Journal of
AIDS & Clinical Research
ISSN: 2155-6113



Research Article Open Access

# Exploring Strategies for Attenuating Changes in Bone Mineral Density in Men who have Sex with Men Using Tenofovir/Emtricitabine Pre-exposure Prophylaxis

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## Abstract

**Objective:** Tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) as HIV pre-exposure prophylaxis (PrEP) decreases bone mineral density (BMD). We explored the impact of vitamin D supplementation, dietary calcium intake, and bone loading exercise on BMD during the first 12 months of daily TDF/FTC PrEP.

**Methods:** All participants from the PREPARATORY-5 pilot demonstration project of gay and bisexual men were eligible for inclusion if they had dual-energy X-ray absorptiometry scans at both baseline and 12 months (n=39). Self-reported vitamin D supplementation was collected prospectively, whereas data on diet and exercise was obtained retrospectively after the trial period, using validated questionnaires. We compared median percent changes in BMD and trabecular bone score (TBS) between those with and without vitamin D supplementation, those with optimal (1000 mg/day) and inadequate (<500 mg/day) dietary calcium intake, and those in the highest versus lowest tertile of contemporaneous bone loading exercise.

**Results:** Median (interquartile range) participant age was 34 (29, 39) years, 74% were White, and most had some post-secondary education. Overall, median BMD declined significantly from baseline to 12 months at all anatomic sites, changing by -2.14% at the lumbar spine (p<0.01), -1.66% at the femoral neck (p<0.01), and -0.85% at the total hip (p<0.01). Compared to those not using vitamin D, those taking any vitamin D supplementation had a smaller median percent decline in the lumbar spine TBS that was not statistically significant (-1.42% versus -3.99%, p=0.06), and no difference in the lumbar spine BMD (-2.11% versus -2.14%, p=0.40), femoral neck BMD (-1.75% versus -1.17%, p=0.34) and total hip BMD (-0.89% versus - 0.69%, p=0.41). There were no significant differences in BMD or TBS according to dietary calcium intake or bone-loading exercise.

**Conclusion:** Vitamin D supplementation may have an attenuating effect on PrEP-related deterioration of lumbar spine bone microarchitecture. The relationship between vitamin D supplementation and PrEP-related changes in BMD warrants further investigation.

**Keywords:** Pre-exposure prophylaxis; Men who have sex with men; Bone mineral density; Trabecular bone score; Vitamin D; Calcium; Bone loading exercise

## Introduction

HIV pre-exposure prophylaxis (PrEP) using daily oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) decreases HIV incidence in men who have sex with men (MSM) when there is high adherence [1]. TDF/FTC PrEP has been approved for the prevention of sexually transmitted HIV in the US since 2012 and in Canada since 2016, but has subclinical adverse effects including modest decreases in renal function and bone mineral density (BMD), primarily driven by the TDF component. For instance, a cohort of 200 MSM using daily TDF PrEP in San Francisco demonstrated declines in BMD over 12 months of -1.1% (-0.4 to -1.9%) at the femoral neck, -0.8% (-0.3 to -1.3%) at the total hip and -0.7% (0.1 to -1.5%) at the lumbar spine (L2-L4) compared to placebo [2]. In the iPrEx study among MSM, BMD loss at the lumbar spine was 1.14% greater (95%CI=0.37%, 1.91%) at 72 weeks among PrEP users and 1.0% greater (95%CI=-0.23%, 2.24%) at 96 weeks compared to placebo recipients, and 8% of PrEP participants versus only 2% of placebo participants lost >5% spine BMD (p=0.003) at week 24, although differences between groups were less pronounced at the hip [3]. Although studies to date have not shown increases in fractures among PrEP users, the long-term impacts of these changes remain unknown.

