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Research Article

Recovery in BMD following recent repeat pregnancy and breastfeeding among African women with and without HIV

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Abstract

Women living with HIV (WLWH) in resource-limited settings have benefited greatly from antiretroviral therapy (ART). However, they have several risk factors that can affect their bone health including being female, high parity/breastfeeding, use of life-long ART, and HIV disease itself. This study assessed bone mineral density (BMD) among recently pregnant WLWH (WLWH-P), and compared BMD changes over 3 yr with WLWH who were not pregnant (WLWH-NP) and with recently pregnant HIV-negative women (HIV-neg-P). We co-enrolled 104 WLWH-P from an ongoing cohort study in Malawi and Uganda, and used linear mixed models to compare standardized BMD values over 30-36 mo with 109 HIV-neg-P women matched for age-group/parity, and 99 age-group/parity matched WLWH who did not plan to become pregnant over 36 mo. BMD was assessed via DXA at baseline, 12, 24, and 30-36 mo. At baseline, median age of WLWH was 33 yr with HIV-neg-P being 32 yr. 91.4% of WLWH-P, and 87.9% of WLWH-NP were on Tenofovir-ART regimens. At baseline, 12, 24, and 30-36 mo postpartum, WLWH-P had lower mean standardized BMD values for FN, LS, and TH compared to HIV-neg-P women, although differences were not statistically significant except for baseline TH. In conclusion, both groups demonstrated BMD increase to above baseline scores by 30-36 mo. For WLWH-P compared to WLWH-NP, mean standardized BMD values trended lower between baseline and 24 mo. Both WLWH-P and HIV-neg-P women showed BMD recovery to baseline for FN, LS, and TH by 30-36 mo postpartum. However, mean BMD standard-scores trended lower throughout follow-up for WLWH-P compared to HIV-neg-P women. WLWH-P and WLWH-NP women had similar BMD standardized scores by 30-36 mo. The BMD recovery results are encouraging, but longer follow-up is needed to assess fracture risk among older WLWH compared to HIV-negative women.

Keywords HIV, BMD, tenofovir disoproxil fumarate, pregnancy, lactation

Lay Summary

Pregnancy and lactation are characteristically associated with loss of bone mass in the mother, but this recovers on weaning in healthy women. Information is sparse among women living with HIV (WLWH), in whom bone mass is typically lower than in non-pregnant/lactating healthy women. The Maternal Bone Health Study conducted in Uganda and Malawi found that BMD decreases during breastfeeding but successfully recovers to baseline levels within 30-36 mo postpartum for both women living

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with HIV and HIV-negative women. Reassuringly, recent pregnancy and breastfeeding did not cause permanent bone loss compared to women with HIV who did not become pregnant. However, while the recovery is encouraging, WLWH maintained consistently lower overall bone density scores compared to their HIV-negative peers throughout the follow-up period. This suggests that while women's BMD recovers from pregnancy-related changes, the combined effects of HIV and antiretroviral therapy require continued monitoring. Women living with HIV should feel empowered to breastfeed, while remaining aware that long-term bone health is a priority as they age.

Introduction

Close to 38 million individuals worldwide are estimated to be living with HIV, including 17.8 million women of childbearing age.[1] Individuals living with HIV are at higher risk of fractures and decreased bone mineral density (BMD) compared to age-matched individuals without HIV, related to a variety of known risk factors including HIV disease itself and antiretroviral therapy (ART).[2,3] ART initiation is associated with decreases in BMD in the first 2 yr of treatment which does not return to baseline levels following virologic suppression.[4,5] Among women living with HIV (WLWH) who have repeated cycles of pregnancy and lactation, the impact of life-long ART on lactation-related BMD loss/recovery remains a critical research and public health gap. These concerns are magnified in resource-limited African settings where gains in life expectancy for individuals with HIV on ART may be associated with increased aging-related bone morbidity including fracture risk, resulting in a sizeable health burden. WLWH on life-long ART are particularly vulnerable to poor bone health, accelerated BMD loss, and longer-term risk of bone fragility and fractures related to several independent risk factors, including poor nutrition, hormonal changes with pregnancy, and lactation.[6]

Pregnancy and breastfeeding are linked to transient decreases in BMD of about 3% and 3%-6% respectively, depending on the skeletal site of measurement.[7,8] This decrease in BMD is generally followed by BMD recovery within 3-6 mo after cessation of lactation.[8,9] However, in resource-limited settings, with high parity and poor nutrition, studies suggest incomplete recovery following repeat pregnancies related to breastfeeding past 12 mo among younger[7,9] women. Women living with HIV in these settings experiencing repeat pregnancies and breastfeeding while on ART, may be a particularly high-risk group for accelerated bone loss. To date, there are limited data on the degree of BMD loss among mothers with HIV on life-long ART; or regarding the potential interaction of ART with repeat pregnancy/lactation episodes on BMD recovery following cessation of breastfeeding.

We conducted a longitudinal follow-up study, "the Maternal Bone Health Study" (MBHS) to compare BMD changes with repeat pregnancy/breastfeeding among mothers with and without HIV at 2 African research sites (Blantyre, Malawi and Kampala, Uganda). Our primary hypothesis was that BMD recovery would be dampened and lower in WLWH on ART after cessation of breastfeeding compared to HIV-negative women of similar age and parity. Additionally, we hypothesized that WLWH-P would have lower mean BMD standardized scores during follow-up through 30-36 mo post baseline after cessation of breastfeeding when compared to non-pregnant WLWH of similar age group and parity who had not experienced a recent pregnancy.

