Safety of tenofovir use during pregnancy: early growth outcomes in HIV-exposed uninfected infants

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Objective: To evaluate the association of tenofovir disoproxil fumarate (TDF) use during pregnancy with early growth parameters in HIV-exposed, uninfected (HEU) infants.

Design: US-based prospective cohort study of HEU children to examine potential adverse effects of prenatal TDF exposure.

Methods: We evaluated the association of maternal TDF use during pregnancy with small for gestational age (SGA); low birth weight (LBW, <2.5 kg); weight-for-age z-scores (WAZ), length-for-age z-scores (LAZ), and head circumference-for-age (HCAZ) z-scores at newborn visit; and LAZ, HCAZ, and WAZ at age 1 year. Logistic regression models for LBW and SGA were fit, adjusting for maternal and sociodemographic factors. Adjusted linear regression models were used to evaluate LAZ, WAZ, and HCAZ by TDF exposure.

Results: Of 2029 enrolled children with maternal antiretroviral information, TDF was used by 449 (21%) HIV-infected mothers, increasing from 14% in 2003 to 43% in 2010. There was no difference between those exposed to combination regimens with vs. without TDF for SGA, LBW, and newborn LAZ and HCAZ. However, at age 1 year, infants exposed to combination regimens with TDF had significantly lower adjusted mean LAZ and HCAZ than those without TDF (LAZ: −0.17 vs. −0.03, $P = 0.04$; HCAZ: 0.17 vs. 0.42, $P = 0.02$).

Conclusion: TDF use during pregnancy was not associated with increased risk for LBW or SGA. The slightly lower mean LAZ and HCAZ observed at age 1 year in TDF-exposed infants are of uncertain significance but underscore the need for additional studies of growth outcomes after TDF use during pregnancy.

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Introduction

Tenofovir disoproxil fumarate (TDF), in combination with other antiretroviral drugs, is recommended as first-line therapy for HIV-infected adults because of its proven safety and efficacy [1]. The recommendation for TDF use in pregnant women for treatment of maternal HIV infections and for prevention of maternal–infant HIV transmission, however, has been limited by concerns about potential detrimental effects of maternal TDF use on fetal growth and bone mineralization [2].

In studies of pregnant Rhesus macaques, administration of tenofovir at high doses beginning in the first trimester resulted in lower crown-rump length, lower body weight, and smaller adrenal glands but no difference in head, arm, or chest circumferences or extremity bone lengths, compared with tenofovir-unexposed control monkeys [3]. Tenofovir-exposed macaque fetuses also exhibited lower circulating insulin-like growth factor-1 (IGF-1) levels and did not demonstrate the normal rise in IGF-1 that occurs during the second and third trimesters [3]. Similarly, high-dose tenofovir administration to infant macaques was associated with infant growth restriction [4]. With administration of lower tenofovir doses to macaques during pregnancy or after birth, however, growth restriction was not observed, suggesting that effects may be dose-dependent [4,5].

Human fetal TDF exposure data are generally limited to TDF initiated around the onset of labor [6]. Efficient transplacental transfer of tenofovir to human fetuses has been demonstrated [7]. In a chart review study, only one of 14 live-born infants whose mothers used TDF during pregnancy was small for gestational age (SGA) [8]. There are no other published studies of infant growth outcomes after prolonged TDF use during pregnancy.

The purpose of this investigation was to evaluate the association of TDF exposure *in utero* with infant size at birth and infant growth at age 1 year.

Methods

Study population and procedures

We conducted an analysis of *in utero* TDF exposure in combination with other antiretroviral drugs based on data collected in the Surveillance Monitoring for Antiretroviral Therapy Toxicities (SMARTT) study of the Pediatric HIV/AIDS Cohort Study (PHACS) network. The SMARTT study enrolled two cohorts: the Static cohort enrolled children aged 1–12 years who were previously enrolled in other prospective cohort studies or who otherwise had detailed information available on maternal antiretroviral exposure by trimester; the Dynamic cohort enrolled newborns and their mothers between 22 weeks gestation and 1 week after birth. The SMARTT protocol was approved by Human Subject Research review boards at each of the participating sites and by the Harvard School of Public Health. Written informed consent was obtained from the parent or legal guardian.

