39, 1.28)			
At-risk	6.20 (−6.05, 18.46)		2.58 (−9.02, 14.18)		0.08 (−0.42, 0.59)				1.02 (0.47, 2.21)			
Weight												
BMI, per 10 kg/m^2	0.33 (−6.46, 7.12)	0.92			0.13 (−0.12, 0.39)	0.30			0.91 (0.59, 1.39)	0.65		
Weight status (ref = Healthy)		0.76				0.57				0.64		
Underweight	−2.19 (−20.00, 15.63)				−0.44 (−1.10, 0.22)				1.67 (0.68, 4.10)			
Overweight	−5.28 (−15.41, 4.84)				−0.06 (−0.42, 0.31)				1.34 (0.70, 2.58)			
Obese	−0.93 (−11.70, 9.84)				0.02 (−0.39, 0.44)				1.08 (0.49, 2.39)			
HIV-related												
CD4 cell count (cells/mm^3), per 100 units	−0.57 (−2.06, 0.92)	0.45			0.04 (−0.01, 0.09)	0.15	0.05 (0.002, 0.09)	0.04	0.93 (0.85, 1.02)	0.12	0.90 (0.83, 0.99)	0.03
Duration of HIV infection, per 10 years	−5.41 (−10.03, −0.78)	0.02			−0.11 (−0.28, 0.05)	0.18			1.29 (0.93, 1.80)	0.13		

(Continues)

TABLE 2 (Continued)

	eGFR (ml/min/1.73 m^2)				$TmPO_4$/eGFR				Significant urinary phosphate wasting (TmPO4/eGFR ratio <2.5)			
	N* = 128** ***Beta **(95% CI)**	***p Value***	***N* = 111** **Adj. *Beta*** **(95% CI)**	***p Value***	***N* = 80** ***Beta*** **(95% CI)**	***p Value***	***N* = 77** **Adj. *Beta*** **(95% CI)**	***p***	***n*/*N* = 35/80** **RR** **(95% CI)**	***p Value***	***n*/*N* = 33/77** **Adj. RR** **(95% CI)**	***p***
Duration of HIV infection ≥20 years (ref = No)		0.19				0.28				0.25		
Yes	−5.45 (−13.59, 2.68)				−0.17 (−0.48, 0.14)				1.42 (0.78, 2.56)			
HBV-related												
HBV DNA (ref = Undetectable)		0.09				0.17				0.90		
Suppressed (10- < 1000 IU/ml)	2.68 (−6.46, 11.83)				0.28 (−0.04, 0.61)				0.94 (0.55, 1.62)			
Not suppressed (≥1000 IU/ml)	12.55 (1.03, 24.07)				0.31 (−0.13, 0.75)				0.83 (0.37, 1.86)			
Advanced fibrosis (ref = No)		0.003		0.02		0.04				0.19		
Yes	−15.86 (−26.00, −5.71)		−10.62 (−19.71, −1.52)		−0.37 (−0.71, −0.03)				1.41 (0.84, 2.35)			
Medication												
TDF use (ref = No)		0.27				0.49				0.69		
Yes	6.31 (−4.90, 17.53)				−0.14 (−0.54, 0.26)				1.16 (0.55, 2.46)			
Integrase inhibitor (ref = No)		0.85				0.04				0.09		
Yes	−0.79 (−8.99, 7.40)				−0.30 (−0.59, −0.01)				1.58 (0.93, 2.66)			

Abbreviations: BMI, body mass index; CD4, CD4, cluster of differentiation 4; CI, confidence interval; DNA, deoxyribonucleic acid; eGFR, estimated glomerular filtration rate; HBV, hepatitis B virus; HIV, human immunodeficiency virus; RR, relative risk; TDF, tenofovir disoproxil fumarate; TmPO4/eGFR, renal threshold phosphate concentration.

mixed models with robust error variance. Values of p for each model are reported.

A series of linear mixed models were used to identify factors related to change in eGFR and renal threshold phosphate concentration, respectively, calculated as follow-up value minus baseline value, as repeated measures. To adjust for covariates, baseline demographics and advanced fibrosis status were considered, as were substance-related, weight-related and HIV-related variables, HBV DNA status and TDF use, entered as repeated measures. Similar methodology was used to identify factors related to change in P1NP, osteocalcin and CTX-1, respectively, with additional time-varying covariates (renal-related, bone-related and medication use variables). Variables with $p < 0.20$ in simple models were entered into a single multivariable model and retained via backward elimination if $p < 0.10$. Results from mixed models are presented as relative risks or regression coefficients with 95% CI and p values.

