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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 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). 100 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 DXA at 2, 14, 26 weeks lactation, and 3 months post-lactation. The primary outcome was the difference between groups in mean % change in LS aBMD between 2 and 14 weeks. Statistical analysis was performed in hierarchical (mixed effects) generalised linear models that corrected for multiple testing. Median age was 23.4 (IQR: 21.0, 26.8) yrs. 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, -5.4] vs REF(n=64) -4.3% [95% CI: -4.8, -3.8]; group*timepoint interaction p=0.008). Decreases in LS aBMD were similar in WWH and REF (2-to-26 weeks: -2.0% [95% CI: -2.5, -1.5]), although there was a tendency towards a smaller decrease in WWH between 2 and 14 weeks (WWH(n=77) -1.8% [95% CI: -2.2, -1.4] vs REF(n=69) -2.9% [95% CI: -3.3, -2.5]; group*timepoint interaction p=0.08). Post-lactation, 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 post-lactation vs week-2: WWH(n=61) -3.1% [95% CI: -3.6, -2.6]; REF(n=29) +0.1% [95% CI: -0.9, +1.1]). These data show accentuated bone loss during lactation and

only partial skeletal recovery by 3 months post-lactation in Ugandan WWH on tenofovir-based ART. Studies are ongoing to understand longer-term consequences for bone health.

Keywords: 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 HIV, and tenofovir disoproxyl 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 under the general umbrella of "Test and Treat"[6]. Also, WWH are advised to breastfeed for 12-24 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-utero and through breastmilk, for offspring bone mineral accretion [15,16]. Bone mineral mobilization can be substantial in the

first 3-6 months of lactation; and is more marked at trabecular rich sites, such as the lumbar spine, compared to cortical sites[12,15,16]. 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 post-lactation 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 naïve); and (2) women without HIV who had never been on ART. The aim of this paper is 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 18.0-39.9 years old, 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 in the following year. In addition, WWH were only eligible if they had a first HIV-diagnosis and initiated ART during the index pregnancy i.e. were previously ART naïve. Exclusion criteria were: non-singleton, high risk pregnancies (hypertension, preeclampsia/eclampsia), diagnosis of bone disease or conditions associated with abnormal bone metabolism and renal function (diabetes mellitus, gestational diabetes, tuberculosis, hepatitis C, proteinuria and renal disease). Later exclusion or loss of mothers from the study included: preterm delivery (<37 weeks gestation), still birth, neonatal death, maternal death, stopping breastfeeding before L14, subsequent pregnancy. Women who stopped breastfeeding before L26 were scheduled for NPNL measurements at least 3 months post-lactation, hence they were not measured at L26.

ART was provided in accordance with the prevailing Uganda national guidelines that recommended routine, opt out HIV testing for all pregnant women at their first antenatal visit, and immediate initiation of a first line ART regimen (TDF/3TC/EFV) in those diagnosed with HIV, under PMTCT Option B-plus guidelines [21]. Participants received routine antenatal, postnatal and HIV care services (HIV-retesting for REF women) from the relevant clinics within Mulago Hospital in accordance with the established standard of care. Ethics and protocol approvals were obtained from the Joint Clinical Research Centre's (JCRC) Institutional Review Board (IRB), Mulago Hospital IRB, and The Uganda National Council for Science and Technology. All women gave informed written consent, by either signing or appending their left thumbprint on the informed consent form.

Study measurements and outcomes

Bone mineral density 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 [Neither Pregnant Nor Lactating]) to investigate skeletal recovery post-lactation. DXA scans of the LS, 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/cm^2), bone mineral content (g), bone area (cm^2) 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 scrutinised and analysed using Hologic Apex software (version 5.6.0.4), 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, medical and reproductive history.

The primary outcome was the difference between the groups in mean % 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 mobilisation in the first 3-6 months of lactation, when both breastmilk 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 timepoint and change between timepoints in total hip (TH) and whole body-less-head (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% (0.4 SD) difference between groups in mean change of LS aBMD between L2 and L14 at 80% power and with a type I error of 0.05 (two tailed)[9,16,24] with aBMD transformed to natural logarithms.

Statistical methods

Data were analysed using DataDesk 6.3.1 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 (mean±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 normalised 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 (%Δ±SE)[25]. A p-value of ≤0.05 was considered significant for all tests.

