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Evaluation of pharmacokinetics of Tenofovir Alafenamide (TAF) and Tenofovir Disoproxil (TDF) in pregnant and postpartum women in South Africa: PrEP-PP PK study

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ABSTRACT

Background: There are few data on tenofovir-diphosphate (TFV-DP) concentrations in pregnant and postpartum women on Tenofovir Disoproxil Fumarate-Emtricitabine (TDF-FTC) or Tenofovir Alafenamide-Emtricitabine (TAF-FTC).

Methods: Eligible pregnant women were randomized to TDF-FTC or TAF-FTC and followed for 16 weeks (8-weeks pregnant, 8-weeks postpartum) with weekly collection of dried blood spot (DBS) and 4-weekly peripheral blood mononuclear cells (PBMC). PrEP dosing was observed daily via asynchronous videos sent via cell phone. We report geometric means (GM) and their ratios (GMR) with 95% confidence intervals (CIs) for TFV-DP in PBMC and DBS from pregnancy and postpartum.

Results: We enrolled N = 39 participants (n = 19 TDF-FTC, n = 20 TAF-FTC): median age was 28 years (IQR:25–34); median gestational age was 24-weeks (IQR:21–28). For TDF-FTC, TFV-DP DBS concentrations at 8-weeks did not differ significantly between pregnancy (GM: 675; 95%CI:537–849) and postpartum (GM: 583; 95% CI:471–722; GMR-TDF = 1.16; 95%CI:0.74–1.80). For TAF-FTC, TFV-DP DBS concentrations at 8-weeks were 44% higher in postpartum (GM: 1199; 95%CI:929–1549) versus pregnancy (GM: 832; 95%CI:751–922; GMR-TAF = 1.44; 95% CI: 1.01–2.06). In PBMC analysis of TDF-FTC, 8-week median TFV-DP (pmol/10^6 cell) was 71 (IQR 44–112) in pregnancy and 73 (IQR 50–102) in postpartum (GMR = 1.04; 95%CI:0.44–2.44). In TAF-FTC, median PBMC at 8-weeks was 580 (IQR:341–985) in pregnancy and 666 (IQR:396–1123) in postpartum (GMR = 1.15; 95%CI:0.30–2.49).

Conclusion: TFV-DP concentrations were overall lower during pregnancy than postpartum for TAF-FTC. We found high concentrations of TFV-DP in PBMC in pregnancy and postpartum on TAF-FTC, suggesting PrEP efficacy is maintained. Efficacy and safety studies are warranted to evaluate TAF-FTC for PrEP in pregnant and postpartum women.

1. Introduction

Oral pre-exposure prophylaxis (PrEP) using Tenofovir Disoproxil Fumarate-Emtricitabine (TDF-FTC) is currently one of the most efficacious biomedical interventions for HIV prevention available, along with injectable PrEP (Celum and Baeten, 2012a; Van Damme et al., 2012;

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Baeten et al., 1999). New formulations of oral PrEP include 25 mg tenofovir alafenamide (TAF) with 200 mg emtricitabine (FTC) (Baeten et al., 1999, 2012; Trang et al., 2016). When used consistently, oral PrEP is over 95% effective at preventing HIV infection (Celum and Baeten, 2012a, 2012b; Baeten and Celum, 2013; Baeten et al., 2016). Although oral PrEP has been shown to be effective across a variety of settings, effectiveness is highly dependent on daily use of the regimen and may differ when pregnant or postpartum (Callahan et al., 1999; Joseph Davey et al., 2019; Joseph Davey et al., 2022; Moran et al., 2022). TAF-FTC is not yet approved for cisgender women to use for exposures related to vaginal intercourse and studies are ongoing to demonstrate efficacy.

PrEP adherence can be assessed through blood concentrations of drugs or drug metabolites, including tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP) (Anderson et al., 2012, 2018). Most pharmacological methods, such as measurement of tenofovir concentrations in plasma and urine or FTC-TP concentrations in dried blood spots (DBS), primarily provide information on recent drug dosing (i.e., dosing in the past 3–5 days) (Adams et al., 1999), while usage to assess adherence is limited by "white coat adherence" or dosing prior to medical visits (Castillo-Mancilla et al., 2013; Gandhi et al., 2015). Alternate methods measuring longer-term cumulative adherence, such as TFV-DP concentrations in peripheral blood mononuclear cells (PBMC) and DBS, offer an assessment of average and dynamic adherence over a longer period (6–8 weeks) (Adams et al., 1999). TFV-DP concentrations, measured in femtomole (fmol)/punch, have strong associations with PrEP efficacy to date (Castillo-Mancilla et al., 2013; Gandhi et al., 2015).

A recent study of men who have sex with men found that ≥4 doses of TDF-FTC a week on average was associated with high PrEP efficacy (Brooks et al., 2019). This relationship is consistent with the IPERGAY trial, which showed an average of 86% efficacy for 3.75 doses/week, as well as iPrEx, which suggested 90% efficacy for approximately 3 doses/week on average (Antoni et al., 2020; Bauer et al., 2020). In terms of oral TAF/FTC PrEP, a 2020 study established adherence interpretations based on steady state TFV-DP concentrations as follows: <450 fmol/punch = <2 doses/week, 450–949 fmol/punch = 2–3 doses/week, 950–1799 fmol/punch = 4–6 doses/week, and ≥1800 = 7 doses/week (Yager et al., 1999).