Sensitivity analysis

Among TDF users we tested whether being boosted with ritonavir or cobicistat (yes/no) was associated with outcomes.

Analyses were conducted using SAS, version 9.4 (SAS Institute Inc., Cary NC; 2000). Reported p values are two-sided and reported to aid interpretation of results.

RESULTS

Baseline/cross-sectional

Baseline demographics and clinical characteristics of the cohort, by analysis sample, are reported in Table 1. For the cross-sectional sample ($n = 134$), median age was 49 years, 92% were male, 52% were non-Hispanic black, median BMI was 26 kg/m^2, with 6.3% underweight, and 59% overweight or obese. Median duration of living with HIV was 20 years. Tenofovir use was reported by 84% of participants and 80% of participants had HBV/HIV suppression. Despite study entry criteria, four participants (3%) reported not being on any HBV therapy at the baseline assessment. Thirteen percent had significant but not advanced fibrosis (stage 2) and 23% had advanced fibrosis (stage 3–4). Four percent reported use of an anticoagulant and no participants reported immunosuppressant use. Three percent reported calcium use, all of whom also reported vitamin D use, which was reported by 25%. Multivitamin use was reported by 33%.

Just over half (52%) of participants had eGFR below normal (<90 ml/min/1.73 m^2) but only 9% were < 60 ml/min/1.73 m^2. Forty-four percent had significant urinary phosphate wasting, 58% had a vitamin D deficiency (an additional 23% had insufficiency) and 33% had hyperparathyroidism. Renal and bone measures for the cross-sectional sample are provided in Table S1.

TmPO4/eGFR was not correlated with PTH ($\rho = -0.10$; $p = 0.97$). Vitamin D insufficiency and deficiency were higher (88% and 65%, respectively) in those not taking vitamin D supplementation versus those taking vitamin D (58% and 35%, respectively; p for both<0.01).

Cross-sectional associations between demographics and clinical characteristics and renal-related outcomes are shown in Table 2. Older age (reflecting the inclusion of age in the CKD-EPI formula), low-risk versus no/minimal drinking and having advanced fibrosis were independently related to eGFR <60 ml/min/1.73m^2. Black versus White race and higher CD4 count were independently related to higher renal threshold phosphate concentration, and significant urinary phosphate wasting, respectively (p for all <0.05). Current TDF use was not related to renal outcomes (at $p < 0.05$) in unadjusted or adjusted models. In unadjusted models, integrase inhibitor use was related to lower $TmPO_4$/eGFR only, but dropped out in the adjusted model.

Cross-sectional associations with markers of bone turnover, P1NP, osteocalcin and CTX-1 are shown in Table S2. Advanced fibrosis and a higher renal threshold phosphate concentration were independently associated with higher P1NP, while being overweight versus normal weight, and TDF use were related to lower P1NP (e.g., P1NP was, on average, 54.8 ng/ml lower with versus without TDF use with adjustment for covariates; $p = 0.04$). There was an indication that TDF use was related to lower osteocalcin (on average, 5.12 ng/ml lower; $p = 0.06$) in an unadjusted model; however, only a higher BMI and lower eGRF were independently related to lower osteocalcin (p for both <0.05). Lower BMI, having HIV at least 20 years versus a shorter duration, and TDF use were independently related to lower CTX-1 (e.g., CTX-1 was, on average, 143.0 ng/ml lower with versus without TDF use with adjustment for covariates; $p = 0.04$).

Longitudinal

Current use of TDF (any, and specifically boosted or unboosted), TAF and any anti-HBV medication by time point is provided in Table S3. TDF use ranged from 79% to 84% in the first year, 84% to 85% in the second year, 83% to 90% in the third year and 91% to 92% in the fourth year. Approximately half of those on TDF were boosted at baseline but the percentage boosted dropped over time to about one-third of those on TDF by week 192 (Table S3). P values for TDF use and for an interaction between TDF use and time were ≥0.20 in all models

