Changes in Bone Mineral Density During and After Lactation in Ugandan Women With HIV on Tenofovir-Based Antiretroviral Therapy

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ABSTRACT

Antiretroviral therapy (ART) in people living with human immunodeficiency virus (HIV) is associated with bone loss, but data are limited in lactation, when physiological bone mineral mobilization is occurring. This research charted changes in areal bone mineral density (aBMD) during and after lactation in Ugandan women with HIV (WWH) initiated onto ART in pregnancy, compared to women without HIV (REF). One-hundred WWH on tenofovir-based ART and 100 REF were enrolled in pregnancy. Lumbar spine (LS), total hip (TH), and whole-body-less-head (WBLH) aBMD were measured by dual-energy X-ray absorptiometry (DXA) at 2, 14, and 26 weeks of lactation, and at 3 months postlactation. The primary outcome was the difference between groups in percent change in LS aBMD between 2 and 14 weeks. Statistical analysis was performed in hierarchical repeated measures ANOVA models that corrected for multiple testing. Median age was 23.4 (IQR, 21.0 to 26.8) years. WWH had lower body weight. aBMD decreased in both groups during lactation, but WWH had greater decreases at TH (2-to-26 weeks: WWH [n = 63] −5.9% [95% CI, −6.4 to −5.4] versus REF [n = 64] −4.3% [95% CI, −4.8 to −3.8]; group*time point interaction p = .008). Decreases in LS aBMD were similar in WWH and REF (2-to-26 weeks: −2.0% [95% CI, −2.5 to −1.5]), although there was a tendency toward a smaller decrease in WWH between 2 and 14 weeks (WWH [n = 77] −2.2% [95% CI, −2.2 to −1.4] versus REF [n = 69] −2.9% [95% CI, −3.3 to −2.5]; group*time point interaction p = .08). Postlactation, LS aBMD was higher relative to week 2 in both groups. TH and WBLH aBMD did not return to week 2 values in WWH but did in REF (TH postlactation versus week 2: WWH [n = 61] −3.1% [95% CI, −3.6 to −2.6]; REF [n = 29] +0.1% [95% CI, −0.9 to +1.1]). These data show accentuated bone loss during lactation and only partial skeletal recovery by 3 months postlactation in Ugandan WWH on tenofovir-based ART. Studies are ongoing to understand longer-term consequences for bone health. © 2020 The Authors. Journal of Bone and Mineral Research published by American Society for Bone and Mineral Research.

KEY WORDS: AFRICAN WOMEN; BONE HEALTH; HIV; LACTATION; TENOFOVIR-BASED ANTIRETROVIRAL THERAPY (ART)

Introduction

Many studies have reported declines in areal bone mineral density (aBMD) following initiation of antiretroviral therapy (ART) in people with human immunodeficiency virus (HIV), and tenofovir disoproxil fumarate (TDF)-containing ART, is associated with greater bone loss than regimens without TDF.1–4 The World Health Organization (WHO) recommends initiation of lifelong triple ART in all pregnant and breastfeeding women with HIV (WWH) at the time of diagnosis, for their own health and to prevent mother-to-child transmission of HIV (PMTCT)—a strategy initially referred to as Option B+.5 Now

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under the general umbrella of “Test and Treat.” Also, WWH are advised to breastfeed for 12 to 24 months while receiving ART as a public health approach to promote HIV-free child survival in resource-limited settings. (7) Globally, 1.1 million pregnant WWH (80%) received antiretroviral agents for PMTCT in 2018, and over 90% were living in Africa. (8) Few longitudinal HIV/ART and bone studies using dual-energy X-ray absorptiometry (DXA) have been conducted in African women, (9-14); hence, data are limited in pregnant and breastfeeding women on triple ART.

Pregnancy and lactation are associated with physiological bone mineral mobilization seen as decreases in maternal aBMD to supply calcium, in uto and through breast milk, for offspring bone mineral accretion. (15,16) Bone mineral mobilization can be substantial in the first 3 to 6 months of lactation, and is more marked at trabecular rich sites, such as the lumbar spine, compared to cortical sites. (11,12,16) To date, most, but not all, (17,18) studies suggest that aBMD is recovered in later months and after lactation in apparently healthy mothers. (15,16,19,20) It is therefore possible that there are combined effects on the maternal skeleton of initiating ART plus physiological bone mineral mobilization in lactation, when demands are greatest on the maternal skeleton, and/or compromised skeletal recovery after lactation in WWH. Preliminary data from two studies suggest greater declines in aBMD in breastfeeding African WWH on ART. (11,12) but neither study measured postlactation changes or had women without HIV as comparative groups.

Therefore, we conducted an observational cohort study involving two groups of Ugandan pregnant mothers: (1) women newly diagnosed with HIV and initiated onto first-line triple ART—TDF, lamivudine (3TC), and efavirenz (EFV)—during the index pregnancy under the Option B+ guidelines (previously ART naive); and (2) women without HIV who had never been on ART. The aim of this work was to chart changes in maternal aBMD during and after lactation, in Ugandan WWH initiated onto TDF-based triple ART in pregnancy compared to reference women without HIV (REF).

Subjects and Methods

Study setting and participant recruitment

Pregnant women were recruited at the Mulago National Teaching and Referral Hospital (Mulago Hospital) antenatal clinic in Kampala, Uganda between January 2015 and February 2016. Eligibility criteria were <36 weeks gestation, aged between 18.0 and 39.9 years, having a documented rapid HIV test from Mulago Hospital during the index pregnancy, planning to breastfeed for at least 6 months, and not planning to move away from Kampala and/or extensions were gently compressed as close to the skull as possible when measuring height. Data were also collected on participant characteristics, breastfeeding practices, and medical and reproductive history.