Birth weight and gestational age were collected retrospectively in the Static cohort; weight, length, and head circumference were obtained at age 1 year only in Static cohort individuals who enrolled in SMARTT by age 1 year. Birth weight, gestational age, current weight, length, and head circumference were obtained at the newborn exam (within 2 weeks after birth) and at each annual visit for Dynamic cohort infants. Weight, length, and head circumference measurements followed standardized protocols, with each measurement performed three times at each visit. Maternal antiretroviral drug use, maternal health status (HIV viral load, CD4 cell count, and CD4%) early during pregnancy and prior to delivery, maternal genital infections and complications during pregnancy were obtained by chart abstraction, and alcohol, marijuana, and other illicit drugs use by self-report [9], both overall and by trimester. All individuals with reported birth weight and maternal antiretroviral exposure information as of 1 January 2011 were included in the current analysis.

Statistical methods

The Centers for Disease Control and Prevention (CDC) 2000 growth standards were used to calculate age-adjusted and sex-adjusted z-scores for birth weight and for weight (WAZ), length (LAZ), and head circumference (HCAZ) for full-term infants at the newborn visit and at age one year [10]. For premature infants, standards developed by Fenton and Suave [11] were used to correct for completed weeks of gestational age in calculation of z-scores. For infants born less than 37 weeks of gestational age, z-scores at age 1 year were corrected by subtracting weeks of prematurity (40 – birth gestational age) from the exact age at the 1-year visit. Infants with birth weight below the 10th percentile for gestational age were considered SGA [12].

Associations of *in utero* TDF exposure with binary outcomes including low birth weight (LBW, <2.5 kg) and SGA at birth were evaluated using logistic regression models to obtain unadjusted odds ratios (ORs) and OR adjusted for potential confounders (aOR). We used multiple linear regression models to evaluate associations of *in utero* TDF exposure with birth WAZ and with WAZ, LAZ, and HCAZ at the newborn visit and at the 1-year study visit (including measurements from children aged 9–18 months) as continuous measures, adjusted for potential confounders. Although the 1-year study visit window was 9–18 months of age, calculation of z-scores was based on the actual age at the time of that visit. We also evaluated low birth length and head circumference

based on z-scores less than −1.50 (<6.7th percentile) and based on newborn visit WAZ, LAZ, and HCAZ less than −1.88 (<3rd percentile). Similarly, we considered binary outcomes of impaired infant growth at the age 1-year study visit based on WAZ, LAZ, and HCAZ less than −1.5 and less than −1.88. We included small size outcomes defined as z-score less than −1.5 in addition to the more standard definition of small size as z-score less than −1.88 in order to have sufficient participants in the 'small' category to be able to assess potential associations of several factors with small size outcomes.

We considered TDF exposure at any time during pregnancy and by TDF duration in months during pregnancy. To reduce potential for selection bias, we restricted our primary models to consider only those exposed *in utero* to combination antiretrovirals (cARV) regimens (≥three drugs from ≥two drug classes) and compared those exposed to cARV, including TDF to those exposed to cARV without TDF. Initial models did not include gestational age due to the possibility of this covariate being on the causal pathway between exposure and birth or growth outcomes. However, sensitivity analyses were conducted to adjust for gestational age, based on the well established association of gestational age with these birth and growth outcomes.

Potential confounders we considered included socio-demographic factors (sex, race, and ethnicity of infant; household income; caregiver education level; marital status), maternal health status during pregnancy (viral load and CD4 cell count measurements and maternal genital infection), and maternal substance use (including smoking) during pregnancy. Univariate models for each potential confounder were first fit for each outcome. Multivariate models were then fit, including TDF exposure and all covariates with *P*-value less than 0.20 in univariate models and then reduced to a core model for each outcome including the TDF variable and only those covariates with *P*-value less than 0.10 or which changed effect estimates for TDF by at least 10%.

SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA) was used to conduct all statistical analyses, and two-sided *P*-values less than 0.05 were considered statistically significant.

Results

Characteristics of study population

Of 2279 individuals enrolled in SMARTT (1240 in the Static cohort and 1039 in the Dynamic cohort), 2029 (89%) had detailed maternal antiretroviral exposure information available, including exposure by trimester. Among these, 2006 had birth weight reported and 1980 had both birth weight and gestational age data allowing identification of SGA. For the Dynamic cohort, 812 had information on length and 800 on head circumference at birth or within 1 month after birth. Growth outcomes at age 1 year, limited to those who reached age 1 year by data freeze date, were available on 677 individuals with maternal antiretroviral information. TDF exposure increased from 14% in 2003 to 43% in 2010; TDF was used by 449 (21%) of 2029 HIV-infected mothers overall, including 263 (13%) who used TDF during the first trimester. The median duration of TDF exposure was 4.8 months (interquartile range: 2.2, 8.0). Maternal and demographic characteristics are summarized in Table 1 within each subgroup forming the basis for analysis of LBW and SGA other birth measurements, and growth measurements at age 1 year.