A new formulation of tenofovir, tenofovir alafenamide (TAF) achieves higher active concentration of tenofovir in lymphocytes than TDF/FTC, resulting in lower concentrations of the active drug in plasma and less toxicity. Clinical trials in HIV-positive patients have shown TAF/FTC (Descovy®) to have similar antiviral efficacy compared to TDF/FTC but to cause smaller decreases in BMD and lower bone turnover [4]. The efficacy of TAF/FTC as PrEP has recently been demonstrated in a large international clinical trial, in which it was non-inferior to TDF/FTC in preventing HIV [5].

Importantly, lower cost generic versions of TDF/FTC are available in some countries and will likely be introduced into others soon. In

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**Received** March 07, 2019; **Accepted** March 22, 2019; **Published** March 29, 2019

**Citation:** Kortenaar J, Cheung AM, Mewa S, Waugh E, Rubin L, et al. (2019) Exploring Strategies for Attenuating Changes in Bone Mineral Density in Men who have Sex with Men Using Tenofovir/Emtricitabine Pre-exposure Prophylaxis. J AIDS Clin Res 10: 789.

contrast, the cost of TAF/FTC is likely to be high, and even if accepted to be as efficacious as PrEP in the future, manufacturing will be protected by patent laws for many years. Thus, if the adverse effects of TDF/FTC can be mitigated by simple, low-cost interventions, generic TDF/FTC might offer a cheaper and comparably safe alternative to TAF/FTC. In particular, vitamin D and calcium supplementation have been shown to mitigate BMD loss in HIV-positive patients on TDF/FTC-containing antiretroviral therapy regimens [6] and bone loading exercise has protective effects on BMD in postmenopausal women [7]. To inform the design of potential interventional trials aimed at mitigating TDF/FTC PrEP-related bone toxicity, we explored the association between vitamin D supplementation, dietary calcium intake and participation in bone loading exercises with changes in BMD in a 12-month pilot demonstration project of MSM using daily TDF/FTC-based PrEP.

## Methods

### Study design

PREPARATORY-5 was a pilot demonstration project of daily oral TDF/FTC use in 52 MSM in Toronto, Canada that took place between November 2014 and June 2016 [8]. The study protocol involved dual energy X-ray absorptiometry (DXA) at the hip and lumbar spine at both baseline and 12 months in all participants. This report includes the results of BMD testing within the trial, and further describes a sub-study within the trial to investigate predictors of BMD change.

The primary objectives were to quantify the change in BMD seen in study participants over 12 months of TDF/FTC PrEP, and to obtain pilot data on the magnitude of effect of vitamin D supplementation on this change. Secondary objectives included obtaining pilot data on the effects of calcium dietary intake and contemporaneous bone-loading exercise on changes in BMD while on PrEP. Exploratory objectives were to analyze the effect of vitamin D supplementation, calcium intake and bone loading exercise on the trabecular bone score (TBS), a measure of bone microarchitecture that complements BMD in determining bone strength and risk of fracture [9]. Rather than testing hypotheses about the impact of these exposures, our ultimate purpose was to inform the design and sample size calculations of future clinical trials.

### Study population and eligibility

Adult (age ≥ 18 years) MSM were eligible for the PREPARATORY-5 trial if they were HIV and hepatitis B seronegative, had no symptoms of HIV seroconversion at enrollment, were at high risk of HIV infection (defined as reporting condomless receptive anal sex in the preceding six months and scoring ≥ 10 on the High Incidence Risk Index for MSM [10], a validated clinimetric tool for quantifying HIV risk), and had an estimated glomerular filtration rate ≥ 60 ml/minute. Full recruitment methods have been previously described [8,11], but involved both self-referrals in response to advertisements on gay social/sexual networking applications and LBGT newspapers/websites, as well as provider referrals from Toronto-area community-based organizations working in gay men's health. Only participants with complete BMD data at baseline and 12 months were included in this analysis. Of these, three participants did not consent to future contact and thus were not contacted for collection of calcium and exercise data.