Materials and methods

Study population and eligibility criteria

Women living with HIV who became pregnant during follow-up in a cohort study called PROMise Ongoing Treatment Evaluation (PROMOTE)[10] and found eligible were co-enrolled into the MBHS study. These pregnant WLWH (WLWH-P) were then age- and parity-matched with 2 comparison groups: (1) non-pregnant WLWH (WLWH-NP) from the PROMOTE study who were not intending to become pregnant over the planned 36 mo of study follow-up and (2) HIV-negative pregnant women (HIV-neg-P) recruited from antenatal clinics.

Study entry criteria included age $\geq$18 yr, documentation of HIV-1 infection status, positive or negative, documented pregnancy status (by ultrasound, clinical exam, or positive urine HCG test). Non-pregnant WLWH were counseled to use an effective method of contraception for the 3-yr duration of study participation. Regardless of their pregnancy and HIV infection status, women were excluded if they (1) had a pre-existing condition known to affect bone metabolism (e.g., thyrotoxicosis, tuberculosis, diabetes mellitus, and liver or renal disease) or (2) were taking medications known to affect bone metabolism (steroids, anticonvulsants, bisphosphonates, and anti-cancer drugs). In addition, women in the non-pregnant group (WLWH-NP) would be excluded if they (1) were intending to get pregnant (2) were currently, or (3) recently breastfeeding in the last 6 mo prior to screening, or (4) had been oophorectomized.

Study procedures

Clinical assessments

Using pre-tested standardized questionnaires, we collected data on baseline demographic characteristics (age, occupation, parity, level of education, etc.), medical history (including known risk factors for low BMD), concomitant medications, contraceptives, lactation and physical activity (using the International Physical Activity questionnaire),[11] anthropometric assessments (weight, height, and BMI) and clinical assessment for gestational age. Physical activity was categorized per World Health Organization physical activity guidelines, which recommend $\geq$150 min of moderate intensity physical activity or $\geq$75 min of vigorous intensity physical activity in a week for adults 18 yr and above.[12] Interviewer administered questionnaires including physical activity, and family planning questionnaires were translated to the primary local language in Kampala, Uganda and Blantyre, Malawi.

Laboratory Procedures

Laboratory evaluations including HIV screening for the negative women, HIV RNA tests for WLWH, and pregnancy tests for all

women were conducted by the Infectious Diseases Institute Core Laboratory, in Uganda, and the Johns Hopkins Research Project Laboratory in Malawi, both accredited by the College of American Pathologists. HIV RNA testing for WLWH was performed every 6 mo using the Roche COBAS AmpliPrep/COBAS TaqMan Assay, version 2.0 in Uganda (reportable range: 20-10 000 000 copies/mL) and the Abbott m2000 RealTime System in Malawi (reportable range: 40-10 000 000 copies/mL).

BMD Assessments

BMD was measured in g/cm^2 according to the International Society for Clinical Densitometry guidelines.[13] BMD of the LS (L1-L4), TH, and FN were measured for the recently pregnant groups (WLHIV-P and HIV-neg-P) at 6 wk, and 12, 24, and 30-36 mo post-delivery. For WLWH-NP, BMD was measured at study entry, 12, 24, and 36 mo. Hologic Discovery DXA machines interfaced with Apex System Software 2.3.2 were used at both the Kampala and Blantyre sites. Towards the end of the study and following mechanical failures occurring with its Hologic Discovery DXA machine, Kampala site switched to the Hologic Horizon, Apex System Software 5.6.1.3, a newer version of the DXA machine manufactured by the same company (Hologic). A validation report showed that the two machines were comparable with no significant effect on interpretation of BMD values.

DXA Quality control

Standardized procedures for obtaining the scans were followed to minimize differences between the 2 study sites—all scanners were Hologic models that had been cross-calibrated with a phantom prior to commencement of the study. Each technician underwent webinar training and quality review of their first scans. Precision errors for the 2 Hologic Discovery DXAs in Kampala and Blantyre were measured using the ISCD Advanced Precision Calculating Tool (30 patients scanned twice) and were within the acceptable range (% coefficient of variation: 0.66%–1.02% at the TH and LS). At the Kampala site, the mean percent differences between the Discovery and the replacement Horizon machines were minimal (TH: 0.135%; LS: −1.357%). The DXA equipment was standardized daily using a phantom as prescribed in the manufacturer's instructions. Co-investigator, J.P., an internationally recognized DXA expert and bone specialist, guided the planning and execution of the DXA investigations; and did a central read of 10% of DXA scans. Scans requiring correction were replaced (less than 3%). J.P. and the DXA technicians were blinded to participants HIV status.

Ethical considerations

All women provided written informed consent for study participation. The protocol was approved by the relevant ethics committees in the collaborating and participating institutions and countries: Johns Hopkins School of Medicine IRB 00183528 (USA), Joint Clinical Research Centre IRB JC3618 (Uganda), Uganda National Council for Science and Technology Hs 2605 (Uganda), and College of Medicine Research Ethics Committee P.10/18/2502 (Malawi).