Birth weight and small for gestational age

LBW was observed in 382 (19%) and very LBW (<1.5 kg) in 51 (2.6%) of the 2006 infants with birth weight data; 162 of 1980 infants (8.6%) were SGA. Among those exposed to maternal cARV ($N = 1582$, 79%), there was no difference in prevalence of LBW by TDF exposure (19.5% for TDF-exposed vs. 19.1% for TDF-unexposed, $P = 0.87$) (Table 2) [12]. After adjusting for high maternal viral load prior to delivery, maternal tobacco use during pregnancy, female sex of infant, low annual household income, and birth cohort, there remained no association of LBW with TDF exposure (aOR = 0.87, $P = 0.40$). More advanced gestational age was strongly associated with a decreased odds of LBW (aOR = 0.40 per week of gestation, $P < 0.001$). However, adjusting for gestational age had little effect on the association of LBW with TDF exposure (aOR = 0.73, $P = 0.14$). We also observed no association of LBW with duration of TDF exposure (aOR per month TDF exposure = 1.00, $P = 0.88$). Birth WAZ among those exposed *in utero* to cARV also showed no association with TDF exposure, with a mean WAZ of −0.58 (SEM = 0.04) for TDF-exposed vs. −0.59 (SEM = 0.03) for TDF-unexposed, after adjustment for potential confounders (Table 3).

The results for SGA were similar to those for LBW (Table 2). There was no difference in prevalence of SGA by TDF exposure (8.3% for TDF-exposed vs. 8.6% for TDF-unexposed, $P = 0.85$); the lack of association persisted after adjustment for nonwhite race, maternal tobacco use during pregnancy, maternal gonorrhea infection, and low-income level (aOR = 1.04, $P = 0.88$), and after additional adjustment for gestational age (aOR = 0.96, $P = 0.85$). There was also no association of duration of TDF exposure with SGA (aOR per month = 1.04, $P = 0.31$), adjusted for the above covariates. Sensitivity analyses fit separately to the Static and Dynamic cohorts yielded results consistent with the overall study population, indicating no association of TDF exposure with LBW or SGA either with or without adjustment for potential confounders (data not shown).

Table 1. Demographic and maternal characteristics for the Surveillance Monitoring for Antiretroviral Therapy Toxicities (SMARTT) participants within subpopulations of interest.

Characteristic	Among those with birth weight information ($N=2006$)	Among those with birth length or head circumference ($N=869$)	Among those with weight, length or head circumference at 1 year of age ($N=677$)
Infant characteristics[a]			
Cohort			
Dynamic	855 (43%)	869 (100%)	480 (71%)
Static	1151 (57%)	0 (0%)	197 (29%)
Birth cohort			
<2002	374 (19%)	0 (0%)	0 (0%)
2002–2004	327 (16%)	0 (0%)	0 (0%)
2005–2007	513 (26%)	110 (13%)	233 (34%)
2008–2010	792 (39%)	759 (87%)	444 (66%)
Female	974 (49%)	422 (49%)	342 (51%)
Black/African-American	1325 (66%)	597 (69%)	440 (65%)
Latino/Hispanic	669 (33%)	264 (30%)	223 (33%)
Cesarean delivery	1078 (54%)	609 (61%)	397 (59%)
Gestational age			
<32 weeks	52 (3%)	23 (3%)	24 (4%)
32 to <37 weeks	351 (17%)	152 (17%)	131 (19%)
≥37 weeks	1516 (76%)	668 (77%)	516 (76%)
Maternal ARV regimens during pregnancy			
Maternal ARV regimen			
Combination with PI and NNRTI	132 (7%)	55 (6%)	44 (6%)
Combination with PI	1277 (64%)	644 (74%)	515 (76%)
Combination with NNRTI	173 (9%)	41 (5%)	26 (4%)
Not on combination ARV	424 (21%)	129 (15%)	92 (14%)
TDF and combination ARV exposure			
Combination with TDF	426 (21%)	293 (34%)	217 (32%)
Combination without TDF	1156 (58%)	447 (51%)	368 (54%)
Maternal/caregiver demographic and socioeconomic characteristics[b]			
Maternal age <25 years at birth of child	687 (34%)	268 (34%)	206 (30%)
Marital status			
Married	523 (26%)	192 (21%)	162 (24%)
Separated/divorced/widowed	225 (11%)	64 (7%)	46 (7%)
Single, never married	1261 (63%)	607 (71%)	456 (67%)
Not reported	8 (<1%)	6 (<1%)	1 (<1%)
Annual household income <$20 000	1271 (63%)	556 (64%)	424 (63%)
Caregiver educational level < high school	691 (34%)	301 (35%)	229 (34%)
Maternal health and substance use during pregnancy[c]			
Maternal RNA >1000 copies/ml early in pregnancy	1052 (52%)	457 (53%)	364 (54%)
Maternal RNA > 1000 copies/ml at delivery	331 (17%)	125 (14%)	92 (14%)
Maternal CD4 cell count <250 cells/μl early in pregnancy	363 (18%)	170 (20%)	137 (20%)
Maternal CD4 cell count <250 cells/μl at delivery	304 (15%)	146 (17%)	125 (18%)
Illicit drug use (marijuana, cocaine, heroin or opiates)	156 (8%)	75 (9%)	46 (7%)
Hard drug use (cocaine, heroin, opiates)	51 (3%)	23 (3%)	19 (3%)
Alcohol use	151 (8%)	83 (10%)	62 (9%)
Tobacco use	337 (17%)	151 (17%)	118 (17%)
Maternal genital infection[d]	392 (20%)	160 (20%)	130 (19%)
Hepatitis B surface antigen-positive	50 (2%)	17 (2%)	15 (2%)