### Data collection

BMD at the lumbar spine (L1-L4), femoral neck and total hip, as well as the trabecular bone score (TBS) for the lumbar spine (L1-L4), was obtained using dual-energy x-ray absorptiometry (DXA; GE Lunar iDXA, GE Healthcare, Mississauga, Canada) at baseline and 12 months. The root-mean-square coefficients of variation were 3%, 4% and 4% for the lumbar spine, femoral neck and total hip respectively. Z-scores for participants were calculated from v.112 of the USA Lunar AP spine and femur reference populations (ages 20-40). T-scores were not calculated because of the young age of the cohort. Study visits were conducted at baseline and months 1, 3, 6, 9 and 12, and included collection of demographic data and information on vitamin D supplementation.

Information on dietary calcium intake and bone loading exercise was gathered retrospectively using an electronic survey administered in March and April, 2017. The Calcium Assessment Tool is a dietary questionnaire that has been validated in the same geographic setting as our study (Toronto, Canada) but in a different population (postmenopausal women) [12]. The tool was modified to ask about dietary calcium intake during the study period. Respondents were categorized as having optimal (1000 mg/day), suboptimal (500-999 mg/day) or inadequate (<500 mg/day) dietary calcium intake as suggested by the Institute of Medicine [13].

Participation in bone loading exercises throughout life and during the study period was assessed using questions from the Bone-Specific Physical Activity Questionnaire (BPAQ) [14]. Participants were asked about their lifetime history and frequency of participation in physical activities, allowing estimation of the lifetime amount of bone loading stimuli. Participants were also asked about their contemporaneous bone-loading physical activity during the study period. Additional questions asked about seasonal participation in these activities. Although not typically part of the BPAQ, seasonal participation is adjusted for in other bone loading questionnaires [15] and is relevant to the Canadian context, where seasonal weather conditions may impact significantly on physical activity. Participants were separated into tertiles based on their cumulative effective load stimulus as calculated by the BPAQ. The BPAQ is a unit-less continuous index, validated as a predictor of current BMD for a number of populations similar to our sample, including men and women aged 18-30 [14], and healthy men above age 50 [16]. The BPAQ increases with the amount of bone loading exercise.

In keeping with standard clinical practice at the study site, all participants were informed at the outset of the trial that TDF/FTC could cause decreases in BMD, and advised to consider taking 1000 IU of vitamin D daily, consuming adequate dietary calcium, and engaging in regular weight-bearing exercise to mitigate these effects. Participants were also offered a voucher for a discounted gym membership at a popular fitness club with multiple branches across the Toronto area to facilitate the latter.

### Laboratory testing

Serum samples were collected at the baseline, 6 and 12 month visits, and analyzed in a batch for 25-hydroxy vitamin D using a noncompetitive electrochemiluminescent immunoassay (Roche Cobas e602 analyzer, Indianapolis, IN), and for parathyroid hormone (PTH) levels using a noncompetitive electrochemiluminescence immunoassay (Roche Cobas e602 analyzer, Indianapolis, IN). Red blood cell concentrations of tenofovir diphosphate (TFV-DP) were determined using dried blood spots at every ensuing study visit after baseline [17].

### Statistical analyses

Participant characteristics were analyzed using descriptive statistics. Percentage change in BMD was calculated as the difference from baseline between baseline values and those at 12 months. Clinically significant change was defined as a decline of ≥ 3.0% for the lumbar spine, or ≥

4% for the femoral neck and total hip. A normal TBS was defined as TBS>1.350, and a partially degraded value as 1.200<TBS<1.350.

The impact of vitamin D supplementation (any supplementation versus no supplementation), calcium dietary intake, and bone loading exercise on median percentage change in BMD and TBS at 12 months was each assessed using Wilcoxon Mann-Whitney tests. Additional analyses examined impact on median change in z-scores. Recognizing that the classification of participants' calcium intake and exercise could be impacted by recall bias, we compared the most extreme categories of calcium dietary intake (optimal versus inadequate) and bone loading exercise (highest versus lowest tertiles). BMD was considered at the lumbar spine, femoral neck and total hip separately. Further, the number of participants experiencing either clinically significant changes in BMD or a change from normal to partially degraded TBS at 12 months was compared for each exposure of interest using chi-square tests.

