Lower Newborn Bone Mineral Content Associated with Maternal Use of Tenofovir Disoproxil Fumarate During Pregnancy

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Key points (40 word limit):

Tenofovir is now widely used by HIV-infected pregnant women. This study demonstrates that infants with in utero tenofovir exposure had significantly lower bone mineral content than unexposed infants, supporting a longstanding concern about adverse fetal bone effects of maternal tenofovir.

ABSTRACT

Background: Fetal bone effects of maternal tenofovir use have not been well studied. We sought to compare whole-body bone mineral content (BMC) of newborns exposed versus not exposed to tenofovir in utero.

Methods: We enrolled participants from April 2011 to June 2013 at 14 US clinical sites. Singleton infants of women with HIV infection who took tenofovir in late pregnancy (tenofovir-exposed) or no tenofovir during pregnancy (tenofovir-unexposed) were enrolled during late pregnancy or within 72 hours of birth. Infants born before 36 weeks gestation or with confirmed HIV infection were excluded. Whole-body BMC was measured in the first month of life and compared between the tenofovir-exposed and tenofovir-unexposed newborns, unadjusted and adjusted for covariates.

Results: Seventy-four tenofovir-exposed and 69 tenofovir-unexposed infants had evaluable BMC measurements. Tenofovir-exposed mothers were more likely to be married (31% vs. 22%, P = 0.04) and use boosted protease inhibitors (84% vs. 62%, P=.004). Tenofovir-exposed newborns did not differ from unexposed newborns on mean gestational age (38.2 vs 38.1 weeks) or mean length (-0.41 vs. -0.18) or weight (-0.71 vs. -0.48) Z-scores. The mean (standard deviation) BMC of tenofovir-exposed infants [56.0 grams (11.8)] was 12% lower than for unexposed infants [56.0 (11.8) vs. 63.8 (16.6) grams; P = 0.002). The adjusted mean bone mineral content was 5.3 grams lower (95% CI -9.5, -1.2, P=0.013) in the tenofovir-exposed infants.

Conclusions: Maternal tenofovir use is associated with significantly lower neonatal BMC. The duration and clinical significance of this finding should be evaluated in longitudinal studies.
INTRODUCTION

Tenofovir disoproxil fumarate (TDF), in combination with other antiretroviral drugs, is preferred initial therapy for adults with HIV infection because of its documented safety and efficacy. However, TDF has been associated with loss of bone mineral density (BMD) in adults and children with HIV infection and in adults taking TDF to prevent HIV infection. The clinical consequences of TDF-associated bone loss are less certain, but TDF use was a risk factor for bone fracture in adults with HIV infection in some studies.

TDF has been increasingly prescribed for pregnant women. The World Health Organization and United States guidelines both recommend TDF as a preferred antiretroviral agent in pregnancy. However, primate studies have demonstrated impaired fetal growth and bone mineralization after maternal tenofovir administration during late pregnancy. Some human studies have suggested a potential adverse effect of maternal TDF use on infant growth, but studies evaluating infant bone outcomes after intrauterine tenofovir exposure have been limited.

The present study aimed to evaluate effects of maternal TDF use on infant bone mineral content (BMC).

METHODS

Study Participants

The source population for the present tenofovir sub-study is the Surveillance Monitoring for ART Toxicities (SMARTT) study of the Pediatric HIV/AIDS Cohort Study (PHACS). SMARTT was designed to study the effect of antenatal and post-natal antiretroviral exposure on childhood outcomes in HIV-exposed but uninfected (HEU) children. Since 2007, SMARTT has
been enrolling HEU infants during pregnancy or within 72 hours of birth from 14 clinical sites in the mainland US and Puerto Rico. Standard of care at all sites is formula-feeding. Infants with HIV infection are excluded.

SMARTT participants were eligible for this cross-sectional tenofovir sub-study if they were singleton pregnancies born at ≥ 36 weeks gestation and met the following criteria for tenofovir exposure: 1) Maternal receipt of TDF for ≥8 weeks in the third trimester of pregnancy (tenofovir-exposed); or 2) No maternal receipt of TDF during the pregnancy (tenofovir-unexposed). Infants not continuing to meet these eligibility criteria at birth were excluded.