Numbers missing are among the 2006 with birth weight information, but percentages missing are similar within the two other subgroups. Percentages in table do not exclude those missing information. ARV, antiretroviral drug; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate.

[a]Information was missing or caregiver chose not to report data for race ($N=132$, 7%), ethnicity ($N=3$, <1%), delivery mode ($N=24$, 1%), and exact gestational age ($N=87$, 4%), although prematurity status was known for 70 of the 87.

[b]Information was missing or caregiver chose not to report data for maternal age at birth of child ($N=27$, 1%), marital status ($N=8$, <1%), household income ($N=142$, 7 %), and caregiver education ($N=8$, <1%).

[c]Information was missing or caregiver chose not to report data for maternal viral load ($N=155$, 8%), maternal CD4 cell ($N=120$, 6%), substance use including alcohol and tobacco during pregnancy ($N=163$, 8%), genital infection ($N=159$, 8%), and hepatitis B status ($N=268$, 13%).

[d]Maternal genital infections among those with birth weight information included gonorrhea, 3.0%; chlamydia, 9.2%; trichomonas, 11.9%; and syphilis, 3.2%.

Birth measurements among infants in the Dynamic study

Infants in the Dynamic cohort overall tended to be small at the newborn visit, with mean (SD) WAZ, LAZ, and HCAZ (adjusted for prematurity, as necessary) of −0.61 (0.89), −0.19 (1.01), and −0.65 (0.86), respectively. Similar mean z-scores were observed within the subset of Dynamic infants exposed *in utero* to cARV (Table 3). All of the above mean z-scores were significantly lower than the standard reference population mean of

Table 2. Effects of tenofovir disoproxil fumarate exposure vs. no tenofovir disoproxil fumarate exposure on newborn outcomes among those on combination antiretroviral regimens during pregnancy, Surveillance Monitoring for Antiretroviral Therapy Toxicities (SMARTT) Study, 2007–2010, USA.

	Percentage with outcome		Unadjusted models			Adjusted models[b]			Fully adjusted models including gestational age		
Outcome[a]	TDF exposed	No TDF	*N*	OR (95% CI)	*P*-value	*N*	aOR (95% CI)	*P*-value	*N*	aOR (95% CI)	*P*-value
LBW	19.5%	19.1%	1582	1.02 (0.77, 1.36)	0.87	1338	0.87 (0.63, 1.20)	0.40	1302	0.73 (0.48, 1.11)	0.14
SGA	8.3%	8.6%	1569	0.96 (0.64, 1.44)	0.85	1189	1.04 (0.65, 1.64)	0.88	1148	0.96 (0.60, 1.52)	0.85
Length z score <-1.5[c]	8.7%	9.0%	701	0.96 (0.56, 1.65)	0.89	614	1.28 (0.73, 2.25)	0.40	609	1.18 (0.66, 2.10)	0.58
HC z score <-1.5[c]	13.9%	16.8%	690	0.80 (0.52, 1.22)	0.30	676	0.81 (0.52, 1.25)	0.34	672	0.82 (0.53, 1.28)	0.39