The primary outcome was the difference between the groups in mean percent change in LS aBMD in early lactation, between L2 and L14. Selection of the primary outcome was informed by previous lactation studies. Most evidence shows greater bone mobilization in the first 3 to 6 months of lactation, when both breast milk output is highest and decreases in aBMD tend to be greater at the LS compared to other skeletal sites. (15,16,23) Secondary outcomes were group differences at each time point and change between time points in TH and WBLH aBMD, and anthropometric measures. The aim was to recruit 100 pregnant WWH and 100 REF women for at least 63 per group to complete study procedures at

Study measurements and outcomes

BMD was measured postpartum at 2 ± 0.5 (L2), 14 ± 1 (L14), and 26 ± 1 (L26) weeks to chart changes during lactation, and at least 3 months after stopping breastfeeding (as soon as possible when NPNL) to investigate skeletal recovery post-lactation. DXA scans of the lumbar spine (LS), total hip (TH), and whole body were performed using the automatic scan mode on a Hologic DXA scanner (Discovery W; Hologic, Inc., Waltham, MA, USA) at the Makerere University Johns Hopkins University (MUJHU) Research Centre in Mulago Hospital. Participants were scanned in light clothing without metal objects/accessories. As per local governance procedures, all participants were offered a pregnancy test before DXA scans at L14, L26, and NPNL visits. The following DXA measures were recorded: aBMD (g/cm²), bone mineral content (g), bone area (cm²), and from whole body scan, total mass (g), fat mass (g), lean mass (g), and percent (%) fat. Manufacturer phantoms were used for daily calibration and monitoring long-term scanner stability. The coefficient of variation on daily calibration scans during the study period was <0.5%. DXA images were scrutinized and analyzed using Hologic Apex software (version 6.0.4.0), and poor quality scans were excluded. Hairstyles containing artificial hair extensions were common and overestimated head aBMD; hence, whole body DXA measures are reported as whole-body-less-head (WBLH). (10) An electronic digital measuring station (SECA 284; SECA GmbH, Hamburg Germany, calibrated daily) was used to measure height and weight (22) with participants in light clothing. Irremovable hair extensions were gently compressed as close to the skull as possible when measuring height. Data were also collected on participant characteristics, breastfeeding practices, and medical and reproductive history.

The primary outcome was the difference between the groups in mean percent change in LS aBMD in early lactation, between L2 and L14. Selection of the primary outcome was informed by previous lactation studies. Most evidence shows greater bone mobilization in the first 3 to 6 months of lactation, when both breast milk output is highest and decreases in aBMD tend to be greater at the LS compared to other skeletal sites. (15,16,23) Secondary outcomes were group differences at each time point and change between time points in TH and WBLH aBMD, and anthropometric measures. The aim was to recruit 100 pregnant WWH and 100 REF women for at least 63 per group to complete study procedures at
L14. This was sufficient to detect at least a 2% difference between groups in mean change in LS aBMD between L2 and L14 at 80% power with a standard deviation of 4% and a type 1 error of 0.05 (two-tailed)\(^{25,16,24}\) with aBMD transformed to natural logarithms.

Statistical methods

Data were analyzed using DataDesk 8.2.1 software (Data Description Inc., Ithaca, NY, USA). Descriptive statistics for discrete data are presented as proportions (%) or median (25 percentile, 75 percentile = interquartile range [IQR]). Contingency tables and chi-square tests were used to test whether the proportions were significantly different between the groups. For all continuous variables, descriptive statistics are presented as mean ± standard deviation (SD) for normally distributed variables and median (IQR) for skewed distributions. Statistical analysis was performed in General Linear Models which combine elements of analysis of variance (ANOVA), analysis of covariance (ANCOVA), and multiple linear regression. Continuous variables were transformed into natural logarithms (\(\log_{e}\)) before analysis in nested models, except for percentage (%) fat because transformation skewed the data. The \(\log_{e}\) transformation normalized positively skewed data, and multiplication of \(\log_{e}\) by 100 enabled reporting of group differences and changes as mean sympercents [(difference (Δ)/mean)*100] ± standard error of the mean (\(\%\Delta \pm SE\)).\(^{22}\) A \(p\) value of ≤0.05 was considered significant for all tests.

Four–time point hierarchical/nested repeated-measures ANOVA and ANCOVA models were constructed for each variable with the individual identifier nested by group, time point, and a group-by-time point interaction term.\(^{26}\) aBMD was first analyzed without adjustment for body size (model 1), then adjusted for body size with aBMD as the dependent variable, and bone area (BA) and weight as covariates (model 2) according to Prentice and colleagues.\(^{127}\) Scheffé post hoc tests were used to account for multiple testing within the hierarchical models, and provided estimates of size and significance of between group differences at each time point, and within-group changes between each time point. Then, separate two–time-point hierarchical models (L2 to L14, L2 to L26, L26 to NPNL, and L2 to NPNL) were fitted using available data for pairwise comparison of changes between the groups and time points. Also, separate three–time-point models (L2-L14-L26) were fit to compare patterns of changes in the first 6 months of lactation. In this paper, data from participants with measurements at only one time point were excluded from all longitudinal statistical analyses. Restricting statistical analysis to participants with data at all visits (rectangular dataset) provided estimates that were comparable to results obtained in four–time point, three–time point, and two–time point models.