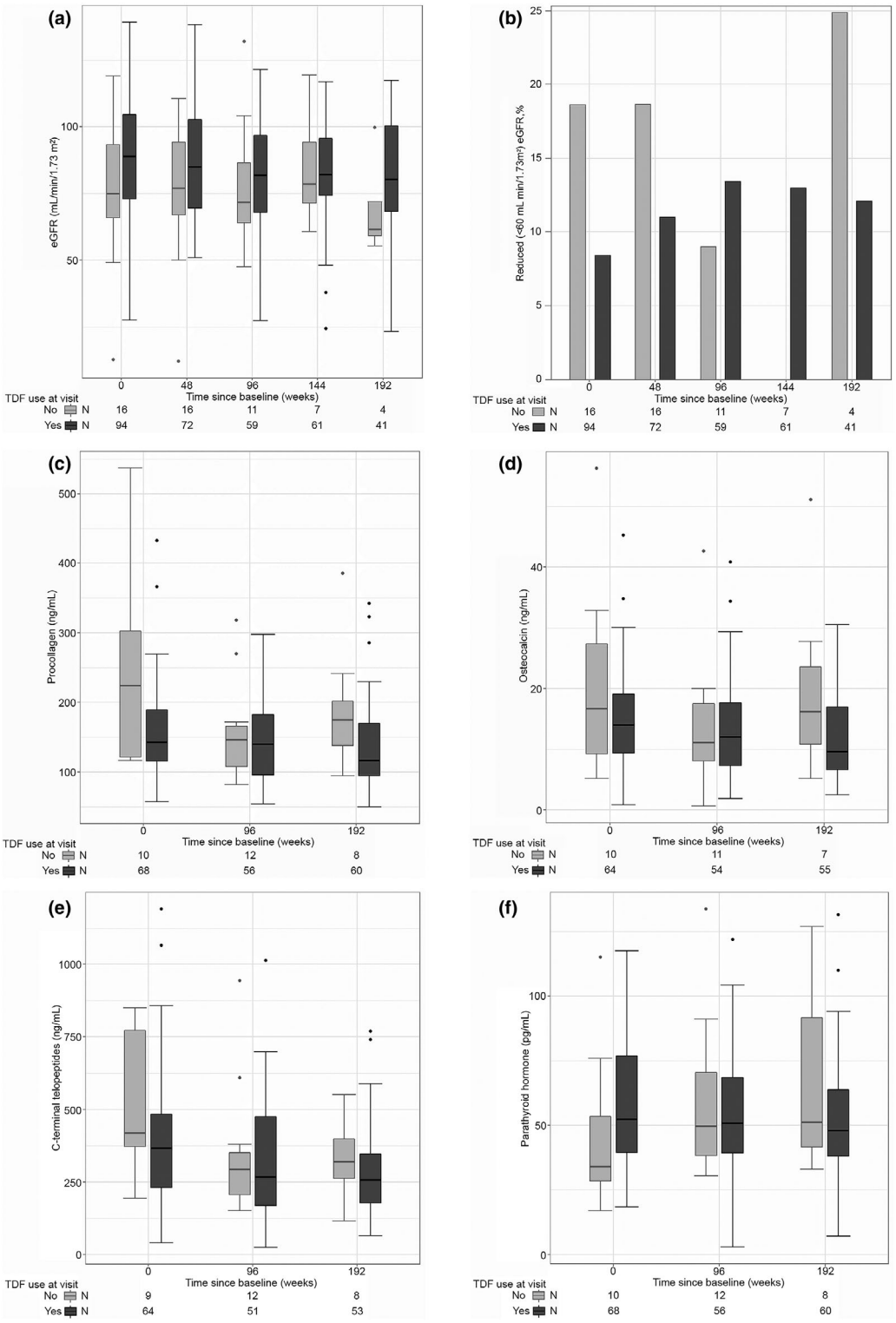

FIGURE 1 Renal and bone-related markers over time by tenofovir disoproxil fumarate (TDF) treatment among a cohort of North American adults with hepatitis B virus (HBV)-HIV co-infection. (a) Estimated glomerular filtration rate (eGFR) (ml/min/1.73 m^2); (b) eGFR <60 ml/min/1.73 m^2; (c) procollagen type I N-terminal propeptide (P1NP) (ng/ml); (d) osteocalcin (ng/ml); (e) C-terminal telopeptides (CTX-1) (ng/ml); (f); parathyroid hormone (PTH) (pg/ml). There was a decrease over time in eGFR ($p < 0.001$; panel a). However, there was no evidence of a change in the prevalence of eGFR <60 ml/min/1.73 m^2 ($p = 0.43$; panel b). There were also decreases over time in P1NP ($p = 0.001$; panel c), osteocalcin ($p < 0.001$; panel d) and CTX-1 ($p < 0.001$; panel e). There was no evidence of a difference in these outcomes by TDF use by time point or over time (p for TDF use and for a time*TDF use interaction >0.20 in all models). PTH decreased among participants with TDF use and increased in those without TDF use (TDF use interaction $p = 0.009$; panel f), but the majority of participants had values in the normal range. Abbreviations: CTX-1, C-terminal telopeptides; eGFR, estimated glomerular filtration rate; P1NP, procollagen type I N-terminal propeptide; PTH, parathyroid hormone; TDF, tenofovir disoproxil fumarate.