aOR, adjusted odds ratio; CI, confidence interval; HC, head circumference; LBW, low birth weight; SGA, small for gestational age; TDF, tenofovir disoproxil fumarate.
[a]LBW defined as <2.5 kg, SGA defined as birth weight less than the 10th percentile for gestational age [12].
[b]The estimates presented above are from separate models for each outcome adjusted for covariates with $P<0.10$ in multivariate models, including for LBW: female infant sex, annual household income <$20 000, maternal viral load >1000 copies/ml prior to labor and delivery, maternal tobacco use during pregnancy, and birth cohort (2002–2004, 2005–2007, and 2008–2010 vs. <2002); for SGA: annual household income <$20 000, nonwhite race, maternal tobacco use during pregnancy, and maternal gonorrhea during pregnancy; for short birth length: female sex and maternal gonorrhea infection; and for small head circumference at birth: female sex, CD4 cell count <250 cells/mm^3 early during pregnancy, low caregiver education, and maternal age <25 years at delivery.
[c]Available only for Dynamic cohort individuals, based on measurements at newborn study visit.

0. The percentage with z-scores less than −1.5 (<6.7th percentile) and less than −1.88 (<3rd percentile), respectively, were 8.7 and 4.7% for LAZ and 15 and 6.1% for HCAZ.

In the Dynamic cohort, 35% of all HIV-infected mothers and 40% of those receiving cARV used TDF during pregnancy. Among those exposed to cARV (85%), there was no difference in mean newborn LAZ by TDF exposure (−0.25 vs. −0.18 for TDF-exposed vs. unexposed); nor was there any difference in mean newborn HCAZ (−0.66 vs. −0.68 for TDF-exposed vs. unexposed) (Table 3). There remained no difference after adjustment for potential confounders (Table 3). Additional analyses based on models using binary outcomes for LAZ and HCAZ less than −1.5 (Table 2) and less than −1.88 (data not shown) yielded similar results.

Age 1 year: weight, length, and head circumference

By 1 year of age, the infants were closer to US growth standards, with mean (SD) WAZ of −0.06 (1.15), mean LAZ of −0.03 (1.06), and mean HCAZ of 0.34 (1.20) for the overall cohort and similar means for the subset of infants with *in utero* cARV exposure (Table 4). There was a slight but statistically significantly lower mean LAZ and HCAZ in infants exposed to cARV with vs. without TDF (Table 4). These differences in adjusted mean z-scores

Table 3. Mean z-scores for birth weight and for weight, length, and head circumference at newborn visit, by exposure to combination antiretroviral regimen with tenofovir disoproxil fumarate vs. combination antiretroviral regimen without tenofovir disoproxil fumarate during pregnancy, unadjusted and adjusted for other covariates.

Gestation or age-adjusted z-score	Among combination ARV-exposed infants *N*, mean (SD)	Exposed to combination regimens with TDF		Exposed to combination regimens without TDF		TDF vs. non-TDF *P*-value[b]
		Unadjusted *N*, mean (SD)	Adjusted[a] mean (SEM)	Unadjusted *N*, mean (SD)	Adjusted[a] mean (SEM)	
z-Scores among all participants at birth: Dynamic ($N=855$) and Static ($N=1151$) cohorts						
Weight	1567, −0.59 (0.85)	421, −0.59 (0.81)	−0.58 (0.04)	1146, −0.60 (0.86)	−0.59 (0.03)	0.77
z-Scores from growth measurements at the newborn examination (within 2 weeks of birth): Dynamic cohort only ($N=815$)						
Weight	702, −0.65 (0.82)	278, −0.62 (0.82)	−0.63 (0.05)	424, −0.66 (0.82)	−0.66 (0.04)	0.58
Length	701, −0.21 (1.00)	277, −0.25 (1.00)	−0.25 (0.07)	424, −0.18 (1.01)	−0.16 (0.05)	0.29
HC	690, −0.67 (0.85)	274, −0.66 (0.84)	−0.66 (0.06)	416, −0.68 (0.85)	−0.65 (0.05)	0.83

ARV, antiretroviral; HC, head circumference; TDF, tenofovir disoproxil fumarate.
[a]The estimates presented above are from separate models for each outcome adjusted for covariates with $P<0.10$ in multivariate models, including birth weight z-score (all individuals): gestational age, female sex, low-income level, maternal use of illicit drugs during pregnancy, and low CD4 cell count early in pregnancy; birth weight z-score (from newborn examination): female sex, nonwhite race, low caregiver education, and maternal gonorrhea infection; birth length z-score: gestational age, female sex, and maternal gonorrhea; birth HC z-score: gestational age, low household income level, and gonorrhea infection.
[b]*P*-value from linear regression model comparing adjusted means for TDF-exposed vs. unexposed.