Statistical analysis

BMD values at 12, 24, and 30-36 mo were standardized against pooled baseline values. Our primary outcomes were changes in standardized BMD score from baseline to 36 mo at the LS, TH, and FN. Standardized BMD was compared between WLWH-P and HIV-neg-P at 6 wk, 12, 24, and 30-36 mo post-delivery. Similarly, standardized BMD of WLWH-NP measured at entry, 12, 24, and 30-36 mo post-enrolment were compared to those from WLWH-P measured at 6 wk, and 12, 24, and 30-36 mo post-delivery. Values were plotted over time, with 95% CIs based on a *t*-distribution.

Linear mixed models were used to model the standardized BMD values from the 3 different sites over time for women from recently pregnant groups (WLWH-P and HIV-neg-P). The models included a second-order polynomial for time of visit (to capture the non-linear relationship between time and BMD), country, HIV status, an interaction term between HIV status and time, a random effect on the intercept for participant, and baseline variables for highest education level attained (less than primary, primary, completed some/all secondary, and tertiary), contraceptive type (3 categories of injectable, other (includes those missing contraceptive type) or none), cumulative breastfeeding before delivery (in years), an indicator for moderate exercise level and BMI. We used F-tests to test for differences in the standardized BMD values between WLWH-P and HIV-neg-P at each time point during study follow-up. We also used a likelihood ratio test (LRT) to test whether the slope of the standardized BMD recoveries varied with HIV status. More specifically, we compared the nested model without the interaction terms between HIV status and time to the proposed model, thus checking if the slope of the BMD recovery varies with HIV status. We compared breastfeeding duration between WLWH-P and HIV-neg-P using a Wilcoxon rank sum test and performed a post-hoc sensitivity analysis re-running the linear mixed model for each country excluding women who ceased breastfeeding before 12 mo.

Similarly, linear mixed models were used to compare standardized BMD values between WLWH-P and WLWH-NP, except that HIV status was replaced with an indicator variable for pregnancy status. The models additionally included baseline variables for undetectable HIV RNA level, ART regimen (dolutegravir, non-nucleoside reverse transcriptase inhibitors, and protease inhibitors) and time on ART (in months). We conducted F-tests to test for differences in the standardized BMD values between WLWH-P and WLWH-NP at each time point during study follow-up.

All *p*-values are two-sided. While statistical significance was defined using a *p*-value of $\leq$.05, clinical significance additionally considered effect sizes. Adjustment for multiple testing was not performed and therefore *p*-values and CIs should not be used to infer definitive effects. All analyses were conducted using SAS, version 9.4 (SAS Institute Inc.).

Results

Figure 1 represents the flow of participants through the study. Between August 2019 and August 2023, 333 women were screened: 108 pregnant WLWH-P, 117 pregnant with no HIV (HIV-neg-P), and 108 non-pregnant women with HIV (WLWH-NP) at research sites in Blantyre, Malawi and Kampala, Uganda (Figure S1). A total of 312 women were enrolled: 104 WLWH-P, 109 HIV-neg-P, and 99 WLWH-NP.

Table 1 presents a summary of participant baseline characteristics. The median age was 33 yr (IQR, 30-36 yr), and 183 (58.6%) had some/completed secondary or tertiary education. The median parity was 3 (IQR, 2-4), with a cumulative median (IQR) duration

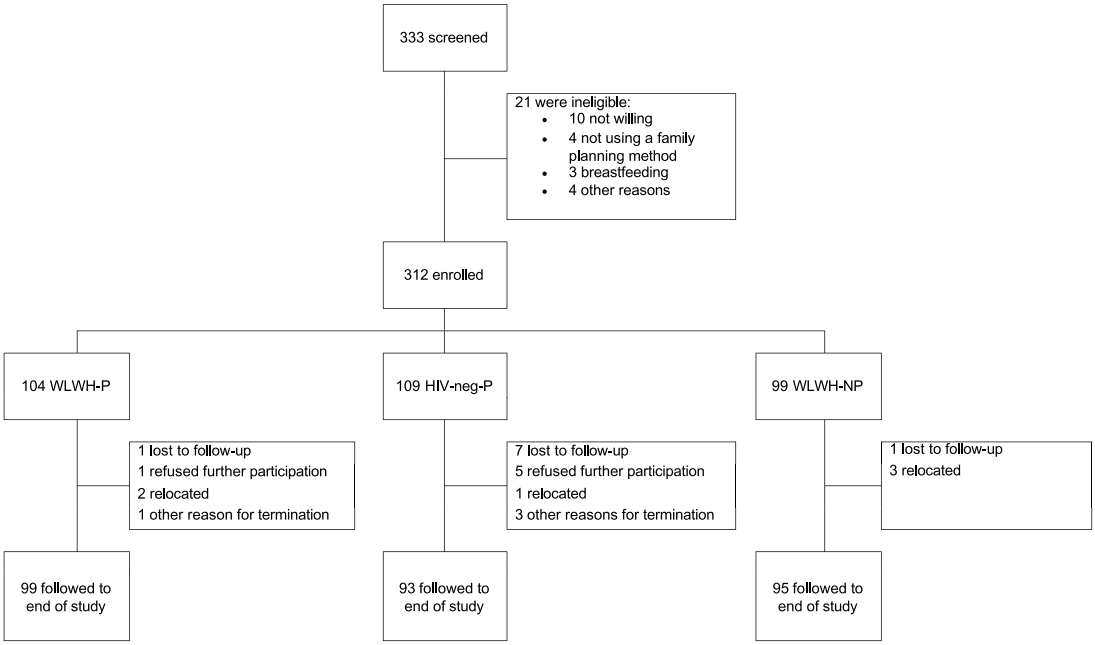

Figure 1 Flow diagram.