TABLE 3 Associations between demographic and time-varying clinical factors with change in renal biomarkers in an adult hepatitis B virus (HBV)-HIV co-infected cohort

	eGFR (ml/min/1.73 m^2) $n = 110$				$TmPO_4$/eGFR $n = 49$			
	Beta (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*	*Beta* (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*
Baseline demographics								
Age, per 5 years	1.36 (0.65, 2.08)	<0.001	1.34 (0.62, 2.05)	<0.001	0.04 (−0.02, 0.10)	0.23		
Biological sex (ref = Male)		0.008		<0.001		0.10		
Female	6.16 (1.61, 10.71)		8.47 (3.85, 13.08)		0.44 (−0.09, 0.98)			
Race (ref = Non-Hispanic White)		0.058				0.002		
Non-Hispanic Black	−2.62 (−5.25, 0.02)				0.11 (−0.09, 0.30)			
Other	−3.70 (−7.08, −0.31)				−0.29 (−0.49, −0.08)			
Current smoker (ref = No)		0.23				0.87		
Yes	2.73 (−1.76, 7.23)				0.02 (−0.22, 0.26)			
Alcohol use (ref = None/minimal)		0.19				0.052		0.02
Low-risk	0.29 (−3.63, 4.20)				−0.22 (−0.44, −0.01)		−0.25 (−0.46, −0.03)	
At-Risk	−6.37 (−13.43, 0.68)				−0.34 (−0.70, 0.03)		−0.43 (−0.81, −0.04)	
Weight								
BMI, per 10 kg/m^2	−2.28 (−4.17, −0.40)	0.02			0.08 (−0.10, 0.27)	0.38		
Weight status (ref = Healthy)		<0.001		<0.001		0.10		0.07
Underweight	11.79 (6.82, 16.76)		11.05 (6.14, 15.95)		−0.43 (−0.86, −0.002)		−0.55 (−0.99, −0.10)	
Overweight	−3.04 (−5.76, −0.33)		−2.93 (−5.60, −0.27)		−0.18 (−0.39, 0.04)		−0.18 (−0.42, 0.06)	
Obese	−1.59 (−4.50, 1.32)		−3.23 (−6.19, −0.27)		−0.01 (−0.26, 0.24)		−0.03 (−0.31, 0.25)	
HIV-related								
CD4 cell count (cells/mm^3), per 100 units	−0.18 (−0.63, 0.28)	0.44			−0.01 (−0.05, 0.02)	0.43		
Duration of HIV infection, per 10 years	−1.07 (−2.35, 0.22)	0.10			−0.05 (−0.15, 0.05)	0.34		
Duration of HIV infection ≥20 years (ref = No)		0.12				0.37		
Yes	−1.87 (−4.26, 0.51)				0.08 (−0.10, 0.27)			
HBV-related								
HBV DNA (ref = Undetectable)		0.34				0.99		
Suppressed (10- < 1000 IU/ml)	−0.48 (−2.89, 1.93)				0.003 (−0.19, 0.19)			

(Continues)

TABLE 3 (Continued)

	eGFR (ml/min/1.73 m^2) $n = 110$				$TmPO_4$/eGFR $n = 49$			
	Beta (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*	*Beta* (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*
Not suppressed (≥1000 IU/ml)	−2.88 (−6.70, 0.95)				0.02 (−0.40, 0.44)			
Baseline advanced fibrosis[a] (ref = No)		0.22				0.73		
Yes	1.74 (−1.01, 4.49)				0.04 (−0.17, 0.24)			
Medication								
TDF use (ref = No)		0.49				0.93		
Yes	−1.10 (−4.25, 2.04)				−0.01 (−0.24, 0.22)			
Integrase inhibitor		0.02				0.94		
Yes	−2.82 (−5.10, −0.54)				0.01 (−0.17, 0.19)			

Abbreviations: BMI, body mass index; CD4, CD4, cluster of differentiation 4; CI, confidence interval; DNA, deoxyribonucleic acid; eGFR, estimated glomerular filtration rate; HBV, hepatitis B virus; HIV, human immunodeficiency virus; TDF, tenofovir disoproxil fumarate; $TmPO_4$/eGFR, renal threshold phosphate concentration.