Table 4. Mean z-scores for weight, length, and head circumference at age 1 year, by exposure to combination antiretroviral regimen with tenofovir disoproxil fumarate vs. combination antiretroviral regimen without tenofovir disoproxil fumarate during pregnancy, unadjusted and adjusted for other covariates.

Gestation or age-adjusted z-score	Among combination ARV-exposed infants *N*, mean (SD)	Exposed to combination regimens with TDF		Exposed to combination regimens without TDF		TDF vs. non-TDF *P*-value[b]
		Unadjusted *N*, mean (SD)	Adjusted[a] mean (SEM)	Unadjusted *N*, mean (SD)	Adjusted[a] mean (SEM)	
Weight	585, −0.07 (1.16)	217, −0.11 (1.20)	−0.09 (0.08)	368, −0.05 (1.14)	−0.04 (0.06)	0.62
Length	582, −0.05 (1.09)	215, −0.17 (1.00)	−0.17 (0.07)	367, 0.02 (1.14)	−0.03 (0.06)	0.04
HC	570, 0.34 (1.20)	209, 0.23 (1.10)	0.17 (0.08)	361, 0.41 (1.25)	0.42 (0.06)	0.02

TDF, tenofovir disoproxil fumarate.
[a]The estimates presented above are from separate models for each outcome adjusted for covariates with $P<0.10$ in multivariate models, including weight z-score at 1 year: gestational age and high maternal viral load prior to delivery; length z-score at 1 year: Latino ethnicity, high maternal viral load prior to delivery, and maternal use of tobacco during pregnancy; head circumference z-score at 1 year: low-income level, low caregiver education level, and maternal use of illicit drugs during pregnancy.
[b]*P*-value from linear regression model comparing adjusted means for TDF-exposed vs. unexposed.

correspond approximately to an average 0.41 cm shorter 1-year length and an average 0.32 cm smaller 1-year head circumference in the TDF-exposed group. The adjusted mean LAZ was slightly below the standard population mean for the TDF group (−0.17), but near 0 for the non-TDF group (−0.03). In contrast, the mean HCAZ was above 0 for TDF-exposed and TDF-unexposed. At 2 year of age, there was no significant difference between those receiving cARV with vs. without TDF for low growth measures defined as WAZ, LAZ, or HCAZ less than −1.5 (Table 5) or less than −1.88 (data not shown) in either crude or adjusted models. The findings at 1 year and at birth for all measures were similar when TDF exposure was further divided into early (first trimester) and later (second and third trimester) exposure vs. no exposure (data not shown).

Other predictors of growth outcomes in the adjusted models

Increased odds of LBW was observed for female infants [aOR = 1.29, 95% confidence interval (CI) 0.98, 1.70, $P = 0.07$], those whose mothers had viral load more than 1000 copies/ml prior to delivery (aOR = 1.57, 95% CI 1.11, 2.21, $P = 0.01$) or used tobacco during pregnancy (aOR = 1.43, 95% CI 1.02, 2.01, $P = 0.04$), and children from families with annual household income less than $20 000 (aOR = 1.31, 95% CI 0.96, 1.79, $P = 0.09$). Odds of LBW were lower for infants born before 2002 vs. those born 2008–2010 (aOR = 0.54, 95% CI 0.33, 0.89, $P = 0.06$). Higher odds of SGA was associated with low income (aOR = 1.95, 95% CI 1.17, 3.25, $P = 0.01$) and with maternal gonorrhea (aOR = 2.78, $P = 0.02$) or tobacco use (aOR = 1.55, $P = 0.08$) during pregnancy. Nonwhite infants had a marginally decreased odds of SGA (aOR = 0.68, 95% CI 0.44, 1.04, $P = 0.08$).