of breastfeeding of 51 (31-72) mo over the previous periods of lactation. Among WLWH, 91% had undetectable HIV RNA levels and had been on ART for a median of 14 (7-38) mo. The majority (89.7%) of WLWH were on a tenofovir (TDF) containing regimen. Median duration of breastfeeding was shorter among WLWH-P compared to HIV-neg-P women (14 vs 20 mo, p-value $< .001$). However, by 24 mo, most (92%) of women had stopped breastfeeding and by 6 mo prior to study exit, all but one woman had ceased breastfeeding. Table S1 provides a clear breakdown of participant characteristics by country.

Comparison of BMD changes among recently pregnant WLWH-P compared to recently pregnant women without HIV (HIV-neg-P)

Mean LS, FN, and TH standardized BMD values for WLWH-P were below those of HIV-neg-P women throughout study follow-up (Figure 2). Results from the linear mixed models showed that, after adjusting for all covariates, standardized BMD values for recently pregnant women without HIV were higher than those of WLWH-P at baseline (6 wk post-delivery), at the TH site by a difference of 0.28 (95% CI: 0.01-0.54), (Table 2, Table 3). Although differences in standardized BMD values were not found to be significant at 12 and 30-36 mo post-delivery, results from the LRT suggested that trajectories of increases in BMD differed by HIV status at the FN (p-value $= .026$) and TH (p-value $< .001$) sites: while predicted standardized BMD values dropped after delivery for both WLWH-P and HIV-neg-P, BMD recovery thereafter began sooner among WLWH-P.

The results from the sensitivity analysis for women who breastfed for 12 mo or longer were consistent with those using the full sample (Tables 2 and 3); differences in standardized BMD values between the two groups were not significant other than at baseline (6 wk post-delivery) at the TH site, and BMD recovery was predicted to occur sooner among WLWH-P compared with HIV-neg-P at the FN (p-value $= .024$) and TH (p-value $< .001$) sites.

Comparison of BMD changes among recently pregnant (WLWH-P) compared to non- pregnant women with HIV (WLWH-NP)

Mean LS, FN, and TH standardized BMD values for WLWH-P were below those of WLWH-NP throughout study follow-up (Figure 2). Results from the linear mixed models showed that WLWH-NP had significantly higher standardized BMD values than WLWH-P at the LS and TH sites prior to 24 mo post-delivery, with a difference of 0.28 (95% CI: 0.0-0.56) and 0.27 (95% CI: −0.01 to 0.55) at the LS and TH sites, respectively (Table 4). However, by 30-36 mo post-delivery, no significant differences in BMD values were noted, indicating recovery of BMD among the WLWH-P group compared to the WLWH-NP group. No significant differences were observed at the FN.

Discussion

African WLWH are a particularly vulnerable group for decreased BMD based on being female, their HIV status, life-long ART, and high parity with repeated episodes of prolonged breastfeeding. We evaluated BMD in this population to characterize changes and recovery in BMD post-cessation of breast feeding attributable to HIV and pregnancy status. The 3-yr follow-up allowed us to assess whether recently pregnant women with HIV had complete BMD recovery back to baseline post-cessation of breastfeeding. The study compared their BMD changes to age and parity matched HIV-negative women with a recent pregnancy; and with WLWH of similar age group and parity who did not become pregnant during 3 yr of follow-up. We found that by 30-36 mo follow-up, recently pregnant women with HIV showed similar standardized

Table 1 Characteristics of women in the Maternal Bone Health Study.

