Bone Mineral Density Changes Among Young, Healthy African Women Receiving Oral Tenofovir for HIV Preexposure Prophylaxis

Brenda G. Mirembe, MBChB, MSc, Clifton W. Kelly, MS,† Nyaradzo Mgodi, MBChB, MMed,‡ Susan Greenspan, MD,§ James Y. Dai, PhD,† Ashley Mayo, MSPH,‖ Jeanna Piper, MD,¶ Carolyne A. Akello, MBChB, MSc,* Flavia M. Kiweewa, MBChB, MSc,* Tsitsi Magure, MBChB, MMed,‡ Clemensia Nakabiito, MBChB, MMed,* Jeanne M. Marrazzo, MD, MPH,# Z. Mike Chirenje, MD, FRCOG,‡ and Sharon A. Riddler, MD, MPH,§ for the MTN-003B Protocol team*

Background: Limited data exist on effect of tenofovir disoproxil fumarate (TDF) when used for preexposure prophylaxis (PrEP) on bone mineral density (BMD) in HIV-negative women. We evaluated the effect of daily oral TDF and emtricitabine/TDF compared with placebo on BMD among women enrolled in an HIV-1 PrEP trial.

Methods: HIV-uninfected women in Uganda and Zimbabwe had BMD measurements of lumbar spine (LS) and total hip (TH) by dual-energy x-ray absorptiometry at baseline and every 24 weeks for 48 weeks of active treatment and for 48 weeks after discontinuation of study medication. Plasma tenofovir levels were assessed every 12 weeks for the first 48 weeks.

Results: Of 518 women enrolled, 432 had dual-energy x-ray absorptiometry results at baseline and week 48. In the primary analysis, no significant differences in percent BMD change in hip or spine between arms observed, likely because of low product adherence. Among the subset with tenofovir detection in 75%–100% of plasma samples, the mean percent BMD change from baseline to week 48 in the LS was 1.4% lower for TDF or emtricitabine/TDF recipients than for placebo ($P = 0.002$) and TH BMD was 0.9% lower ($P = 0.018$). BMD changes from end of active treatment to 48 weeks were significantly greater in the active arm participants compared with placebo participants with a net difference of approximately +0.9% at the LS ($P = 0.007$) and +0.7% ($P = 0.003$) at the TH.

Conclusions: TDF-containing oral PrEP resulted in small but significant reversible decreases in hip and spine BMD among young African women.

Key Words: preexposure prophylaxis, tenofovir, bone density, African women, HIV prevention

(*J Acquir Immune Defic Syndr* 2016;71:287–294)

INTRODUCTION

Tenofovir disoproxil fumarate (TDF) has proven efficacy for prevention of HIV-1 infection alone or in combination with emtricitabine (FTC) for heterosexual men and women, serodiscordant couples, men who have sex with men (MSM), and injection drug users, especially among individuals with high adherence.[1–4]

Modest decreases in bone mineral density (BMD) have been observed in antiretroviral therapy naive HIV-infected persons taking TDF as part of their antiretroviral therapy regimen[5–9] and some studies in HIV-infected persons have shown increased risk for fractures or osteomalacia from TDF use.[10–13] The exact mechanism of TDF-induced BMD loss is not well understood although studies have shown that it could be due to effects on bone metabolism[14,15] or renal tubular dysfunction.[13,16]

Recent studies have reported the effects of oral TDF used for preexposure prophylaxis (PrEP) on BMD in HIV-uninfected individuals,[17–19] however data from African women are limited. The impact of TDF on bone is especially important in HIV-negative women in sub-Saharan Africa who may receive PrEP for several years during young adulthood and who also may be concurrently impacted by other factors affecting bone density including contraception, pregnancy, and lactation.[20–24]

Received for publication July 1, 2015; accepted September 7, 2015.

From the *Makerere University—Johns Hopkins University Research Collaboration, Kampala, Uganda; †Statistical Center for HIV/AIDS Research & Prevention (SCHARP)-Fred Hutchinson Cancer Research Center, Seattle, WA; ‡Department of Obstetrics and Gynaecology, University of Zimbabwe-University of California San Francisco, Harare, Zimbabwe; §Department of Medicine, University of Pittsburgh, Pittsburgh, PA; ‖FHI 360, Durham, NC; ¶Division of AIDS/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD; and #Department of medicine and Global Health, University of Washington, Seattle, WA.

Supported by the National Institute of Allergy and Infectious Diseases (UM1AI068633, UM1AI068615, UM1AI1106707) with cofunding from Eunice Kennedy Shriver National Institute of Child Health and Human Development and the National Institute of Maternal Health, all components of the US National Institutes of Health. Additional funding to the clinical sites was provided by NIH grants UM1AI069436 (UZ-UCSF CTU) and UM1AI069530 (MU-JHU CTU), and P30AG024827 (Osteoporosis Research Center at the University of Pittsburgh). Study medications were provided by Gilead Sciences.