[a]Fibrosis status based on liver biopsy was unavailable for the full sample at follow-up, so only baseline status was evaluated.

evaluating change in renal and bone markers over time with one exception. PTH (51.2 [37.1–75.9] pg/ml at entry) slightly decreased over time with TDF use but increased without (interaction $p = 0.002$). However, the majority of participants with and without TDF use, respectively, had values in the normal range.

Boxplots of eGFR by time point, stratified by TDF use, are shown in Figure 1 (panel a). There was a decrease in eGFR over time from 87.1 (70.8–104.6) at entry to 79.9 (63.7–99.6) at week 192 ($p < 0.001$). However, median renal threshold phosphate concentration ($p = 0.34$) and the prevalence of eGFR <60 ml/min/1.73m^2 (Figure 1 panel b; $p = 0.43$) and significant urinary phosphate wasting ($p = 0.81$) appeared similar over time (Table S4).

Boxplots of P1NP, osteocalcin, CTX-1 and PTH by time point, stratified by TDF use, are also shown in Figure 1 (panels c–f). Study entry and week 192 values of P1NP were 146.7 (116.3–207.9) and 130.5 (95.4–179.9) ng/ml, osteocalcin were 14.4 (9.2–21.5) and 10.2 (6.8−17.6) ng/ml and CTX-1 were 373 (235–511) and 273 (186–351) pg/ml, respectively (p for all ≤0.001). In contrast, 25(OH)D was 10.5 (5.0–18.5) ng/ml at entry with no indication of change over time ($p = 0.55$) (Table S5).

Factors associated with change in eGFR and renal threshold phosphate concentration are shown in Table 3. Younger age, male sex and being normal weight versus underweight were independently related to a decrease in eGFR. Alcohol consumption (moderate or at-risk) versus none/low and underweight versus normal weight were independently related to a decrease in renal threshold phosphate concentration.

Factors associated with change in P1NP, osteocalcin and CTX-1, respectively, are shown in Table 4. Factors independently related to a decrease in P1NP included Black versus White race, HBV DNA undetected versus ≥1000 IU/ml, advanced fibrosis, insufficient versus sufficient vitamin D and lower levels of PTH. HBV DNA undetected versus ≥1000 IU/ml and not having hyperparathyroidism were independently related to a decrease in osteocalcin. Factors independently related to a decrease in CTX-1 included non-Hispanic Black versus non-Hispanic White, normal weight versus underweight, a lower CD4 cell count, a duration of HIV infection <20 years, and HBV DNA undetected versus ≥1000 IU/ml.

Sensitivity analysis

Cross-sectional and longitudinal associations between boosted versus unboosted TDF use with renal and bone

TABLE 4 Associations between demographic and time-varying clinical factors and change in markers of bone turnover in an adult hepatitis B virus (HBV)-HIV co-infected cohort

	P1NP (ng/ml) $n = 78$				Osteocalcin (ng/ml) $n = 74$				CTX-1 (ng/ml) $n = 73$			
	Beta (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*	*Beta* (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*	*Beta* (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*
Demographics												
Age, per 5 years	−4.44 (−12.38, 3.49)	0.27			0.39 (−0.44, 1.22)	0.35			−4.96 (−28.47, 18.55)	0.68		
Biological sex (ref = Male)		0.85				0.79				0.58		
Female	5.40 (−49.72, 60.53)				0.85 (−5.56, 7.27)				44.52 (−115.75, 204.79)			
Race (ref = Non-Hispanic White)		0.03		<0.001		0.98				0.01		0.005
Non-Hispanic Black	−39.34 (−68.50, −10.19)		−56.11 (−83.27, −28.95)		−0.31 (−3.44, 2.82)				−116.05 (−205.55, −26.55)		−94.60 (−180.74, −8.46)	
Other	−23.70 (−63.69, 16.28)		−15.11 (−51.77, 21.55)		−0.24 (−4.42, 3.94)				8.57 (−109.57, 126.72)		65.34 (−41.93, 172.62)	
Current smoker (ref = No)		0.35				0.25				0.50		
Yes	17.02 (−18.94, 52.99)				2.17 (−1.52, 5.86)				35.70 (−68.52, 139.93)			
Alcohol use (ref = None/minimal)		0.34				0.46				0.39		
Low-risk	22.29 (−7.82, 52.40)				1.77 (−1.34, 4.88)				−9.33 (−100.67, 82.02)			
At-risk	10.47 (−38.77, 59.71)				1.90 (−3.10, 6.90)				97.98 (−52.95, 248.90)			
Weight												
BMI, per 10 kg/m^2	−5.03 (−27.25, 17.19)	0.66			−0.87 (−3.26, 1.52)	0.47			5.13 (−64.39, 74.66)	0.88		
Weight status (ref = Healthy)		0.19				0.31				0.04		0.009
Underweight	5.98 (−47.89, 59.85)				3.49 (−2.31, 9.29)				198.52 (42.14, 354.89)		237.31 (98.87, 375.74)	
Overweight	30.95 (0.19, 61.70)				2.50 (−0.69, 5.70)				93.66 (−0.39, 187.71)		55.50 (−34.25, 145.24)	
Obese	−0.28 (−34.29, 33.73)				0.05 (−3.64, 3.74)				78.22 (−23.51, 179.94)		17.14 (−82.6, 116.88)	