*Medical history and anthropometrics*

Maternal socio-demographic data and substance use during the current pregnancy were obtained by self-report. Maternal medical history abstracted from the medical chart included pregnancy complications, infections, antiretroviral drugs and dates received, first and last CD4+ T-lymphocyte (CD4) count/percent and HIV viral load during the current pregnancy, delivery mode, and gestational age. Infant medical history and birth weight were abstracted from the infant’s chart.

Infant weight and length were measured at the entry visit by trained personnel using standard methods previously described. Each measurement was obtained three times and the average computed. Weight and length Z-scores were calculated based on the CDC 2000 normative data for infants born ≥ 37 weeks gestation and based on preterm infant normative data for infants born < 37 weeks gestation.
Dual-energy X-ray absorptiometry (DXA)

Infant DXA scans were attempted within four (allowed up to five) weeks of birth on a Hologic scanner (Delphi A, Discovery A, Discovery W, QDR4500A) using infant whole-body mode. DXA results included whole-body and whole-body less head measures of BMC, BMD, and total mass. To generate whole-body less head results, a custom sub-region was created to divide the head from the rest of the body. Acceptable images were free of artifacts, contained the entire body of the infant, and had minimal movement distortion. A European Spine Phantom (ESP) was circulated to all sites to ensure calibration among scanners. All scans were analyzed centrally by an ISCD-certified bone densitometry technologist at the Tufts University Body Composition Analysis Center using Hologic APEX software version 13.4.

One DXA trainer traveled to sites without prior experience in performing infant DXA scans (13/14) to train site technicians. Detailed instructions for performing infant whole-body DXA scans were provided in a manual of operations. The undressed infant (cotton shirt and diaper allowed) was swaddled and not sedated. Up to three scan attempts were permitted.

Outcome and exposure measures

The primary objective was to compare the mean BMC of tenofovir-exposed infants to that of tenofovir-unexposed infants. The primary BMC outcome was whole-body BMC (with head), but whole body BMC less head was also evaluated.

Potential confounders

Potential confounding maternal factors included age, race/ethnicity, tobacco use in pregnancy, delivery in summer/fall vs. winter/spring, CD4% and HIV viral load before starting
antiretroviral regimen, and other antiretroviral medications received during the pregnancy. Infant factors considered as risk factors for the outcome were sex, race/ethnicity, body length and weight, gestational age, age at DXA, whole-body mass with and less head (by DXA), and whole-body lean mass with and less head (by DXA). The gestational age at birth and age at DXA were included because bone mineral content accrues rapidly during late fetal and neonatal periods.

Statistical analysis

Target enrollment was set at 75 per group to ensure at least 63 evaluable participants per group. A sample size of 63 per group would allow detection of a difference of 0.5 standard deviations (SD) of mean whole-body BMC between groups, assuming 80% power and a 2-sided alpha of 0.05.

Differences in continuous variables and categorical variables between the groups were compared by Wilcoxon and Chi-square tests, respectively. For unadjusted BMC analyses, we compared mean BMC (with and less head) between groups. We then performed multivariable linear regression analysis to evaluate differences in BMC between groups, adjusted for potential confounders. All models were adjusted for clinical site, infant length, gestational age, age at DXA, and maternal tobacco use anytime in pregnancy. In addition, we considered the following potential covariates: infant whole-body mass with and less head from DXA, whole body lean mass with and less head from DXA, and maternal height, pre-pregnancy weight and BMI. These potential confounders were included if the univariate association with BMC was $P < 0.2$ and retained in the model if the effect estimate changed by $> 10\%$. We fit models separately for whole-body BMC with and less head. We also conducted subgroup and sensitivity analyses to explore potential effects of other drugs in the maternal antiretroviral regimen.
We were unable to adjust for confounding by indication because maternal CD4 and viral load values before ART initiation were missing for many women and reasons for maternal regimen choice were not collected. We did not include CD4 and viral load values from the third trimester of pregnancy in the models because these might be the result of the exposure and on the causal pathway between maternal TDF use and infant BMC. However, we evaluated the associations of CD4% and viral load with the BMC outcomes.

All analyses were performed in SAS 9.2.

