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ORIGINAL ARTICLE

Associations between vitamin D metabolites, antiretroviral therapy and bone mineral density in people with HIV

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Abstract
Summary Rationale: To see if vitamin D and antiretroviral therapy are associated with bone mineral density (BMD) in people with HIV. Result: Lower hip BMD was associated with tenofovir (an antiretroviral medicine) in those with 25(OH)D ≥50 nmol/L. Significance: The relationship between antiretroviral therapy and hip BMD differs depending on vitamin D status.
Introduction People with HIV have an increased risk of low BMD and fractures. Antiretroviral therapy contributes to this increased risk.

The aim of this study was to evaluate associations between vitamin D metabolites and antiretroviral therapy on BMD.
Methods The simplification of antiretroviral therapy with tenofovir-emtricitabine or abacavir-lamivudine trial (STEAL) was an open-label, prospective randomised non-inferiority study that compared simplification of current nucleoside reverse transcriptase inhibitors (NRTIs) to fixed-dose combination tenofovir-emtricitabine (TDF-FTC) or abacavir-lamivudine. Serum 25(OH)D and $1,25(OH)_2D$ were measured in 160 individuals (90 receiving TDF-FTC, 70 receiving other NRTIs) at baseline from this study. Multivariable linear regression models were constructed to evaluate the covariates of $1,25(OH)_2D$ and BMD.
Results Protease inhibitor use ($p = 0.02$) and higher body mass index (BMI) ($p = 0.002$) were associated with lower $1,25(OH)_2D$ levels in those with 25(OH)D <50 nmol/L. However, TDF-FTC use ($p = 0.01$) was associated with higher $1,25(OH)_2D$ levels, but only in those with 25(OH)D ≥50 nmol/L.

White ethnicity ($p = 0.02$) and lower BMI ($p < 0.001$) in those with 25(OH)D <50 nmol/L and with TDF-FTC use ($p = 0.008$) in those with 25(OH)D ≥50 nmol/L were associated with lower hip BMD. TDF-FTC use, higher serum calcium and serum βCTX, winter, and lower bone-specific alkaline phosphatase (BALP) and BMI were associated with lower lumbar spine BMD.
Conclusion TDF-FTC use (versus non-TDF-FTC use) was associated with lower hip BMD, and this difference was more pronounced in those with 25(OH)D ≥50 nmol/L. Serum 25(OH)D <50 nmol/L was associated with lower hip BMD in all participants. Therefore, the associations between antiretroviral therapy and hip BMD differ depending on vitamin D status.

Keywords Antiretroviral therapy · Bone · HIV · Vitamin D

Abbreviations

25(OH)D	25-Hydroxyvitamin D
$1,25(OH)_2D$	1,25-Dihydroxyvitamin D

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ABC-3TC	Abacavir-lamivudine
BALP	Bone-specific alkaline phosphatase
βCTx	C-terminal cross-linking telopeptide of type 1 collagen
BMD	Bone mineral density
BTM	Bone turnover marker
CI	Confidence interval
ART	Antiretroviral therapy
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NtRTI	Nucleotide reverse transcriptase inhibitor
P1NP	Procollagen type 1N-terminal propeptide
PI	Protease inhibitor
TDF-FTC	Tenofovir-emtricitabine
VDR	Vitamin D receptor

Introduction

People living with HIV are at an increased risk of developing metabolic conditions, including osteoporosis [1]. HIV infection itself and some antiretroviral therapies (ART) are associated with reduced bone mineral density (BMD) and an increased risk of fracture [2–5]. The use of tenofovir (TDF), a nucleotide reverse transcriptase inhibitor (NtRTI), in particular, has been associated with reduced BMD and an increased fracture risk [6]. TDF use has been inconsistently with an increased fracture risk and should be replicated with larger long-term studies before clinical decisions around altering ART as a result of fracture risk are made.