Four-timepoint 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-timepoint interaction term [26]. aBMD was analysed firstly 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 et al. [27]. 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 timepoint, and within group changes between each timepoint. Then, separate two-timepoint 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 timepoints. Also, separate three-timepoint 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 timepoint 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-, three- and two- timepoint 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 timepoints 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 vs primiparity], previous and current use of depot medroxyprogesterone acetate [DMPA, yes vs no], gestation age at birth [weeks], sex of the infant [male vs female], exclusive breastfeeding [yes vs no], resumption of menses [yes vs no], weeks postpartum, total duration of breastfeeding[weeks], duration post-lactation 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 paper, 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 Figure 1. Overall, 426 pregnant women (210 WWH and 216 REF) were screened and 200 enrolled in the study (100 per group). A total of 4 pre-term births, 5 stillbirths and 4 neonatal deaths were reported and 22 women had a subsequent pregnancy; hence, the affected mothers were discontinued from the study. Nine women (7 WWH; 2 REF) were not measured at L26 because they had stopped

breastfeeding and scheduled for NPNL measurements. Twenty-four women (5 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 (5 WWH; 3 REF) with DXA measurement at only one timepoint, and 17 poor quality DXA images (LS: 3 WWH, 7 REF; TH: 1 WWH 0, REF; WBLH: 4 WWH, 2 REF) were excluded. Supplemental Table 1 gives a detailed breakdown of the final number of DXA images and aBMD measurements by skeletal site and timepoint. Maternal characteristics were not associated with having DXA measurements at NPNL or at least two DXA measurements throughout the study (Supplemental 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 CD_4 counts ($\geq$ 500cells/cm^3). 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, 26.8), and was comparable between the groups at enrolment. Fewer WWH were primiparous, married, and had attained post-secondary school education. Other participant characteristics (demographic, health and socio-economic 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 vs 65.6 weeks, $p \leq 0.001$), but a longer duration post-lactation at NPNL measurement (median [lower, upper quartile]: 3.4 months [3.3, 4.4] vs 3.3 months [3.1 vs 3.5], p=0.04). The proportion of women who had resumed 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 Supplemental Table 3, respectively. LS aBMD was comparable between the groups, but both 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 % 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 vs no [mean difference±SE]: LS +2.5±1.1%, p=0.02); TH +5.3±1.2%, $p \leq 0.0001$; WBLH +3.4±0.7%, $p \leq 0.0001$), but the associations were not significant after adjusting for body size. Body weight was positively associated with parity (multiparae vs primiparae [mean difference±SE]: +4.6±1.6kg, p=0.004), previous use of DMPA (yes vs no [mean difference±SE]: +5.7±1.7kg, $p \leq 0.0001$) and gestation age at parturition (β= +0.8±0.3kg per week, p=0.05). Adjusting body weight and BMI for parity,

previous use of DMPA and gestation age at parturition increased the mean differences between the groups (WWH vs REF [mean difference±SE]: weight -8.6±2.3%, p≤0.0001; 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=0.02; WBLH +2.1±0.7%, p=0.001).

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, -5.4] vs REF: -4.3% [95% CI: -4.8, -3.8], three timepoint group*timepoint interaction term p=0.01). Decreases in LS aBMD were comparable between the groups, though there was a tendency towards a smaller decrease in WWH at L14 (WWH -1.8% [95% CI: -2.2, -1.4] vs REF -2.9% [95% CI:-3.3, -2.5]; two timepoint group*timepoint interaction p=0.08). 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*timepoint interaction terms from three timepoint models (p>0.1, Table 3 and Supplemental Table 4).

Previous exposure to DMPA was associated with increases in TH aBMD between L2 and 26 independent of maternal HIV-status, in a two timepoint model with change in aBMD as the dependent variable and group and prior DMPA exposure as covariates (previous DMPA yes

vs no [mean difference±SE]: +1.4±0.7%, p=0.04); but the association was not significant after adjusting for changes in weight and BA (previous DMPA yes vs no [mean difference±SE]: +0.9±0.6%, p=0.12). Overall, decreases in TH and WBLH aBMD were greater in WWH in the first 6 months of lactation, before and after adjusting for body size.

After lactation (L26 to NPNL and L2 to NPNL)

LS and TH aBMD increased in both groups between L26 and NPNL, and there were tendencies towards smaller increases in WWH (Table 4). LS aBMD was higher at NPNL compared to L2 in both groups. TH and WBLH aBMD did not return to L2 values at NPNL in WWH (L2 to NPNL: -3.1% [95% CI: -3.6, -2.6]), but did in REF women (L2 to NPNL: +0.1% [95% CI: -0.9, +1.1]). Body weight and lean mass decreased in both groups between L2 and NPNL. However, fat mass and % fat increased only in WWH between L2 and NPNL. DMPA exposure, breastfeeding practices, and other measured potential confounders were not associated with changes in aBMD after adjusting for body weight and BA.

Overall patterns of changes during and after lactation (L2-L14-L26-NPNL)

Figure 3 shows overall changes in aBMD in WWH and REF. In summary, WWH started with higher TH and WBLH aBMD at L2, 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 towards smaller aBMD reduction at L14, and smaller increase in aBMD post-lactation. 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 (Figure 3). Restricting statistical analysis to participants with data at all timepoints (rectangular dataset) did not change interpretation of the results (Supplemental 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 (Supplemental 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 studies in Caucasian and African women [16,23]. However, WWH had greater decreases in TH and WBLH aBMD between L2 and L26 than reference women, and a tendency towards a smaller decrease in LS aBMD in early lactation (L2 and L14). At 3 months post-lactation, LS aBMD was higher relative to L2 in both groups, and TH 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 % 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 post-lactation.

