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In Utero Tenofovir Exposure Is not Associated With Fetal Long Bone Growth

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Background. Despite widespread use of tenofovir disoproxil fumarate (TDF) in pregnant and breastfeeding women, few data exist on fetal bone development after in utero TDF exposure. We evaluated fetal long bone growth in human immunodeficiency virus (HIV)–infected pregnant woman/fetus dyads in Cape Town, South Africa.

Methods. Women were recruited from primary care antenatal services and underwent ultrasonography to determine femur (FLZ) and humerus (HLZ) length *z* scores. The duration of in utero TDF exposure was calculated in weeks. Linear regression models were applied to assess the associations between the duration of in utero TDF exposure and change in FLZ and HLZ.

Results. A total of 646 woman/fetus dyads contributed 1376 ultrasonographic scans to this analysis: 132 dyads with ≥25 weeks, 326 with 10–24 weeks, and 188 with <10 weeks of TDF exposure. Women receiving TDF for ≥25 weeks were older than those receiving TDF for 10–24 or <10 weeks (median age, 31 vs 28 and 28 years, respectively; $P < .01$), and had lower HIV RNA levels (median $\log_{10}$ HIV RNA level, 1.59 vs 4.08 and 3.83, respectively; $P < .01$). Throughout gestation, overall median FLZ and HLZ were 0.30 (interquartile range, −0.03 to 0.63) and 0.22 (−0.26 to 0.59) respectively. In multivariate analysis, there was no association between duration of in utero TDF exposure per 1-week increment and change in FLZ (ß = .00; $P = .51$) or change in HLZ (ß = .00; $P = .40$). Results were similar using mixed-effects models.

Conclusions. Although longer follow-up is needed, these in utero data are reassuring and support the continued use of TDF in pregnancy.

Keywords. pregnancy; tenofovir; fetal; femur; humerus.

With recent World Health Organization recommendations endorsing combination antiretroviral therapy (cART) to prevent mother-to-child transmission of human immunodeficiency virus (HIV) [1], the use of cART during pregnancy has greatly expanded in resource-constrained settings. In parallel, the designation of tenofovir disoproxil fumarate (TDF) as part of first-line cART by the World Health Organization [2] has increased the use of TDF in pregnancy dramatically, nearly doubling its use in developing countries [3, 4]. Although the safety of in utero antiretroviral exposure has received attention with other agents [5–7], there are relatively few data on the safety of TDF in pregnancy, and continued monitoring remains necessary because the intrauterine interval is a critical period in which fetal growth and development influences the future health of a child [8–11].

TDF has been reported to affect bone health in both animal [12] and human studies [13, 14]. TDF-containing regimens have been associated with decreased bone density in HIV-infected adults [13, 14] and children [15, 16]. However, much less has been published regarding in utero TDF exposure and its impact on fetal or early infant bone development. Studies in pregnant rhesus macaques have demonstrated compromised intrauterine growth and slightly decreased fetal bone porosity in infants born to high-dose (30 mg/kg) TDF-treated simian immunodeficiency virus–infected and uninfected monkeys [12, 17], raising concerns regarding possible detrimental effects on fetal and infant bone health. In humans, there have been few studies measuring fetal growth under TDF exposure [18, 19]. To address this issue, we examined the association between the duration of in utero TDF exposure and fetal long bone growth in a cohort of HIV-infected pregnant woman/fetus dyads.

MATERIALS AND METHODS

Study Population

As part of a larger study of optimization of antiretroviral therapy (ART) in pregnant and postpartum women (Maternal Child Health-Antiretroviral Therapy study; NCT01933477), we recruited and followed up a cohort of ART-eligible pregnant women seeking antenatal care in a large primary care facility in the peri-urban community of Gugulethu, Cape Town, South Africa, from 2013 to 2015. The local antenatal service, which includes interventions to prevent mother-to-child transmission, is available

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free of charge and provides care to >4000 women annually. ART has been available through local public sector services since 2004 and the antenatal HIV seroprevalence is approximately 30%. The analysis included women aged ≥18 years who were receiving on TDF-containing cART, had ≥2 ultrasonographic (US) scans, and documented cART start dates. Pregnancies ending in miscarriage, abortion, intrauterine fetal demise, or stillbirth were excluded, as were those complicated by multiple gestations, gestational diabetes, or preeclampsia/eclampsia. Informed consent was obtained for all enrolled participants. This study was approved by the institutional review boards of the University of Cape Town as well as ICAP at Columbia University.