Presented in part at the HIV Research for Prevention Conference, October 27–31, 2014, Cape Town, South Africa.

The authors have no conflicts of interest to disclose.

The content is solely the responsibility of the authors and does not necessarily represent the official views of National Institutes of Health.

Correspondence to: Brenda G. Mirembe, MBChB, MSc, Makerere University—Johns Hopkins University Research Collaboration, Upper Mulago Hill Road, P.O. Box 23491, Kampala, Uganda (e-mail: bgati@mujhu.org).

It is also important to know whether the effect of TDF on bone is reversible. Limited existing data suggest that the effect of TDF on BMD is reversible after discontinuation in HIV-infected individuals.[25–27] In MSM and transgendered women, BMD decreases observed with FTC/TDF were partially reversed when measured a median of 24 weeks after discontinuation in the iPrEX study.[19] Reversibility of bone changes has not been studied in HIV-negative women and is very critical information for determining recommendations for long-term use of oral TDF for PrEP.

The National Institutes of Health sponsored PrEP trial, the Microbicides Trial Network (MTN)-003 (VOICE) assessed the safety and efficacy of daily oral TDF, oral FTC/TDF, and tenofovir gel in HIV-uninfected heterosexual women in 3 countries in sub-Saharan Africa.[28] To determine the bone effects of TDF in this population, a subset of participants randomized to the oral arm in the VOICE study were offered enrollment into a bone density substudy.

METHODS

Study Population

From September 2009 to June 2011, 5029 women were enrolled into the VOICE trial.[28] Women randomized to the VOICE oral arm at sites in Zimbabwe and Uganda were invited to participate in the bone density study, MTN-003B. Eligible women were aged 18–45 years, not pregnant or breastfeeding, and were generally healthy with normal renal function. Use of an effective method of contraception was required. Additionally, women were excluded if they reported any condition known to affect bone (eg, hyperparathyroidism and bone cancer) or were taking any medication known to affect bone (eg, glucocorticoids, heparin, warfarin, cyclosporine, cancer chemotherapy, and thyroid hormone).

Study Procedures

The enrollment visit was conducted within 14 days of initiation of study treatment in VOICE. Subsequent MTN-003B study visits occurred every 24 weeks during study treatment until the end of active treatment and 24 and 48 weeks after discontinuation of active treatment. At the enrollment visit, demographics, contraceptive, and lactation history were obtained by self-report using an interviewer-administered questionnaire. The following evaluations were completed at each study visit: updated medical history including contraceptive use, pregnancy testing, height and weight, and physical assessment for malnutrition. Dietary assessment using an abbreviated food frequency questionnaire tailored to calcium-rich foods available at the sites, and physical activity estimation using the International Physical Activity Questionnaires short form (August 2002; available at www.ipaq.ki.se)[29] were completed at every study visit. Physical activity was categorized as low, medium, or high according to the International Physical Activity Questionnaire algorithm. Site staff provided nutritional counseling and/or calcium supplementation for participants with T-score or Z-score less than −2.0 SD at study entry or at any study follow-up visit. Urine human chorionic gonadotropin was performed before each BMD assessment described below.

Tenofovir Measurements

Tenofovir concentration was measured by mass spectrometry on plasma samples collected every 3 months as part of the VOICE study.[28] Only samples from the participants in the active TDF and FTC/TDF arms were tested. The lower limit of detection for tenofovir in plasma was 0.31 ng/mL.

Bone Mineral Density

BMD of the lumbar spine (LS, L1–L4) and total hip (TH) were measured at each study visit by dual-energy x-ray absorptiometry (DXA) using identical densitometers at the 2 research sites (Software version 2.3.2; Hologic explorer, Bedford, MA) using conventional protocols as previously described.[21] To reduce measurement error, all DXA scans were performed in duplicate and the average of the 2 scans was calculated for a given visit. DXA was repeated if a reduction in T-score or Z-score at any site was observed to be greater than 1 SD from the baseline. All DXA analyses were reviewed by a central quality control facility to ensure appropriate and consistent analyses at the Osteoporosis Prevention and Treatment Center, University of Pittsburgh Medical Center, Pittsburgh, PA. DXA scans were discontinued in women who became pregnant on study but could be resumed after completion of the pregnancy.

Study Oversight

The study was funded by the National Institutes of Health (ClinicalTrials.gov number NCT00729573). Written informed consent was obtained from each study participant. Study conduct adhered to international guidelines, and the study was approved initially and annually by an institutional review board or ethics committee at each site and corresponding collaborating institutions in the United States.