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 PROMISE (Promoting Maternal and Infant Survival Everywhere) trial, conducted in Uganda, Zimbabwe, Malawi and South Africa, randomized asymptomatic WWH with high CD_4 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 sub-study (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 [12]. However, neither studies [11,12] 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 [14,15,23] shows greater changes in aBMD at the LS – a

trabecular rich site – compared to other skeletal sites . We, therefore, hypothesised 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 3-6 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[11]. However, the reasons for lack of a difference at LS are unclear.

Mobilization of maternal bone mineral during lactation may be influenced by several factors including: exclusivity and duration of breastfeeding, infant breastmilk intake, duration of lactation, maternal height, resumption of menses, and duration of amenorrhoea.[16,23,29]. Furthermore, changes in body weight and bone area (BA) may affect interpretation of aBMD results in longitudinal studies [27,30]. 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 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 post-lactation to investigate recovery in bone mineral density consistent with previous lactation studies [17,19,23]. We used DXA and adjusted aBMD for body size and measured potential confounders. All WWH were previously ART naïve 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 CD_4 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 in 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 post-lactation. These data show accentuated decrease in hip and whole body aBMD during the first 6 months of lactation, and only partial skeletal recovery post-lactation in Ugandan WWH initiated on triple ART during pregnancy. The clinical implication of these findings are unclear at this stage as 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.

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Authors roles: Study design: FN, AP, MMH, AK, MGF, JB and GRG. Study conduct: FN, AK, MGF, and JB. Data collection: FN. Data analysis: FN and AP. Data interpretation: FN, AP, MMH, AK, MGF, JB and GRG. Drafting manuscript: FN. Revising manuscript content: AP, GRG, AF, MMH, AK, MGF and JB. Approving final version of manuscript: all authors. FN and AP take responsibility for the integrity of the data analysis.

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FIGURE LEGENDS

Figure 1: Flow chart of women in the two study groups from recruitment to final DXA measurements. DXA was performed postpartum at 2 (L2), 14 (L14), 26 (L26) weeks of lactation, and at least 3 months after stopping breastfeeding when the women were neither pregnant nor lactating (NPNL). Women who stopped breastfeeding between L14 and L26 were excluded from L26 measurement, but were scheduled for final measurements at NPNL.

Figure 2: Percent changes in aBMD during and after lactation from L2. Data are mean percent changes (%Δ) and 95% confidence intervals (CI). LS = lumbar spine, TH= total hip, WBLH = whole body-less-head, aBMD = areal bone mineral density (g/cm^2), WWH = women with HIV initiated on lifelong triple ART during pregnancy (TDF-3TC-EFV, previously ART naïve), REF = women without HIV (reference group, ART naïve), CI= confidence intervals. L2, L14, L26 = 2, 14 and 26 weeks postpartum, respectively, NPNL = Neither Pregnant Nor Lactating. 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 6.3.1 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 (loge) and multiplied by 100 before data analysis. P-values are for comparison of overall patterns of changes between the groups (WWH vs. REF).

Figure 3: Changes in adjusted aBMD during and after lactation, from REF at L2. Data are mean percent changes (%Δ) ± standard errors (SE). LS = lumbar spine, TH= total hip, WBLH = whole body-less-head, aBMD = areal bone mineral density (g/cm^2), WWH = women with HIV initiated on lifelong triple ART during pregnancy (TDF-3TC-EFV, previously ART naïve), REF = women without HIV (reference group, ART naïve), CI= confidence intervals. L2, L14, L26 = 2, 14 and 26 weeks postpartum, respectively, NPNL = Neither Pregnant Nor Lactating. 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 6.3.1 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 (loge) and multiplied by 100 before data analysis. aBMD was adjusted for body weight and bone area in nested linear regression models. P-values are for comparison of overall patterns of changes between the groups (WWH vs REF).

TABLES AND FIGURES

Table 1: Summary of participant characteristics and medical history at L2, L14, L26 and NPNL