ART is also associated with altered vitamin D metabolism. Several cross-sectional studies have found that serum 25-hydroxyvitamin D [25(OH)D] levels modify the effect of ART on other vitamin D metabolites or calciotropic hormones, such as parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] [7]. Tenofovir-emtricitabine (TDF-FTC) in particular is associated with increased serum levels of $1,25(OH)_2D$ when compared with other NRTIs [7] and other ART [8]. $1,25(OH)_2D$ is the active metabolite of vitamin D, which is generated in the proximal renal tubules [9] where TDF-FTC is excreted. Some cross-sectional studies found that individuals with optimal 25(OH)D (>75 nmol/L) and receiving TDF-FTC had higher $1,25(OH)_2D$ levels compared with those on other ART. However, no difference in serum $1,25(OH)_2D$ was seen following TDF-FTC exposure in patients with suboptimal 25(OH)D levels [7, 10, 11].

A randomised placebo-controlled trial found that vitamin D supplementation reduced PTH levels, only in those on TDF-FTC, but not in those on other ART. PTH levels reduced with supplementation in those receiving TDF-FTC even in those with sufficient vitamin D levels at baseline, further supporting an interaction between TDF-FTC and vitamin D metabolites or calciotropic hormones [12]. Other cross-sectional studies found that those with vitamin D deficiency or insufficiency and on TDF-FTC had higher PTH levels when compared with those on other ART [13, 14], or other or no ART[15]. One longitudinal study evaluated those starting either TDF-FTC or ABC-3TC and found that PTH levels were higher at weeks 4–36, but not week 48 in those started on TDF-FTC [16]. The findings from these studies are difficult to interpret, and the exact mechanism behind the interaction between vitamin D status and TDF-FTC use is yet unclear. It is also unknown if there is a relationship between the increased serum levels of PTH and $1,25(OH)_2D$ and bone mineral density, and furthermore, how 25(OH)D status might further affect this relationship. $1,25(OH)_2D$ is the biologically active vitamin D metabolite which maintains calcium and phosphate homeostasis and low levels stimulate PTH production. The effects on bone of low $1,25(OH)_2D$ and high PTH are particularly evident in patients with chronic kidney disease as the renal production of $1,25(OH)_2D$ is reduced, thereby increasing PTH levels. This combination results in high bone turnover and bone loss [17].

This study aimed to evaluate vitamin D status and its association with bone mineral density in HIV-infected antiretroviral-experienced adults and to explore interaction effects between vitamin D status and ART on bone mineral density, biochemical bone turnover markers, and $1,25(OH)_2D$ levels.

Methods

Study design The simplification of antiretroviral therapy with tenofovir-emtricitabine or abacavir-lamivudine trial (STEAL) was an open-label, prospective, randomised, non-inferiority study that compared simplification of current nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) to a fixed-dose combination TDF-FTC or ABC-3TC over 96 weeks in 357 adults with plasma HIV viral load ≤50 copies/mL [18]. The objective of the study was to test the hypothesis that switching to antiretroviral therapy including TDF-FTC would be virologically non-inferior to antiretroviral therapy including ABC-3TC over 96 weeks in HIV-infected adults with sustained suppression of HIV replication but that TDF-FTC and ABC-3TC would have different safety profiles. Participants were aged ≥18 years receiving stable antiretroviral therapy including ≥2 NRTIs for ≥12 weeks, had plasma HIV loads <50 copies/mL for ≥12 weeks and had an estimated glomerular filtration rate of ≥70 mL/min/1.73 m^2. In addition to 2 NRTIs, participants were also receiving either protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitor (NNRTI)

therapy as HIV infection is treated with a combination of at least two different classes of antiretroviral therapy (ART).

Ethics The study was approved by each site's Human Research and Ethics Committee (30 sites) and registered at Clinicaltrials.gov (NCT00192634). Each participant signed a written informed consent before enrolment.

Bone mineral density, vitamin D metabolites and biochemical bone turnover markers Dual-energy X-ray absorptiometry (DXA) of the lumbar spine and right hip were performed for each participant at baseline using a standardised protocol. BMD scans were not centrally analysed. DXA instruments varied between sites (GE-Lunar in 72 % of sites). For the analysis of clinically relevant low BMD and to ensure comparability with other studies, low BMD (or osteopenia) was defined as T-score between −1 and −2.5, and osteoporosis was defined as T-score ≤−2.5 in accordance with WHO diagnostic thresholds [19]. T-scores are derived using normative data from the NHANES reference database on Caucasian women aged 20–29 years [20].