While previous literature has evaluated TDF-FTC concentrations and adherence/efficacy relationships, pharmacokinetic research is limited in women and especially in women who are pregnant or postpartum (Anderson et al., 2020; Stranix-Chibanda et al., 2021a). One such study, the IMPAACT 2009 study in Malawi, South Africa, Uganda, and Zimbabwe, found that among N = 40 (n = 20 pregnant and n = 20 postpartum) African adolescent girls and young women, TFV-DP was 31–37% lower in pregnant women compared to postpartum women – indicating that strict adherence to PrEP is needed in pregnancy (Stranix-Chibanda et al., 2021a). There are limited data on TAF-FTC for PrEP efficacy nor pharmacokinetics in pregnant and postpartum women (Bekker et al., 2024). Pregnancy and postpartum are periods of profound physiological change. Altered metabolism, volume changes, and increased renal clearance during these periods may result in reduced tenofovir concentrations in plasma and DBS. Given the gaps identified from the literature, this study aims to describe the pharmacokinetics of TFV-DP and FTC-TP in pregnant women followed through postpartum period and randomized to TDF-FTC or TAF-FTC for PrEP.

2. Methods

2.1. Study design and participants

This study was conducted between June 2022 and April 2023. The study enrolled participants from a large, urban clinic outside of Cape Town, South Africa. Participation was strictly voluntary and required written informed consent. Overall, 60 consecutive eligible pregnant women presenting for antenatal care were enrolled between June 2022 and October 2022. Inclusion criteria required the women to: (1) be ≥ 18 years old; (2) test HIV-negative (via rapid HIV 4th generation antigen and antibody test); (3) be between 20 and 30 weeks of gestation; (4) own a smart phone; (5) agree to daily video observations of taking a PrEP pill; (6) have no psychiatric or medical contraindications to PrEP; (7) have an estimated creatinine clearance (CrCl) greater than 60 mL/min; (8) have a negative Hepatitis B surface antigen test (HBsAg); (9) reside close to the clinic (<10 km); and (10) intend to give birth at the facility of study enrolment.

2.2. Sample size

A target sample size of at least 15 women per group was consistent with similar studies of directly observed PrEP that estimated steady-state concentrations of TFV-DP, providing adequate precision for outcome estimates of steady-state concentrations of TFV-DP when comparing pregnancy vs. postpartum samples [12]. We increased the sample size to 30 participants per group to account for potential attrition and pregnancy loss (total N = 60 women). This study was not powered to assess for safety or efficacy.

2.3. Study procedures

Once enrolled, participants were randomized 1:1 to receive a particular formulation of PrEP, i.e., assigned to either to TDF-FTC or TAF-FTC. Participants were then observed for 16 weeks of daily PrEP dosing, including 8 weeks during pregnancy and 8 weeks in postpartum. Once participants had completed their 8 weeks of in-pregnancy observation, observation of therapy was paused until they entered the first week of the postpartum phase (median 7 days from infant birth date to the start of the postpartum study phase, IQR 5–12 days postpartum). PrEP dosing was not observed or measured during this pause, but participants were supplied with sufficient pills during this time and were encouraged to continue with daily PrEP use.

Participants were observed daily via video observed therapy (VOT) during the periods of observation from study weeks 1–8 in pregnancy and again in postpartum period to confirm daily dosing. Participants had a choice to record themselves taking their PrEP pill and send this video to study staff via WhatsApp or to receive a video call from the study staff at a pre-decided time daily. Study staff then recorded each dose as either observed or unobserved via REDCap, an electronic data platform (Harris et al., 2009). If an asynchronous video was sent, the participant would verbally confirm the date of recording in the video. Additionally, staff members would also compare a newly received video to previous ones to ensure that videos were not reused. As part of the quality control, each VOT observer was assigned a maximum of 10 participants at a given time. Study staff observed the videos of these participants and also conducted the study interviews with them during study visits. This enabled each staff member to have a good recognition of the participant and to ensure that the video was from that participant (and recorded on a separate date).

Demographic information such as age, gestational age, socioeconomic status, and education was recorded at baseline. Safety laboratory testing (e.g., liver function, creatinine, haematocrit) was conducted at baseline, at first postpartum visit and end of study.

Blood for PBMC samples was collected every 4 weeks (twice in pregnancy, twice postpartum), whereas plasma and fingerpick DBS samples were collected weekly. For DBS, 50 μL blood was applied to Whatman 903 protein saver cards. Cards were dried at room temperature, stored in labelled plastic bags with desiccant at −80 °C. DBS and PBMC samples were assayed with validated liquid chromatography tandem mass spectrometry methods (Anderson et al., 2018; Castillo-Mancilla et al., 2013; Yager et al., 1999). Breast milk samples were collected at the final study visit via 5 mL aliquots (Stranix-Chibanda et al., 2021b, 2021c). Breastmilk was also spotted onto Whatman 903