Several socioeconomic and maternal health measures also showed significant associations with z-scores at birth and age 1 year: female infants had significantly lower newborn visit WAZ and LAZ, lower caregiver education was associated with significantly lower newborn visit WAZ and HCAZ at age 1 year, and low household income was associated with significantly lower z-scores for WAZ and HCAZ at newborn visit and HCAZ at age 1 year. Maternal gonorrhea infection was associated with lower z-scores for all newborn visit measures (WAZ, LAZ, and HCAZ), whereas high maternal viral load prior to delivery was paradoxically associated with significantly higher WAZ and LAZ at age 1 year. Although illicit drug use was relatively uncommon in our cohort

Table 5. Effects of exposure to combination antiretroviral regimen with tenofovir disoproxil fumarate vs. combination antiretroviral regimen without tenofovir disoproxil fumarate during pregnancy on infant growth outcomes at age 1 year, Surveillance Monitoring for Antiretroviral Therapy Toxicities (SMARTT) Study, 2007–2010, USA.

Gestation-adjusted or age-adjusted z-score<−1.5	Percentage with outcome TDF exposed	No TDF	Unadjusted models			Adjusted models[a]			Fully adjusted models including gestational age		
			N	OR (95% CI)	*P*-value	*N*	aOR (95% CI)	*P*-value	*N*	aOR (95% CI)	*P*-value
Weight	11.5%	9.5%	585	1.24 (0.72, 2.13)	0.44	582	1.25 (0.72, 2.15)	0.42	578	1.25 (0.73, 2.17)	0.42
Length	7.9%	8.2%	582	0.97 (0.52, 1.79)	0.91	561	0.94 (0.50, 1.78)	0.86	558	0.95 (0.50, 1.79)	0.87
HC	6.2%	5.8%	570	1.07 (0.53, 2.19)	0.84	545	1.15 (0.56, 2.39)	0.70	541	1.17 (0.57, 2.42)	0.67

aOR, adjusted odds ratio; CI, confidence interval; TDF, tenofovir disoproxil fumarate.
[a]Logistic regression model adjusted for the following covariates: low weight at 1 year: maternal age <25 years at delivery, high maternal viral load early in pregnancy, genital infection during pregnancy, and maternal use of hard drugs during pregnancy; for short length at 1 year: female sex; and for small head circumference at 1 year: maternal use of hard drugs during pregnancy.

(approximately 8%), its use was associated with significantly lower HCAZ at age 1 year, and maternal tobacco use was associated with significantly lower LAZ at age 1 year.

Discussion

The increasing use of TDF by HIV-infected pregnant women warrants careful evaluation of the safety of this agent. Over 40% of pregnant mothers in our study used TDF during pregnancy in 2010, more than doubling TDF use in the last 5 years. TDF exposure was associated with significantly lower mean LAZ and lower HCAZ at age 1 year but not at birth, an unexpected finding of uncertain significance. The magnitudes of these differences were quite small – corresponding to an average difference of less than 0.5 cm for mean length and mean head circumference – and the biologic mechanisms underlying a delayed effect on infant growth outcomes after *in utero* TDF exposure are not readily explained. Later growth differences, especially for length in which mean z-scores were less than 0 in the TDF group, should be evaluated in other cohorts. The overall findings of this extensive analysis, however, are highly reassuring. The proportion of children at age one year with low LAZ and low HCAZ ($z<-1.5$ and $z<-1.88$) did not differ by TDF exposure. Furthermore, there was no association of TDF exposure with lower weight, shorter length, or smaller head circumference in the newborn period, whether these outcomes were defined based on mean z-scores or on z-scores below thresholds of -1.5 and -1.88. Analyses of longer-term growth and neurodevelopmental outcomes are underway in the SMARTT protocol.

The association of maternal TDF use with lower length and head circumference at 1 year but not in the newborn period was not predicted by animal studies. This observation suggests that maternal TDF use does not affect fetal growth but could lead to a delayed effect on infant growth in the first year, after ongoing exposure to maternal TDF has ceased. Adjustment for maternal HIV disease, demographic factors, and substance use suggests that the impaired infant growth is not related to confounding of maternal TDF use by these well known influences on infant outcomes. In addition, more than 99% of all infants received zidovudine prophylaxis, of whom 10% were given additional antiretroviral drugs for prophylaxis (data not shown), making it unlikely that infant growth differences were related to different neonatal antiretroviral drug exposures. Although newborn length and gestational age at birth are important and often interrelated predictors of length at 1 year, the association of maternal TDF with lower infant length at 1 year persisted despite adjusting for these factors. Women in this US-based study would have been counseled to not breastfeed their infants, eliminating the potential for ongoing infant TDF exposure through breast milk or nutritional differences due to feeding type (breastfeeding vs. formula feeding) in first year of life. Thus, the association of maternal TDF use and lower mean infant length at 1 year does not appear attributable to these cofactors.