	L2		L14		L26		NPNL	
	WWH (n=84)	REF (n=78)	WWH (n=83)	REF (n=81)	WWH (n=69)	REF (n=72)	WWH (n=67)	REF (n=32)
Weeks postpartum	2.2±0.5	2.1±0.4	14.3±0.6	14.2±0.8	26.5±0.9	26.7±1.0	65.1±14.1[a]	81.1±18.4
Months post-lactation	-	-	-	-	-	-	3.4 (3.3, 4.4)[c]	3.3 (3.1, 3.5)
Age (years)	23.7(21.4,27.4)	23.3(20.9,27.1)	23.9(21.8,27.6)	23.6(20.1,27.4)	24.1(22.1,27.8)[c]	23.7(21.4,27.5)	25.0(23.0,28.7[c]	24.3(22.6,28.4)
Parity	2 (1, 3)	1 (1, 2)	2 (1, 3)	1 (1, 2)	2 (1, 3)	1 (1, 2)	2 (1, 3)	1 (1, 2)
Primiparous, %	36.5 [c]	53.8	36.1[c]	55.6	37.1 [c]	54.2	33.8	53.1
Weeks on ART	17.6±5.5	-	29.5±5.1	-	42.0±5.7	-	80.6±15.2	-
CD_4 cell count	400 (301, 516)	-	405 (294, 525)	-	487 (331, 672)	-	471 (338, 688)	-
% pills taken[1]	99.3±2.0	-	99.7±1.6	-	99.4±3.3	-	99.3±3.8	-
EBF, %	82.1 [a]	59.7	88.1 [a, 2]	65.4 [2]	81.6 [a]	42.9	-	-
BF duration, weeks	2.2±0.5	2.1±0.4	14.3±0.6	14.2±0.8	26.5±0.9	26.7±1.0	47.8±13.4[a]	65.6±18.1
Resumed menses, %	-	-	39.8	34.6	56.5	53.9	-	-
Current DMPA, %	-	-	30.1 [b]	13.6	34.7	21.7	55.8	43.5
Prior DMPA, %	41.5[b]	19.5	38.3 [b]	18.8	36.2	22.5	43.9 [b]	16.1

Values are means±standard deviations, medians (25 percentiles, 75 percentiles), or percentage (%) of participants reporting “yes”).

L2, L14, L26 = 2, 14 and 26 weeks postpartum, respectively; NPNL= measurement made at least 3 months post-lactation when women were neither pregnant nor lactating; WWH = women with HIV initiated on lifelong triple ART during pregnancy (TDF-3TC-EFV, previously ART naïve), REF = women without HIV (reference group, ART naïve); ART= antiretroviral therapy; CD_4 cell count = cells/cm^3; BF =breastfeeding; EBF = exclusive breastfeeding; DMPA= depot medroxyprogesterone acetate

[1] 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].

[2] Proportions are higher than L2 because some babies received pre-lacteal feeds at L2 but were exclusively breastfed at L14.

[a,b,c] P values for differences between the groups obtained from chi-square tests in DataDesk 6.3.1 software: [a] $p \leq 0.001$, [b] $p \leq 0.01$, [c] $p \leq 0.05$.

Table 2: Summary of anthropometry, body composition and bone measures

	L2		L14		L26		NPNL	
	WWH (n=84)	REF (n=78)	WWH (n=83)	REF (n=81)	WWH (n=69)	REF (n=72)	WWH (n=67)	REF (n=32)
Lumbar spine	*n=78*	*n=71*	*n=82*	*n=77*	*n=68*	*n=72*	*n=62*	*n=31*
aBMD, g/cm^2	0.909±0.095	0.911±0.111	0.889±0.090	0.884±0.104	0.887±0.086	0.893±0.104	0.915±0.092[c]	0.957±0.109
BA, cm^2	54.4±4.7	54.1±5.2	54.2±4.9[b]	53.8±5.3	54.2±4.9	54.2±5.2	55.5±5.0	54.7±4.7
BMC, g	49.5±7.4	49.5±9.1	48.3±7.0[b]	47.8±8.6	48.2±7.0	48.7±8.7	50.8±7.4	52.5±8.6
Total hip	*n=78*	*n=74*	*n=82*	*n=80*	*n=68*	*n=72*	*n=65*	*n=32*
aBMD, g/cm^2	0.946±0.119[a]	0.917±0.093	0.905±0.120[c]	0.896±0.108	0.883±0.115	0.886±0.111	0.904±0.132	0.925±0.108
BA, cm^2	28.2±2.4[a]	28.8±2.9	28.2±2.5[a]	28.9±2.9	28.4±2.6[c]	28.8±2.6	28.5±2.6[a]	29.1±3.1
BMC, g	26.7±4.1	26.5±4.1	25.6±4.1	25.9±4.1	25.1±4.3	25.5±4.0	25.8±4.5[c]	27.0±4.2
WBLH	*n=78*	*n=73*	*n=80*	*n=79*	*n=68*	*n=71*	*n=64*	*n=32*
aBMD, g/cm^2	0.935±0.066[a]	0.919±0.064	0.919±0.061[c]	0.915±0.069	0.915±0.065	0.919±0.068	0.908±0.065	0.918±0.073
BA, cm^2	1579.8±109.1[c]	1595.6±138.9	1574.3±95.3	1590.4±137.6	1566.9±105.0	1588.2±133.0	1596.9±112.1	1598.6±127.7
BMC, g	1479.3±169.3	1470.1±13.0	1450.1±156.1	1460.0±202.0	1437.7±174.6	1462.9±197.5	1454.5±182.3	1471.8±197.6
Lean, kg	33.5 (30.1, 36.6)[c]	34.7 (31.1, 40.0)	32.5 (30.0 35.5)[b]	33.5 (30.8, 37.5)	32.7 (29.6, 35.6)	33.3 (29.8, 37.1)	31.2 (28.1, 34.0)	32.8 (29.7, 35.1)
Fat, kg	18.7 (14.9, 22.9)	19.3 (15.3, 25.8)	19.1 (14.7, 23.1)	19.6 (15.3, 26.0)	19.3 (15.3, 22.9)	18.9 (15.3, 25.7)	19.3 (14.6, 25.0)	20.8 (15.9, 27.0)
% Fat	34.5±8.1	35.4±8.5	36.2±7.1	35.9±8.5	35.5±6.7	36.4±7.9	36.9±8.4	38.3±8.2
Anthropometry	*n=79*	*n=73*	*n=83*	*n=80*	*n=69*	*n=70*	*n=63*	*n=32*
Height, cm	157.0±4.6	158.6±5.7	156.9±4.4	158.5±5.8	157.1±4.4	158.7±5.6	157.4±4.7	157.8±4.6
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)	56.9 (53.0, 63.2)[a]	59.9 (52.9, 68.8)	56.9 (51.4, 63.1)[a]	58.7 (54.2, 67.1)
BMI, kg/cm^2	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)	23.2 (21.8, 26.0)	23.4 (21.1, 27.2)	23.3 (20.7, 25.7)[c]	24.7 (21.7, 27.2)