Primary Outcome

We measured fetal long bone (femur and humerus) growth using a commercially available US system (General Electric LOGIC C Premium). US was performed by trained research ultrasonographers blinded to the status of TDF exposure. Using a longitudinal view of the fetal thigh closest to the probe, femur lengths were measured with the femur as close as possible to the horizontal plane so that the angle of the ultrasound beam was approximately 90° with the full length of the bone visualized. Electronic calipers were placed on the outer edges of the femoral diaphysis. Humerus lengths were measured in similar fashion. Fetal long bone growth was assessed according to (1) fetal femur length *z* score (FLZ), (2) fetal humerus length *z* score (HLZ), (3) changes in FLZ, and (4) changes in HLZ. The *z* scores were calculated using published standards [20]. Change in FLZ and HLZ were calculated as the difference in *z* scores between the final and the initial US measurements. Data on length at birth, as recorded by clinical staff at delivery facilities, was abstracted from birth records; formulas from the INTERGROWTH study were used to estimate length-for-age z scores (LAZ) [21].

Primary Exposure of Interest

We evaluated the duration of in utero TDF exposure by medical record review and self-report from participants. Self-report was used as the primary source of the date the participant started taking ART. If there was a discrepancy or the self-reported date was implausible, we used the date ART medication was first dispensed, as documented in the medical record. Duration of TDF exposure was calculated in weeks and defined as the number of weeks of TDF exposure before the final US scan for each woman/fetus dyad. We further categorized the duration of TDF exposure as: (1) ≥25 weeks, (2) 10–24 weeks, or (3) <10 weeks before the final scan as the primary categorization of the exposure variable. In secondary analyses, we also categorized TDF exposure as (1) since conception (women who started therapy before pregnancy), (2) ≥4 weeks (initiated after the first trimester), (3) or <4 weeks.

Measurements

Information on potential confounders, including maternal age, gestational age (GA), gravidity, sociodemographics, maternal CD4 cell count and HIV RNA levels, and maternal height, were collected at the time of the initial US scan. GA at each scan was calculated using regression equations described by Hadlock et al [22], both with and without femur length measurements. A composite socioeconomic status variable (based on an aggregate of asset ownership, income, education, and employment status) was categorized as low, medium, or high. Marital status was defined as married or living with a partner versus not married or not living with a partner.

Statistical Analysis

Characteristics of women and fetuses at enrollment were compared between TDF exposure groups using Kruskal–Wallis and χ^2 tests, as appropriate. Mean response profile plots were fit to compare mean femoral and humerus lengths over time between TDF exposure groups. Because calculated GA at each US scan did not differ significantly when we used the equations from Hadlock et al [22] with or without femur length measurements, we used GA calculated by the full formulas. Linear regression models were applied to assess the association between duration of in utero TDF exposure and change in FLZ or HLZ while adjusting for confounders. For these models, we assessed TDF exposure as both a continuous and a categorical variable. In secondary analyses, mixed-effects models with a random intercept and slope were used to evaluate the association of TDF exposure with FLZ and HLZ where TDF exposure was considered a time-varying covariate for both the primary and secondary methods of TDF exposure categorization listed above. We did not impute missing data because <5% had missing (at random) data once inclusion and exclusion criteria were met. Statistical analyses were performed using SAS 9.3 software (SAS Institute)

RESULTS

After exclusion of 29 pregnancies resulting in miscarriage, 25 with multiple gestations, 32 resulting in intrauterine fetal demise or stillbirth, 19 complicated by preeclampsia/eclampsia or gestational diabetes, a total of 646 HIV-infected pregnant woman/fetus dyads contributing 1376 fetal US scans were available for this analysis: 132 dyads with ≥25 weeks, 326 with 10–24 weeks, and 188 with <10 weeks of TDF exposure. Eighty-four women contributed >2 scans each. Overall, median age in the cohort was 28 years (interquartile range [IQR], 25–33 years] and GA at enrollment was 22 weeks (17–27 weeks). The range of total in utero TDF exposure was 2–40 weeks (median, 15 weeks; 9–22 weeks) before the date of the final scan. The median time between the first and last scans was 12 weeks (IQR, 7–15 weeks), and the median GA at the final scan was 34 weeks (33–35 weeks).