In sensitivity analyses, we compared changes in BMD and TBS only between those taking ≥ 1000 IU daily versus no vitamin D, by excluding those taking doses <1000 IU/day and those in whom the vitamin D dosage information was missing. Another sensitivity analysis included only those that had their final DXA within a month of stopping PrEP, as recent evidence suggests that there is a quick recovery of BMD after discontinuation of TDF/FTC PrEP [18]. We used SAS version 9.4 (Cary, NC) for all analyses.

### Sample size considerations

Sample size for this exploratory study was constrained by the parent study. Assuming 80% power, alpha=0.05 and the standard deviation of percent change in BMD to be 3.95% in the lumbar spine and 2.34% in the total hip based on published data, [2,3] the estimated minimum detectable difference in median percent change in BMD was 3.75% in the lumbar spine and 2.22% in the total hip [19].

## Ethics

Ethical approval was obtained from St. Michael's Hospital's Research Ethics Board (REB) for all study activities. Written informed consent had been obtained from all participants in the parent PREPARATORY-5 study. Implied consent, as approved by the REB, was assumed for responses to the retrospective questionnaire.

## Results

Of the 52 PREPARATORY-5 pilot trial participants, 39 met the inclusion criteria for this sub-study. Participants were relatively young (median age of 34, IQR 29-40) and predominantly White (74.4%), and most had finished post-secondary education (Table 1), with no significant differences compared to trial participants that were not included in this analysis (data not shown). Twelve participants (30.8%) had at least one risk factor for osteoporosis, including five with multiple risk factors. The most common risk factor was current smoking (n=10). One participant had a history of corticosteroid use and hypogonadism, and another participant had a history of parental hip fracture. Eleven participants had a prior fracture, but none were fragility fractures. No participant self-reported drinking three or more alcoholic beverages per day.

Adherence to PrEP was high. TFV-DP dried blood spot concentrations averaged at time-points after steady-state (3, 6, 9 and 12 months) corresponded with 27 participants (69.2%) taking 7 doses/week and 10 (25.6%) taking 4-6 doses/week; such adherence levels have been associated with an estimated HIV risk reduction in MSM of 100% (95%CI=86-100%) [17]. Two participants had TFV-DP dried blood spot concentrations averaging only 2-3 doses/week. No participants acquired HIV during the study.

At baseline, the median (IQR) BMD at the lumbar spine (L1-L4), femoral neck and total hip was 1.197 g/cm$^2$ (1.006, 1.291), 1.029 g/cm$^2$ (0.924, 1.133), and 1.044 g/cm$^2$ (0.908, 1.142) respectively in the cohort overall. The median BMD z-scores at these sites were -0.2 (-1.8, 0.6), -0.2 (-1.0, 0.8), and -0.3 (-1.3, 0.4) respectively. Two participants, aged 24 and 40, had baseline z-scores below -2.5 at the lumbar spine, femoral neck or total hip; both were White and neither had any risk factors for osteoporosis.

Twenty-four participants (61%) were taking vitamin D supplementation during the entire study; 15 were taking ≥1000 IU per day while the others were taking a lower (n=2) or unspecified (n=7) dose. Of those on supplementation, 13 (54%) had been taking vitamin D even before beginning the trial. Median (IQR) plasma 25-hydroxy vitamin D levels at baseline were 71 (52, 79) and 68 (43, 76) nmol/L in those taking and not taking vitamin D supplements respectively, and rose modestly only in those taking supplementation. In contrast to other studies of TDF/FTC PrEP [20], serum parathyroid hormone (PTH) levels did not change appreciably during the study, regardless of supplementation status (data not shown).