RESULTS

Study population

Of 195 enrolled infants, 21 were no longer eligible at birth for the following reasons: gestational age less than 36 weeks (N=16, including one fetal demise), born at another hospital (N=1), stillborn (N=1), loss of maternal custody (N=1), disqualifying change in maternal antiretroviral regimen during pregnancy (N=2) (Figure 1). No infant was excluded because of HIV infection. Of the 174 who were eligible, 126 enrolled before delivery, 37 were enrolled by age 2 days, 24 between ages 3 and 14 days, and 8 between ages 15 and 22 days. Evaluable DXA scans were obtained on the first scan attempt in 81 infants, the second attempt in 38 infants, and the third attempt in 24 infants. DXA scans were performed at a median age of 15 days overall, but at a median of 13 days in tenofovir-exposed infants and 19 days in unexposed infants (P < 0.001).
Characteristics of mothers and infants (Table 1)

Mothers who used TDF were more likely to be married (31% vs. 22%, \( P = 0.035 \)) and use a boosted protease inhibitor (84% vs. 62%, \( P = 0.004 \)). There were significant differences in the distribution of participants by tenofovir exposure across clinical sites (\( P < .001 \)). The two groups of mothers did not differ on race/ethnicity, summer/fall delivery, high school education, income, or substance use during pregnancy (Table 1). Mothers received diverse combinations of antiretroviral drugs, including 13 combinations for women who used TDF and 18 combinations for women who did not use TDF (Table 2).

Gestational age at birth was 36 to less than 37 weeks for 5 (7%) tenofovir-exposed infants and 4 (6%) tenofovir-unexposed infants. Tenofovir-exposed infants did not differ from unexposed infants with respect to race/ethnicity, gestational age at birth, or length or weight Z-scores (Table 1).

Mean bone mineral content by tenofovir exposure

The mean (SD) whole-body BMC of infants exposed to tenofovir was 56.0 (11.8) g, compared to 63.8 (16.6) g for unexposed infants (\( P = 0.002 \)) (Table 3). This unadjusted difference of 7.8 grams in BMC represents a difference of 12.2% and 0.5 SD between exposure groups. For whole-body BMC less head, the mean (SD) was 33.3 (7.3) g in the tenofovir-exposed group, compared to 36.3 (9.7) g in the unexposed group (\( P = 0.038 \)), representing a difference of 8.3% and 0.4 SD between exposure groups.
Adjusted difference in whole-body BMC by Tenofovir exposure

We fit a linear regression model to determine the mean difference in whole-body BMC between the tenofovir-exposed and unexposed infants, adjusted for potential cofactors. Our final model was adjusted for maternal age at delivery, ever use of tobacco in pregnancy, infant race (black vs. non-black), gestational age, length, age at DXA scan, and clinical site (Table 4). The adjusted mean whole-body BMC (with head) was 5.3 g lower (95% CI -9.5, -1.2, p=0.013) in the tenofovir-exposed compared to unexposed. When we fit the model for whole-body BMC less head, adjusted for the same factors, the mean whole-body BMC was 1.9 g lower (95% CI -4.5, 0.7, P =0.15) in the tenofovir-exposed compared to the unexposed infants.

Including boosted PI exposure in the models had a minimal impact on the estimate of the tenofovir effect. Duration of maternal TDF use was not associated with mean whole-body BMC in a multivariate model limited to tenofovir-exposed infants (p=0.81).

Because triple NRTI therapy was a non-conventional ART regimen and was limited to the tenofovir-unexposed, we refit our model adjusting for covariates without these 16 participants. In this model, BMC with head was 6.5 g lower in the tenofovir-exposed (95%CI -10.9, -2.2, P = 0.004). To exclude the possibility that atazanavir, a drug often prescribed with TDF, might drive the observed negative effect on BMC, we also performed a subgroup analysis including only infants without atazanavir exposure (n=91). In this smaller sample, the tenofovir effect persisted with an even larger effect size of -7.5g (95%CI -13.3, -1.7, P=0.012).

Maternal CD4 values and viral load

CD4 values were available for 107 women and viral load for 112 women in the third trimester. There were no differences between the groups in the proportion with CD4 ≥ 500
cells/mm³ (57% vs. 41%, p=0.11) or with viral load ≥ 400 copies per mL (10% vs. 10%, p=0.95) (Table 1). There was also no significant correlation between CD4% or viral load in the third trimester and BMC. For whole-body BMC with head, the correlations were r=0.12 (P=0.21) and r=0.15 (P=0.13) for CD4% and viral load, respectively. For whole-body BMC less head, the correlations were r=0.12 (P=0.21) and r=0.09 (P=0.33) for CD4% and viral load, respectively.