Values are means±standard deviations or medians (25 percentiles, 75 percentiles).

L2, L14, L26 = 2, 14 and 26 weeks postpartum, respectively; NPNL= measurement made at least 3 months post-lactation when women were neither-pregnant nor-lactating; WWH = women with HIV initiated on lifelong triple ART during pregnancy (TDF-3TC-EFV, previously ART naïve), REF = women without HIV (reference group, ART naïve); aBMD= areal bone mineral density; BA= bone area; BMC = bone mineral content; WBLH = whole body-less-head; BMI=body mass index;
a,b,c P-values for mean percent differences between the groups: a $p \leq 0.001$, b p-value ≤ 0.01, c p-value ≤ 0.05. See Supplemental Table 3 for actual p-values and associated mean differences between the groups.

Table 3: Mean changes in aBMD, body composition and weight during lactation (L2-L14-L26)

	L2 to L14			**L2 to L26**			**Three timepoint model (L2-L14-L26) group*visit p-value[2]**
	WWH %Δ [95% CI]	REF %Δ [95% CI]	group*visit p-value[1]	WWH %Δ [95% CI]	REF %Δ [95% CI]	group*visit p-value[1]	
Lumbar spine	*n=77*	*n=69*		*n=63*	*n=63*		
aBMD, g/cm^2	-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*	*n=64*		
aBMD, g/cm^2	-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]	0.008	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	0.0001
WBLH	*n=76*	*n=71*		*n=64*	*n=63*		
aBMD, g/cm^2	-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	0.001
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	0.001
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
% Fat	+1.9 [+1.1, +2.7]	+0.9 [+0.04, +1.8]	0.39	+1.7[+0.7, +2.7]	+1.2 [+0.2, +2.2]	0.51	0.63
Anthropometry	*n=78*	*n=74*		*n=63*	*n=64*		
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% confidence intervals (CI) [lower, upper confidence limits]. The + or - signs show the direction of within group changes (increase or decrease, respectively). Mean %Δ ± standard errors (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 standard errors from Scheffé post hoc tests and associated sample sizes were used to calculate the confidence intervals using OpenEpi Epidemiological calculator.

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

[1] p-value for group*interaction term in two timepoint hierarchical repeated measures ANOVA models.

[2] p-value for overall group*interaction term in three timepoint hierarchical repeated measures ANOVA models (L2-L14-L26).

Table 4: Mean changes in aBMD, body composition and weight after lactation (L26/L2 to NPNL)