		Total	WLWH-P ($N = 104$)	HIV-neg-P ($N = 109$)	WLWH-NP ($N = 99$)
Baseline					
Age in years, median (IQR)		33 (30-36)	33 (30-36)	32 (29-35)	33 (30-36)
Parity, median (IQR)		3 (2-4)	3 (2-4)	3 (2-4)	3 (3-4)
Highest education level attained, *N* (%)	Primary	35 (11.2)	12 (11.5)	9 (8.3)	14 (14.1)
	Some/ all secondary	158 (50.6)	53 (51.0)	55 (50.5)	50 (50.5)
	Tertiary	25 (8.0)	5 (4.8)	14 (12.8)	6 (6.1)
BMI, median (IQR)		26.9 (23.2-31.4)	26.3 (22.9-30.3)	26.9 (24.2-30.6)	27.5 (23.2-32.3)
Alcohol history, *N* (%)	Yes	38 (12.2)	13 (12.5)	13 (11.9)	12 (12.1)
Smoking history, *N* (%)	Yes	1 (0.3)	0 (0)	0 (0)	1 (1.0)
Vigorous activity (>150 min per week), *N* (%)	Yes	80 (25.6)	28 (26.9)	22 (20.2)	30 (30.3)
Moderate activity (>75 min per week), *N* (%)	Yes	240 (76.9)	79 (76.0)	87 (79.8)	74 (74.7)
Cumulative breastfeeding duration in prior pregnancies (mo), median (IQR)		51 (31-72)	43.5 (26-61)	55 (35-74)	53 (31-78)
Grip strength (mean of right and left grip), median (IQR)		23 (20-27.5)	22.25 (19.5-27)	24 (19.5-28)	24 (20.5-28)
Contraception type (at 6-12 mo), *N* (%)	3-mo injectable	91 (32.6)	36 (35.6)	23 (25.3)	32 (36.8)
	Oral	9 (3.2)	6 (5.9)	3 (3.3)	0 (0)
	Male condom	10 (3.6)	3 (3.0)	1 (1.1)	6 (6.9)
	Progestin only	4 (1.4)	2 (2.0)	1 (1.1)	1 (1.1)
	IUD	20 (7.2)	3 (3.0)	10 (11.0)	7 (8.0)
	Implant	40 (14.3)	12 (11.9)	10 (11.0)	18 (20.7)
	Lactation amenorrhoea	7 (2.5)	2 (2.0)	5 (5.5)	0 (0)
	Sterilization (self/partner)	36 (12.9)	8 (7.9)	8 (8.8)	20 (23.0)
	None	53 (19.0)	26 (25.7)	24 (26.4)	3 (3.4)
Months on ART, median (IQR)		14 (7-38)	16 (6-43)	NA	12 (7-28)
ART regimen, *N* (%)	Non-nucleoside reverse transcriptase inhibitors (NNRTI)	44 (23.0)	28 (28.6)	NA	16 (17.2)
	Protease inhibitor (PI)	36 (18.9)	20 (20.4)	NA	16 (17.2)
	Dolutegravir	111 (58.1)	50 (51.0)	NA	61 (65.6)
Regimen contains TDF, *N* (%)	Yes	182 (89.7)	95 (91.4)	NA	87 (87.9)
HIV viral load, *N* (%)	<40 cps/mL or undetectable	175 (91.6)	90 (90.9)	NA	85 (92.4)
BMD, median (IQR)	Femoral neck	0.85(0.77-0.93)	0.85(0.76-0.92)	0.85(0.78-0.94)	0.85(0.79-0.92)
	Lumbar spine	0.92(0.85-1.01)	0.884(0.82-0.97)	0.93(0.84-1.01)	0.945(0.87-1.03)
	Total hip	0.95(0.88-1.04)	0.93(0.865-1.01)	0.95(0.9-1.05)	0.97(0.88-1.05)

(*Continued*)

Table 1 Continued

		Total	WLWH-P ($N = 104$)	HIV-neg-P ($N = 109$)	WLWH-NP ($N = 99$)
BMD (standardized), median (IQR)	Femoral neck	−0.041(−0.692-0.61)	−0.041(−0.773-0.529)	−0.041(−0.61-0.692)	−0.041(−0.529-0.529)
	Lumbar spine	−0.096(−0.709-0.694)	−0.411(−0.973-0.343)	−0.008(−0.797-0.694)	0.124(−0.534-0.869)
	Total hip	−0.098(−0.683-0.653)	−0.265(−0.808-0.402)	−0.098(−0.516-0.736)	0.068(−0.683-0.736)
Follow-up					
Contraception type (at 6-12 mo), *N* (%)	3-mo injectable	91 (33)	36 (36)	23 (25)	32 (37)
	Oral	9 (3)	6 (6)	3 (3)	0 (0)
	Male condom	10 (4)	3 (3)	1 (1)	6 (7)
	Progestin only	4 (1)	2 (2)	1 (1)	1 (1)
	IUD	20 (7)	3 (3)	10 (11)	7 (8)
	Implant	40 (14)	12 (12)	10 (11)	18 (21)
	Lactation amenorrhoea	7 (3)	2 (2)	5 (6)	0 (0)
	Sterilization (self/partner)	36 (13)	8 (8)	8 (9)	20 (23)
	None	53 (19)	26 (26)	24 (26)	3 (3)
Cumulative breastfeeding duration in prior pregnancies (mo), median (IQR)		15 (12-20)	14 (11-20)	20 (15-21)	NA

Abbreviations: HIV-neg-P, recently pregnant HIV-negative women; WLWH-P, recently pregnant women living with HIV; WLWH-NP, women living with HIV who were not recently pregnant. Missing values: 6 (2%) BMI, 1 (0.3%) grip strength, 33 (11%) contraceptive type, 10 (3%) time on ART, 12 (4%) ART regimen and 12 (4%) viral load values, and 27 (9%) BMD values were missing.