Finally, General Linear Models were set-up to adjust changes in aBMD for body size and other potential confounders; and to investigate if maternal factors were associated with baseline aBMD at L2. For changes in aBMD, fully adjusted General Linear Models were established by calculating sympercent changes between the time points of interest (L2 to L14, L2 to L26, L26 to NPNL and L2 to NPNL) in order to include in the models variables that did not change over time. Baseline (L2) aBMD and changes in BA and body weight were maintained as a covariates in the models on a \(\log_{e}\) scale; then other potential confounders (both continuous and categorical variables) were added as covariates without transformation (maternal age [years], parity [multiparity versus primiparity], previos and current use of depot medroxyprogesterone acetate [DMPA, yes versus no], gestation age at birth [weeks], sex of the infant [male versus female], exclusive breastfeeding [yes versus no], resumption of menses [yes versus no], weeks postpartum, total duration of breastfeeding [weeks], duration postlactation at NPNL [months]). However, most of these potential confounders did not have significant effects in the models containing weight and BA (\(p\) values ≥0.05), and for those that were significant, they did not have a material effect on the effect size and significance of the aBMD results. Therefore, in this work, we only report aBMD results, before and after adjustment for body size.

Results

Participant characteristics

The flow of participants through the study is presented in Fig. 1. Overall, 426 pregnant women (210 WWH and 216 REF) were screened and 200 enrolled in the study (100 per group). A total of four preterm births, five stillbirths, and four neonatal deaths were reported, and 22 women had a subsequent pregnancy; hence, the affected mothers were discontinued from the study. Nine women (seven WWH; two REF) were not measured at L26 because they had stopped breastfeeding and scheduled for NPNL measurements. Twenty-four women (five WWH; 19 REF) were not measured at NPNL because they were still breastfeeding when the study closed. Overall, 162 women (84 WWH; 78 REF) were measured at L2, 164 (83 WWH; 81 REF) at L14 (the primary endpoint for the study), 141 (69 WWH; 72 REF) at L26, and 99 (67 WWH; 32 REF) at NPNL. For the current analysis, eight women (five WWH; three REF) with DXA measurement at only one time point, and 17 poor-quality DXA images (LS: three WWH, seven REF; TH: one WWH, no REF; WBLH: four WWH, two REF) were excluded. Supplementary Table 1 gives a detailed breakdown of the final number of DXA images and aBMD measurements by skeletal site and time point. Maternal characteristics were not associated with having DXA measurements at NPNL or at least two DXA measurements throughout the study (Supplementary Table 2).

Table 1 presents a summary of participant characteristics and medical history. All WWH had been recently initiated first-line ART regimen at enrolment, and the majority had preserved CD4 counts (≥500cells/cm⁴). The mean duration on ART in WWH at L2, L14, L26, and NPNL was 17.6 ± 5.5, 29.5 ± 5.1, 42.0 ± 5.7, and 80.6 ± 15.2 weeks, respectively; and mean adherence to ART was >99% at all visits, based on the pill count method used in routine clinical care. All REF women remained HIV-negative throughout the study. Median age was 23.4 years (IQR, 21.0 to 26.8), and was comparable between the groups at enrolment. Fewer WWH were primiparous, married, and had attained postsecondary school education. Other participant characteristics (demographic, health, and socioeconomic status) were comparable between the groups (data not presented).

All women breastfed their babies and 71.9% reported initiation of breastfeeding within an hour of birth. Self-reported rates of exclusive breastfeeding were higher among WWH compared to REF women at L2, L14, and L26. Mean total...
duration of breastfeeding was 54.4 ± 17.5 weeks. WWH compared to REF had a shorter duration of breastfeeding (mean 47.8 versus 65.6 weeks, \( p \leq .001 \)), but a longer duration postlactation at NPNL measurement (median [lower, upper quartile]: 3.4 months [3.3, 4.4] versus 3.3 months [3.1 versus 3.5], \( p = .04 \)). The proportion of women who had resumed
| Characteristic                | L2 (WWH (n = 84), REF (n = 78)) | L14 (WWH (n = 83), REF (n = 81)) | L26 (WWH (n = 69), REF (n = 72)) | NPNL (WWH (n = 67), REF (n = 32)) |
|------------------------------|---------------------------------|----------------------------------|---------------------------------|----------------------------------|
| Weeks postpartum             | 2.2 ± 0.5                       | 2.1 ± 0.4                        | 26.5 ± 0.9                      | 65.1 ± 14.1^a                    |
| Months postlactation         | -                               | -                                | -                               | 3.4 (3.3, 4.4)^c                 |
| Age (years)                  | 23.7 (21.4, 27.4)               | 23.3 (20.9, 27.1)                | 23.9 (21.0, 27.4)               | 25.0 (23.0, 28.7)^c              |
| Parity                       | 2 (1, 3)                        | 1 (1, 2)                         | 2 (1, 3)                        | 2 (1, 3)                         |
| Primiparous, %               | 36.5^a                          | 53.8                             | 36.1^c                          | 55.6                             |
| CD4 cell count (cells/cm^3)  | 400 (301, 516)                  | 405 (294, 525)                   | 487 (331, 672)                  | 471 (338, 688)                   |
| % Pills taken^d              | 99.3 ± 2.0                      | 99.7 ± 1.6                       | 99.4 ± 3.3                      | 99.3 ± 3.8                       |
| BF duration (weeks)          | 2.2 ± 0.5                       | 2.1 ± 0.4                        | 14.3 ± 0.6                      | 42.9                             |
| Resumed menses, %            | -                               | -                                | 39.8                            | -                                |
| Current DMPA, %              | -                               | -                                | 30.1^b                          | 34.7                             |
| Prior DMPA, %                | 41.5^b                          | 19.5                             | 38.3^b                          | 36.2                             |

Values are mean ± SD, median (25 percentile, 75 percentile), or percentage (%) of participants reporting "yes".