Plasma samples were collected at baseline (following a 10-h overnight fast) and stored at −70 °C. Serum calcium, phosphate and biochemical bone turnover markers (BTMs) including markers of bone resorption (C-terminal cross-linking telopeptide of type 1 collagen, [βCTX] and bone formation (procollagen type 1 N-terminal propeptide [P1NP]; bone-specific alkaline phosphatase [21]) were measured. Serum βCTX and P1NP were assayed by an electrochemiluminescence immunoassay (E170 immunoassay analyser; Roche, Mannheim, Germany; reference ranges βCTX 170–600 ng/L, P1NP 16.3–78.2 μg/L). Bone-specific alkaline phosphatase (BALP) was assayed by Immunoenzymetric Assay (Manual with Plate Reader; Immunodiagnostic Systems, Boldon, UK; reference ranges BALP 8–21.3 μg/L). Both BTMs and vitamin D metabolites were batch-tested after study completion in one laboratory. Coefficients of variation were within accepted standard limits.

Serum 25(OH)D and $1,25(OH)_2D$ levels were both assayed using the automated IDS-iSYS chemiluminescence immunoassays (IDS Ltd, Boldon, United Kingdom). The dynamic range for the 25(OH)D assay is 13.8–350 nmol/L and for the $1,25(OH)_2D$ assay is 15.6–504 pmol/L. The reference range for $1,25(OH)_2D$ is 50–250 pmol/L. 25(OH)D was categorised into clinically meaningful definitions and vitamin D status refers to these categories. Severe vitamin D deficiency was defined as 25(OH)D <25 nmol/L, vitamin D deficiency was defined as 25(OH)D <50 nmol/L and vitamin D sufficiency was defined as 25(OH)D ≥50 nmol/L [22, 23].

Body mass index (BMI) was calculated by weight (kilogrammes) divided by height (metres) squared. BMI categories were defined according to the WHO guidelines [24]: BMI <18.50 was considered underweight, BMI 18.50–24.99 was considered a healthy weight, BMI 25.00–29.99 was considered overweight and BMI ≥30.00 was considered obese.

Statistical analyses For the present study, in order to have >80 % power to detect a mean difference of 8 pg/mL in $1,25(OH)_2D$ levels between TDF-FTC and other NRTI use, we calculated a sample of 160 was necessary. A 5 % significance level is assumed with a standard deviation of 10.9 pg/mL. The variation and effect size was calculated from a previous study of adults with ART on NRTIs and with $1,25(OH)_2D$ and 25(OH)D measurements collected [7]. The baseline sample was chosen from those participants who had frozen plasma available.

Baseline variables (including demographic, HIV-related factors, ART, body composition, BTMs and vitamin D metabolites) were evaluated as potential predictors of both $1,25(OH)_2D$ and BMD measures using linear regression.

Multivariable models were built using backward, stepwise methods. Predictors that achieved a p value <0.2 in univariable analysis were assessed for inclusion in multivariable models. TDF-FTC and vitamin D deficiency were potentially included in all multivariable models regardless of their significance level in univariable analysis in order to achieve the study aims.

Statistical significance was defined as a 2-sided α of 0.05. Statistical analyses were performed with STATA, version 13 (StataCorp LP, College Station, TX, USA).

Results

Description of study participants Baseline characteristics of the sample analysed were similar to those of the main study population. The baseline characteristics of the study sample are described in Table 1. Most participants were male (99 %), 138 (86 %) were white, and 53 (33 %) and 90 (56 %) were receiving abacavir-3TC (ABC-3TC) or TDF-FTC, respectively, at baseline. The mean duration of TDF-FTC and other NRTI use was 4.5 and 7.8 years prior to the baseline visit, respectively; 39 (24 %) were receiving protease inhibitors (PIs) and the remaining 121 (76 %) were receiving an NNRTI. The majority (53 %) of participants had a healthy BMI, 3 (2 %) had a low BMI, 61 (38 %) were overweight and a further 12 (8 %) were obese.

25(OH)D Multivariable linear regression models were constructed to identify variables associated with serum 25(OH)D and $1,25(OH)_2D$ levels and BMD of the hip and spine.