Statistical Analysis

Baseline characteristics were compared across arms with proportions tested by χ^2 tests and medians tested by the Kruskal–Wallis test. The primary analysis assessed changes in BMD from baseline to 48 weeks of follow-up between each oral active treatment arm (oral TDF, oral FTC/TDF) and the oral placebo arm. Participants were included in the primary analysis when a BMD measure was available at both baseline and the 48-week visit. Participants were excluded from the analysis if HIV-1 seroconversion occurred before the 48-week visit, or if the 48-week visit occurred after January 2, 2012 for participants in the oral TDF arm. This date, January 2, 2012, was chosen for censoring to allow final DXA scan data collection up to 13 weeks after the date that participants began coming off oral TDF because of VOICE Data Safety Monitoring Board recommendation (October 3, 2011). When comparing the oral TDF and oral placebo arms, we limit both arms to BMD measurements from 48-week DXA scans that

occurred on or before January 2, 2012. When the active arms are combined for comparison with the oral placebo arm, all arms are censored to include DXA scans that occurred on or before January 2, 2012.

Student t-test was used to compare the mean percentage change in TH and total spine BMD from baseline to 48 weeks on study between each active oral treatment arm and the oral placebo arm in the primary analysis. The proportion of participants in each arm with at least a 3% decrease in BMD observed at any time in follow-up was compared using a Fisher exact test.

The analysis was repeated to evaluate change in BMD among participants in active arms with detectable plasma tenofovir (greater than the lower limit of detection) in 75%–100% of quarterly plasma samples compared with change in BMD among participants in the placebo arm. Because of this subsetting, the presumed benefit of randomization may be lost. For *t* tests found to be statistically significant, a regression model was used to adjust for potential confounders such as country, age, baseline body mass index (BMI), baseline physical activity level, baseline contraceptive use [depot medroxyprogesterone acetate (DMPA), oral contraceptive pills (OCPs), other], and history of ever breastfeeding at baseline.

A secondary analysis was performed using the Student *t* test to compare mean percentage change in BMD of total spine and TH from the end of active study product use to 48 weeks after study product discontinuation between each oral active treatment arm and the oral placebo arm.

This study was designed based on an expected standard deviation of 4.5% for the percentage change in BMD from baseline to week 48, and a sample size of 100 women per arm would allow the detection of a difference as small as 2.1% with 90% power and type I error rate of 5%. All statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC).

RESULTS

Study Participants

A total of 576 women were randomized into the oral arms of the VOICE study at the BMD study sites, and of these, 518 women (331 in Zimbabwe and 187 in Uganda) were enrolled into MTN-003B between September 2009 and June 2011 (Fig. 1). Most women who did not enroll were in Zimbabwe and the main reason was the time required to travel to DXA machine. There were no statistically significant differences for baseline characteristics between treatment arms (Table 1), and no differences between those who enrolled and those who did not (data not shown). At baseline, median age was 29 years and BMI was 24.8 kg/m^2. A history of DMPA use was reported by 367 participants (71%). At baseline, contraceptive methods included DMPA (52% of