ART = antiretroviral therapy; BF = breastfeeding; DMPA = depot medroxyprogesterone acetate; EBF = exclusive breastfeeding; L2 = 2 weeks lactation; L14 = 14 weeks lactation; L26 = 26 weeks lactation; NPNL = measurement made at least 3 months postlactation when women were neither pregnant nor lactating; REF = women without HIV (reference group, ART naive); WWH = women with HIV initiated on lifelong triple ART during pregnancy (TDF-3TC-EFV, previously ART naive).

^a,b,c Values of p for differences between the groups obtained from chi-square tests in DataDesk 6.3.1 software.

^a p ≤ 0.001.
^b p ≤ 0.01.
^c p ≤ 0.05.

^d Mean % adherence to ART based on the pill count method used in routine clinical care = 100*[number of pills taken/number of pills dispensed for the duration].

^e Proportions are higher than L2 because some babies received pre-lacteal feeds at L2 but were exclusively breastfed at L14.
Table 2. Summary of Anthropometry, Body Composition, and Bone Measures

| Parameter          | L2 (n = 84) | L14 (n = 83) | L26 (n = 69) | NPNL (n = 67) | P-value    |
|--------------------|------------|-------------|-------------|--------------|------------|
| Lumbar spine       |            |             |             |              |            |
| aBMD (g/cm²)       | 0.909 ± 0.095 | 0.911 ± 0.111 | 0.889 ± 0.090 | 0.884 ± 0.104 |            |
| BA (cm²)           | 54.4 ± 4.7  | 54.1 ± 5.2  | 54.2 ± 4.9b | 53.8 ± 5.3  |            |
| BMC (g)            | 49.5 ± 7.4  | 49.5 ± 9.1  | 48.3 ± 7.0b | 47.8 ± 8.6  |            |
| Total hip          |            |             |             |              |            |
| aBMD (g/cm²)       | 0.946 ± 0.119a | 0.917 ± 0.093 | 0.905 ± 0.120c | 0.896 ± 0.108 |            |
| BA (cm²)           | 28.2 ± 2.4a | 28.8 ± 2.9  | 28.2 ± 2.5a | 28.9 ± 2.9  |            |
| BMC (g)            | 26.7 ± 4.1  | 26.5 ± 4.1  | 25.6 ± 4.1  | 25.9 ± 4.1  |            |
| WBLH               | n = 78      | n = 74      | n = 82      | n = 80       |            |
| aBMD (g/cm²)       | 0.935 ± 0.066a | 0.919 ± 0.064 | 0.919 ± 0.061c | 0.915 ± 0.069 |            |
| BA (cm²)           | 1579.8 ± 109.1c | 1595.6 ± 138.9 | 1574.3 ± 95.3 | 1590.4 ± 137.6 |            |
| BMC (g)            | 1479.3 ± 169.3 | 1470.1 ± 13.0 | 1450.1 ± 156.1 | 1460.0 ± 202.0 |            |
| Lean (kg)          | 33.5 (30.1, 36.6)c | 34.7 (31.1, 40.0) | 32.5 (30.0, 35.5)c | 33.5 (30.8, 37.5) |            |
| Fat (kg)           | 18.7 (14.9, 22.9) | 19.3 (15.3, 25.8) | 19.1 (14.7, 23.1)c | 19.6 (15.3, 26.0) |            |
| % Fat              | 34.5 ± 8.1  | 35.4 ± 8.5  | 36.2 ± 7.1  | 35.9 ± 8.5  |            |
| Anthropometry      | n = 79      | n = 73      | n = 83      | n = 80       |            |
| Height (cm)        | 157.0 ± 4.6 | 158.6 ± 5.7 | 156.9 ± 4.4 | 158.5 ± 5.8 |            |
| Weight (kg)        | 59.2 (53.7, 65.5)a | 61.2 (56.3, 67.1) | 56.9 (53.0, 64.5)a | 60.5 (54.0, 67.9) |            |
| BMI (kg/m²)        | 23.8 (22.2, 26.2)a | 24.1 (22.4, 27.9) | 23.1 (21.6, 26.2)a | 23.7 (21.8, 27.4) |            |

Values are mean ± SD or median (25 percentile, 75 percentile).

aBMD = areal bone mineral density; BA = bone area; BMC = bone mineral content; BMI = body mass index; L2 = 2 weeks lactation; L14 = 14 weeks lactation; L26 = 26 weeks lactation; NPNL = measurement made at least 3 months postlactation when women were neither pregnant nor lactating; REF = women without HIV (reference group, ART naive); WBLH = whole body-lean; WWH = women with HIV initiated on lifelong triple ART during pregnancy (TDF-3TC-EFV, previously ART naive).

Values of $p ≤ .001$. $p ≤ .01$. $p ≤ .05$. $p ≤ .001$. $p ≤ .01$. $p ≤ .05$. $p ≤ .001$. $p ≤ .01$. $p ≤ .05$. $p ≤ .001$. $p ≤ .01$. $p ≤ .05$.
menses was comparable between the groups. However, more WWH were on DMPA contraception before and after the index pregnancy.