(Continues)

TABLE 4 (Continued)

	P1NP (ng/ml) $n = 78$				Osteocalcin (ng/ml) $n = 74$				CTX-1 (ng/ml) $n = 73$			
	Beta (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*	*Beta* (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*	*Beta* (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*
HIV-related												
CD4 cell count (cells/mm^3), per 100 units	2.96 (−2.12, 8.03)	0.25			0.08 (−0.44, 0.61)	0.75			12.74 (−1.69, 27.16)	0.08	21.82 (9.38, 34.25)	<0.001
Duration of HIV infection, per 10 years	8.64 (−6.90, 24.19)	0.27			1.63 (−0.10, 3.36)	0.06	1.67 (−0.02, 3.35)	0.053	25.40 (−20.88, 71.68)	0.28		
Duration of HIV infection ≥20 years (ref = No)		0.09				0.09				0.01		0.02
Yes	22.76 (−3.74, 49.26)				2.41 (−0.39, 5.21)				99.76 (22.20, 177.33)		91.74 (15.15, 168.34)	
HBV-related												
HBV DNA (ref = Undetectable)		0.02		0.001		0.02		0.008		0.03		0.02
Suppressed (10- < 1000 IU/ml)	−5.48 (−31.99, 21.04)		−14.53 (−39.34, 10.29)		−1.18 (−3.96, 1.59)		−0.29 (−3.13, 2.55)		−2.08 (−82.72, 78.56)		1.51 (−74.40, 77.42)	
Not suppressed (≥1000 IU/ml)	60.61 (14.28, 106.95)		66.62 (24.22, 109.01)		5.84 (0.95, 10.73)		7.28 (2.47, 12.08)		182.81 (42.18, 323.44)		181.07 (52.09, 310.05)	
Baseline advanced fibrosis (Ishak ≥3)[a] (ref = No)		0.001		<0.001		0.56				0.27		
Yes	−50.99 (−81.44, −20.54)		−56.28 (−85.49, −27.06)		−1.00 (−4.42, 2.42)				53.93 (−42.43, 150.29)			
Renal-related												
eGFR (ml/min/1.73 m^2), per 100 units	−20.31 (−92.42, 51.81)	0.58			−4.44 (−12.04, 3.15)	0.25			−31.68 (−237.99, 174.63)	0.76		
Reduced (<60 ml/min/1.73 m^2) eGFR (ref = No)		0.47				0.59				0.12		0.055
Yes	−18.07 (−67.27, 31.13)				−1.34 (−6.26, 3.57)				−106.51 (−241.58, 28.56)		−143.18 (−289.75, 3.40)	
$TmPO_4$/eGFR, per 1 unit	10.69 (−14.24, 35.62)	0.39			1.15 (−1.58, 3.89)	0.40			33.93 (−47.85, 115.71)	0.41		

TABLE 4 (Continued)