**DISCUSSION**

Infants with *in utero* tenofovir exposure had a significantly lower BMC than infants without *in utero* tenofovir exposure. The magnitude and significance of this difference remained robust despite adjustment for factors expected to affect neonatal BMC. This finding is consistent with observations in animal studies and strongly supports a longstanding concern about potential adverse effects of maternal TDF on offspring bone mineral status.

The magnitude of the mean BMC difference (12% or 0.5 SD) between tenofovir-exposed and unexposed infants should be considered relative to other studies of bone effects of tenofovir and other exposures. In a double-blind, placebo-controlled randomized clinical trial of TDF-emtricitabine in HIV-uninfected adults, spine BMD declined 0.13 SD in the TDF-emtricitabine arm but increased by 0.13 SD in the placebo arm by week 24, for a net difference of 0.26 SD. In a trial that randomized HIV-infected adults to initiate a TDF-containing regimen vs. an abacavir-containing regimen (without TDF), spine BMD declined by 0.22 SD in the TDF arm but only by 0.11 SD in the abacavir arm at week 48. In a trial that randomized preterm infants to receive preterm formula vs. standard term-infant formula, whole-body BMC was 18% and 19% higher in the preterm formula-fed infants after two and four months, respectively; the
corresponding differences when BMC was corrected for body weight were 9% and 7%, respectively. Thus, the magnitude of difference in the present study appears to be larger than the effect sizes reported for adults who take TDF and in infants with nutritional interventions.

While the association of maternal TDF during pregnancy with lower infant BMC in our study is concerning, the need to modify current WHO and US recommendations for TDF in pregnancy will depend on whether the effect persists and whether there are clinical consequences. The present study provides strong evidence of a biologic effect of maternal TDF on infant bone; however, the lack of infant BMC reference standards makes it difficult to determine if the lower BMC in tenofovir-exposed infants is abnormal. In a large study of healthy, full-term (37-42 weeks gestation) Canadian infants in which whole-body BMC measurements were obtained within 2-4 weeks of birth using Hologic scanners, the mean (SD) whole-body BMC (with head) was 76.0 (14.2) grams and mean (SD) whole-body BMC per body weight was 20.7 (2.5) grams per kilogram, substantially greater than the corresponding unadjusted measurements for both exposure groups in the present study (Table 3). These relatively large differences are unlikely the result of the lower gestational age in the present study (mean 38.2 weeks vs. 39.3 weeks in the Canadian study); in our study, each additional week of gestational age was associated with an adjusted mean BMC increase of 2.1 grams (Table 4). The difference in participant racial composition between these studies is also not likely to explain the differences since BMC is similar for black and white young infants.

Lower BMC and BMD are well established risk factors for bone fracture in adults and children. Low BMC at birth may increase fracture risk in infancy and early childhood and also have longer-term implications. Failure to reach expected peak bone mass in early adulthood is a risk factor for osteoporosis in later life. Preterm birth and low birthweight increase the risk
of lower adult bone mass and fracture, suggesting that intrauterine exposures may have lifelong bone consequences. Maternal sun exposure, vitamin D status, magnesium intake and other dietary factors during pregnancy correlate with BMC and BMD outcomes in offspring nine years later, providing evidence that time-limited exposures during pregnancy can lead to long-term bone consequences. The potential clinical consequences of lower BMC in the present study will require longitudinal evaluation.

Although tenofovir was the primary exposure of interest, the potential effect on neonatal bones of other antiretroviral drugs was considered. As a class, protease inhibitors have been associated with low BMD in some, but not all, studies of children and adults with HIV infection. In addition, higher tenofovir levels (but lower PI levels) occur when tenofovir is co-administered with atazanavir or other PIs. However, adjustment for protease inhibitors as a class did not affect the relationship of tenofovir exposure to infant BMC in the present study. Furthermore, subgroup analysis provided evidence that atazanavir alone, the PI most commonly used with TDF in this study, did not explain the association between maternal TDF and lower infant BMC. The relatively small sample size, heterogeneity of antiretroviral drug regimens, and imbalance in antiretroviral drugs and drug classes by concurrent tenofovir exposure prevented further analysis of the effect of these other drugs on infant BMC outcomes. More data are needed about the potential for protease inhibitors in pregnancy to affect neonatal BMC.