### Anthropometry and bone measures

#### Two weeks of lactation (L2)

Mean aBMD values and mean percent differences between the groups are presented in Table 2 and Supplementary Table 3, respectively. LS aBMD was comparable between the groups, but TH and WBLH aBMD were higher in WWH at L2. TH and WBLH BA, body weight, BMI, and lean mass were lower in WWH. BMC, height, fat mass, and percent fat were comparable between the groups at L2.

In a cross-sectional ANCOVA model, previous use of DMPA was independently associated with higher aBMD at L2 (previous DMPA yes versus no [mean difference ± SE]: LS +2.5 ± 1.1%, p = .02; TH +5.3 ± 1.2%, p ≤ .0001; WBLH +3.4 ± 0.7%, p ≤ .0001), but the associations were not significant after adjusting for body size. Body weight was positively associated with parity (multiparae versus primiparae [mean difference ± SE]: +4.6 ± 1.6 kg, p = .004), previous use of DMPA (yes versus no [mean difference ± SE]: +5.7 ± 1.7 kg, p ≤ .0001) and gestation age at parturition (β = +0.8 ± 0.3 kg per week, p = .05). Adjusting body weight and BMI for parity, previous use of DMPA and gestation age at parturition increased the mean differences between the groups (WWH versus REF [mean difference ± SE]: weight −8.6 ± 2.3%, p ≤ .0001; BMI −6.5 ± 2.3%). However, mean TH and WBLH aBMD remained higher in WWH at L2 after adjustment for BA and body, parity and previous DMPA exposure [mean difference ± SE]: TH +2.8 ± 1.2%, p = .02; WBLH +2.1 ± 0.7%, p = .001).

#### First 6 months of lactation (L2, L14, L26)

LS and TH aBMD decreased in both groups, but WBLH aBMD decreased only in WWH in the first 6 months of lactation (Table 3). Overall, changes in aBMD were greater at TH, compared to LS and WBLH. However, WWH had greater decreases in WBLH and TH aBMD (WWH −5.9% [95% CI, −6.4 to −5.4] versus REF −4.3% [95% CI, −4.8 to −3.8], three–time point group*time point interaction term p = .01). Decreases in LS aBMD were comparable between the groups, though there was a tendency toward a smaller decrease in WWH at L14 (WWH −1.8% [95% CI, −2.2 to −1.4] versus REF −2.9% [95% CI, −3.3 to −2.5]; two–time point group*time point interaction p = .08). Lean mass decreased in both groups, but body weight decreased only in REF women by L26. Even so, changes in all anthropometric, body composition, and BA measures were comparable between the groups in the first 6 months of lactation as shown by group*time point interaction terms from three–time point models (p > .1, Table 3 and Supplementary Table 4).

Previous exposure to DMPA was associated with increases in TH aBMD between L2 and L26 independent of maternal HIV status, in a two–time point model with change in aBMD as the dependent variable and group and prior DMPA exposure as

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### Table 3. Mean Changes in aBMD, Body Composition, and Weight During Lactation (L2-L14-L26)

| Parameter          | L2 to L14 | L2 to L26 | Three–time point model (L2-L14-L26) |
|--------------------|-----------|-----------|-----------------------------------|
| Lumbar spine       | n = 77    | n = 69    | n = 63                            |
| aBMD (g/cm²)       | −1.8 (−2.2, −1.4) | −2.9 (−3.3, −2.5) | 0.08 | −2.0 (−2.5, −1.5) | −2.0 (−2.5, −1.5) | 0.93 | 0.06 |
| aBMD adjusted      | −1.7 (−2.1, −1.3) | −2.6 (−3.0, −2.2) | 0.14 | −2.0 (−2.5, −1.5) | −1.6 (−2.1, −1.1) | 0.34 | 0.08 |
| Total hip          | n = 77    | n = 73    | n = 63                            |
| aBMD (g/cm²)       | −3.9 (−4.3, −3.5) | −2.8 (−3.2, −2.4) | 0.02 | −5.9 (−6.4, −5.4) | −4.3 (−4.8, −3.8) | 0.08 | 0.01 |
| aBMD adjusted      | −3.8 (−4.1, −3.5) | −2.7 (−3.1, −2.3) | 0.02 | −5.8 (−6.3, −5.3) | −3.7 (−4.2, −3.2) | ≤0.0001 | 0.0010 |
| WBLH               | n = 76    | n = 71    | n = 64                            |
| aBMD (g/cm²)       | −1.2 (−1.5, −0.9) | −0.5 (−0.8, −0.2) | 0.01 | −1.7 (−2.1, −1.3) | −0.6 (−1.0, −0.2) | 0.001 | 0.0010 |
| aBMD adjusted      | −1.2 (−1.5, −0.9) | −0.6 (−0.9, −0.3) | 0.03 | −1.8 (−2.2, −1.4) | −0.6 (−1.0, −0.2) | 0.0008 | 0.0010 |
| Lean (kg)          | −3.0 (−4.1, −1.9) | −2.3 (−3.6, −1.0) | 0.65 | −3.3 (−4.7, −1.9) | −3.7 (−5.2, −2.2) | 0.95 | 0.90 |
| Fat (kg)           | +6.9 (+4.2, +9.6) | +2.5 (+0.6, +5.6) | 0.29 | +5.5 (+1.9, +9.2) | +2.3 (+1.4, +6.0) | 0.34 | 0.45 |
| Anthropometry      | n = 78    | n = 74    | n = 63                            |
| Weight (kg)        | −0.5 (−1.1, +0.1) | −1.2 (−1.8, −0.6) | 0.55 | −0.8 (−1.5, −0.1) | −2.2 (−3.0, −1.4) | 0.16 | 0.29 |