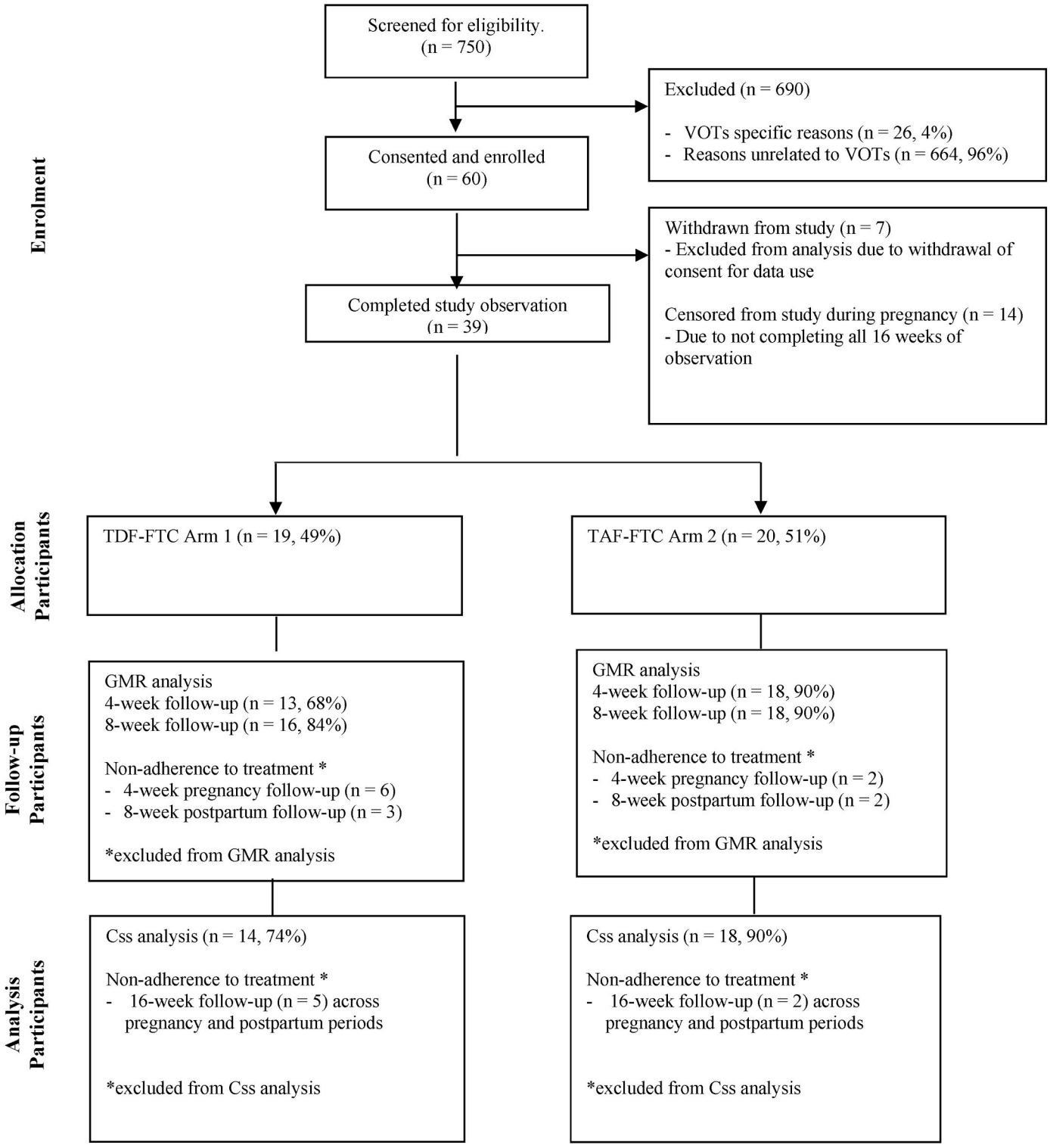

Fig. 1. Consort diagram illustrating the patient flow through screening, recruitment, enrollment and follow-up in PrEP-PP PK randomized control trial, Cape Town, South Africa (2022–2023).

protein saver cards for dried milk samples (DMS). DMS and milk aliquots were stored at −80 °C. FTC-TP concentrations in PBMC at week 8 were analysed for pregnancy and postpartum periods within both study arms. Supplementary Fig. 1 depicts the study visit schedule and sample schedule.

2.4. Statistical methods

In each of the TDF-FTC and TAF-FTC arms, clinical characteristics and safety lab results from participants were summarized and compared at baseline during pregnancy and after 4-weeks of postpartum follow-up. A paired *t*-test was used to estimate the differences between the continuous clinical measures during pregnancy and postpartum.

At each 4-week interval, geometric means and 95% CIs were

Table 1
Baseline socio-demographic and clinical characteristics of pregnant women randomized to study arm in pharmacokinetic study of TDF-FTC and TAF-FTC in Cape Town, South Africa (June 2022–June 2023; N = 39).