Table 1 Baseline characteristics of 160 participants in the STEAL study by N(t)RTI use

Characteristic*	Baseline TDF-FTC ($n = 90$)	Baseline other NRTI ($n = 70$)	p value
Age, years	44 (±8)	48 (±9)	0.003
Male (%)	90 (100)	69 (98)	0.26
Ethnicity—white (%)	77 (86)	61 (87)	0.77
HIV duration, years	9 (±6)	12 (±6)	0.003
ART exposure			
PI (*n*, %)	25 (28)	14 (20)	0.26
NNRTI (*n*, %)	65 (72)	56 (80)	
Prior N(t)RTI use, years	4.5 (±3.3)	7.8 (±4.3)	<0.001
Body mass index (BMI), kg/m^2	25 (±4)	25 (±3)	0.85
Body fat mass, % of total body mass	22 (±9)	21 (±7)	0.63
Android fat mass, g, % of total body mass	25 (±10)	26 (±8)	0.83
BMD			
Right hip, g/cm^2	1.01 (±0.13)	1.05 (±0.15)	0.12
Spine, g/cm^2	1.16 (±0.16)	1.23 (±0.15)	0.005
Right hip T-score	−0.57 (±0.98)	−0.29 (±1.13)	0.09
Spine T-score	−0.54 (±1.28)	0.12 (±1.15)	0.0008
Right hip z-score	−0.10 (±0.98)	0.18 (±1.02)	0.08
Spine z-score	−0.32 (±1.20)	0.32 (±1.14)	0.0009
Osteopenia or osteoporosis of spine (*n*, %)	37 (41)	10 (15)	<0.001
Osteopenia or osteoporosis of hip (*n*, %)	29 (32)	17 (25)	0.30
CD4+ cell count, cells/μL	590 (±256)	620 (±304)	0.50
Calcium, mmol/L Reference range 2.15–2.55 mmol/L	2.34 (±0.11)	2.32 (±0.10)	0.22
Phosphate, mmol/L Reference range 0.81–1.45 mmol/L	1.03 (±0.17)	1.01 (±0.15)	0.43
Hypophosphatemia, serum phosphate <0.81 mmol/L (*n*, %)	10 (11)	4 (6)	0.21
TC/HDL Reference range >5	4.29 (±1.76)	4.45 (±1.44)	0.54
Creatinine clearance, mL/min	112 (±28)	108 (±24)	0.33
bALP, μg/L Reference range 8–21.3 μg/L	23.8 (±11.9)	18.6 (±10.2)	0.004
P1NP, μg/L Reference range 16.3–78.2 μg/L	67.2 (±24.9)	50.2 (±22.4)	<0.001
βCTx, ng/L Reference range 170–600 ng/L	298 (±163)	253 (±133)	0.06
25-Hydroxyvitamin D, nmol/L	52 (±20)	49 (±19)	0.33
Vitamin D deficiency (<50 nmol/L) (*n*, %)	50 (56)	35 (50)	0.49
1,25-Dihydroxyvitamin D, pmol/L Reference range 50–250 pmol/L	136 (±47)	125 (±43)	0.13
Month of baseline measurement *n* (%)			0.06
January–March (summer)	15 (17)	23 (33)	
April–May (autumn)	28 (31)	18 (26)	
June–August (winter)	47 (52)	29 (41)	

Mean (±S.D.) reported unless otherwise specified

Eighty-five (53 %) participants were vitamin D deficient (defined as 25(OH)D <50 nmol/L), and there was no difference in the proportion with vitamin D deficiency between TDF-FTC and other NRTI use. Serum 25(OH)D was significantly associated with winter (coefficient −11; 95 % confidence interval (CI) −16 to −6; $p < 0.001$), white ethnicity (20; 95 % CI 13 to 28; $p < 0.001$) and PI use (16; 95 % CI 10 to 22; $p < 0.001$) compared with NNRTI use, after adjusting for age.

1, 25(OH)$_2$D An interaction effect of vitamin D deficiency and ART was seen in the multivariable models for 1,25(OH)$_2$D and hip BMD; therefore, models stratified by 25(OH)D

Table 2 Multivariable models of $1,25(OH)_2D$ levels including all variables, and models stratified by 25(OH)D deficiency status