Values are within–group mean percent changes (%Δ) and 95% CI (lower, upper confidence limits). The + or − signs show the direction of within group changes (increase or decrease, respectively). Mean %Δ ± SE, and p values for group–visit interaction terms were obtained from Scheffe post hoc tests for group*visit (time point) interaction terms in hierarchical repeated–measures ANOVA and ANCOVA models, that included subject (nested by group), group, visit, and group*visit interaction in DataDesk software. Variables, except % fat, were transformed into natural logarithms and multiplied by 100 before data analysis. Mean changes and SEs from Scheffe post hoc tests and associated sample sizes were used to calculate the CIs using OpenEpi Epidemiological calculator.

aBMD = areal bone mineral density; aBMD adjusted = adjusted for bone area and body weight; BA = bone area; BMC = bone mineral content; BMI = body mass index; L2 = 2 weeks lactation; L14 = 14 weeks lactation; L26 = 26 weeks lactation; NPNL = measurement made at least 3 months postlactation when women were neither pregnant nor lactating; REF = women without HIV (reference group, ART naive); WBLH = whole body–less–head; WWH = women with HIV initiated on lifelong ART during pregnancy (TDF–3TC–EFV, previously ART naive).

*Value of p for group*interaction term in two–time point hierarchical repeated–measures ANOVA models.

Value of p for overall group*interaction term in three–time point hierarchical repeated measures ANOVA models (L2-L14-L26).
covariates (previous DMPA yes versus no [mean difference ± SE]: +1.4 ± 0.7%, p = .04); but the association was not significant after adjusting for changes in weight and BA (previous DMPA yes versus no [mean difference ± SE]: +0.9 ± 0.6%, p = .12). Overall, decreases in TH and WBLH aBMD at L2, and experienced greater bone loss between L2 and L26. Hence, mean TH and WBLH aBMD values were lower at NPNL relative to L2. Mean LS aBMD values were comparable between the groups at L2, but WWH had tendencies toward smaller aBMD reduction at L14, and smaller increase in aBMD postlactation. Therefore, LS mean aBMD values were lower in WWH compared to REF women at NPNL, but were comparable between the groups after adjusting for their lower body size. Mean TH and WBLH aBMD values, before or after adjusting for body size, were not significantly different between the groups at NPNL (Table 3).

Therefore, the overall patterns of changes in aBMD during and after lactation were different for WWH and REF, before and after adjustment for changes in body size (weight and BA). Allowing for cross-sectional differences between the groups at L2 further reduced the mean differences between the groups at NPNL (Fig. 3). Restricting statistical analysis to participants with data at all time points (rectangular dataset) did not change interpretation of the results (Supplementary Table 5). Further sensitivity analyses showed comparable magnitudes of changes in aBMD in the first 6 months of lactation in women with and without measurements at NPNL (Supplementary Table 2).

**Discussion**

This study showed a consistent pattern of decreases in aBMD in Ugandan women both with (on TDF-based ART) and without HIV in the first 6 months of lactation, consistent with prior lactation

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**Table 4. Mean Changes in aBMD, Body Composition, and Weight After Lactation (L26/L2 to NPNL)**

| Parameter          | L26 to NPNL | L2 to NPNL | Four-time point model |
|--------------------|-------------|------------|-----------------------|
|                    | WWH %Δ (95% CI) | REF %Δ (95% CI) | Group* visit p | WWH %Δ (95% CI) | REF %Δ (95% CI) | Group*visit p |
| Lumbar spine       | n = 52      | n = 29     |                       | n = 59      | n = 28      |                       |
| aBMD (g/cm²)       | +3.8 (+3.2, +4.4) | +5.2 (+4.2, +6.2) | .07 | +1.7 (+1.2, +2.2) | +3.2 (+2.2, +4.2) | .10 | .003 |
| aBMD adjusted      | +3.5 (+2.9, +4.1) | +5.0 (+4.0, +6.0) | .07 | +1.6 (+1.1, +2.1) | +3.3 (+2.3, +4.3) | .06 | .002 |
| Total hip          | n = 55      | n = 30     |                       | n = 61      | n = 29      |                       |
| aBMD (g/cm²)       | +2.8 (+2.3, +3.3) | +4.4 (+3.4, +5.4) | .06 | −3.1 (−3.6, −2.6) | +0.1 (−0.9, +1.1) | .0008 | ≤.001 |
| aBMD adjusted      | +2.9 (+2.4, +3.4) | +4.2 (+3.3, +5.1) | .20 | −2.9 (−3.4, −2.4) | +0.5 (−0.4, +1.4) | ≤.001 | ≤.001 |
| WBLH               | n = 55      | n = 30     |                       | n = 61      | n = 28      |                       |
| aBMD (g/cm²)       | −0.7 (−1.1, −0.3) | +0.5 (−0.2, +1.2) | .03 | −2.4 (−2.8, −2.0) | −0.1 (−0.8, +0.6) | .002 | ≤.001 |
| aBMD adjusted      | −0.5 (−0.9, −0.1) | +0.5 (−0.2, +1.2) | .03 | −2.3 (−2.7, −2.0) | −0.1 (−0.8, +0.6) | .009 | ≤.001 |
| Lean (kg)          | −3.4 (−5.2, −1.6) | −3.3 (−6.1, −0.5) | .60 | −6.7 (−8.2, −5.2) | −7.0 (−9.7, −4.2) | .62 | .93 |
| Fat (kg)           | +1.6 (−2.6, +5.8) | −0.6 (−7.5, +6.3) | .54 | +7.0 (+3.2, +10.8) | +1.7 (−5.3, +8.7) | .51 | .65 |
| % Fat              | +1.2 (+0.1, +2.3) | +0.7 (−1.1, +2.6) | .52 | +2.9 (+1.9, +3.9) | +1.9 (−0.2, +3.8) | .74 | .81 |
| Anthropometry      | n = 52      | n = 29     |                       | n = 57      | n = 30      |                       |
| Weight (kg)        | −1.4 (−2.4, −0.4) | −0.6 (−2.2, +1.0) | .34 | −2.2 (−3.1, −1.3) | −2.8 (−4.3, −1.3) | .53 | .54 |