Vitamin D, parathyroid hormone, and diet were not assessed in the mothers in the present study. Vitamin D insufficiency is common in people with HIV infection and TDF leads to parathyroid hormone elevations that are mitigated by vitamin D supplementation. Recent data suggest that vitamin D supplementation in adults initiating ART can prevent ART-associated BMD loss. The role of vitamin D levels in the association between maternal TDF and infant
bone outcomes should be explored in future studies. Maternal or infant vitamin D supplementation may be a strategy to counteract the infant bone effects of intrauterine tenofovir exposure, though infant vitamin D supplementation has not improved bone outcomes in trials of preterm infants or healthy breastfeeding infants.\textsuperscript{40,41} Infant physical therapy interventions may be another option for improving postnatal bone growth and bone mass accrual.\textsuperscript{42}

Limitations of this study include the potential for residual confounding and the lack of criteria for defining abnormal and normal BMC in infants. While preterm birth has not been associated with tenofovir exposure and therefore would not be on the causal pathway, we excluded preterm infants because preterm birth results in lower BMC. Thus, findings may not be generalizable to preterm infants. Very few women in this study used NNRTIs, preventing evaluation of potentially different effects of TDF when used in a regimen with NNRTIs. This cross sectional study does not provide information about longitudinal BMC changes or later risk of bone fragility. However, since infants in this sub-study are part of the ongoing SMARTT cohort study, further follow up will be possible as they grow and develop. The International Maternal Pediatric AIDS Adolescent Clinical Trials (IMPAACT) network has completed enrollment in the multi-site study (P1084s) in Africa that measures BMC at birth and again at age 26 weeks in infants whose mothers were randomized to receive ART regimens with or without tenofovir during pregnancy. Results from this study are expected in 2016.\textsuperscript{43}

TDF remains an important part of highly efficacious antiretroviral regimens for treating women with HIV infection and for preventing infant HIV. The association of maternal TDF with lower infant BMC in the present study will need confirmation in other studies that can also evaluate persistence and clinical implications of lower infant BMC before recommendations for TDF use in pregnancy are modified.
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**Author Contributions:**

Dr. Jacobson has had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: All authors

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Siberry, Jacobson, Wu.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Jacobson, Wu.

Administrative, technical, or material support: All authors. Study supervision: All authors.
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Figure 1 Legend:

Figure 1. Participants were enrolled 2-April-2011 to 4-June-2013 during pregnancy (gestation age ≥ 23 weeks) through 2 weeks after birth. Eligibility was confirmed at birth for those enrolled before birth; 21 infants were excluded for the following reasons: gestational age less than 36 weeks (N=16, one of which was fetal demise), born at another hospital (N=1), still born (N=1), mother lost custody of child (N=1), disqualifying change in maternal antiretroviral regimen during pregnancy N= 2). DXA, dual-energy X-ray absorptiometry.
TABLE 1: Characteristics of mothers and infants by tenofovir exposure group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Tenofovir-unexposed N= 69</th>
<th>Tenofovir-exposed N = 74</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td><strong>MATERNAL</strong></td>
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</tr>
<tr>
<td>Median age, years</td>
<td>28</td>
<td>30</td>
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</tr>
<tr>
<td>Married</td>
<td>15 (22%)</td>
<td>23 (31%)</td>
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<td>High school or higher education</td>
<td>47 (70%)²</td>
<td>51 (69%)</td>
<td>0.87</td>
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<td>Annual income ≤$10,000</td>
<td>33 (49%)</td>
<td>46 (62%)</td>
<td>0.12³</td>
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<tr>
<td>Substance use during pregnancy</td>
<td>25 (37%)</td>
<td>19 (26%)</td>
<td>0.19</td>
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<tr>
<td>Black non-Hispanic</td>
<td>40 (59%)</td>
<td>51 (70%)</td>
<td>0.17</td>
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<td>Summer/fall delivery</td>
<td>39 (56%)</td>
<td>43 (58%)</td>
<td>0.85</td>
</tr>
<tr>
<td>Boosted protease inhibitor use</td>
<td>43 (62%)</td>
<td>62 (84%)</td>
<td>0.004</td>
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<td>3rd trimester CD4 ≥ 500 cells/mcL</td>
<td>25/44 (57%)</td>
<td>26/63 (41%)</td>
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<td>3rd trimester viral load ≥ 400 copies/mL</td>
<td>5/50 (10%)</td>
<td>6/62 (10%)</td>
<td>0.95</td>
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<tr>
<td><strong>INFANT</strong></td>
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<tr>
<td>Mean gestational age at birth, weeks</td>
<td>38.1</td>
<td>38.2</td>
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<tr>
<td>Mean length Z-score at birth (SD)</td>
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<td>-0.41 (1.02)</td>
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<td>Mean weight Z-score at birth (SD)</td>
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<tr>
<td>Black non-Hispanic</td>
<td>40 (58%)</td>
<td>52 (70%)</td>
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<tr>
<td>Female sex</td>
<td>34 (49%)</td>
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<tr>
<td>Median age at DXA, days</td>
<td>19</td>
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<td>&lt;0.001</td>
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</tbody>
</table>