Baseline variable	$1,25(OH)_2D$ (pmol/L)		$1,25(OH)_2D$ (pmol/L)			
	Full model B coefficient (95 % CI)	*P*	25(OH)D <50 nmol/L B coefficient (95 % CI)	*P*	25(OH)D ≥50 nmol/L B coefficient (95 % CI)	*P*
non TDF-FTC NRTIs ($n = 90$)	Reference		Reference	–	Reference	–
TDF-FTC ($n = 70$)	14.2 (0.3 to 28.2)	0.05	2.6 (−15.5 to 20.7)	0.78	28.6 (7.2 to 50.1)	0.01
NNRTIs ($n = 121$)	Reference		Reference	–	Reference	–
PIs ($n = 39$)	−19.4 (−36.7 to −2.2)	0.03	−35.9 (−64.8 to −7.1)	0.02	−13.3 (−35.4 to 8.7)	0.23
25(OH)D ≥50 nmol/L	Reference		–		–	
25(OH)D <50 nmol/L	−14.7 (−29.4 to 0.5)	0.05				
BMI (kg/m^2)	−3.3 (−5.5 to −1.1)	0.004	−4.2 (−6.8 to −1.6)	0.002		
Creatinine clearance (min/mL)	43.6 (8.4 to 78.8)	0.02				
CD4 (cells/μL)	0.3 (−0.0 to 0.0)	0.06	0.03 (−0.01 to 0.06)	0.10	0.01 (−0.03 to 0.05)	0.55
Creatinine clearance (min/mL)	43.6 (8.4 to 78.8)	0.02				

status were constructed and presented. In those with vitamin D deficiency, PIs (coefficient −35.9; 95 % CI −64.8, −7.1; $p = 0.02$) were associated with lower $1,25(OH)_2D$ levels. However, in those with vitamin D sufficiency, only TDF-FTC use was associated with higher $1,25(OH)_2D$ levels (coefficient 28.6; 95 % CI 7.2 to 50.1; $p = 0.01$) (Tables 2). Figure 1a, b demonstrates the