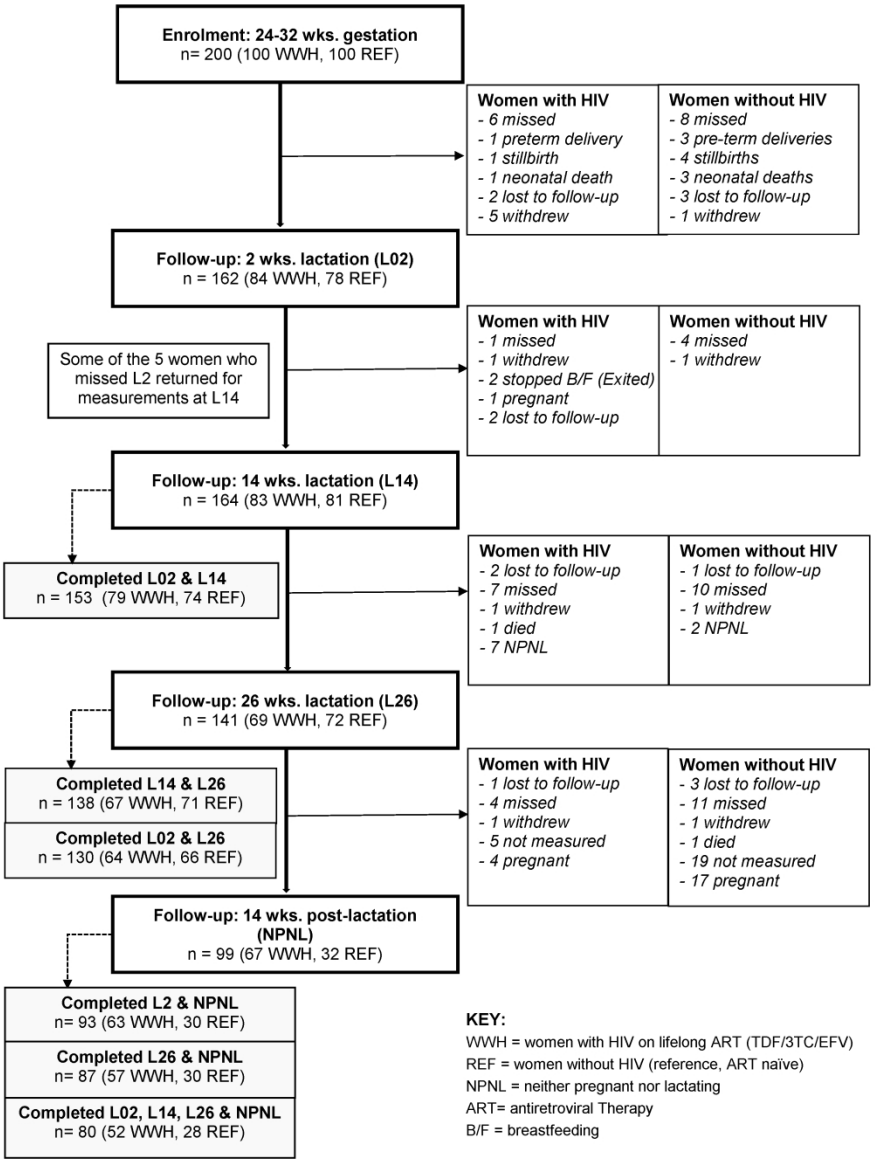
	L26 to NPNL			**L2 to NPNL**			**Four timepoint model**
	WWH	REF	group*visit	WWH	REF	group*visit	**L2-L14-L26-NPNL**
	%Δ [95% CI]	%Δ [95% CI]	p-value[1]	%Δ [95% CI]	%Δ [95% CI]	p-value[1]	**group*visit p-value[3]**
Lumbar spine	*n=52*	*n=29*		*n=59*	*n=28*		
aBMD, g/cm^2	+3.8 [+3.2, +4.4]	+5.2 [+4.2, +6.2]	0.07	+1.7 [+1.2, +2.2]	+3.2 [+2.2, +4.2]	0.10	0.003
aBMD adjusted	+3.5 [+2.9, +4.1]	+5.0 [+4.0, +6.0]	0.07	+1.6 [+1.1, +2.1]	+3.3 [+2.3, +4.3]	0.06	0.002
Total hip	*n=55*	*n=30*		*n=61*	*n=29*		
aBMD, g/cm^2	+2.8 [+2.3, +3.3]	+4.4 [+3.4, +5.4]	0.06	-3.1 [-3.6, -2.6]	+0.1 [-0.9, +1.1]	0.0008	≤0.001
aBMD adjusted	+2.9 [+2.4, +3.4]	+4.2 [+3.3, +5.1]	0.20	-2.9 [-3.4, -2.4]	+0.5 [-0.4, +1.4]	≤0.001	≤0.001
WBLH	*n=55*	*n=30*		*n=61*	*n=28*		
aBMD, g/cm^2	-0.7 [-1.1, -0.3]	+0.5 [-0.2, +1.2]	0.03	-2.4 [-2.8, -2.0]	-0.1 [-0.8, +0.6]	0.002	≤0.001
aBMD adjusted	-0.5 [-0.9, -0.1]	+0.5 [-0.2, +1.2]	0.03	-2.3 [-2.7, -2.0]	-0.1 [-0.8, +0.6]	0.009	≤0.001
Lean, kg	-3.4 [-5.2, -1.6]	-3.3 [-6.1, -0.5]	0.60	-6.7 [-8.2, -5.2]	-7.0 [-9.7, -4.2]	0.62	0.93
Fat, kg	+1.6[-2.6, +5.8]	-0.6 [-7.5, +6.3]	0.54	+7.0 [+3.2, +10.8]	+1.7 [-5.3, +8.7]	0.51	0.65
% Fat	+1.2 [+0.1, +2.3]	+0.7 [-1.1, +2.6]	0.52	+2.9 [+1.9, +3.9]	+1.9 [-0.02, +3.8]	0.74	0.81
Anthropometry	*n=52*	*n=29*		*n=57*	*n=30*		
Weight, kg	-1.4 [-2.4, -0.4]	-0.6 [-2.2, +1.0]	0.34	-2.2 [-3.1, -1.3]	-2.8 [-4.3, -1.3]	0.53	0.54