Women with ≥25 weeks of TDF exposure were older than those with 10–24 or <10 weeks of exposure (median age, 31 vs 28 and 28 years, respectively; $P < .01$) (Table 1). In addition, women with ≥25 weeks of TDF exposure had higher gravidity (median gravida, 3 vs 2 and 2, respectively; $P < .01$), were more likely to be married or living with a partner (52.3% vs 42.9%

Table 1. Characteristics of Women and Fetuses by Duration of Tenofovir Disoproxil Fumarate Exposure[a]

Maternal and Fetal Characteristics	TDF Exposure Before Final US Scan			*P* Value[b]
	≥25 wk (n = 132)	10–24 wk (n = 326)	<10 wk (n = 188)	
Maternal age, y	31 (28–34)	28 (24–32)	28 (25–32)	<.01
GA, wk	20 (16–28)	18 (15–21)	29 (25–32)	<.01
Total TDF exposure in pregnancy, wk	34 (33–35)	16 (13–19)	5 (3–8)	<.01
Gravidity	3 (2–3)	2 (2–3)	2 (2–3)	<.01
Married or living with partner				<.01
Yes	69 (52.3)	140 (42.9)	62 (33.0)	
No	63 (47.7)	186 (57.1)	126 (67.0)	
Socioeconomic status				.09
Low	32 (24.2)	84 (25.8)	67 (35.6)	
Medium	54 (40.9)	120 (36.8)	66 (35.1)	
High	46 (34.9)	122 (37.4)	55 (29.3)	
Previous LBW infant (<2500 g)	5 (4.8)	4 (1.9)	1 (0.8)	.11
History of tuberculosis	8 (6.7)	11 (3.9)	5 (3.1)	.30
Maternal height, cm	159 (154–163)	157 (153–162)	158 (153–162)	.20
CD4 cell count, cells/mm^3				.05
≥500	43 (32.6)	86 (26.4)	44 (23.4)	. . .
200–499	77 (58.3)	177 (54.3)	106 (56.4)	. . .
<200	12 (9.1)	63 (19.3)	38 (20.2)	. . .
Log_{10} HIV RNA level	1.59 (1.59–1.60)	4.08 (3.36–4.56)	3.83 (3.26–4.58)	<.01
cART regimen in pregnancy				<.01
TDF/XTC/EFV	89 (67.4)	326 (100.0)	188 (100.0)	. . .
TDF/XTC /NVP	34 (25.8)	0 (0.0)	0 (0.0)	. . .
TDF/ XTC/LPV/r	9 (6.8)	0 (0.0)	0 (0.0)	. . .
Fetal *z* score at initial US scan				
Abdominal circumference	0.67 (0.30–1.16)	0.77 (0.24–1.36)	0.79 (0.31–1.19)	.74
Femur length	0.29 (−0.09 to 0.62)	0.33 (−0.11 to 0.81)	0.23 (−0.07 to 0.62)	.44
Humerus length	−0.03 (−0.35 to 0.42)	−0.15 (−0.57 to 0.30)	0.00 (−0.33 to 0.37)	.13

Abbreviations: cART, combination antiretroviral therapy; EFV, efavirenz; GA, gestational age; HIV, human immunodeficiency virus; LBW, low birth weight; LPV/r, lopinavir/ritonavir; NVP, nevirapine; TDF, tenofovir disoproxil fumarate; US, ultrasonographic; XTC, lamivudine or emcitritabine.

[a] Data are reported as median (interquartile range) for continuous variables and No. (%) for categorical variables.

[b] *P* values determined with Kruskal–Wallis tests for continuous variables and χ^2 or Fisher exact tests for categorical variables.

and 33.0%; $P < .01$), were less likely to have CD4 cell counts <200 cells/mm^3 (9.1% vs 19.3% and 20.2%; $P = .05$), and had lower HIV RNA levels (median log_{10} value, 1.59 vs 4.08 and 3.83; $P < .01$). Finally, women with <10 weeks of TDF exposure presented at later GA than those with 10–24 or ≥25 weeks of exposure (29 vs 18 vs 20 weeks, respectively; $P < .01$).