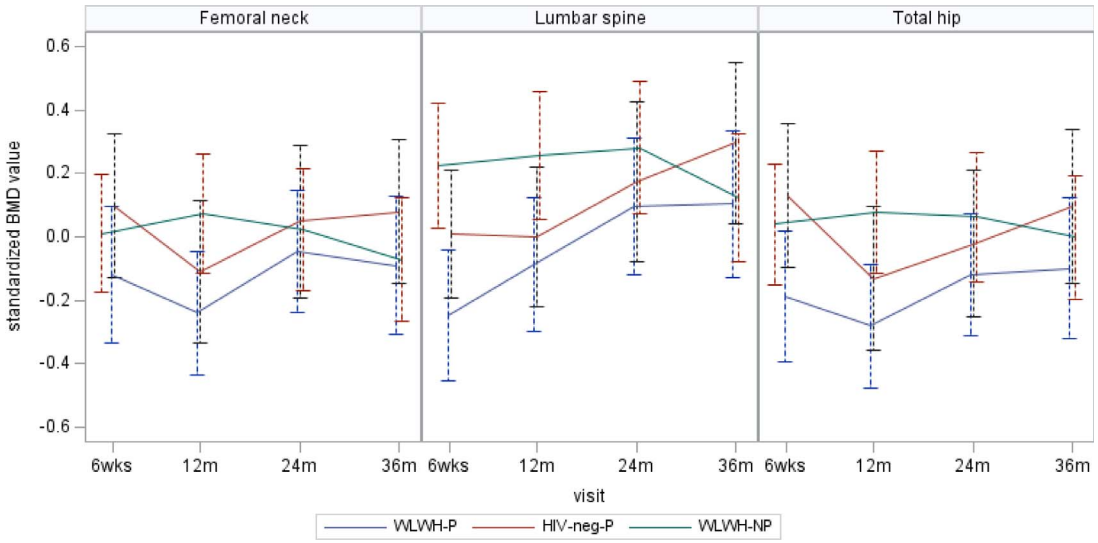

Figure 2 Mean standardized BMD values and 95% CIs, stratified by HIV/pregnancy status for FN, LS, and TH sites.

Table 2 Estimated difference (95% CI) in standardized BMD values and associated *p*-value between study groups by time of visit and BMD site using linear mixed models.

		All women who were recently pregnant		All women who were recently pregnant excluding women who breastfed for <12 mo		All women living with HIV	
Time of visit	**BMD site**	**Difference (HIV-neg-P minus WLWH-P) (95% CI)**	***p*-value**	**Difference (HIV-neg-P minus WLWH-P) (95% CI)**	***p*-value**	**Difference (WLWH-NP minus WLWH-P) (95% CI)**	***p*-value**
Enrolment/6 wk post-delivery	Femoral neck	0.19 (−0.09 to 0.47)	.173	0.21 (−0.10 to 0.52)	.186	0.24 (−0.04 to 0.52)	.092
	Lumbar spine	0.15 (−0.14 to 0.44)	.303	0.21 (−0.12 to 0.55)	.212	0.52 (0.23-0.81)	<.001
	Total hip	0.28 (0.01 to 0.54)	.043	0.31 (0.00-0.62)	.051	0.30 (0.02-0.58)	.037
12 mo	Femoral neck	0.08 (−0.18 to 0.33)	.545	0.10 (−0.18 to 0.38)	.489	0.26 (0.00-0.53)	.050
	Lumbar spine	0.05 (−0.22 to 0.33)	.718	0.11 (−0.20 to 0.43)	.473	0.42 (0.14-0.69)	.003
	Total hip	0.09 (−0.16 to 0.33)	.487	0.14 (−0.14 to 0.42)	.323	0.34 (0.06-0.61)	.016
24 mo	Femoral neck	0.04 (−0.21 to 0.29)	.725	0.05 (−0.22 to 0.33)	.706	0.18 (−0.09 to 0.44)	.186
	Lumbar spine	0.04 (−0.24 to 0.32)	.761	0.11 (−0.21 to 0.42)	.511	0.28 (0.00-0.56)	.046
	Total hip	0.02 (−0.22 to 0.27)	.849	0.08 (−0.20 to 0.36)	.563	0.27 (−0.01 to 0.55)	.057
30/36 mo	Femoral neck	0.09 (−0.17 to 0.35)	.483	0.07 (−0.22 to 0.36)	.627	−0.02 (−0.29 to 0.26)	.914
	Lumbar spine	0.13 (−0.16 to 0.42)	.383	0.19 (−0.14 to 0.51)	.266	0.11 (−0.17 to 0.39)	.443
	Total hip	0.09 (−0.17 to 0.35)	.507	0.13 (−0.17 to 0.42)	.392	0.10 (−0.19 to 0.39)	.496

BMD scores to the non-pregnant group with HIV. We also found that both recently pregnant women with and without HIV demonstrated nadir hip BMD mean scores at 12 mo post-delivery followed by increase in BMD. However, mean BMD scores for FN, LS, and TH trended lower at each time point (6 wk postpartum, 12, 24, and 30-36 mo) for the recently pregnant women with HIV compared to the recently pregnant group without HIV, albeit with overlapping 95% CI.

Overall, the results demonstrating BMD recovery following cessation of breastfeeding for the WLWH with a recent pregnancy are encouraging, although the group mean scores were consistently lower for recently pregnant women with HIV compared to the pregnant group without HIV. Likewise, the finding that recently pregnant women with HIV had similar BMD scores at 30-36 mo compared to women with HIV who did not have a pregnancy during 3 yr of follow-up indicates that pregnancy and breastfeeding did not negatively impact BMD recovery for recently pregnant women with HIV following pregnancy/lactation.

Our findings build on prior work within the PROMISE bone and kidney sub study[14] which followed BMD among women with HIV through 18 mo postdelivery of both mothers living with HIV and their HIV-ARV exposed infants in a large randomized trial conducted in east and southern Africa. PROMISE found decreased BMD levels at 18 mo postdelivery for mothers living

Table 3 Linear mixed model fixed effect parameters and *p*-values for modeling of standardized BMD values among recently pregnant women, using the full sample and a subset including women who breastfed for 12 or more months.