*Values are within-group mean percent change (%Δ) and 95% CI (lower, upper confidence limits). The + or – signs show the direction of within group changes (increase or decrease, respectively). Mean %Δ ± SE, and p values for group-visit interaction terms were obtained from Scheffé post hoc tests for group*visit (time point) interaction terms in hierarchical repeated-measures ANOVA and ANCOVA models, that included subject (nested by group), group, visit, and group*visit interaction in DataDesk software. Variables, except % fat, were transformed into natural logarithms and multiplied by 100 before data analysis. Mean changes and SEs from Scheffé post hoc tests and associated sample sizes were used to calculate the CIs using OpenEpi Epidemiological calculator.

aBMD = areal bone mineral density; aBMD adjusted = adjusted for bone area and body weight; BA = bone area; BMC = bone mineral content; BMI = body mass index; L2 = 2 weeks lactation; L14 = 14 weeks lactation; L26 = 26 weeks lactation; NPNL = measurement made at least 3 months postlactation when women were neither pregnant nor lactating; REF = women without HIV (reference group, ART naive); WBLH = whole body-less-head; WWH = women with HIV initiated on lifelong triple ART during pregnancy (TDF-3TC-EFV, previously ART naive).

aValue of p for group*interaction term in two–time point hierarchical repeated measures ANOVA models.
bValue of p for overall group*interaction term in four–time point hierarchical repeated measures ANOVA models (L2-L14-L26-NPNL).
studies in white and African women. However, WWH had greater decreases in TH and WBLH aBMD between L2 and L26 than reference women, and a tendency toward a smaller decrease in LS aBMD in early lactation (L2 and L14). At 3 months postlactation, TH aBMD was higher relative to L2 in both groups, and LS aBMD returned to L2 values in REF women consistent with previous studies. (16, 19, 23) However, WWH had lower TH and WBLH aBMD (−3.1% and −2.4%, respectively) at NPNL relative to L2. WWH had lower body weight at all visits, and significant increases in both fat mass and percent fat. Adjusting for body size and measured potential confounders did not have material effects on the results. These data show accentuated mobilization of hip and WBLH bone mineral during lactation in Ugandan WWH initiated on lifelong triple ART during pregnancy, and only partial skeletal recovery by 3 months postlactation.

To the best of our knowledge, this is the first study to describe changes in aBMD during and after lactation in a longitudinal cohort of lactating African WWH on triple ART compared to those without HIV. The multicentre Promoting Maternal and Infant Survival Everywhere (PROMISE) trial, conducted in Uganda, Zimbabwe, Malawi, and South Africa, randomized asymptomatic WWH with high CD4 counts (who did not meet country treatment criteria at the time of the study enrollment) to either ART or zidovudine (ZDV) antepartum, and then randomized postpartum to either TDF-based ART through up to 18 months of breastfeeding or infant nevirapine (NVP) prophylaxis. Preliminary results from the DXA substudy (P1048s) suggest greater decreases in maternal TH and LS aBMD in mothers between 1 and 74 weeks postpartum with maternal ART provision. The mean duration of breastfeeding in PROMISE was 16 months (70 weeks) and 34.3% of the women were still breastfeeding at 18 months postpartum. (28) Unlike PROMISE, the current study recruited women initiated on Option B+ ART during pregnancy, as per contemporary ART guidelines for pregnant and breastfeeding women at the time of the study, now under the general umbrella of “Test and Treat.” Preliminary results from another...
study in Ugandan WWH, showed no differences in changes in aBMD T-scores at the hip and spine between 2 weeks and 9 months of lactation in women on triple ART (initiated in pregnancy) compared to infant prophylaxis. However, neither studies included breastfeeding women without HIV as a comparative group.

The sample size for the current study was calculated for the LS aBMD because most evidence from lactation studies shows greater changes in aBMD at the LS—a trabecular-rich site—compared to other skeletal sites. We, therefore, hypothesized that if there is an additive effect of lactation and ART on bone mineral mobilization, the changes in aBMD would be more accentuated at the LS. Contrary to our hypothesis, overall aBMD changes in the first 3 to 6 months were smaller at the LS compared to TH in both groups. In fact, WWH had greater bone loss at the TH, but LS aBMD decreased by the same magnitude in WWH and REF. Previous DMPA exposure was not associated with changes in LS aBMD. The finding of greater bone loss at the TH in Ugandan WWH in the current study is consistent with data from the PROMISE study. However, the reasons for lack of a difference at the LS are unclear.