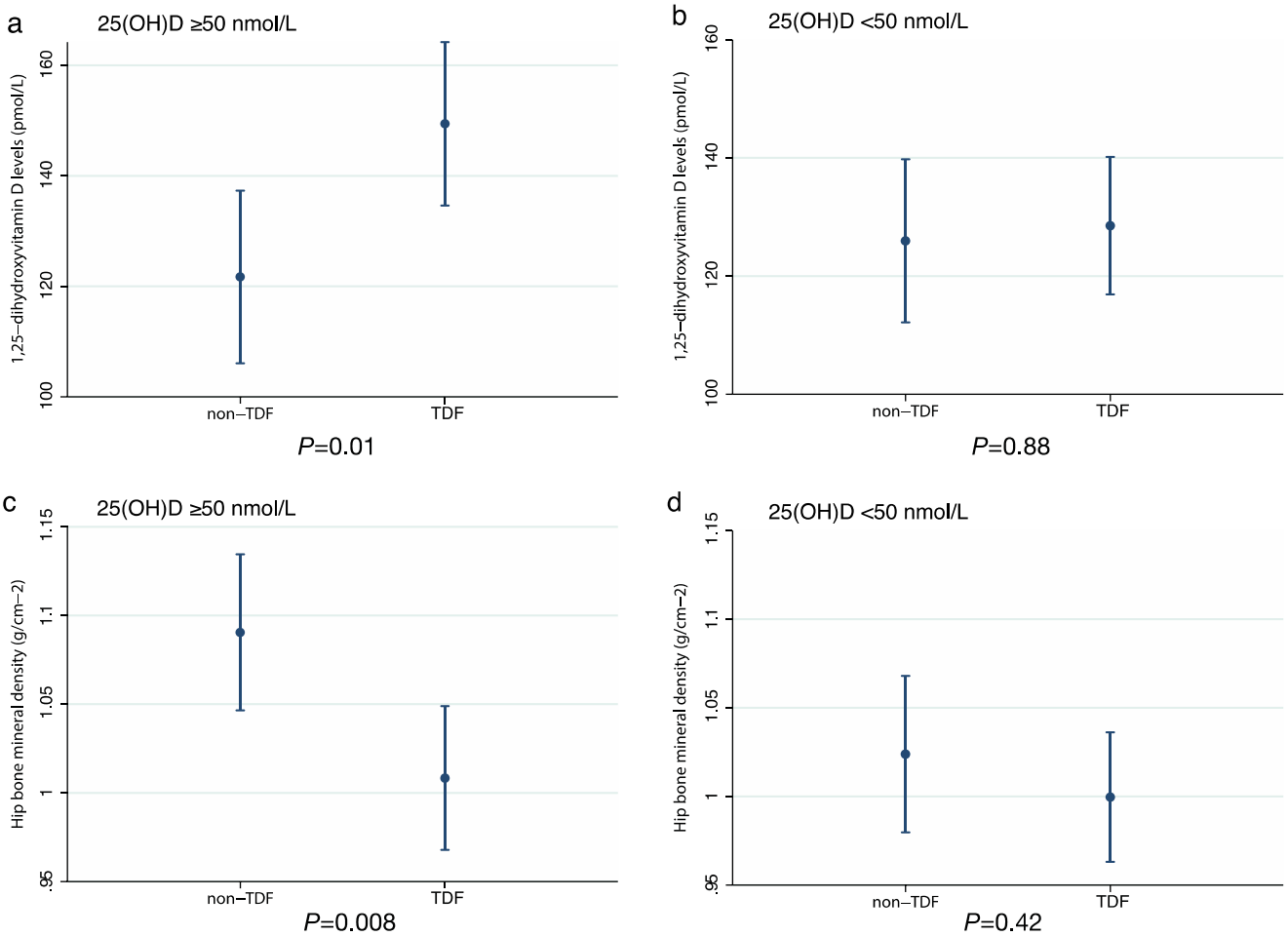

Fig. 1 Effect modification of 25(OH)D status on TDF-FTC use on 1,25-dihydroxyvitamin D and hip BMD

Table 3 Multivariable models of hip bone mineral density, including all variables, and models stratified by 25(OH)D deficiency status

Baseline variable	Hip BMD (g/cm^2)		Hip BMD (g/cm^2)			
	Full model		25(OH)D <50 nmol/L		25(OH)D ≥50 nmol/L	
	B coefficient (95 % CI)	*P*	B coefficient (95 % CI)	*P*	B coefficient (95 % CI)	*P*
Non-TDF-FTC NRTIs (*n* = 70)	Reference	–	Reference	–	Reference	–
TDF-FTC (*n* = 90)	−0.05 (−0.09 to −0.01)	0.02	−0.02 (−0.08 to −0.03)	0.42	−0.08 (−0.14 to −0.02)	0.008
25(OH)D ≥50 nmol/L (*n* = 75)	Reference	–	–	–	–	–
25(OH)D <50 nmol/L (*n* = 85)	−0.05 (−0.09 to −0.01)	0.02				
Age (by 10 years)	−0.03 (−0.05 to −0.01)	0.02	−0.03 (−0.06 to −0.00)	0.06	−0.00 (−0.00 to 0.00)	0.24
Ethnicity white (*n* = 138)	Reference	–	Reference	–	Reference	–
Ethnicity—non-white (*n* = 22)	−0.07 (−0.13 to −0.00)	0.04	−0.08 (−0.15 to −0.01)	0.02	0.00 (−0.18 to 0.20)	0.93
Body mass index (kg/m^2)	0.02 (0.01 to 0.02)	<0.001	0.02 (0.01 to 0.03)	<0.001	0.01 (−0.00 to 0.02)	0.06

interaction effect between TDF-FTC use and vitamin D deficiency by estimating the marginal means of 1,25(OH)$_2$D levels using the model parameters.

Bone mineral density Forty-six (29 %) individuals had osteoporosis or osteopenia of the hip. In multivariable analysis (see Table 3), in those with vitamin D deficiency, non-white ethnicity was associated with lower hip BMD (coefficient −0.08; 95 %CI −0.15 to 0.01; $p = 0.02$), while a higher BMI was associated with higher hip BMD (coefficient 0.02; 95 % CI 0.01 to 0.03; $p < 0.001$). In those with vitamin D sufficiency, TDF-FTC use was negatively associated with hip BMD (coefficient −0.08; 95 % CI −0.14 to −0.02; $p = 0.008$) (see Figure 2). Other biochemical markers of bone turnover were not significant predictors of hip BMD. Figures 1c, d demonstrate the interaction effect between TDF-FTC use and vitamin D deficiency by estimating the marginal means of hip BMD using the model parameters. These figures also demonstrate that those who were vitamin D deficient and not on TDF-FTC had lower mean hip BMD (1.02 g/cm^2) when compared with those who were vitamin D sufficient (mean hip BMD of 1.10 g/cm^2, $p = 0.008$). Vitamin D deficiency was associated with lower bone density in those not receiving TDF-FTC. Conversely, vitamin D status had no association with bone density in those receiving TDF-FTC.