¹Distribution of clinical research sites also differed significantly (P<0.001) between the tenofovir-unexposed and tenofovir-exposed participant groups. ²Missing data on educational level for two women in tenofovir-unexposed group. ³Missing income data for 9 tenofovir-unexposed and 2 tenofovir-exposed. SD = standard deviation
TABLE 2: Most recent antiretroviral drug regimens during pregnancy by study arm

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Maternal antiretroviral regimen</th>
<th>N (% within tenofovir exposure group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenofovir-exposed (n=74)</td>
<td>TDF-FTC-ATVr</td>
<td>39 (53%)</td>
</tr>
<tr>
<td></td>
<td>TDF-FTC-DRVr</td>
<td>12 (16%)</td>
</tr>
<tr>
<td></td>
<td>TDF-FTC-RAL</td>
<td>6 (8%)</td>
</tr>
<tr>
<td></td>
<td>TDF-FTC-LPVr</td>
<td>4 (5%)</td>
</tr>
<tr>
<td></td>
<td>TDF-FTC-RAL-ATVr</td>
<td>3 (4%)</td>
</tr>
<tr>
<td></td>
<td>TDF-FTC-RPV</td>
<td>2 (3%)</td>
</tr>
<tr>
<td></td>
<td>TDF-FTC-ZDV-RPV</td>
<td>2 (3%)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>6 (8%)</td>
</tr>
<tr>
<td>Tenofovir-unexposed (n=69)</td>
<td>ZDV-3TC-LPVr</td>
<td>27 (39%)</td>
</tr>
<tr>
<td></td>
<td>ABC-3TC-ZDV</td>
<td>16 (23%)</td>
</tr>
<tr>
<td></td>
<td>ZDV-3TC-DRVr</td>
<td>5 (7%)</td>
</tr>
<tr>
<td></td>
<td>ABC-3TC-ATVr</td>
<td>3 (4%)</td>
</tr>
<tr>
<td></td>
<td>ZDV-3TC-NFV</td>
<td>3 (5%)</td>
</tr>
<tr>
<td></td>
<td>ABC-3TC-DRVr</td>
<td>2 (3%)</td>
</tr>
<tr>
<td></td>
<td>ZDV-3TC-RAL</td>
<td>2 (3%)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>11 (16%)</td>
</tr>
</tbody>
</table>

1In the tenofovir-exposed group, 29 (39%) women were already receiving their TDF-containing antiretroviral regimen when they became pregnant, while 11 (15%), 30 (41%), and 4 (5%) started that regimen in the first, second and third trimesters, respectively.

2In the tenofovir-unexposed group, 18 (26%) women were already receiving their antiretroviral regimen prior to pregnancy, while 17 (25%), 31 (45%), and 3 (4%) started their pregnancy regimen in the first, second and third trimesters, respectively.
Other regimens used by 1 (1%) woman in tenofovir-exposed group included: TDF-FTC-ABC-RAL, TDF-FTC-FPVr, TDF-FTC-NVP, TDF-FTC-SQVr, TDF-ZDV-ATVr, TDF-FTC-RAL-DRVr

Other regimens used by 1 (1%) woman in tenofovir-unexposed group included: ABC-3TC-ATV, ZDV-3TC-ATVr, ZDV-3TC-NVP, ZDV-3TC-MVC, ETR-RAL-DRVr, ABC-3TC-ZDV-NVP, ABC-3TC-ZDV-RAL, ZDV-3TC RAL-LPVr, ZDV-3TC-RAL-MVC-DRVr, ZDV-3TC-ETR-DRVr, 3TC-LPVr