		Femoral neck				**Lumbar spine**				**Total hip**			
		All ($N = 661$)		**Breastfeed >12 ($N = 551$)**		**All ($N = 671$)**		**Breastfeed >12 ($N = 560$)**		**All ($N = 661$)**		**Breastfeed >12 ($N = 551$)**	
		Estimate	***p*-value**	**Estimate**	***p*-value**	**Estimate**	***p*-value**	**Estimate**	***p*-value**	**Estimate**	***p*-value**	**Estimate**	***p*-value**
Intercept		−1.84	<.001	−2.46	<.001	−1.37	.008	−1.66	.004	−2.13	<.001	−2.61	<.001
HIV status (reference: HIV−)	HIV+	−0.39	.040	−0.39	.066	−0.34	.068	−0.40	.069	−0.59	<.001	−0.58	.004
Site (reference: Uganda)	Malawi	0.23	.158	0.35	.064	0.22	.174	0.18	.350	0.37	.029	0.46	.021
Time (years starting at 1)		−0.31	<.001	−0.32	<.001	−0.06	.423	−0.07	.340	−0.48	<.001	−0.48	<.001
Time^2 (years starting at 1)		0.07	<.001	0.07	<.001	0.04	.006	0.04	.005	0.10	<.001	0.10	<.0001
Time*HIV status		0.24	.017	0.21	.052	0.24	.021	0.23	.039	0.38	<.001	0.33	<.001
Time^2*HIV status		−0.04	.026	−0.03	.100	−0.05	.013	−0.04	.028	−0.06	<.001	−0.05	.003
Highest education (reference: Tertiary)	Below Primary	0.23	.363	0.49	.049	−0.19	.493	0.19	.487	0.37	.107	0.61	.021
	Primary	0.19	.505	0.38	.207	−0.16	.572	0.14	.660	0.44	.128	0.66	.061
	Some/ all secondary	0.28	.208	0.51	.024	0.11	.663	0.46	.064	0.33	.105	0.53	.031
Contraception type (reference: None)	Injectable	−0.15	.399	−0.08	.702	−0.30	.089	−0.16	.461	−0.07	.682	−0.01	.980
	Other	−0.13	.450	−0.04	.828	−0.08	.642	−0.05	.809	0.01	.974	0.09	.639
Cumulative breastfeeding (years)		−0.05	.049	−0.05	.043	−0.03	.254	−0.03	.319	−0.05	.044	−0.05	.047
Moderate activity per week (reference: <150 min)	>150 min	−0.31	.073	−0.22	.267	−0.30	.074	−0.29	.135	−0.21	.235	−0.16	.423
BMI		0.09	<.001	0.09	<.001	0.07	<.001	0.06	<.001	0.09	<.001	0.10	<.001

Table 4 Linear mixed model fixed effect parameters and *p*-values for modeling of standardized BMD values among women living with HIV.

		Femoral neck ($N = 604$)		Lumbar spine ($N = 613$)		Total hip ($N = 604$)	
		Estimate	***p*-value**	**Estimate**	***p*-value**	**Estimate**	***p*-value**
Intercept		−2.01	.001	−1.53	.011	−2.44	.001
Pregnancy status (reference: not recently pregnant)	Recently pregnant	−0.11	.550	−0.59	.002	−0.16	.357
Site (reference: Uganda)	Malawi	0.32	.028	0.32	.036	0.40	.015
Time (years starting at 1)		0.14	.027	0.14	.022	0.11	.064
Time^2 (years starting at 1)		−0.03	.012	−0.03	.018	−0.02	.069
Time*Pregnancy status		−0.18	.071	0.05	.580	−0.19	.029
Time^2*Pregnancy status		0.05	.005	0.02	.358	0.05	.001
Highest education (reference: Tertiary)	Less than primary	0.08	.843	−0.34	.418	0.53	.178
	Primary	0.10	.810	−0.34	.443	0.46	.291
	Some/all secondary	−0.08	.830	−0.09	.832	0.31	.412
Contraception type (reference: none)	Injectable	−0.17	.404	−0.62	.001	−0.25	.271
	Other	−0.48	.015	−0.52	.007	−0.46	.035
Cumulative breastfeeding (yr)		−0.05	.029	−0.07	.013	−0.05	.017
Moderate activity per week (reference: <150 min)	>150 min	0.03	.840	−0.12	.470	0.13	.429
BMI		0.08	<.001	0.08	<.001	0.08	<.001
HIV viral load	<40 cps/mL or undetectable	−0.01	.985	0.16	.501	−0.07	.764
ART regimen (reference: PI)	Dolutegravir	0.03	.888	0.25	.232	0.23	.311
	NNRTI	0.35	.090	0.07	.703	0.56	.007
Time on ART (mo)		−0.003	.587	0.01	.066	−0.002	.641

with HIV who did not meet treatment initiation criteria at the time of the study and who had been randomized to receive ART during breastfeeding compared to those whose infants received infant nevirapine prophylaxis (no maternal ART). However, PROMISE lacked an HIV-negative comparison group. Another study, the GUMBA study, done in Uganda by Nabwire et al.[15] included recently pregnant women and without HIV with BMD measured at 6 wk and then through 3 mo post-cessation of breastfeeding. As in our study, Nabwire et al. reported similar trends of lower standardized BMD scores for women with HIV compared to HIV-negative women from 3 mo postdelivery through 3 or more months post-lactation, with nadir BMD in the first year postdelivery. In addition, for HIV-negative women in the GUMBA study, there was full recovery to baseline BMD after cessation of breastfeeding for both LS and TH, whereas for women with HIV, recovery for LS was at or above baseline, whereas TH BMD scores did not show full recovery. Nabwire et al. found statistically significant differences in BMD scores between women with HIV and HIV-negative women for both LS and TH following end of breastfeeding, whereas our study showed similar trends but with overlapping 95% CI between groups. One other study from Soweto, South Africa, by Hamill et al.[16] also examined women of child bearing age (39 HIV-negative, 28 women with HIV not on ART, and 43 WLWH initiating ART) and followed them for 24 mo. They reported that the women with HIV who initiated ART had decreased BMD scores for LS during the first 12 mo after ART initiation and did not achieve the same BMD levels as HIV-negative women based on DXA BMD scores at 24 mo.