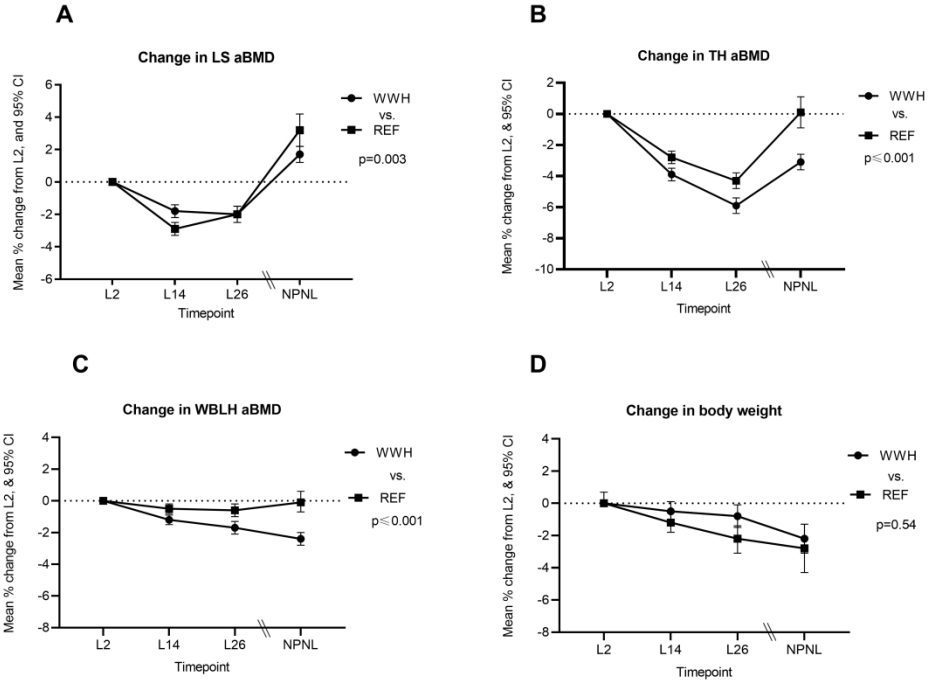
Values are within group mean percent changes (%Δ) and 95% confidence intervals (CI) [lower, upper confidence limits]. The + or - signs show the direction of within group changes (increase or decrease, respectively). Mean %Δ ± standard errors (SE), and p-values for group-visit interaction terms were obtained from Scheffé post hoc tests for group*visit (time point) interaction terms in 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 standard errors from Scheffé post hoc tests and associated sample sizes were used to calculate the confidence intervals using OpenEpi Epidemiological calculator.

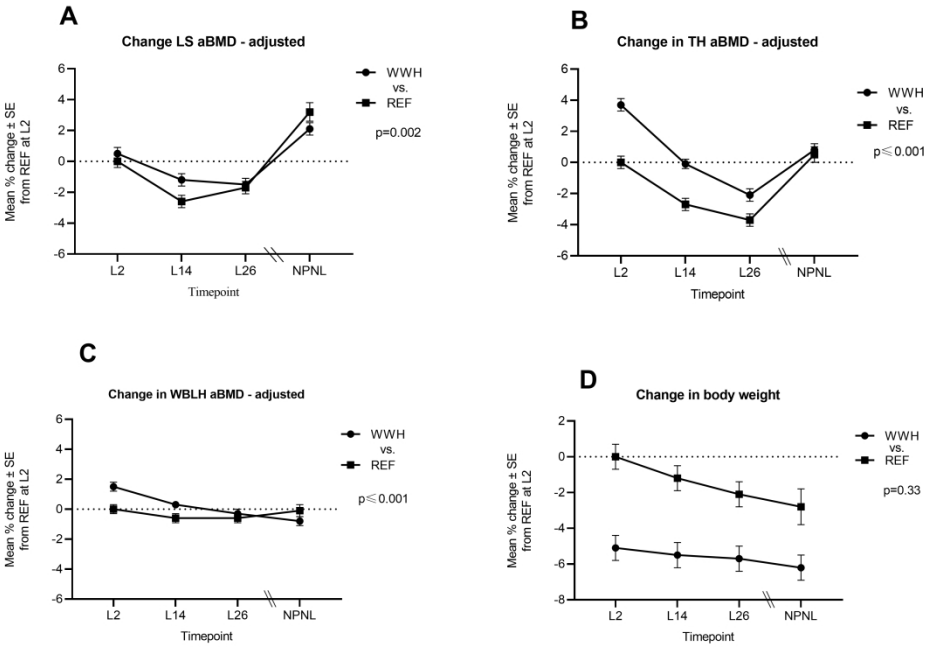
L2, L14, L26 = 2, 14 and 26 weeks postpartum, respectively; NPNL= measurement made at least 3 months post-lactation when women were neither-pregnant nor-lactating; WWH = women with HIV initiated on lifelong triple ART during pregnancy (TDF-3TC-EFV, previously ART naïve), REF = women without HIV (reference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