In the study cohort overall, median BMD declined significantly

| Characteristic | Value[a] |
|---|---|
| **Age** | 34 (29, 40) |
| **Ethnicity** | |
| White | 29 (74.4%) |
| Other | 10 (25.6%) |
| **Education** | |
| High school diploma or some college | 9 (23.1%) |
| College or undergraduate degree | 18 (46.1%) |
| Graduate or professional degree | 12 (30.7%) |
| **Season of Enrolment** | |
| Fall | 11 (28.2%) |
| Winter | 15 (38.5%) |
| Spring | 11 (28.2%) |
| Summer | 2 (5.1%) |
| **BMI (kg/m$^2$)** | 26.2 (23.0 - 27.9) |
| **Any risk factors for osteoporosis[b]** | 12 (30.8%) |
| **Baseline serum 25 (OH) vitamin D** | |
| Adequate (≥ 75 nmol/L) | 12 (30.8%) |
| Inadequate (31-74 nmol/L) | 21 (53.9%) |
| Deficient (≤ 30 nmol/L) | 1 (2.9%) |
| Missing | 5 (12.8%) |
| **Calcium Dietary Intake (post-baseline)** | |
| Optimal ≥ 1000 mg/day | 14 (52%) |
| Suboptimal 999 - 500 mg/day | 6 (22%) |
| Inadequate <500 mg/day | 7 (26%) |
| Missing | 12 (31%) |
| **Bone Physical Activity Questionnaire score (post-baseline)** | |
| Lifetime (n=25) | 18.2 (9.2, 48.4) |
| During study period (n=24) | 3.9 (1.3, 7.8) |

[a] Values are median (interquartile range) and frequency (proportion)
[b] Ten participants were current smokers, one had a parent who had experienced a hip fracture and one had a history of both chronic corticosteroid use and hypogonadism.

**Table 1:** Baseline characteristics of study population and questionnaire responses.

from baseline to 12 months at all three anatomic sites, with a -2.14% change at the lumbar spine (p<0.01), -1.66% change at the femoral neck (p<0.01), and -0.85% change at the total hip (p<0.01). Of the 39 participants, 14 saw clinically significant decreases in BMD at one (n=12), two (n=1) or all three (n=1) anatomic sites: nine in the lumbar spine, six in the femoral neck and two in the total hip.

BMD was marginally higher in those on vitamin D supplementation at baseline (Table 2). Participants using any vitamin D supplementation experienced a similar median percent change in BMD compared to their counterparts not using any vitamin D supplementation, at -2.11% versus -2.14% at the lumbar spine (p=0.40), -1.75% versus -1.17% at the femoral neck (p=0.34), and -0.89% versus -0.69% at the total hip (p=0.41). Results were similar when examining z-scores rather than percentage changes in BMD (data not shown). Compared to those not on supplementation, those using vitamin D were similarly likely to experience a clinically significant decrease in BMD at any site, with an odds ratio of 0.83 (95%CI=0.22 to 3.23).

Twenty-seven participants (69%) completed the retrospective survey about calcium intake and exercise. Just over half (14/27) had optimal levels of calcium dietary intake (≥ 1000 mg/day) while 7/27 had inadequate intake (<500 mg/day). Modest differences in the magnitude of BMD change were observed between the optimal and inadequate calcium intake groups, though none of these differences were statistically significant (Table 3). The direction of these small differences varied according to anatomic site, with findings at the lumbar spine and total hip appearing to favour calcium supplementation, and findings at the femoral neck and TBS results suggesting the opposite. Compared to those that had inadequate calcium dietary intake, those with optimal intake were similarly likely to experience a clinically significant decrease in BMD at any site, with an odds ratio of 1.35 (95%CI=0.21 to 8.62).