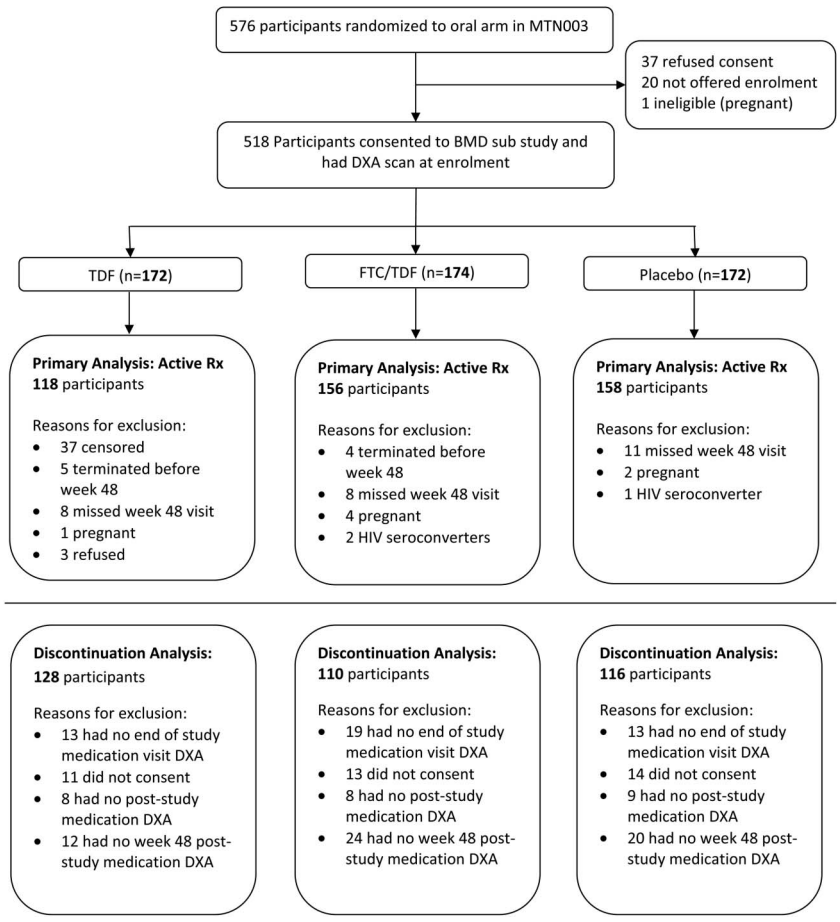

FIGURE 1. Participant flow diagram. "Primary analysis: Active Rx" displays the number of participants who completed DXA scan at week 48 of follow-up. "Discontinuation analysis" displays the number of participants who completed DXA scans at end of study medication and at week 48 after study medication. BMD, bone mineral density; DXA, dual-energy x-ray absorptiometry; FTC, emtricitabine; MTN003, the Microbicides Trial Network; TDF, tenofovir disoproxil fumarate.

TABLE 1. Baseline Characteristics and BMD of the Study Population

Baseline Characteristics	Baseline Cohort				P†
	Total (N = 518)	TDF* (N = 172)	FTC/TDF (N = 174)	Placebo (N = 172)	
Demographics					
Uganda	187 (36%)	63 (37%)	60 (34%)	64 (37%)	0.86
Zimbabwe	331 (64%)	109 (63%)	114 (66%)	108 (63%)	
Age group					0.78
18–24	122 (24%)	41 (24%)	43 (25%)	38 (22%)	
25–34	339 (65%)	111 (65%)	116 (67%)	112 (65%)	
35–39	57 (11%)	20 (12%)	15 (9%)	22 (13%)	
Educational level					0.80
None or primary	149 (29%)	52 (30%)	47 (27%)	50 (29%)	
Secondary or higher	369 (71%)	120 (70%)	127 (73%)	122 (71%)	
Married					0.45
Yes	410 (79%)	131 (76%)	142 (82%)	137 (80%)	
No	108 (21%)	41 (24%)	32 (18%)	35 (20%)	
Alcohol use, past 3 mo					0.69
Never	382 (74%)	130 (76%)	131 (75%)	121 (70%)	
Once a week or less	77 (15%)	22 (13%)	22 (13%)	33 (19%)	
2–6 times per week	48 (9%)	16 (9%)	17 (10%)	15 (9%)	
Everyday	11 (2%)	4 (2%)	4 (2%)	3 (2%)	
Medical and reproductive history					
Median BMI, kg/m^2 (IQR)	24.8 (22.2–28.6)	24.4 (22.1–28.5)	24.9 (21.9–28.9)	24.6 (22.6–29.0)	0.64
Physical activity level					0.38
Low	29 (6%)	10 (6%)	10 (6%)	9 (5%)	
Moderate	166 (32%)	46 (27%)	64 (37%)	56 (33%)	
High	323 (62%)	116 (67%)	100 (57%)	107 (62%)	
Median total daily calcium, mg (IQR)	368 (243–568)	373 (248–560)	352 (237–562)	392 (243–603)	0.84
Median parity (IQR)	2 (2–3)	2 (2–3)	2 (1–3)	2 (2–3)	0.09
Ever breastfed					0.60
Yes	510 (98%)	170 (99%)	172 (99%)	168 (98%)	
No	8 (2%)	2 (1%)	2 (1%)	4 (2%)	
Contraception, ever used					
Oral contraceptives	420 (81%)	138 (80%)	139 (80%)	143 (84%)	0.62
Injection (DMPA)	367 (71%)	118 (69%)	132 (76%)	117 (68%)	0.22
Implant	139 (27%)	46 (27%)	43 (25%)	50 (29%)	0.64
Median (IQR) duration of contraceptive use, mo					
Oral contraceptives	39 (18–84)	46 (19–92)	42 (23–80)	36 (14–84)	0.64
Injection (DMPA)	12 (1–36)	11 (3–36)	12 (1–36)	12 (2–48)	0.55
Implant	0.5 (0–11)	0 (0–14)	1 (0–12)	1 (0–4)	0.51
BMD Measurements [median (IQR)]					
Median BMD at baseline (g/cm^2)					
LS	0.97 (0.90–1.04)	0.98 (0.90–1.03)	0.96 (0.90–1.03)	0.98 (0.90–1.05)	0.61
TH	0.96 (0.88–1.03)	0.96 (0.87–1.02)	0.96 (0.87–1.03)	0.97 (0.89–1.03)	0.57

*Censored on 02 January, 2012, 13 weeks (3 months) after the date participants began coming off oral TDF because of early stoppage recommendations by Data Safety Monitoring Board (03 October, 2011).