	P1NP (ng/ml) $n = 78$				Osteocalcin (ng/ml) $n = 74$				CTX-1 (ng/ml) $n = 73$			
	Beta (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*	*Beta* (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*	*Beta* (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*
Bone-related												
25(OH)D (ng/ml), per 10 units	−8.23 (−17.86, 1.41)	0.09			−0.60 (−1.60, 0.40)	0.24			2.08 (−26.87, 31.03)	0.89		
Vitamin D status (ref = Sufficient)		0.17		0.09		0.38				0.92		
Insufficient (12- < 20 ng/ml)	−26.32 (−66.47, 13.82)		−36.97 (−73.23, −0.71)		−1.80 (−5.95, 2.36)				24.83 (−101.77, 151.43)			
Deficient (<12 ng/ml)	8.51 (−21.26, 38.28)		−0.54 (−30.12, 29.04)		0.84 (−2.32, 4.00)				12.00 (−77.77, 101.76)			
Parathyroid hormone (pg/ml), per 100 unit	61.67 (9.41, 113.94)	0.02	58.06 (6.79, 109.34)	0.03	6.02 (0.49, 11.55)	0.03			55.22 (−104.25, 214.7)	0.49		
Hyperparathyroidism (ref = No)		0.03				0.02		0.02		0.61		
Yes	31.76 (3.12, 60.40)				3.60 (0.57, 6.64)		3.68 (0.60, 6.76)		−22.65 (−110.14, 64.84)			
Medication												
TDF use (ref = No)		0.37				0.18				0.28		
Yes	16.46 (−20.06, 52.98)				−2.63 (−6.51, 1.24)				58.57 (−48.09, 165.24)			
Anticoagulant use (ref = No)		0.53				0.53				0.43		
Yes	28.35 (−59.84, 116.54)				2.82 (−6.13, 11.76)				102.25 (−153.67, 358.16)			
Calcium use (ref = No)		0.48				0.60				0.14		
Yes	18.80 (−33.29, 70.88)				1.58 (−4.37, 7.54)				112.61 (−37.98, 263.21)			
Multivitamin and vitamin D use (Ref = Neither)		0.55				0.31				0.65		
Multivitamin	−21.58 (−54.89, 11.72)				−2.56 (−5.97, 0.85)				19.98 (−81.24, 121.20)			

(Continues)

TABLE 4 (Continued)

	P1NP (ng/ml) $n = 78$			Osteocalcin (ng/ml) $n = 74$			CTX-1 (ng/ml) $n = 73$		
	Beta (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*Beta* (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*Beta* (95% CI)	*p Value*	*Adj. Beta* (95% CI)	*p Value*
Vitamin D	−17.52 (−50.82, 15.79)			−1.31 (−4.87, 2.25)			−50.41 (−152.8, 51.97)			
Multivitamin and Vitamin D	−16.47 (−61.50, 28.56)			−3.61 (−8.19, 0.97)			−29.75 (−165.57, 106.08)			

Abbreviations: 25(OH)D, 1,25-dihydroxy vitamin D levels; BMI, body mass index; CD4, cluster of differentiation 4; CI, confidence interval; CTX-1, C-terminal telopeptides; DNA, deoxyribonucleic acid; eGFR, estimated glomerular filtration rate; HBV, hepatitis B virus; HIV, human immunodeficiency virus; P1NP, procollagen type I N-terminal propeptide; TDF, tenofovir disoproxil fumarate; TmPO4/eGFR, renal threshold phosphate concentration.

[a]Fibrosis status based on liver biopsy was unavailable for the full sample at follow-up, so only baseline status was evaluated.

outcomes are provided in supplemental material (Tables S6 and S7, respectively). No associations had a $p < 0.10$.

DISCUSSION

In this prospective cohort of adults with HBV-HIV followed for ~4 years, the substantial majority of whom were on TDF, on average eGFR decreased over time, although the prevalence of eGFR indicative of chronic kidney disease remained fairly stable. We also observed a decrease in bone turnover markers, particularly bone formation and resorption markers like P1NP, osteocalcin and CTX-1. TDF use was negatively related to P1NP and CTX-1, but not osteocalcin or renal markers, at study entry. We did not detect an association between TDF use and change in any bone or renal markers across follow-up; however, statistical power to detect an association with TDF use was limited due to the small number of participants not on TDF.

In studies to evaluate the impact of TDF-containing ART regimens, TDF use has been associated with kidney tubulopathy [42] and decline in eGFR in some [15] but not all studies [43]. A published case report implicated TDF use with significant kidney damage and occurrence of Fanconi syndrome [44], though post-market studies did not reveal this. Older age, low body weight, pre-existing kidney dysfunction and concomitant nephrotoxic drug use were identified as risk factors for developing TDF-associated nephrotoxicity [10]. In our study, being underweight versus normal weight, but not older age, was independently related to a decrease in renal threshold phosphate concentration, although we could not determine if these factors interacted with TDF use. Unlike most other studies [19, 20], we did not observe a difference in renal function between boosted versus unboosted TDF.