Several studies of antiretroviral-exposed infants born to HIV-infected mothers demonstrate the potential for late adverse effects that may be attributable to perinatal antiretroviral exposure. In the Women and Infants Transmission Study (WITS), the significant difference in $CD8^+$ cell counts by antiretroviral exposure status did not appear until 6–24 months of age, even after adjustment for potential confounders [13]. In a cohort of children with apparent mitochondrial dysfunction after perinatal exposure to zidovudine with or without lamivudine, neurologic and developmental problems did not develop until age 4–14 months [14]. Similarly, febrile seizures were significantly more common in antiretroviral-exposed infants than HIV-exposed, antiretroviral-unexposed infants; however, this difference did not appear until age 6–12 months of age [15]. Among antiretroviral-exposed French infants, the overall rate of cancer in long-term follow-up was no different from population-based rates, but there was a higher risk of central nervous system cancer at 1–8 years of age [16]. These examples emphasize the importance of evaluating outcomes both at birth and at later time points when assessing the safety of *in utero* exposure to antiretroviral drugs.

Suggested mechanisms by which fetal/neonatal antiretroviral exposure could result in persistent or delayed abnormalities have focused on nucleoside reverse transcriptase inhibitor (NRTI) toxicity to nuclear DNA of hematopoietic stem cells and NRTI damage to mitochondrial DNA [17,18]. TDF does not appear to have as much potential to cause mitochondrial dysfunction, as zidovudine and other NRTIs in in-vitro studies [19,20], but adverse effects on host DNA are plausible based on its nucleotide structure and mechanism of action. After oral administration, TDF is converted in the systemic circulation to tenofovir, which crosses the placenta. Tenofovir undergoes phosphorylation intracellularly to its active form, tenofovir diphosphate (TDP), which competitively inhibits HIV reverse transcriptase and causes DNA chain termination. The long intracellular half-life of TDP contributes to convenient dosing of TDF and its potent anti-HIV effect. Circulating tenofovir is renally cleared through glomerular filtration and tubular secretion, but renal elimination in the fetus would be expected to be much slower than in adults. As a result, the fetus may accumulate substantially more intracellular TDP, resulting in high, potentially more toxic levels as well as much longer persistence of intracellular TDP, exerting effects beyond the end of

exposure to maternal TDF at birth. If these effects include reduced bone mass accrual, as suggested by some studies of TDF in adults and children [21–24], the end result may be attainment of smaller head circumference and length. However, there is currently no direct evidence from animal or human studies that can confirm the potential for maternal TDF exposure to cause a delayed effect on infant growth.

The strengths of our investigation include large sample size, the prospective data collection of antiretroviral medications during pregnancy within the Dynamic cohort, and the evaluation of growth outcomes at both birth and age 1 year. The size of the study provides 80% power to detect differences in mean z-scores ranging from 0.18 (newborn) to 0.29 (at age 1 year). The study is also well powered to detect increased odds of LBW or SGA, with 80% power to detect ORs of 1.5–1.8. The use of a comparison group exposed to combination regimens without TDF reduces the chance results could be compromised by selection bias and controls for association of maternal combination regimens with LBW observed in some, though not all, studies [25–28].

Like all cohort studies, a limitation of this study is the nonrandom assignment of TDF to women during pregnancy, which may result in unmeasured confounding, despite the adjustment for covariates expected to be important. None of the comparisons presented would be significant if adjusted for the three to four comparisons made per outcome (e.g., LBW, WAZ at birth, WAZ at age 1); however, because this was a safety study with a limited number of comparisons addressing a single antiretroviral drug, our concern for maintaining low type I error rates was balanced with equally high concern for minimizing type II error rates (i.e., minimizing the chance of not detecting true associations with TDF).

On the whole, these data provide reassurance about the lack of major detrimental effects on fetal and infant growth when TDF is used in combination antiretroviral regimens in pregnancy. The unexpected observation of lower mean length and head circumference at 1 year of age warrants further studies monitoring longer term growth outcomes of TDF-exposed infants in SMARTT and other large HIV-exposed, uninfected cohorts.

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Conflicts of interest

There are no conflicts of interest.

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