After adjusting for age and ethnicity, the statistically significant predictors of lumbar spine BMD at baseline included TDF-FTC use, BMI, serum calcium, serum βCTX and serum BALP (data not shown). The interaction between TDF-FTC use and vitamin D deficiency was not significant in this model.

Discussion

To our knowledge, this is the first study to evaluate the interaction effect between vitamin D status and ART on bone health. We confirmed previous findings [7, 8] that there is an interaction between vitamin D status and TDF-FTC use on 1, 25(OH)$_2$D levels. Those who were vitamin D sufficient and on TDF-FTC had the highest 1,25(OH)$_2$D levels. Furthermore, 25(OH)D <50 nmol/L (vitamin D deficiency) modified the association between ART and BMD of the hip. Vitamin D deficiency was associated with low hip BMD in those not on TDF-FTC, while there was no association found in those on TDF-FTC.

Individuals on TDF-FTC had higher 1,25(OH)$_2$D levels compared with those not on TDF-FTC. Particularly, those who were vitamin D sufficient and on TDF-FTC had the highest 1,25(OH)$_2$D levels. Approximately half of participants were vitamin D deficient which falls within the large

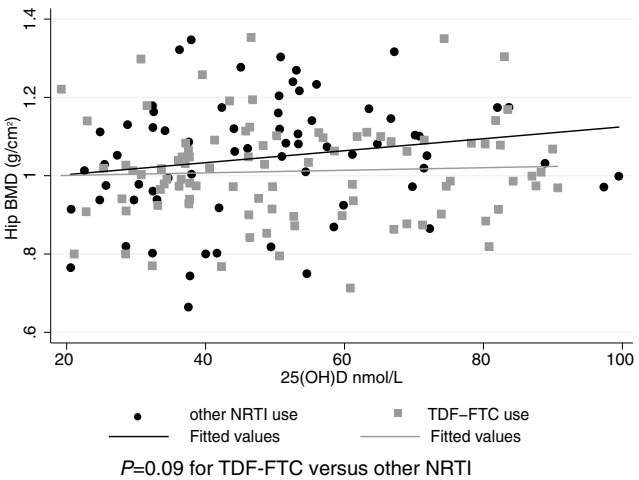

Fig. 2 The relationship between hip bone mineral density and 25(OH)D levels with lines for TDF-FTC and other NRTI use predicted from linear regression

variation of vitamin D deficiency reported internationally and in Australia [25, 26] after considering the vitamin D assay used for the measurement of 25(OH)D levels. The higher $1,25(OH)_2D$ levels in individuals using TDF-FTC were also associated with lower hip BMD in this cross-sectional analysis. Although individuals on TDF-FTC had higher bone turnover markers (BTMs) compared with other NRTIs, BTMs were not significantly associated with hip BMD in the adjusted linear regression model. Although there was a negative association between BALP and hip BMD ($p = 0.20$), the relationship between bone turnover markers (PINP and βCTx) and spine BMD was stronger. We have reported T-scores and osteoporosis proportions in Table 1 to provide the reader with a standardised method of reporting BMD in which the reader can compare BMD levels from this manuscript with others. As T-scores were derived from young women and are used primarily to diagnose osteoporosis in postmenopausal women and men over 50 years old [20], absolute BMD measurements (in g/cm^2) were used for multivariable analysis.

In the previously published longitudinal analysis of all study participants, randomisation to TDF-FTC was associated with increased BMD loss from both the hip and the spine at 96 weeks [27]. In our cross-sectional analysis of baseline hip BMD, TDF-FTC was only associated with lower hip BMD in those with vitamin D sufficiency. This finding is unexpected as vitamin D deficiency is often associated with lower bone mineral density [28] and fracture occurrence [29] and is what we found in those not on TDF-FTC. In an analysis of a multicultural group of American HIV-infected individuals, vitamin D deficiency was a strong predictor of femoral neck bone mineral density [30, 31]. However, few other studies have reported the relationship between vitamin D deficiency and BMD in HIV-infected individuals. It is possible that the methods we used to assess 25(OH)D and $1,25(OH)_2D$ levels were not fully reflective of the participant's "functional" vitamin D status. Therefore, those who are 25(OH)D "sufficient" may not actually have sufficient levels of free $1,25(OH)_2D$ and vitamin D-binding protein, as found in one study [32]. Exploring the interaction effect of other metabolites of vitamin D and TDF-FTC on bone outcomes will help to further clarify this relationship.