Similar to a large meta-analysis of 17 studies including 10 889 HIV participants (percentage with HBV unknown) which showed a modest decrease in eGFR over time, particularly in participants with low kidney function at baseline [45], our study showed a decline in eGFR across 4 years, the majority of whom were taking TDF. Since age is a component of eGFR, its decline over time is expected. However, we were surprised to observe a positive association between age and change in eGFR over time, that is, younger age was associated with a decrease. One explanation is that serum creatinine may not be a good biomarker of eGFR among older adults as it can be low in those with low muscle mass. Also contrary to expectation, we observed that being normal weight versus underweight was associated with a decrease in eGFR. Given

that only eight participants were underweight, this requires further study.

Despite high prevalence of significant phosphate wasting in our cohort at baseline, renal threshold phosphate concentration and prevalence of significant phosphate wasting remained fairly stable across 4 years of follow-up. This observation is similar to most other studies showing higher rate of phosphate wasting measures in HIV participants but no worsening after being on TDF [14, 46–48]. Unlike other studies [18], TmPo4/eGFR was not correlated with PTH at baseline and over time in our cohort. This difference may be related to the differences in demographics of the study populations or duration of observation. TDF use has been associated with decreased BMD in HIV patients treated with cART [49–56], which could be a result of subclinical phosphate wasting [45]. However, there are few data regarding these effects in HBV-HIV co-infected populations. Bone mineral turnover (BMT) markers may mediate the BMD decline observed with TDF-containing regimens as evidenced by high BMT markers associated with bone resorption markers, PTH levels and negatively associated with bone formation markers [57].

Our study measured decreases in all bone formation and resorption markers such as P1NP, osteocalcin and CTX-1, which may indicate accelerated bone remodelling. Because TDF was used in most participants throughout our observation, without a control (no TDF) group we could not determine the impact TDF on renal function and bone turnover markers. Prior studies demonstrated a higher level of PTH associated with TDF use and resultant disruption of physiologic relationship between vitamin D (25(OH)D) to PTH in participants using TDF [57]. Overall, our study is in line with others which suggest monitoring of bone markers in HIV patients on TDF is not essential with 25(OH)D status, hyperparathyroidism or BMT markers [58]. However, our finding that vitamin D supplementation was related to lower vitamin D insufficiency and deficiency prevalence supports its utility in this population. Finally, unlike most other studies [22], we did not see an impact of boosted versus unboosted TDF on bone health.

The strengths of our study were the prospective nature, close follow-up, the systematic collection of renal and bone studies, and the availability of liver history at baseline to determine the impact of liver fibrosis. However, our study also had several limitations. Our cohort consisted of only those who were willing to undergo initial liver biopsy from eight sites in North America and therefore may not reflect all adults with HBV-HIV. Furthermore, since most of the cohort was on cART including TDF before study entry, and cART use was not mandated by the protocol, we were unable to evaluate its use with appropriate statistical power. Furthermore, because the effect on renal function may occur soon after initiating TDF containing cART and remain relatively stable thereafter [43], our study was not able determine the impact of initiating cART. We also could not rule out that treating clinicians took participants' renal function and bone turnover into account when deciding if and how to treat patients (such as putting patients with lower eGFR on non-TDF containing regimens). In addition, our sample size was moderate and our follow-up only 4 years thus limiting the observations for multivariate analyses. Therefore, larger studies of longer duration, with greater variation in TDF use, will be needed to confirm our findings. In addition, we did not include other markers of renal injury, such as cystatin-C or dual X-ray absorptiometry (DEXA) scans, to monitor changes in BMD. Lastly, we did not have a HIV or HBV mono-infected cohort on TDF for comparison.

In conclusion, in this prospective cohort study of adults with HBV-HIV, the majority of whom used TDF, several biomarkers of renal function and bone turnover indicated worsening status over ~4 years highlighting the importance of clinical awareness, especially in at-risk groups.

AUTHOR CONTRIBUTIONS

AG contributed to analysing the data and drafting the manuscript. WCK performed the data analysis, contributed to analysis and interpretation of results, drafted the manuscript, and revised the manuscript critically for content. ASH performed the data analysis, contributed to analysis and interpretation of results, and revised the manuscript critically for content. RC, ML-M, MarcGG, MK, MKJ, DEK, MS and DKW contributed data collection and revised the manuscript critically for content. JG and TS-S performed the bone and renal assays, helped interpret the results, and revised the manuscript critically for content. RKS contributed to data collection, analysis, and interpretation of results, drafted the manuscript, and revised the manuscript critically for content. All authors approved the final manuscript for submission.

CONFLICTS OF INTEREST

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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