Small numerical differences in median percent change in BMD were also seen between participants in the highest tertile of contemporaneous bone loading exercise during the study period compared to those in the lowest tertile, but again these differences did not reach statistical significance (Table 4). Findings varied according to anatomic site, appearing to favour bone loading exercise only for BMD at the lumbar spine. Compared to those in the lowest tertile of contemporaneous bone loading, those in the highest tertile were similarly likely to experience a clinically significant decrease in BMD at any site, with an odds ratio of

| | Median (IQR) baseline value | | Median (IQR) percentage change at 12 months | | |
|---|---|---|---|---|---|
| | Vitamin D supplement (n=24) | No vitamin D supplement (n=15) | Vitamin D supplement (n=24) | No vitamin D supplement (n=15) | p-value [a] |
| **BMD lumbar spine (g/cm²)** | 1.232 (1.001, 1.315) | 1.111 (1.035, 1.244) | -2.114 (-2.613, 1.404) | -2.142 (-4.006, -1.451) | 0.40 |
| **BMD femoral neck (g/cm²)** | 1.044 (0.927, 1.184) | 1.022 (0.905, 1.067) | -1.749 (-2.690, -0.300) | -1.173 (-2.878, 0.783) | 0.34 |
| **BMD total hip (g/cm²)** | 1.062 (0.918, 1.186) | 0.987 (0.905, 1.097) | -0.889 (-2.762, -0.092) | -0.691 (-2.229, 1.576) | 0.41 |
| **TBS Lumbar Spine** | 1.489 (1.406, 1.542) | 1.421 (1.391, 1.495) | -1.423 (-2.586, 0.347) | -3.988 (-4.888, -1.397) | 0.06 |

[a] Comparison between participants taking versus not taking a vitamin D supplement

**Table 2:** Bone mineral density and trabecular bone score at baseline and percentage change from baseline according to vitamin D supplementation.

| | Median (IQR) baseline value | | Median (IQR) percentage change at 12 months | | |
|---|---|---|---|---|---|
| | Optimal (n=14) | Inadequate (n=7) | Optimal (n=14) | Inadequate (n=7) | p-value [a] |
| **BMD lumbar spine (g/cm²)** | 1.244 (1.078, 1.342) | 1.197 (1.006, 1.288) | -2.372 (-3.285, -0.246) | -2.512 (-4.006, -1.471) | 0.60 |
| **BMD femoral neck (g/cm²)** | 1.022 (0.835, 1.112) | 0.971 (0.936, 1.190) | -1.724 (-2.878, 0.783) | -1.173 (-1.774, 0.855) | 0.54 |
| **BMD total hip (g/cm²)** | 1.015 (0.905, 1.157) | 0.924 (0.908, 1.093) | -0.691 (-2.719, 1.024) | -1.211[a] (-2.805, 0.000) | 0.79 |
| **TBS Lumbar Spine** | 1.503 (1.430, 1.547) | 1.464 (1.394, 1.485) | -4.590 (-5.658, 0.263) | -1.588 (-2.527, 0.272) | 0.25 |

[a] Comparison between participants with optimal versus inadequate dietary calcium intake

**Table 3:** Bone mineral density and trabecular bone score at baseline and percentage change from baseline according to dietary calcium intake.

| | Median (IQR) baseline value | | Median (IQR) percentage change at 12 months | | |
|---|---|---|---|---|---|
| | Highest tertile (n=8) | Lowest tertile (n=8) | Highest tertile (n=8) | Lowest tertile (n=8) | p-value [a] |
| **BMD lumbar spine (g/cm²)** | 1.293 (1.059, 1.323) | 1.251 (1.059, 1.304) | -1.709 (-3.320, 1.883) | -2.338 (-2.929, -0.364) | 0.72 |
| **BMD femoral neck (g/cm²)** | 1.088 (0.955, 1.263) | 1.012 (0.958, 1.086) | -1.946 (-5.702, -0.686) | -0.630 (-1.492, 1.059) | 0.16 |
| **BMD total hip (g/cm²)** | 1.100 (0.910, 1.269) | 1.038 (0.925, 1.125) | -0.955 (-2.491, 0.898) | -0.427 (-0.770, 0.937) | 0.51 |
| **TBS Lumbar Spine** | 1.486 (1.431, 1.543) | 1.493 (1.423, 1.520) | -1.737 (-2.143, 0.803) | -1.207 (-4.636, 0.907) | 0.96 |

[a] Comparison between participants in highest versus lowest tertile of bone loading activity

**Table 4:** Bone mineral density and trabecular bone score at baseline and percentage change from baseline in the highest and lowest tertiles of contemporaneous bone loading activity.