Abbreviations: 3TC, lamivudine; ABC, abacavir; ATV, atazanavir; DRV, darunavir; ETR, etravirine; FPV, fosamprenavir; LPV, lopinavir; MVC, maraviroc; NFV, nelfinavir; NVP, nevirapine; r, ritonavir (boosting dose); RAL, raltegravir; RPV, rilpivirine; SQV, saquinavir; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.
TABLE 3: Unadjusted mean whole-body bone mineral content, whole-body bone mineral content per body weight, whole-body bone mineral density, and whole-body body mass from DXA by tenofovir exposure

<table>
<thead>
<tr>
<th>Whole-body DXA measure</th>
<th>Tenofovir-unexposed N = 69 Mean (SD)</th>
<th>Tenofovir-exposed N = 74 Mean (SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone mineral content (grams)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with head</td>
<td>63.8 (16.6)</td>
<td>56.0 (11.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>less head</td>
<td>36.3 (9.7)</td>
<td>33.3 (7.3)</td>
<td>0.038</td>
</tr>
<tr>
<td><strong>Bone mineral content per body weight (grams/kg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with head</td>
<td>17.0 (2.3)</td>
<td>16.1 (1.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>less head</td>
<td>9.7 (1.5)</td>
<td>9.5 (1.2)</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>Bone mineral density (grams/cm²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with head</td>
<td>0.19 (0.02)</td>
<td>0.18 (0.02)</td>
<td>0.047</td>
</tr>
<tr>
<td>less head</td>
<td>0.15 (0.02)</td>
<td>0.15 (0.02)</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Whole body mass (grams)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with head</td>
<td>3755.2 (678.5)</td>
<td>3497.3 (531.4)</td>
<td>0.038</td>
</tr>
<tr>
<td>less head</td>
<td>2757.9 (521.9)</td>
<td>2605.7 (452.3)</td>
<td>0.12</td>
</tr>
</tbody>
</table>
TABLE 4: Adjusted differences in whole-body BMC in tenofovir-exposed compared to tenofovir-unexposed infants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unadjusted</th>
<th>Adjusted</th>
<th>P value</th>
<th>Unadjusted</th>
<th>Adjusted</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean difference (grams) in whole body BMC (with head)</td>
<td>Mean difference (95%CI)</td>
<td>P value</td>
<td>Mean difference (95%CI)</td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td>Primary exposure</td>
<td>Tenofovir vs. No tenofovir exposure</td>
<td>-7.8(-12.6, -3.1)</td>
<td>0.001</td>
<td>-5.3 (-9.5, -1.2)</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Maternal characteristics</td>
<td>Age, per year</td>
<td>0.08(-0.3, 0.5)</td>
<td>0.69</td>
<td>0.04 (-0.24, 0.33)</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Did not smoke in pregnancy</td>
<td>2.5(-3.8, 8.7)</td>
<td>0.43</td>
<td>1.1 (-3.4, 5.7)</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4 count &gt; 500 cells/mm3 in 3rd trimester</td>
<td>1.7(-4.4, 7.8)</td>
<td>0.58</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral load &gt; 400 copies/mL in 3rd trimester</td>
<td>0.4(-9.1, 9.8)</td>
<td>0.94</td>
<td>--</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant characteristics</td>
<td>Female sex</td>
<td>-2.6(-7.5, 2.3)</td>
<td>0.30</td>
<td>-0.20 (-3.4, 3.8)</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth, per week</td>
<td>3.8(1.8, 5.9)</td>
<td>0.0003</td>
<td>2.1 (0.50, 3.7)</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at DXA, days</td>
<td>0.5(0.2, 0.9)</td>
<td>0.004</td>
<td>0.53 (0.23, 0.82)</td>
<td>0.0006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-black vs. black, non Hispanic</td>
<td>8.7(3.8, 13.6)</td>
<td>0.0006</td>
<td>3.2 (-1.2, 7.6)</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body length (cm)</td>
<td>3.0(2.3, 3.8)</td>
<td>&lt;0.0001</td>
<td>2.4 (1.7, 3.2)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Model also adjusted for clinical site in addition to age, smoking, CD4 count, viral load, sex, gestational age, age at DXA, race and body length.
Figure 1: Enrollment

Enrolled: 195

Confirmed Eligible at birth: 174

DXA scan performed: 150

DXA scan evaluable: 143

TDF-unexposed Arm: 69

TDF-Exposed: 74