†*P*-value for treatment arm difference from χ^2 test for categories and from Kruskal–Wallis test for medians.

IQR, interquartile range.

participants), OCPs (27%), and implants (24%) with limited rates of switching through week 48. Nearly, all participants (98%) reported a history of breastfeeding, with 30% having a cumulative lactation history of greater than 5 years. Most participants had moderate [136 (31%)] to high [272 (63%)] levels of physical activity. Use of supplemental calcium or vitamin D was infrequent: at baseline, 1 participant (0.2%) each reported calcium and vitamin D use in the last 6 months; at 48 weeks, 6/432 (1.4%) reported calcium and 4/432 (0.9%) reported vitamin D use.

All 518 women completed the baseline DXA scan, and 432 (83%) participants had DXA results available at week 48 and were included in the primary analysis. This included 156 in the TDF/FTC group, 158 in the placebo group, and 118 in the TDF group. The TDF arm of the VOICE study was stopped early because of futility resulting in the censoring of

37 participants who had not reached week 48 visit before discontinuation of study medication; the reasons for the remaining discontinuations were similar across arms (Fig. 1).

At baseline, a Z-score of <-2.0 was observed for LS in 30 (5.8%) participants and for TH in 3 (0.6%) participants with no statistically significant differences by treatment arm.

Change in Bone Density During Active Treatment

At the LS, a small decrease in BMD between baseline and week 48 was observed for the 2 active arms, TDF and FTC/TDF, and a small increase was seen in the placebo recipients (Table 2). Small increases, less than 0.5%, in the BMD at the TH were observed in all 3 arms. No significant differences were observed in the primary analysis comparing the mean percent change in TH BMD and LS BMD from baseline to week 48 between the TDF or FTC/TDF arms compared with placebo (all $P > 0.05$). Additionally, there was no difference when the active arms were pooled (n = 235) compared with placebo (Table 2 and Fig. 2A). Low BMI at baseline was not associated with an increase in BMD among women in the placebo group.

Plasma tenofovir drug levels were available for 342 (98.8%) of the 346 participants on the oral active arms (173/174 on oral FTC/TDF and 169/172 on oral TDF). Overall, tenofovir was detected in at least one plasma sample from 194/342 (57%) of participants. Including specimens that were collected until the week 48 DXA scan, plasma tenofovir drug levels were available from 4 visits for most participants, 247 (71%); from more than 4 visits for 17 (5%); and from 1 to 3 quarterly follow-up visits for 78 (23%). In the subset of women from the active arms with tenofovir detected in 75%–100% of plasma samples (n = 81 for the combined active arms), the net change in LS BMD was on average −1.0 to −1.4% for the TDF, FTC/TDF, and combined active drug recipients compared with placebo (all $P < 0.05$, Table 2). For the TH, the net change in BMD was on average −0.7 to −0.9% from baseline to week 48 for the active treatment participants compared with placebo ($P < 0.05$ for FTC/TDF and combined active arms vs placebo, Table 2 and Fig. 2B). These differences remained significant after adjusting for baseline factors known to be associated with BMD including country, age, baseline BMI, baseline physical activity level, baseline histories of contraceptive use (DMPA, OCP, and other), and breastfeeding.

Fractures and 3% Decrease in BMD

No bone fractures were reported in the study. Among the 432 participants with DXA scan at 48 weeks, greater than a 3% decrease in BMD was observed in 105 (24%) and 72 (17%) participants for spine and hip, respectively, at any time in follow-up and did not differ significantly between the

TABLE 2. Change in BMD of LS and Hip After 48 Weeks of Active Treatment for Each Oral Active Product vs Oral Placebo