0.81 (0.27 to 2.43).

At baseline, all participants were deemed to have a normal TBS, except one who had a partially degraded score (TBS<1.350). The median (IQR) baseline TBS was 1.469 (1.392, 1.519) at the lumbar spine. At 12 months, the median (IQR) TBS had declined to 1.430 (1.368, 1.483), with four participants' TBS classified as partially degraded. Vitamin D supplementation was associated with smaller numerical median percent changes in TBS over 12 months, at -1.42% compared to -3.99%, although this did not quite reach statistical significance (p=0.06).

The results of the sensitivity analysis comparing those taking ≥ 1000 IU of vitamin D daily with those taking no vitamin D at all produced results that were similar to the primary analyses (data not shown). However, the estimated magnitude of the difference in TBS was numerically larger, with a decline of -1.29% over 12 months associated with vitamin D ≥ 1000 IU compared to -3.99% for those taking none, although this was not statistically significant (p=0.13). The results of the sensitivity analysis excluding participants who underwent their final DXA over a month after discontinuing PrEP were similar to those of the primary analysis (data not shown).

## Discussion

In this pilot study of 39 MSM on TDF/FTC PrEP for 12 months, we observed a significant decline in BMD at the lumbar spine, femoral neck and total hip overall, and we generated preliminary estimates of the impact of vitamin D supplementation, calcium intake and bone loading exercise on BMD as well as the lumbar spine TBS. Supplementation was associated with a potentially smaller median percent decline in the lumbar spine TBS, although this did not quite reach statistical significance (-1.42% versus -3.99%, p=0.06). Importantly, the primary purpose of our pilot study was to generate initial estimates of effect size to inform the design of future studies, rather than to ascertain definitive evidence of benefit. While the absence of a signal that vitamin D supplementation could be beneficial was contrary to our hypothesis, there are several potential explanations that could also inform the design of future research.

First, the vitamin D dosages in the study might not have been sufficient to achieve the desired changes. The most common dosage used was 1000 IU daily, although data on some participants' dosages were missing. That higher dosages of vitamin D may be needed is supported by our sensitivity analyses, in which the magnitude of the favourable effect on TBS appeared larger when changing the definition of supplementation to taking ≥ 1000 IU or higher daily. Several factors may influence the effect of supplementation on vitamin D serum levels, including genetic factors that were not controlled for in this study [21]. The increase in vitamin D levels might also be due to the season in which participants were recruited; most participants were recruited in the winter and spring, when vitamin D levels are lowest. Vitamin D dosages even greater than 1000 IU may be needed to appreciate a significant change in BMD; a previous clinical trial found that 4000 IU of vitamin D and 1000 mg of calcium supplementation daily attenuates BMD loss in HIV-positive patients using TDF/FTC-containing regimens [6]. Over 48 weeks, those in the vitamin D and calcium supplementation arm of that trial had a median percent change in their total hip BMD of -1.36%, compared to a -3.22% change in the control arm (p=0.004), while median percent changes in the lumbar spine BMD were -1.23% and -4.87%, respectively (p=0.033). Higher dose vitamin D supplementation should be considered in future interventional work.

Second, vitamin D use was not randomized in this cohort, and all participants were advised to consider taking a vitamin D supplement at trial outset. Hence there was likely some degree of confounding by indication in our study, in that those who took supplements may have been more health-conscious than their counterparts. Indeed, several (13/24) participants taking vitamin D supplementation had been doing so even prior to enrollment. In those that did supplement, median BMD was higher at baseline for all anatomic sites and for TBS, although not to a statistically significant degree. A definitive trial should employ a randomized design.