By definition all women in the sample were using TDF plus either lamivudine or emcitritabine. Overall 93.3% (n = 603) of women were using efavirenz, and 1.4% (n = 9) and 5.2% (n = 34) were using lopinavir/ritonavir (LPV/r) or nevirapine (NVP), respectively. All women using LPV/r or NVP were in the group of mother/fetus dyads exposed to TDF for ≥25 weeks.

Fetal anthropometric measurements at the initial US scan did not differ significantly by TDF exposure group (Table 1), and mean fetal and humerus lengths did not differ by groups over time (Figure 1). At enrollment, the overall median FLZ and HLZ were 0.29 (IQR, −0.11 to 0.63) and −0.10 (−0.45 to 0.3), respectively, and these scores did not differ between groups. Throughout gestation, the overall median FLZ and HLZ were 0.30 (IQR, −0.03 to 0.63) and 0.22 (−0.26 to 0.59), respectively. After adjustment for maternal age and height, gravidity, socioeconomic and marital status, maternal CD4 cell count and HIV RNA level at enrollment, and GA at the initial US scan, there was no association between duration of in utero TDF exposure per 1-week increment and change in FLZ (ß = .00; $P = .51$) or change in HLZ (ß = .00; $P = .40$) (Table 2). Results were similar when we assessed TDF exposure as a categorical variable.

In secondary analyses using mixed-effects models, we also did not observe an association between duration of in utero TDF exposure and FLZ (ß = .02 and $P = .19$ for fetuses exposed to TDF for ≥25 vs <10 weeks and ß = −.01 and $P = .45$ for those exposed to TDF for 10–24 vs <10 weeks) or HLZ (ß = .03 and $P = .12$ for fetuses exposed to TDF for ≥25 vs <10 weeks and ß = .00 and $P = .57$ for those exposed to TDF 10–24 vs <10 weeks) over time (Supplementary Table). Our results did not change in a sensitivity analysis when we excluded women receiving LPV/r-based or NVP-based cART.