	Mean BMD (SD) at Baseline, g/cm²	Mean (SD) % Change at 48 weeks	Difference %, Active Minus Placebo (95% CI)	P*
Primary analysis: all study participants				
LS				
Oral TDF (n = 118) vs oral placebo (n = 119)†	0.976 (0.104) vs 0.978 (0.104)	−0.359 (3.09) vs 0.201 (2.99)	−0.559 (−1.338 to 0.220)	0.159
Oral FTC/TDF (n = 156) vs oral placebo (n = 158)	0.968 (0.097) vs 0.979 (0.111)	−0.104 (2.85) vs 0.238 (2.92)	−0.342 (−0.982 to 0.298)	0.294
Oral actives—FTC/TDF and TDF (n = 235) vs oral placebo (n = 119)†	0.971 (0.099) vs 0.978 (0.104)	−0.360 (2.83) vs 0.201 (2.99)	−0.561 (−1.200 to 0.078)	0.085
TH				
Oral TDF (n = 118) vs oral placebo (n = 119)†	0.958 (0.117) vs 0.959 (0.109)	0.203 (2.67) vs 0.492 (2.58)	−0.289 (−0.961 to 0.383)	0.397
Oral FTC/TDF (n = 156) vs oral placebo (n = 158)	0.959 (0.114) vs 0.968 (0.119)	0.161 (2.19) vs 0.368 (2.40)	−0.207 (−0.717 to 0.304)	0.426
Oral actives—FTC/TDF and TDF (n = 235) vs oral placebo (n = 119)†	0.957 (0.111) vs 0.959 (0.109)	0.189 (2.48) vs 0.492 (2.58)	−0.303 (−0.859 to 0.252)	0.283
Subset analysis (active arm participants with drug detection at 75%–100% of visits)				
LS				
Oral TDF (n = 43) vs oral placebo (n = 119)†	0.989 (0.116) vs 0.978 (0.104)	−0.994 (3.40) vs 0.201 (2.99)	−1.194 (−2.286 to −0.103)	0.032
Oral FTC/TDF (n = 51) vs oral placebo (n = 158)	0.969 (0.099) vs 0.979 (0.111)	−0.762 (3.08) vs 0.238 (2.92)	−0.999 (−1.939 to −0.060)	0.037
Oral actives—FTC/TDF and TDF (n = 81) vs oral placebo (n = 119)†	0.984 (0.104) vs 0.978 (0.104)	−1.173 (3.07) vs 0.201 (2.99)	−1.373 (−2.233 to −0.514)	0.002
TH				
Oral TDF (n = 43) vs oral placebo (n = 119)†	0.962 (0.140) vs 0.959 (0.109)	−0.214 (2.98) vs 0.492 (2.58)	−0.706 (−1.639 to 0.227)	0.137
Oral FTC/TDF (n = 51) vs oral placebo (n = 158)	0.964 (0.114) vs 0.968 (0.119)	−0.374 (1.97) vs 0.368 (2.40)	−0.742 (−1.474 to −0.010)	0.047
Oral actives—FTC/TDF and TDF (n = 81) vs oral Placebo (n = 119)†	0.970 (0.125) vs 0.959 (0.109)	−0.383 (2.52) vs 0.492 (2.58)	−0.875 (−1.599 to −0.151)	0.018

*P-value from Student *t* test.

†Censored on 02 January, 2012, 13 weeks (3 months) after the date participants began coming off oral TDF because of early stoppage recommendations by Data Safety Monitoring Board (03 October, 2011).

CI, confidence interval; LS, lumbar spine; TH, total hip.