Third, median plasma levels of 25-hydroxy vitamin D were similar in both groups at baseline (71 and 68 nmol/L), and those in the non-supplementation category might have been getting adequate vitamin D through means other than supplementation, such as sun exposure, dietary intake and tanning. Twenty-four participants (61.5%) had desirable 25-hydroxy vitamin D levels of >75 nmol/L. These findings contrast with the high prevalence of vitamin D deficiency in the general Canadian population [22-24] and may have limited the potential benefit of additional supplementation. Arguing against this possibility is the hypothesis that TDF-induced bone loss may result from functional rather than absolute vitamin D deficiency [20]. That hypothesis is supported by the increase in vitamin D binding protein and decline in fibroblast growth factor 23 with TDF initiation in both HIV-positive and negative individuals [25,26]. The rise in PTH seen with TDF initiation in some PrEP studies [26-28] further supports a mechanistic role of vitamin D deficiency in PrEP-related bone loss. Interventional studies should include nested laboratory evaluations to help elucidate the mechanisms by which TDF causes BMD decline.

Fourth, data on vitamin D supplementation was self-reported and thus subject to social desirability bias. This bias may be particularly relevant given that all participants were told to consider this intervention, but could be avoided in a randomized trial.

Finally, BMD has limitations as a measure of bone health. BMD represents areal mineral content but not microarchitecture of bone [9]. However, it is noteworthy that those using vitamin D experienced a smaller PrEP-related decline in TBS, a measure of spinal integrity. Additional studies should consider including this measure when monitoring the bone health of PrEP users.

Our secondary analyses suggested that neither calcium dietary intake nor bone-loading exercise was significantly associated with median percent changes in BMD, although modest differences were seen that varied by anatomic site. Participants reporting ≥ 1000 mg of calcium intake per day appeared to have numerically higher baseline BMD at two of the three anatomic sites assessed, and those within the highest tertile of contemporaneous bone-loading exercise had numerically higher baseline TBS and BMD at every anatomical site, suggesting that larger studies should be conducted to more definitively assess for benefit.

There are limitations to this study that warrant consideration. Most notably, the small sample size limited the power of our analyses and precluded our ability to perform multivariable regression modeling. However, the purpose of this pilot work was to generate preliminary estimates of effect size to inform the design of more definitive trials, and our findings should thus be considered hypothesis-generating only. Further, the BPAQ and CAT questionnaires have not been validated for retrospective use as was done in our study, although it is unclear in what direction this issue might bias the results.

There are compelling reasons to believe that vitamin D and calcium supplementation could have mitigating effects on TDF-related

changes in bone health among PrEP users, including their efficacy in HIV-positive patients on TDF-containing therapy, and the above-mentioned hypotheses that the mechanism of TDF-related bone loss relates to functional vitamin D deficiency. A larger interventional trial is warranted to determine the effect of different dosages of vitamin D and calcium supplementation, and should include prospective measurement of 25-hydroxy vitamin D levels, calcium dietary intake and bone-loading exercise. Such low-cost interventions with TDF/FTC PrEP might be both an economical and non-inferior alternative to a potential TAF/FTC PrEP regimen, if approved as PrEP in the future.

### Acknowledgement

We would like to acknowledge Alexandre Schnubb for his assistance in contacting the participants for the retrospective questionnaire.

### Funding

This work was supported by grants from the Ontario HIV Treatment Network (OHTN) and the Canadian Institutes of Health Research (CIHR). Pre-exposure prophylaxis study drug was provided by Gilead Sciences Inc. DHST is supported by a New Investigator Award from the CIHR/OHTN. Funders had no role in the study design, data collection, analysis, interpretation or manuscript-writing.

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