	All women N (%)	TDF-FTC Arm n, %	TAF-FTC Arm n, %	p-value [a]
Total participants	39	19 (49%)	20 (51%)	
Socio-demographic characteristics				
Maternal age (median, IQR) years	28 (25, 34)	29 (25, 34)	28 (25, 32)	>0.9
18–25	12 (31%)	6 (32%)	6 (30%)	>0.9
>25	27 (69%)	13 (68%)	14 (70%)	
BMI (median, IQR) kg/m^2	32 (28, 37)	30 (28, 36)	34 (28, 39)	0.5
Gravidity				0.7
Primigravida	7 (18%)	4 (21%)	3 (15%)	
Multigravida	32 (82%)	15 (79%)	17 (85%)	
Education level completed				>0.9
Primary	29 (74%)	14 (74%)	15 (75%)	
Secondary or Tertiary	10 (26%)	5 (26%)	5 (25%)	
Employment				0.2
Employed	14 (36%)	9 (47%)	5 (25%)	
Unemployed/Student	25 (64%)	10 (53%)	15 (75%)	
Socioeconomic status				0.3
Low SES	12 (31%)	4 (21%)	8 (40%)	
Moderate/high SES	27 (69%)	15 (79%)	12 (60%)	

TDF-FTC, tenofovir disoproxil fumarate and emtricitabine; TAF-FTC, tenofovir alafenamide-emtricitabine; μmol/L, micromole per Liter; UL, units per Liter; L/L, liter of cells per liter of blood; GA: gestational age; BMI, body mass index; kg, kilograms; m, meters.
[a] Wilcoxon rank sum test or Fisher's exact test.

reported for TFV-DP and FTC-TP measures obtained using PBMC and DBS. Geometric mean ratios (GMR) were then used to compare TFV-DP and FTC-TP measures during pregnancy and postpartum after 4- and 8-weeks of follow-up. The GMR is a parameter of the observed effect size between all concentrations in each group. GMR and 95% confidence intervals (CIs) were used to interpret if there is a clinically significant difference in drug exposure between the two phases. Participants with concentrations below the limit of quantification (BLQ) due to poor adherence at 4- and 8-week follow-up were excluded from the GMR calculations, which represented an "as treated" analysis.

TFV-DP measures were obtained by DBS once a week for 16 weeks. The midpoint of the lower limit quantification value of TFV-DP concentrations (15.7) was allocated to participants with BLQ TFV-DP concentrations from DBS samples as to include all participants in subsequent analysis, which represented an "intention to treat" analysis. Non-linear regression, specifically a one-phase exponential model, was used to describe the pattern of TFV-DP concentrations over time by the equation; observed TFV-DP concentration = Css x $(1 - e^{(-k \times time)})$.

This pharmacokinetic modelling was used to determine fitted values of steady-state concentrations at the 25th-75th percentiles as well as the half-life of TFV-DP. Comparisons were only made between pregnancy and postpartum states within each arm and no concentration comparisons were made across arms. We adjusted for participants' body mass index (BMI), haematocrit, and creatinine levels at baseline and for haematocrit and creatinine only at 4-week follow-up in postpartum. The model provided steady-state concentration estimates (Css) and an elimination rate constant (k). Half-life in weeks was calculated by dividing 0.693 by k. The exponential model projections were fitted on a scatter plot of TFV-DP concentration over time in pregnant and postpartum groups within each arm. Fitted 25th and 75th percentiles were generated using bootstrap resampling with initial Css and k estimates as starting points, resulting in a distribution of Css estimates. All analyses were performed in RStudio version 4.3.1.

2.5. Ethics

The PrEP-PP PK study was approved by the University of Cape Town Human Research Ethics Committee (UCT HREC:108/2021). The study was also granted provincial approval by the Western Cape government to conduct study activities at a public health care facility (WC_202,103_029).

3. Results

Between June 2022 and October 2022, we enrolled N = 60 eligible, consenting pregnant women in the study. Over the course of the study, n = 21 participants were censored due to pregnancy loss, loss to follow up or withdrawal from the study and were excluded from analysis. Overall, n = 39 participants completed all 16 weeks of observation, with 19 participants in the TDF-FTC arm and 20 participants in TAF-FTC arm. Whilst 19 participants in the TDF-FTC arm completed the study period, for the pharmacokinetic analysis of TFV-DP metabolite concentrations, participants that had a 'below limit of quantification' (BLQ) measure in either the PBMC or DBS samples were excluded based on nonadherence to the protocol. This resulted in a sample size of n = 13 at week 4 and n = 16 at week 8 within the TDF-FTC arm. For the TAF-FTC arm, although 20 participants had completed the study, for pharmacokinetic analysis

Table 2
Clinical characteristics of pregnant women at baseline and postpartum women in TDF-FTC or TAF-FTC study arm in Cape Town, South Africa (PrEP-PP PK Study, June 2022–June 2023; N = 39).

	TDF Arm		p-value[a]	TAF-FTC Arm		p-value[a]
	Pregnancy	Postpartum		Pregnancy	Postpartum	
Total participants	N = 19			N = 20		
	N = 19, 100% Baseline	N = 19, 100% 4 weeks postpartum		N = 20, 100% Baseline	N = 20, 100% 4 weeks postpartum	
GA (median, IQR) weeks	24 (23, 26)	4.1 (3.5, 4.6)		24 (21, 28)	4.1 (3.8, 5.6)	
Creatinine (μmol/L)	40 (38, 46)	69 (61, 75)	**<0.001**	43 (40, 48)	70 (65, 73)	**<0.001**
Alanine transaminase (U/L)[b]	12 (10, 16)	17 (15, 22)	0.2	12 (9, 23)	14 (11, 19)	0.4
Haemoglobin (g/dL)	11.1 (10.4, 11.9)	12.4 (11.1, 13.0)	0.4	11.4 (10.8, 11.8)	11.70 (11.4, 13.3)	**0.017**
Haematocrit (%)	33 (32, 36)	37 (35, 41)	**<0.001**	35 (33, 37)	37 (35, 40)	0.3
Platelet Count (× 10^9/L)	279 (215, 296)	303 (276, 351)	**0.010**	210 (197, 259)	312 (237, 351)	**0.011**