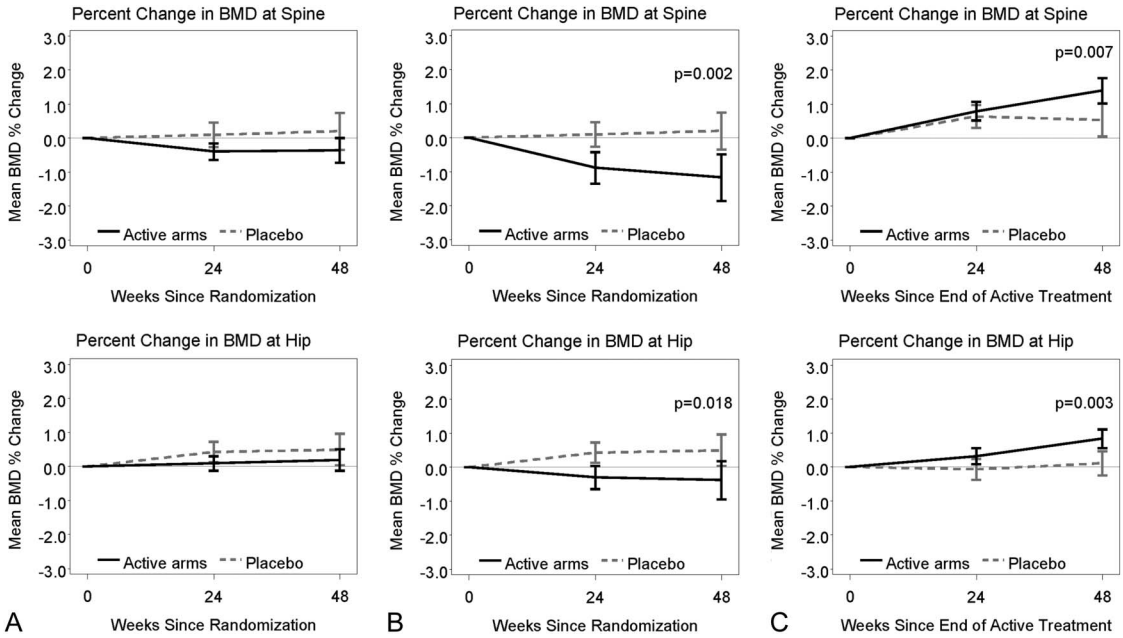

FIGURE 2. Percent change in spine and TH BMD for during and after study treatment TDF-containing PrEP or placebo. The solid line shows the combined results for the TDF and FTC/TDF groups and the dashed line for the placebo group. When presented *P*-values come from Student t-test of difference in mean percentage change between the 2 groups at 48 weeks. The first panel (A) shows the percentage change in BMD by study week from baseline to week 48 of active treatment for all study participants (active arms n = 235, placebo n = 119). In panel (B), the percentage change in BMD from baseline to week 48 for the subset of participants with good adherence defined as plasma tenofovir detected in 75%–100% of samples obtained during the first 48 weeks, combined TDF, and FTC/TDF groups (n = 81) compared with placebo (n = 119). In panel (C), percentage change in BMD during the 48 weeks from end of active treatment for the combined TDF and FTC/TDF groups (n = 238) and placebo (n = 116). BMD, bone mineral density.

active arms and placebo. For the subset of women from the active arms with tenofovir detected in 75%–100% of plasma samples, greater than a 3% decrease in spine BMD was observed in 17 (40%) of TDF participants, 13 (25%) on FTC/TDF, 29 (36%) for the combined active arm, and 22 (18%) on placebo ($P = 0.012$ for TDF vs placebo and $P = 0.008$ for the combine active arms vs placebo). No statistically significant differences between groups were observed for the proportion with greater than 3% decrease in hip BMD.

Bone Outcomes After Discontinuing Active Treatment With Tenofovir

Among the 518 enrolled participants, 354 (68%) contributed data to the analysis of the change in BMD after discontinuation of active treatment with tenofovir, including 38/128 (30%) in the TDF arm who received less than 48 weeks of study medication. These participants had DXA scan results within 3 months of the end of study treatment and again 48 weeks later. BMD increases at both the spine and the hip were observed after stopping study medication. The BMD changes were significantly greater in the active arm participants compared with the placebo participants with a net difference of approximately 0.9% at the LS ($P = 0.007$) and 0.7% at the TH ($P = 0.003$; Table 3 and Fig. 2C). Significant differences remained in models adjusted by country, age, BMI, physical activity level, and history of DMPA use. In all arms for both TH and LS, the mean BMD level at 48 weeks after active treatment discontinuation was at least as high as the mean BMD level at baseline. The changes for the subgroup who had detectable plasma tenofovir levels in 75% or greater of samples from the first year of study treatment were similar to the overall group.

DISCUSSION

In this well-powered study of bone density changes in African women on TDF and FTC/TDF for PrEP, we observed no significant differences in net BMD change over the first 48 weeks of active treatment, mainly because of low product adherence that was observed among the VOICE participants.[28] However, in the subset analysis limited to tenofovir detected in 75%–100% of plasma samples, TDF use resulted in small but significantly greater net decrease in BMD from baseline to 48 weeks compared with placebo. At the LS, net change was −1.0 to −1.4% and hip net change was −0.7 to −0.9%.

Our study findings are consistent with earlier studies of the impact of TDF used for PrEP on BMD in various populations. In the Centers for Disease Control MSM PrEP study conducted among HIV-negative men in San Francisco,[18] a 0.8% net decline at the TH was observed in TDF (compared with placebo) with no significant differences for spine BMD. The lack of impact of TDF at the spine may be related to differences in the study population (all male, mostly

TABLE 3. Change in BMD of LS and Hip at 48 Weeks After Active Treatment Discontinuation for Each Oral Active Product vs Oral Placebo

	Mean BMD* (SD) at End of Study Medication Visit, g/cm^2	Mean (SD) % Change at 48 weeks After End of Study Medication Visit	Difference %, Active Minus Placebo (95% CI)	*P**
LS				
Oral TDF (n = 128) vs oral placebo (n = 116)	0.966 (0.111) vs 0.977 (0.120)	1.569 (2.59) vs 0.533 (2.60)	1.037 (0.382 to 1.691)	0.002
Oral FTC/TDF (n = 110) vs oral placebo (n = 116)	0.975 (0.110) vs 0.977 (0.120)	1.201 (3.19) vs 0.533 (2.60)	0.668 (−0.093 to 1.430)	0.087
Oral actives—FTC/TDF and TDF (n = 238) vs oral placebo (n = 116)	0.970 (0.106) vs 0.977 (0.120)	1.399 (2.88) vs 0.533 (2.60)	0.866 (0.244 to 1.488)	0.007
TH				
Oral TDF (n = 128) vs oral placebo (n = 115)	0.950 (0.122) vs 0.977 (0.129)	0.954 (2.06) vs 0.109 (1.92)	0.845 (0.340 to 1.350)	0.001
Oral FTC/TDF (n = 110) vs oral placebo (n = 115)	0.965 (0.125) vs 0.977 (0.129)	0.672 (2.24) vs 0.109 (1.92)	0.563 (0.016 to 1.109)	0.044
Oral actives—FTC/TDF and TDF (n = 238) vs oral placebo (n = 115)	0.957 (0.123) vs 0.977 (0.129)	0.823 (2.14) vs 0.109 (1.92)	0.715 (0.252 to 1.178)	0.003