Mobilization of maternal bone mineral during lactation may be influenced by several factors, including: exclusivity and duration of breastfeeding, infant breast milk intake, duration of lactation, maternal height, resumption of menses, and duration of amenorrhea. Furthermore, changes in body weight and BA may affect interpretation of aBMD results in longitudinal studies. In the current study, more WWH reported use of DMPA contraception (prior to and after the index pregnancy), and exclusive breastfeeding in the first 6 months. In addition, the total duration of breastfeeding was shorter in WWH. However, the time period between stopping breastfeeding and NPNL measurement, and the proportion of women who had resumed menses by 6 months postpartum were comparable between the groups. Overall, the mean differences between the groups for changes in aBMD persisted after adjusting for body size (weight and BA), DMPA exposure (both previous and postpartum) and

Fig 3. Changes in adjusted aBMD during and after lactation, from REF at L2. Data are mean percent changes (%Δ) ± SEs. NPNL measurements were scheduled at least 3 months after lactation. Results were obtained from Scheffé post hoc tests for group*visit (time point) interaction terms in hierarchical repeated-measures ANOVA and ANCOVA models that included subject (nested by group), group, visit, and group*visit interaction in DataDesk 8.2.1 software. Numbers included in the models were: LS (L2: 78 WWH, 71 REF; L14: 82 WWH, 77 REF; L26: 68 WWH, 72 REF; NPNL: 62 WWH, 31 REF); TH (L2: 78 WWH, 74 REF; L14: 82 WWH, 80 REF; L26: 68 WWH, 72 REF; NPNL: 65 WWH, 32 REF); WBLH (L2: 78 WWH, 73 REF; L14: 80 WWH, 79 REF; L26: 68 WWH, 71 REF; NPNL: 64 WWH, 32 REF), body weight (L2: 79 WWH, 73 REF; L14: 83 WWH, 80 REF; L26: 69 WWH, 70 REF; NPNL: 63 WWH, 32 REF). All variables were transformed into natural logarithms (log e) and multiplied by 100 before data analysis. aBMD was adjusted for body weight and bone area in nested linear regression models. Values of p are for comparison of overall patterns of changes between the groups (WWH versus REF). aBMD = areal bone mineral density (g/cm²); CI = confidence interval; L2 = 2 weeks of lactation; L14 = 14 weeks of lactation; L26 = 26 weeks of lactation; LS = lumbar spine; NPNL = neither pregnant nor lactating; SE = standard error; TH = total hip; REF = women without HIV (reference group, ART naive); WBLH = whole body-less-head; WWH = women with HIV initiated on lifelong triple ART during pregnancy (TDF-3TC-EFV, previously ART naive). (A) LS; (B) TH; (C) WBLH; (D) body weight.
breastfeeding practices, suggesting that ART may have accentuated bone loss in WWH.

The Ugandan WWH initiated on lifelong triple ART during pregnancy had higher TH and WBLH aBMD at L2. This could be that, by chance, such women were recruited who had higher aBMD, or may reflect a bone remodeling transient.\(^{(31)}\) Alternatively, higher aBMD in WWH could be explained by bone recovery following discontinuation of DMPA. DMPA use is independently associated with bone loss, and aBMD recovery after discontinuation.\(^{(32)}\) In the current study, a greater proportion of WWH reported previous DMPA use. DMPA exposure was associated with higher body weight, higher TH and WBLH aBMD at L2, and an increase in aBMD (recovery) between L2 and L26 despite lactational bone loss. The association between DMPA and aBMD were not significant after adjustment, suggesting that the effects of prior DMPA exposure on aBMD were mediated by its effect on body weight regardless of HIV/ART status. Biochemistry data and further longitudinal studies with aBMD measured preconception are needed to confirm this finding.

The current study has several strengths. It has a contemporaneous group of women without HIV for comparison of both cross-sectional and longitudinal outcomes. Participants were measured at least 3 months postlactation to investigate recovery in BMD consistent with previous lactation studies.\(^{(17,19,21)}\) We used DXA and adjusted aBMD for body size and measured potential confounders. All WWH were previously ART naive and initiated onto TDF-based ART during pregnancy (TDF/3TC/EFV, standard first-line regimen at the time of the study); and the majority reported good adherence based on the pill count method used in routine clinical care. It is not possible to say how generalizable the results are, but the lactation changes in women without HIV are very similar to those reported from women in different countries and settings.\(^{(15,16,23)}\) Limitations of the current study include the inability to acquire baseline aBMD measurements before ART was started or during pregnancy and lack of data on HIV viral loads or seroconversions during pregnancy. Although fewer women without HIV were measured at NPNL, sensitivity analysis showed that changes in aBMD from L2 to 26 were comparable between women with and without measurements at NPNL. Only one set of NPNL measurements were obtained, so it is unknown whether hip and WBLH aBMD would have returned to baseline (L2) values in the WWH group after longer follow-up. Finally, results in WWH might not be generalizable to women initiated on ART during pregnancy at low CD4 counts, newer ART regimens with and without TDF (for example dolutegravir and tenofovir alafenamide, respectively), or enter pregnancy while on ART.

In conclusion, Ugandan WWH initiated on lifelong triple ART during pregnancy experienced greater decreases in TH and WBLH aBMD within the first 6 months of lactation than women without HIV. TH and WBLH aBMD were lower in WWH relative to L2, but returned to L2 values in REF women, and size-adjusted mean aBMD values were comparable between the groups at 3 months postlactation. These data show accentuated decrease in hip and whole body aBMD during the first 6 months of lactation, and only partial skeletal recovery postlactation in Ugandan WWH initiated on triple ART during pregnancy. The clinical implication of these findings are unclear at this stage because both maternal ART and breastfeeding are critical for maternal health and child survival in resource-limited settings. Further studies are ongoing to investigate the mechanisms and longer-term consequences for bone health of the mother.

Disclosures

All authors state that they have no conflicts of interest.

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PEER REVIEW

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