TDF-FTC, tenofovir disoproxil fumarate and emtricitabine; TAF-FTC, tenofovir alafenamide-emtricitabine; PBMC, peripheral blood mononuclear cells; DBS, dried blood spots; fmol, femtomole; pmol, picomole.
Bold: $p < 0.05$.
[a] Paired *t*-test.
[b] One participant missing Alanine result at baseline.

Table 3
Geometric mean levels of TFV-DP and FTC-TP in TAF-FTC and TDF arms in pregnant vs. postpartum periods with log transformed geometric mean ratios (GMR) in Cape Town, South Africa (June 2022–June 2023).

	Pregnancy (4-week follow-up) GM (95% CI)	Postpartum (4-week follow-up) GM (95% CI)	GMR (95% CI) comparing log transformed Postpartum vs. Pregnant concentrations (4 week follow-up)	Pregnancy (8-week follow-up) GM (95% CI)	Postpartum (8-week follow-up) GM (95% CI)	GMR (95% CI) comparing log transformed Postpartum vs. Pregnant concentrations (8 week follow-up)
TDF-FTC Arm (N = 19)	n = 13	n = 13		n = 16	n = 16	
GA (median, IQR) weeks	28 (27–30)	4.1 (3.5–4.6)		32 (31–34)	8.43 (7.50–9.00)	
TFV-DP PBMC (fmol/10^6 cells)	67 (47–94)	69 (48–98)	1.04 (0.51, 2.09)	71 (44–112)	73 (50–108)	1.04 (0.44, 2.44)
FTC-TP in PBMC (pmol/10^6 cell)	7 (5–10)	5.1 (3.3–7.9)	0.76 (0.33, 1.73)	8 (5–13)	4.9 (2.9–8.2)	0.60 (0.22, 1.60)
TFV-DP DBS (fmol/3 mm punch)	431 (358–517)	579 (465–721)	1.34 (0.90, 2.01)	583 (471–722)	675 (537–849)	1.16 (0.74, 1.80)
TAF-FTC Arm (N = 20)	**n = 18**	**n = 18**		**n = 18**	**n = 18**	
GA (median, IQR) weeks	28 (25–31)	4.1 (3.8–5.6)		31 (29–35)	8.71 (7.96–9.93)	
TFV-DP PBMC (fmol/10^6 cells)	712 (537–944)	996 (797-1245)	1.40 (0.84, 2.32)	580 (341–985)	666 (396-1123)	1.15 (0.30, 2.49)
FTC-TP in PBMC (pmol/10^6 cells)	8 (6–11)	12 (10–14)	1.49 (0.89, 2.51)	7 (4–13)	9 (6–12)	1.25 (0.49, 3.21)
TFV-DP DBS (fmol/7 mm punch)	632 (575–694)	1032 (887–1202)	**1.63 (1.28, 2.51)**	832 (751–922)	1199 (929–1549)	**1.44 (1.01, 2.06)**

Bold: $p < 0.05$.

of TFV-DP metabolite concentrations, participants that had a BLQ measure in either the PBMC or DBS samples were similarly excluded. This resulted in a sample size of n = 18 at week 4 and n = 18 at week 8 for the TAF-FTC arm. The consort diagram in Fig. 1 illustrates the flow of participants through screening, enrolment and completion of analysis per study arm.

The baseline sociodemographic characteristics of these participants are presented in Table 1. All participants were Black/African females, median age was 28 years (IQR: 25, 34), and median gestational age at enrolment was 24 weeks (IQR: 21, 28 weeks). The majority (74%) of all participants had only completed a primary education and most (64%) were unemployed at the time of enrolment. There were no significant differences in baseline characteristics between participants randomized to the TDF-FTC and TAF-FTC arms.

Participant baseline pregnancy and postpartum clinical measurements are summarized in Table 2. In both TDF-FTC and TAF-FTC arms, the median creatinine (μmol/L) was significantly higher postpartum compared to during pregnancy (TDF/FTC: 69 vs. 40, $p < 0.001$; TAF/FTC: 70 vs. 43, $p < 0.001$). Median haematocrit (L/L) was lower during pregnancy in the TDF/FTC arm (median = 0.33; IQR: 0.32, 0.35) than postpartum (0.37; IQR: 0.35, 0.40; p-value <0.001), but no significant difference was found in pregnancy versus postpartum in the TAF/FTC arm. Platelet count was also lower in pregnancy than postpartum in both arms. These changes reflect expected physiological changes from pregnancy to postpartum and were expected. No differences were found in alanine transaminase.