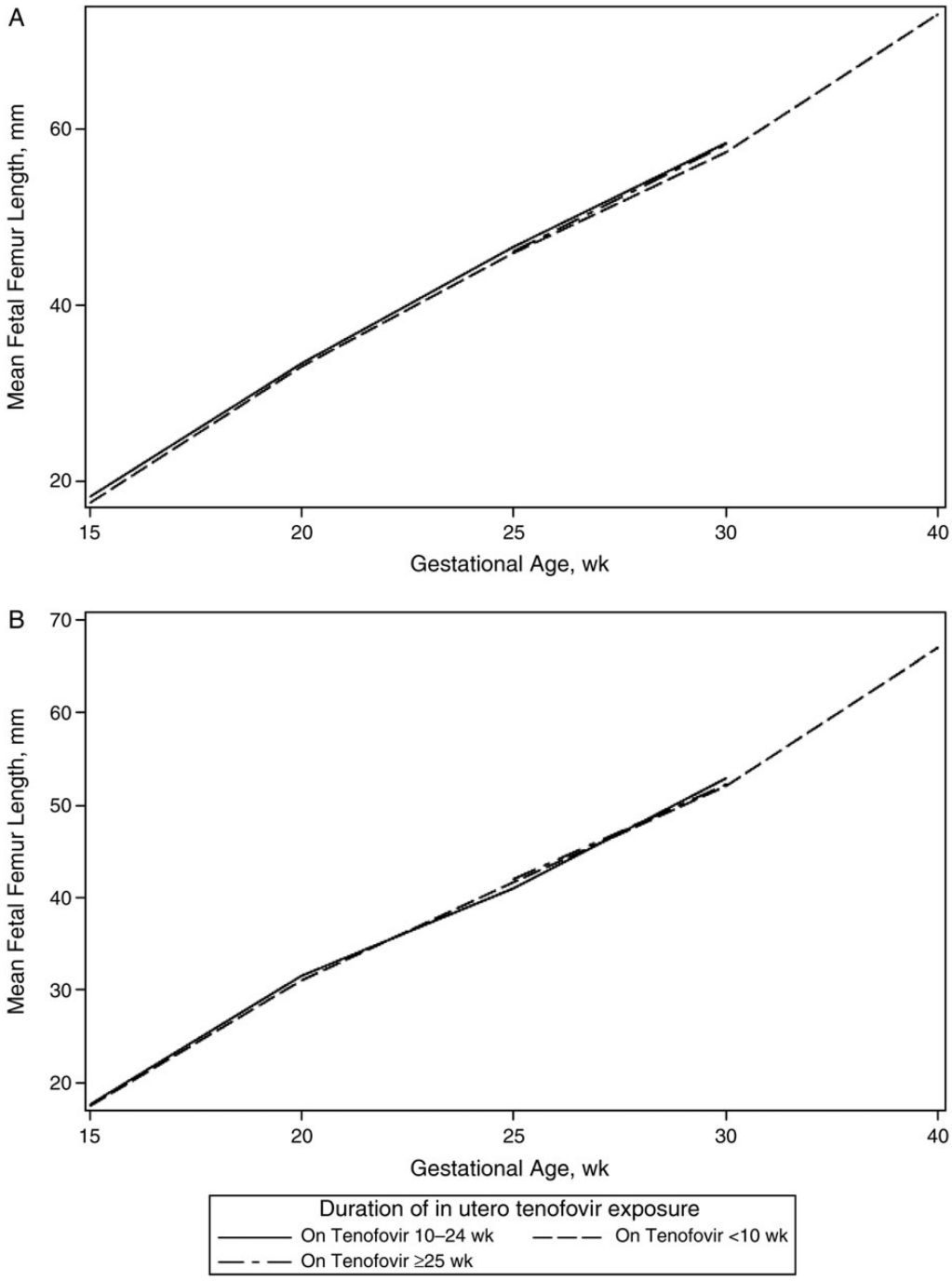

Figure 1. Mean response profiles of mean *A*, femur and *B*, humerus lengths over time by in utero tenofovir exposure.

Finally, we used mixed-effects modeling to also assess whether our secondary method of TDF exposure categorization (TDF exposure since conception vs TDF exposure for ≥4 weeks initiated after the first trimester vs TDF exposure for <4 weeks) was associated with fetal long bone growth, and we found no association between TDF exposure and FLZ (ß = .04 and $P = .56$ for exposure to TDF since conception vs <4 weeks and ß = .01 and $P = .96$ for exposure to TDF ≥4 weeks initiated after the first trimester vs <4 weeks) or HLZ (ß = .01 and $P = .91$ for exposure to TDF since conception vs <4 weeks and ß = −.01 and $P = .89$ for exposure to TDF ≥4 weeks initiated after the first trimester vs <4 weeks) (data not shown). Furthermore, birth lengths were available for 569 pregnancies (88%), and data availability did not differ by TDF exposure category ($P = .20$). Newborns with ≥25 weeks of TDF exposure in utero were longer (mean LAZ, 0.33) than those with 10–24 or <10 weeks of TDF exposure

Table 2. Linear Regression Models for Change in Femur Length z Score and Humerus Length z Score[a]

Effect	Change in FLZ		Change in HLZ	
	Coefficient (95% CI)	*P* Value	Coefficient (95% CI)	*P* Value
Model Assessing TDF Exposure as Continuous Variable				
TDF exposure in pregnancy, per 1-wk increment before last US scan	0.00 (−.01 to .01)	.51	0.00 (−.01 to .01)	.40
Model Assessing TDF Exposure as Categorical Variable				
TDF exposure in pregnancy, wk before last US scan				
≥25	0.05 (−.13 to .23)	.56	−0.21 (−.43 to .03)	.07
10–24	0.08 (−.05 to .21)	.23	−0.11 (−.27 to .06	.21
<10	Reference	. . .	Reference	. . .

Abbreviations: CI, confidence interval; FLZ, femur length *z* score; HLZ, humerus length *z* score; TDF, tenofovir disoproxil fumarate; US, ultrasonographic.

[a] All models adjusted for gestational age at first US scan, maternal age, gravidity, socioeconomic status, marital status, maternal height, and CD4 cell count and human immunodeficiency virus RNA level at enrollment.

(mean LAZ, 0.31 and 0.19, respectively), but this difference did not reach statistical significance ($P = .71$).

DISCUSSION

In this large South African cohort of HIV-infected pregnant woman/fetus dyads with a generally homogenous background of cART, we found no association between duration of in utero TDF exposure and fetal long bone growth. These are novel findings; no published studies, to our knowledge, have specifically used US to evaluate TDF exposure and long bone growth trajectories in mother/fetus dyads from sub-Saharan Africa.