[1] p-value for group*interaction term in two timepoint hierarchical repeated measures ANOVA models.

[3] p-value for overall group*interaction term in four timepoint hierarchical repeated measures ANOVA models (L2-L14-L26-NPNL).


Enrolment: 24-32 wks. gestation
n= 200 (100 WWH, 100 REF)
Women with HIV
- 6 missed
- 1 preterm delivery
- 1 stillbirth
- 1 neonatal death
- 2 lost to follow-up
- 5 withdrew
Women without HIV
- 8 missed
- 3 pre-term deliveries
- 4 stillbirths
- 3 neonatal deaths
- 3 lost to follow-up
- 1 withdrew
Follow-up: 2 wks. lactation (L02)
n = 162 (84 WWH, 78 REF)
Some of the 5 women who missed L2 returned for measurements at L14
Women with HIV
- 1 missed
- 1 withdrew
- 2 stopped B/F (Exited)
- 1 pregnant
- 2 lost to follow-up
Women without HIV
- 4 missed
- 1 withdrew
Follow-up: 14 wks. lactation (L14)
n = 164 (83 WWH, 81 REF)
Completed L02 & L14
n = 153 (79 WWH, 74 REF)
Women with HIV
- 2 lost to follow-up
- 7 missed
- 1 withdrew
- 1 died
- 7 NPNL
Women without HIV
- 1 lost to follow-up
- 10 missed
- 1 withdrew
- 2 NPNL
Follow-up: 26 wks. lactation (L26)
n = 141 (69 WWH, 72 REF)
Completed L14 & L26
n = 138 (67 WWH, 71 REF)
Completed L02 & L26
n = 130 (64 WWH, 66 REF)
Women with HIV
- 1 lost to follow-up
- 4 missed
- 1 withdrew
- 5 not measured
- 4 pregnant
Women without HIV
- 3 lost to follow-up
- 11 missed
- 1 withdrew
- 1 died
- 19 not measured
- 17 pregnant
Follow-up: 14 wks. post-lactation (NPNL)
n = 99 (67 WWH, 32 REF)
Completed L2 & NPNL
n= 93 (63 WWH, 30 REF)
Completed L26 & NPNL
n= 87 (57 WWH, 30 REF)
Completed L02, L14, L26 & NPNL
n= 80 (52 WWH, 28 REF)
KEY:
WWH = women with HIV on lifelong ART (TDF/3TC/EFV)
REF = women without HIV (reference, ART naïve)
NPNL = neither pregnant nor lactating
ART= antiretroviral Therapy
B/F = breastfeeding


A
Change in LS aBMD
Mean % change from L2, and 95% CI
6
4
2
0
-2
-4
-6
L2
L14
L26
NPNL
Timepoint
WWH
vs.
REF
p=0.003
B
Change in TH aBMD
Mean % change from L2, & 95% CI
2
0
-2
-4
-6
-8
-10
L2
L14
L26
NPNL
Timepoint
WWH
vs.
REF
p≤0.001
C
Change in WBLH aBMD
Mean % change from L2, & 95% CI
4
2
0
-2
-4
-6
-8
L2
L14
L26
NPNL
Timepoint
WWH
vs.
REF
p≤0.001
D
Change in body weight
Mean % change from L2, & 95% CI
4
2
0
-2
-4
-6
-8
L2
L14
L26
NPNL
Timepoint
WWH
vs.
REF
p=0.54


A
Change LS aBMD - adjusted
Mean % change ± SE from REF at L2
WWH vs. REF
p=0.002
L2
L14
L26
NPNL
Timepoint
B
Change in TH aBMD - adjusted
Mean % change ± SE from REF at L2
WWH vs. REF
p≤ 0.001
L2
L14
L26
NPNL
Timepoint
C
Change in WBLH aBMD - adjusted
Mean % change ± SE from REF at L2
WWH vs. REF
p≤ 0.001
L2
L14
L26
NPNL
Timepoint
D
Change in body weight
Mean % change ± SE from REF at L2
WWH vs. REF
p=0.33
L2
L14
L26
NPNL
Timepoint