Daily PrEP dosing was asynchronously observed via video recordings or video calls (depending on participant preference). Overall, out of 4077 total expected video doses, 3749 (92%) videos were received by study staff. A further 241 (6%) doses were not observed but were reported by participants as having been taken. In the TDF-FTC arm, 1828 (92%) doses were observed out of an expected 1979. Similarly, in the TAF-FTC arm, 1921/2098 (92%) of videos were received. Overall, 1981/2181 (91%) videos were received during pregnancy and 1768/1896 (93%) during postpartum observation.

3.1. Pharmacokinetics of TFV-DP

Concentrations accumulated to steady state concentrations at week 8 of observation in both the pregnancy and postpartum periods in the same women. Observed concentrations of TFV-DP in DBS and PBMC at weeks 4 and 8 among those in both the TDF-FTC and TAF-FTC arm are shown in Table 3. How these concentrations compare to the previously published IMPAACT 2009 adherence categories (Stranix-Chibanda et al., 2021a) for TDF-FTC in pregnancy and postpartum are reflected in Supplementary Table 1.

3.2. TDF/FTC arm

The geometric mean TFV-DP concentrations in DBS (fmol/3 mm punch) at week 8 were 583 during pregnancy (95% CI: 71, 722) and 675 in postpartum (95% CI: 537, 849), with a geometric mean ratio (GMR) of 1.16 (95% CI 0.74, 1.80) at 8-week follow-up (Table 3). TFV-DP PBMC sampling showed a similar pattern, with geometric mean concentrations of TFV-DP also being lower during pregnancy (71; 95% CI: 44, 112) compared to postpartum (73; 95% CI: 50, 108; GMR = 1.04; 95% CI: 0.44, 2.44), (Table 3). Geometric mean concentrations of FTC-TP in PMBC (picomole [pmol]/10^6 cell) were 8 in pregnancy (95% CI: 5, 13) compared to 4.9 in postpartum (95% CI: 2.9, 8.2; GMR = 0.60, 95% CI: 0.22, 1.60).

The fitted 8-week median TFV-DP steady-state concentration was 603 (IQR 549–672) fmol/3 mm punch in pregnancy compared to 611 (IQR 555–646) fmol/3 mm punch in postpartum, indicating 1.3% increase postpartum (Table 4). TFV-DP accumulated with a median (IQR)

Table 4
Tenofovir diphosphate (TFV-DP) in dried blood spots (DBS) at steady state during pregnancy and postpartum (N = 39).

	Pregnancy (8 weeks follow-up) [a]		Postpartum (8 weeks follow-up) [b]	
	Observed TFV-DP concentration	Fitted 50th (25th and 75th percentiles	Observed TFV-DP concentration	Fitted 50th (25th and 75th percentiles
TDF-FTC Arm TFV-DP DBS (fmol/3 mm punch)	**n = 19**		**n = 19**	
Css	602	603 (549–672)	613	611 (555–646)
Half-life (weeks)	2.8	2.8 (2.4–3.4)	1.4	1.4 (1.2–1.8)
Median	410.0	–	518.3	–
Min/ Max	8.3/1208.0	–	8.3/1377.4	–
Q1/Q3	271.0/571.1	–	285.7/780.1	–
TAF-FTC Arm TFV-DP DBS (fmol/7 mm punch)	**n = 20**		**n = 20**	
Css	754	754 (704–813)	1154	1149 (1086–1231)
Half-life (weeks)	2.4	2.4 (2.1–2.8)	1.6	1.5 (1.4–1.9)
Median	611.3	–	940.5	–
Min/ Max	8.3/1282.7	–	8.3/2481.1	–
Q1/Q3	396.6/754.5	–	630.2/1259.3	–

Participants with levels below limit of quantification (BLQ) replaced with midpoint of LLQ (16.6/2 = 8.3).
TDF-FTC: 19 participants with 16 observations = 304 total observations. BLQ replacement of 10 observations (3%).
TAF-FTC: 20 participants with 16 observations = 320 total observations. BLQ replacement of 23 observations (7%).
[a] Adjusted for BMI, haematocrit, and creatinine at baseline.
[b] Adjusted for haematocrit and creatinine at 4 weeks of postpartum follow-up.

half-life of 2.8 weeks (2.4–3.4) in pregnancy and 1.4 weeks (1.2–1.8) postpartum.

3.3. TAF-FTC arm

The geometric mean of TFV-DP DBS concentrations (fmol/7 mm punches) at 8-week follow-up during pregnancy (832; 95%CI: 751, 922) was lower than in postpartum (1199; 95% CI: 929, 1549; GMR: 1.44m95%CI: 1.01, 2.06) (Table 3). The geometric mean of TFV-DP concentrations measured in PBMC samples at 8-week follow-up were580 (95%CI: 341, 985) compared to 666 in postpartum (95% CI: 396, 1123: GMR = 1.15; 95%CI: 0.30, 2.49). In contrast to the TDF-FTC arm, the geometric mean of FTC-TP concentrations in PBMC (pmol/10^6 cell) samples at 8-week follow-up were 7 pregnancy (95% CI: 4, 13) compared to 9 in postpartum (95% CI: 6, 12), with a GMR of 1.25 (95% CI: 0.49, 3.21). In adjusted pharmacokinetic modelling, the 8-week median TFV-DP steady state concentration was 52% higher postpartum (1149 fmol/3 mm punch; IQR: 1086,1231) compared to pregnancy (754 fmol/3 mm punch; IQR: 704, 813). TFV-DP accumulated with a median half-life of 2.4 weeks (2.1–2.8) in pregnancy and 1.5 weeks (1.4–1.9) postpartum.