Our results add to the body of literature that describes the short-term safety of TDF during the intrauterine period with regards to fetal growth. Although no studies have reported specifically on fetal long bone growth trajectories, several have reported on fetal weight growth by assessing low birth weight (<2500 g) and small-for-gestational-age outcomes [18, 23, 24] and fetal length growth by assessing birth/neonatal LAZ. All such studies have found no increased risk of impaired fetal growth and/or fetal weight associated with in utero TDF exposure [18, 23, 24]. Beyond intrauterine growth, few studies have evaluated postnatal growth and its association with exposure to in utero TDF [18, 23, 24]. Of these, 2 studies did not report any differences in postnatal growth [23, 24], but 1 reported lower LAZ at 1 year of age in TDF-exposed infants, compared with those not exposed to TDF [18].

Although bone lengthening is correlated with bone formation, cellular activities involved in bone remodeling throughout life better determine the overall strength, thickness, and fragility of bone [25]. Therefore, other studies have evaluated bone health by also assessing bone mineral content and other markers of bone metabolism after in utero TDF exposure. Animal studies in rhesus macaques have shown lower overall body weights and crown-rump lengths than age-matched controls, decreased fetal bone porosity, and reductions of insulin-like growth factor in newborns exposed to in utero TDF [12]. Differences between these results in animal studies and our findings may be explained in part by the differences in TDF dosing, because these pregnant rhesus macaques received 30 mg/kg/d, approximately 7 times the recommended human dose (4–5 mg/kg/d in a 70-kg individual). Studies in humans have reported lower bone mineral content in TDF-exposed than in unexposed neonates [26], which may suggest increased bone fragility with in utero TDF exposure. However, a study in 68 HIV-exposed uninfected children used quantitative US to evaluate total bone resistance in the tibia as an indicator of bone mineral density, cortical thickness, and microstructure and found no difference in bone structure between TDF-exposed and unexposed groups. Biochemical parameters of bone metabolism were also no different between groups [27].

In addition, studies in HIV-uninfected pregnant woman/fetus pairs have demonstrated that late pregnancy growth in fetal femur length and abdominal circumference is positively associated with postnatal bone size and density at 4 years of age [28]. Although we have no postnatal data in this analysis on eventual stature or bone mineral content, the fact that we found largely normal fetal long bone growth trajectories throughout pregnancy indicates that perhaps bone size and density in TDF-exposed children would not likely be severely affected in early childhood.

Our study has several limitations. We lacked a comparison group of HIV-uninfected pregnant woman. We were also not able to compare TDF against other nucleoside reverse-transcriptase inhibitors, given our study design. As a result, because all women were receiving TDF, we would not have been able to detect differences in fetal long bone growth if the effect of TDF on long bone growth was idiosyncratic and not dose or duration dependent. Although we were able to measure in utero bone growth, we were not able to assess bone development at a cellular level or the presence of pathophysiologic abnormalities within the bone during the fetal period.

In conclusion, we did not observe an association between duration of in utero TDF exposure and fetal long bone growth. These results seem reassuring and support the continued use of TDF as part of cART during pregnancy. Further studies are warranted to evaluate long-term postnatal bone health, including bone growth, bone mineral content, and bone metabolism, because long-term effects of in utero TDF exposure are still unknown and TDF use in pregnancy remains increasingly widespread across the world.

Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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References

1. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. Geneva, Switzerland: World Health Organization Press, **2013**. Available at: http://www.who.int/hiv/pub/guidelines/arv2013/en/. Accessed 30 October 2014.
2. World Health Organization. Antiretroviral therapy for HIV infections in adults and adolescents: recommendations for a public health approach. 2010 Revision. Geneva, Switzerland: World Health Organization, **2010**.
3. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector. Progress report 2009. Available at: http://www.who.int/hiv/pub/2009progressreport/en/. Accessed 10 May 2013.
4. World Health Organization. Progress report 2011: global HIV/AIDS response. Available at http://www.who.int/hiv/pub/progress_report2011/en/. Accessed 6 November 2014.
5. Stek AM. Antiretroviral treatment in pregnancy. Curr Opin HIV AIDS **2008**; 3:155–60.
6. Thorne C, Newell ML. The safety of antiretroviral drugs in pregnancy. Expert Opin Drug Saf **2005**; 4:323–35.
7. Watts DH, Covington DL, Beckerman K, et al. Assessing the risk of birth defects associated with antiretroviral exposure during pregnancy. Am J Obstet Gynecol **2004**; 191:985–92.
8. Geremia C, Cianfarani S. Insulin sensitivity in children born small for gestational age (SGA). Rev Diabet Stud **2004**; 1:58–65.
9. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. Lancet **1989**; 2:577–80.
10. Jaquet D, Collin D, Levy-Marchal C, Czernichow P. Adult height distribution in subjects born small for gestational age. Horm Res **2004**; 62:92–6.
11. Levy-Marchal C, Jaquet D. Long-term metabolic consequences of being born small for gestational age. Pediatr Diabetes **2004**; 5:147–53.
12. Tarantal AF, Castillo A, Ekert JE, Bischofberger N, Martin RB. Fetal and maternal outcome after administration of tenofovir to gravid rhesus monkeys (*Macaca mulatta*). J Acquir Immune Defic Syndr **2002**; 29:207–20.
13. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. JAMA **2004**; 292:191–201.
14. Jones S, Restrepo D, Kasowitz A, et al. Risk factors for decreased bone density and effects of HIV on bone in the elderly. Osteoporos Int **2008**; 19:913–8.
15. Gafni RI, Hazra R, Reynolds JC, et al. Tenofovir disoproxil fumarate and an optimized background regimen of antiretroviral agents as salvage therapy: impact on bone mineral density in HIV-infected children. Pediatrics **2006**; 118: e711–8.
16. Purdy JB, Gafni RI, Reynolds JC, Zeichner S, Hazra R. Decreased bone mineral density with off-label use of tenofovir in children and adolescents infected with human immunodeficiency virus. J Pediatr **2008**; 152:582–4.
17. Tarantal AF, Marthas ML, Shaw JP, Cundy K, Bischofberger N. Administration of 9-[2-(R)-(phosphonomethoxy)propyl]adenine (PMPA) to gravid and infant rhesus macaques (*Macaca mulatta*): safety and efficacy studies. J Acquir Immune Defic Syndr Hum Retrovirol **1999**; 20:323–33.
18. Siberry GK, Williams PL, Mendez H, et al. Safety of tenofovir use during pregnancy: early growth outcomes in HIV-exposed uninfected infants. AIDS **2012**; 26:1151–9.
19. Himes SK, Wu JW, Jacobson DL, et al. Meconium tenofovir concentrations and growth and bone outcomes in prenatally tenofovir exposed HIV-uninfected children. Pediatr Infect Dis J **2015**; 34:851–7.
20. Chitty LS, Altman DG. Charts of fetal size: limb bones. BJOG **2002**; 109:919–29.
21. Villar J, Cheikh Ismail L, Victora CG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. Lancet **2014**; 384:857–68.
22. Hadlock FP, Deter RL, Harrist RB, Park SK. Estimating fetal age: computer-assisted analysis of multiple fetal growth parameters. Radiology **1984**; 152: 497–501.
23. Ransom CE, Huo Y, Patel K, et al. Infant growth outcomes after maternal tenofovir disoproxil fumarate use during pregnancy. J Acquir Immune Defic Syndr **2013**; 64:374–81.
24. Gibb DM, Kizito H, Russell EC, et al. Pregnancy and infant outcomes among HIV-infected women taking long-term ART with and without tenofovir in the DART trial. PLoS Med **2012**; 9:e1001217.
25. Bronner F, Farach-Carson M. Bone formation. London, United Kingdom, and New York, NY: Springer, **2004**.
26. Siberry GK, Jacobson DL, Kalkwarf HJ, et al. Lower newborn bone mineral content associated with maternal use of tenofovir disoproxil fumarate during pregnancy. Clin Infect Dis **2015**; 61:996–1003.
27. Vigano A, Mora S, Giacomet V, et al. In utero exposure to tenofovir disoproxil fumarate does not impair growth and bone health in HIV-uninfected children born to HIV-infected mothers. Antivir Ther **2011**; 16:1259–66.
28. Harvey NC, Mahon PA, Robinson SM, et al. Different indices of fetal growth predict bone size and volumetric density at 4 years of age. J Bone Miner Res **2010**; 25:920–7.