NOTE: The changes for the subgroup who had detectable plasma tenofovir levels in 75% or greater of samples from the first year of study treatment were similar to the overall group and are not presented.

*P-value from Student t test.

CI, confidence interval; LS, lumbar spine; TH, total hip.

white) or the sample size for this study. In the iPrEX study[19] of FTC/TDF in a diverse population of HIV-seronegative men and transgendered women, small but statistically significant net decreases in BMD from baseline in both hip and spine were observed in participants taking FTC/TDF compared with placebo.

The TDF2 study conducted in Botswana[17] evaluated effects of FTC/TDF on bone among heterosexual men and women (N = 68, 30 women). The net decrease in BMD from baseline to month 30 was of −1.62% (P = 0.0002) at the spine and −1.51% (P = 0.003) at the hip in the FTC/TDF group when compared with placebo. In our subset of women with detectable tenofovir in most samples, we show similar results with clear evidence of an effect of TDF taking into account baseline factors including contraceptive method.

We observed a rebound in bone density after stopping FTC/TDF and TDF. The increase in BMD was significantly greater in the active arm participants compared with placebo at 48 weeks after stopping study medication. The difference between the active and placebo arms was evident among the entire population, not only the adherent subset, indicating some impact of TDF on bone even with suboptimal adherence. This reversible effect of TDF on bone has been observed in HIV-positive populations as evidenced by improvement in bone density after switch from TDF to an alternate antiretroviral agent.[25–27] The recently published iPrEX study also showed partial reversal of bone loss in the spine for FTC/TDF recipients compared with placebo 24 weeks after discontinuation of PrEP. In our study, we observed continued net increases in bone density at both the spine and hip in the period from 24 to 48 weeks after the end of active treatment, suggesting that longer follow-up is needed to fully assess the trajectory of bone changes. The reversibility of the bone effects of TDF is of particular concern for PrEP use in young adults, especially young women, who have not achieved peak bone mass and who would be eligible for PrEP for many years because of ongoing high risk of HIV acquisition.

MTN-003B is the largest study of bone density changes with TDF-based PrEP in women. Our results are limited by overall low adherence to TDF and FTC/TDF in the parent study—VOICE[28] and this likely affected the results of the primary analysis. We used plasma tenofovir levels to define a subset with good adherence to more clearly identify drug effects on bone. After considering differences in populations and study design, the impact of TDF-based PrEP on bone has been remarkably similar across studies to date.[17–19] Additionally, our study was affected by stoppage of the TDF arm in VOICE earlier than expected because of futility. Because of this, the active treatment component was shortened, which led to reduction of follow-up time in the analyses of the combined oral active arms.

Our study has several strengths including the large sample size and excellent retention, resulting in adequate power to determine the impact of TDF on bone in HIV-negative African women. The DXA scans for our study were conducted according to international standards with centralized review of each scan by an independent group and high-quality control. Finally, our study evaluated reversibility of the bone effects of TDF used for PrEP in healthy HIV-negative sub-Saharan women. Our results demonstrating that BMD increased after stopping TDF are very encouraging, but additional longitudinal studies are needed to determine the impact of longer exposures to TDF-containing PrEP among all populations, including daily and intermittent use strategies.

In conclusion, TDF use resulted in a small but significant decrease in BMD in HIV-negative premenopausal African women, and more importantly, the observed decrease in BMD was reversible. Demonstration projects and PrEP roll-out programs may provide the opportunity for further assessment of the impact of TDF/FTC on bone density.

ACKNOWLEDGMENTS

The authors acknowledge Karen Patterson and Edward Livant for their dedication in quality control of the DXA data.

The authors are grateful to the study participants for participating in the study. The authors also acknowledge the support of the staff of Osteoporosis Research Center at University of Pittsburgh. The authors thank the Microbicides Trial Network (MTN)-003B protocol team, the MTN-003 (VOICE) protocol team, and the UZ-UCSF and MUJHU research teams for sample and data collection.

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