Figs. 2 and 3 demonstrate the one-phase exponential formula for the TFV-DP measured ruing DBS in the TDF-FTC arm (fmol/3 mm punch) and TAF-FTC arm (fmol/7 mm punches) during pregnancy (blue) and postpartum (green) and the linear relationship adjusted for age and haematocrit at baseline by pregnancy vs postpartum periods in both study arms.

3.4. TFV concentrations in breastmilk

Breastmilk samples were obtained at the final study visit at week 8 of postpartum observation. Dried milk spot samples were obtained from 36 participants. Across both study arms TFV-DP concentrations in breast milk were found to be < 0.25 pmol/30 μL (or <8.3 pmol/mL). Sample concentrations were all classified as either below the limit of detection where the TFV-DP chromatographic peak was not distinguishable from the assay background or as below the limit of quantification (i.e., samples where the TFV-DP is detectable, but is not resolved from the interfering peak and has a signal response below our lowest calibrator of 0.25 pmol/sample).

4. Discussion

Our study assessed the pharmacokinetics of two different formulations of PrEP (i.e., TDF-FTC and TAF-FTC) in women during pregnancy and postpartum periods in an urban setting in South Africa. Comparisons were made between concentrations of TFV-DP measured in women during pregnancy and postpartum phases. We found that the geometric mean TFV-DP concentration in DBS did not differ statistically when compared to postpartum, among women taking a daily dose of TDF-FTC and were slightly higher in postpartum compared to pregnancy (GMR = 1.44; 95% CI = 1.01–2.06) among women taking TAF-FTC. We found similar patterns of TFV-DP concentrations in PBMC samples in both study arms, but differing patterns of FTC-TP concentrations, in that geometric mean concentrations did not differ in pregnancy compared to postpartum in the TDF-FTC arm, but higher in postpartum versus pregnancy in the TAF-FTC arm. We did not observe any appreciable accumulation of TFV-DP in breast milk. Our findings of women having lower concentrations of tenofovir metabolite in the TAF-FTC arm during pregnancy compared to postpartum are consistent with previous literature (Anderson et al., 2020). These findings are also supported by the fact that women have an altered metabolism and increased volume and renal clearance during pregnancy which may result in reduced tenofovir concentrations in plasma, PBMC, and DBS as observed (Anderson et al., 2018). Whilst the TFV-DP concentration findings of this study was expected due to the previous IMPAACT 2009 study (Stranix-Chibanda et al., 2021a), the reductions during pregnancy were generally not statistically significant (except for TFV-DP in DBS arising from TAF). Thus the implication for PrEP efficacy require further study as it is still unclear if pregnancy reduces efficacy levels of daily oral PrEP and requires stricter adherence measures.

Our study was not designed to compare FTC-TP between the TDF and TAF arms. In the TAF arm the concentrations of FTC-TP are increased postpartum, as might be expected. No significant differences were found in FTC-TP concentrations in the TDF arm between pregnancy and postpartum. This may be due to issues with adherence and not necessarily a biological difference in FTC-TP levels, which this study was not powered to determine.

A strength of this study the combined use of pharmacologic measures (DBS and PBMC) with daily video observed therapy which allowed assessment of average adherence whilst also providing detail of dosing patterns (e.g., when doses were missed or resumed) for both TDF-FTC and TAF-FTC. The high proportion of expected doses that were observed (94%) indicate good adherence over the 16 weeks of observation with similar proportion of doses observed during pregnancy and postpartum periods; however, we noted several cases of DBS or PBMC TFV-DP that were below the limit of quantification, highlighting some possible weakness of the video DOT method compared to traditional DOT observed by a clinician that will be discussed in a later manuscript.