In addition to reduced BMD, TDF-FTC use has also been associated with renal impairment and proximal tubulopathy [33] which may alter the 1α-hydroxylation of 25(OH)D to $1,25(OH)_2D$ as this also occurs of the proximal tubules in the kidneys. TDF-FTC does not alter 25(OH)D levels themselves however. It is yet unclear how higher $1,25(OH)_2D$ levels would impact BMD, but this study found that those with vitamin D sufficiency [25 (OH)D >50 nmol/L] had higher $1,25(OH)_2D$ levels and lower hip BMD if they were on TDF-FTC. It is also unclear whether renal impairment and altered PTH or $1,25(OH)_2D$ is related and, furthermore, whether they may be contributing to the bone loss commonly found in patients on TDF-FTC. One analysis found no association between proximal renal tubulopathy in TDF-FTC users in influencing BMD [34]; however, this analysis was cross-sectional and did not include measures of urinary phosphate or calcium excretion, both of which are related to both proximal tubulopathy and bone mineralisation. Other research has indicated that those on TDF-FTC have lower fractional excretion of calcium [7], but it is unknown how this influences BMD. A more detailed analysis of biochemical parameters relating to proximal renal tubulopathy and vitamin D metabolism over time in relation to BMD would be required to clarify the relationship between renal side effects and bone loss associated with TDF-FTC. TDF-FTC will most likely soon be replaced with emtricitabine and tenofovir alafenamide (F/TAF), a novel nucleotide reverse transcriptase inhibitor with an improved bone and kidney side effect profile to TDF-FTC [35]. Once this new compound is in use, its long-term side effects will need to be re-examined.

Body mass index (BMI) and ethnicity were also significant predictors of hip BMD. We found that higher BMI was associated with higher hip BMD and that this was independent of vitamin D status. The relationship between BMI and BMD is not always linear. People with a very low BMI often have lower BMD [36, 37]; however, there have been variable findings in people who are overweight or obese [38, 39]. In our study, the mean BMI was 25, with the majority of participants being of a healthy weight, 12 % being obese and only 2 % having low BMI. Therefore, the relationship between BMI and BMD is a reflection of those who are of a normal or overweight status. This suggests that in our participants, increased body mass was associated with bone mass through a mechanical loading effect [40]. In those with vitamin D deficiency, white ethnicity was associated with lower hip BMD when compared with those of non-white ethnicity. Ethnicity did not appear to be a predictor of hip BMD in the model for those with vitamin D sufficiency; however, only two individuals of non-white ethnicity were also vitamin D sufficient. Ethnicity influences both vitamin D status [41] and BMD [42]; however, it was included in the multivariable model for BMD as its effect is thought to be vitamin D-independent.

A further limitation of our study is that we were unable to measure parathyroid hormone levels on frozen plasma due to its instability and that urine samples were not available to analyse markers of calcium and phosphate excretion. Including PTH would have enabled us to see if vitamin D status modified the association between TDF-FTC and several different calciotropic hormones along the vitamin D pathway. In spite of this, the inclusion of clinically relevant outcomes, such as bone mineral

density, 25(OH)D, 1,25$(OH)_2$D, serum calcium and phosphate levels, are helpful in identifying clinically relevant associations.

Conclusions

The significance of these findings for clinicians managing patients with HIV is that TDF-FTC is associated with lower BMD. Vitamin D supplementation might ameliorate bone loss seen in patients with HIV, and the effect of TDF-FTC on the effects of supplementation should continue to be evaluated in longitudinal studies. Vitamin D and calcium supplementation combined have minimised BMD loss in people on TDF-FTC [43]; however, it is still unclear how serum 25(OH)D, 1,25 $(OH)_2$D and PTH levels may influence bone mineral density changes.

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Compliance with ethical standards

Conflicts of interest None.

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