In terms of clinical implications, both our study and the Nabwire study were conducted among younger women of childbearing age with a recent pregnancy and there were no osteoporotic related fractures noted. It is unclear what the future clinical implications of the lower BMD scores seen in our study among recently pregnant women with HIV compared to pregnant women without HIV. Specifically, our findings do not let us to predict whether women with HIV will be at increased fracture risk as they go through menopause. However, several recent studies among older women with HIV suggest there is cause for concern. A number of studies including in resource limited settings in older women with HIV showed higher risk of fracture and abnormal bone microarchitecture as well as low BMD compared to HIV-negative older women.[17–21] A recent cross sectional study from Zimbabwe, by Madanhire et al.,[17] evaluated DXA scores among an older group (40-60 yr) of 393 Zimbabwean women, of whom 49% were women with HIV. They found that older Zimbabwean women with HIV did demonstrate significantly higher rates of osteoporosis level z scores (≤2 SD) based on DXA BMD measures of LS and FN. They also reported a doubling of history of fracture events for the WHWH group (14%) compared to the HIV-negative group (7%); and that women with HIV had a significantly higher 10-yr risk of major osteoporotic fracture compared to the HIV-negative group.

There are a number of strengths to this study. These include the longitudinal follow-up over 3 yr with high retention, as well as 2 comparison groups to compare changes in BMD scores by both HIV and recently pregnant status. Other strengths include the standardization and QA of the DXA reads by a DXA expert, as well as DXA machines by the same company which were serviced regularly. A further strength of the study was inclusion of women from both Malawi and Uganda which increases the generalizability of the findings. There are also some limitations. The study was designed to detect at least a 30% difference in BMD, so we lacked power to detect smaller, yet potentially clinically relevant differences. Given that the first DXA scans were performed 6 wk postpartum, we are not able to assess pregnancy related BMD changes. In addition, another limitation was that despite use of similar Hologic DXA units at the Malawi and Kampala Uganda sites and use of a common phantom for standardization at both sites, there was no direct cross-calibration between the new DXA unit installed in Kampala site and the unit utilized in Malawi. Another limitation is that we had limited information on the diet or nutrition status of the mothers in the study except for BMI. We did not collect detailed data on the frequency or intensity of breastfeeding, which is a known determinant of the nadir of BMD reached during lactation. Lastly, although there may be slight differences in lifestyle and clinical factors between the 2 sites most notably alcohol use, physical activity levels, and use of protease inhibitor ART regimen (Table S1), to ensure these differences did not bias our findings, we included these factors as well as country as covariates in our adjusted models. This approach allows us to report generalized findings while respecting the unique sociodemographic profile of each cohort.

Conclusions

Further research in resource-limited international settings is urgently needed on women with HIV on life-long ART through menopause, to identify whether the lower BMD scores of younger women with HIV compared to HIV-negative women will later place them at increased risk for osteoporosis, and related hip and spine fracture post menopause. This seems plausible based on multiple risk factors including HIV disease progression and related inflammatory markers, current TDF-based ART regimens, and high parity/lactation for many WLHIV in resource-limited African settings. Among those women with HIV and HIV-negative older women who do show evidence of low bone mass with resultant increased fracture risk, interventions need to be tested that are cost effective and deliverable within the context of available health care infrastructure and resources.

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Author contributions

Flavia Matovu Kiweewa (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing—original draft, Writing—review & editing), Lara Lewis (Formal analysis, Software, Validation, Visualization, Writing—review & editing), Lynda Stranix-Chibanda (Investigation, Methodology, Writing—review & editing), Sufia Dadabhai (Investigation, Methodology, Writing—review & editing), Patience Atuhaire (Investigation, Methodology, Writing—review & editing), Felluna Chauwa (Investigation, Methodology, Writing—review & editing), Jim Aizire (Investigation, Methodology, Writing—review & editing), Taha E. Taha (Conceptualization, Investigation, Writing—review & editing), John M. Pettifor (Conceptualization, Investigation, Methodology, Writing—review & editing), Sean S. Brummel (Conceptualization, Data curation, Formal analysis, Validation, Writing—review & editing), Todd T. Brown (Conceptualization, Investigation, Methodology, Writing—review & editing), and Mary Glenn Fowler (Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing—review & editing)

Supplementary material

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Conflicts of interest

None of the authors has financial, consultant, institutional, or other relationships that might lead to a bias or conflict of interest.

Data availability

Data can be shared with qualifying researchers who submit a proposal with a valuable research question as assessed by the Protocol Co-Chairs. Specific Requests should be directed to mfowler5@jhmi.edu, ttaha1@jhu.edu, and fmatovu@mujhu.org.

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