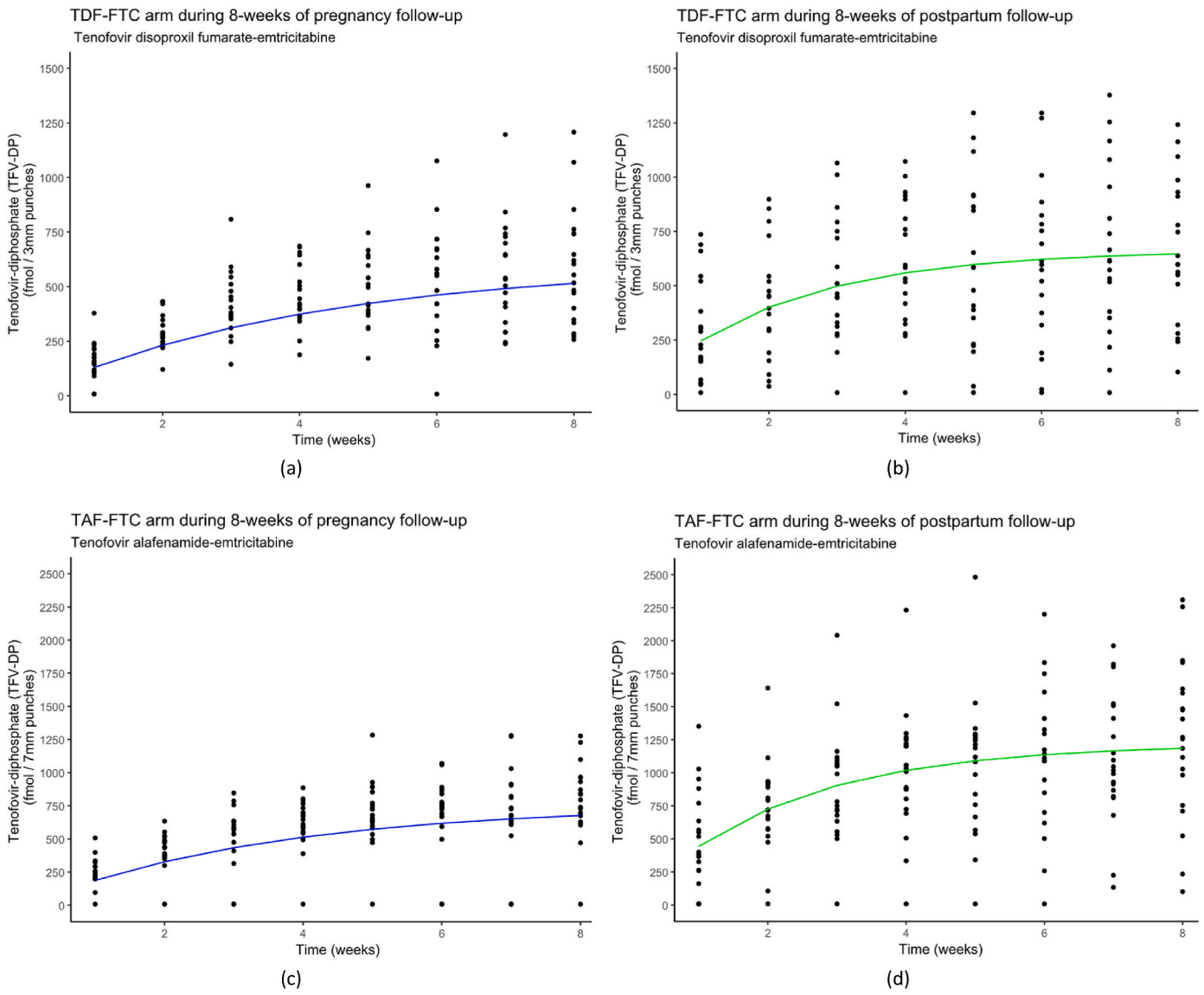

Fig. 2. TFV-DP (fmol/3 mm punch) measured using DBS during a) pregnancy and b) postpartum in the TDF-FTC arm. In TAF-FTC arm is measured in TFV-DP (fmol/7 mm punch) during c) pregnancy and d) postpartum over an observational period of 16 weeks in total. A one-phase exponential formula is shown during pregnancy (blue) and postpartum (green).

Women in this study were recruited during pregnancy and then observed through to their postpartum phase, so participants were their own comparators for the two phases of observation. This reduced the potential for inter-individual variability to act as a confounder. Although studies commonly focus on DBS measurements to assess TFV-DP metabolite concentrations, red blood cells are not the target site for tenofovir drug action (Anderson et al., 2018). We included assessment of PBMC concentrations to provide insight into PrEP pharmacokinetics in target cells in pregnancy and postpartum.

Our study found lower concentrations of TFV-DP in DBS among pregnant women when compared to the IMPAACT 2009 study in Malawi, South Africa, Uganda, and Zimbabwe. The IMPAACT 2009 study found that TFV-DP in DBS was 31–37% lower in pregnant women compared to postpartum women (Anderson et al., 2020). Their benchmark concentrations were also higher in pregnancy and postpartum, highlighting better adherence to the video DOT protocol, population variation or BMI differences. For context, Supplemental Table 1 compares our results to the IMPAACT 2009 adherence categories.

We found high concentrations of TFV-DP in PBMC in pregnancy and postpartum on TAF-FTC (above TDF-FTC levels), suggesting PrEP efficacy may be retained. Efficacy and safety studies are warranted to evaluate TAF-FTC for PrEP in pregnant and postpartum women. The PURPOSE-1 trial results demonstrated equivalent efficacy of TAF compared to TDF in cisgender women, including some pregnant women. There was no evidence of a meaningful difference in HIV incidence was observed between TAF and TDF (incidence rate ratio, 1.20; 95% CI, 0.67 to 2.14), though adherence to TAF and TDF was low and declined over time. Pregnant women were included in the study follow up and we expect that safety and efficacy will be reported in women using TAF in a sub-analysis.

A limitation of this study was nonadherence to the VOT protocol as observed in some participants. This increased variability in the benchmark estimates. Additionally, participants were advised to continue PrEP after birth prior to the postpartum PK assessment. This could result in slightly higher TFV-DP accumulation during the postpartum phase, although 8 weeks of dosing represents 90% of eventual steady-state. Our modelling suggested that fitted Css values were similar to observed week 8 values indicating that Css was essentially reached, and the half-lives were consistent with previous studies (Anderson et al., 2018; Yager et al., 1999). Another limitation of this study is that the sample consisted

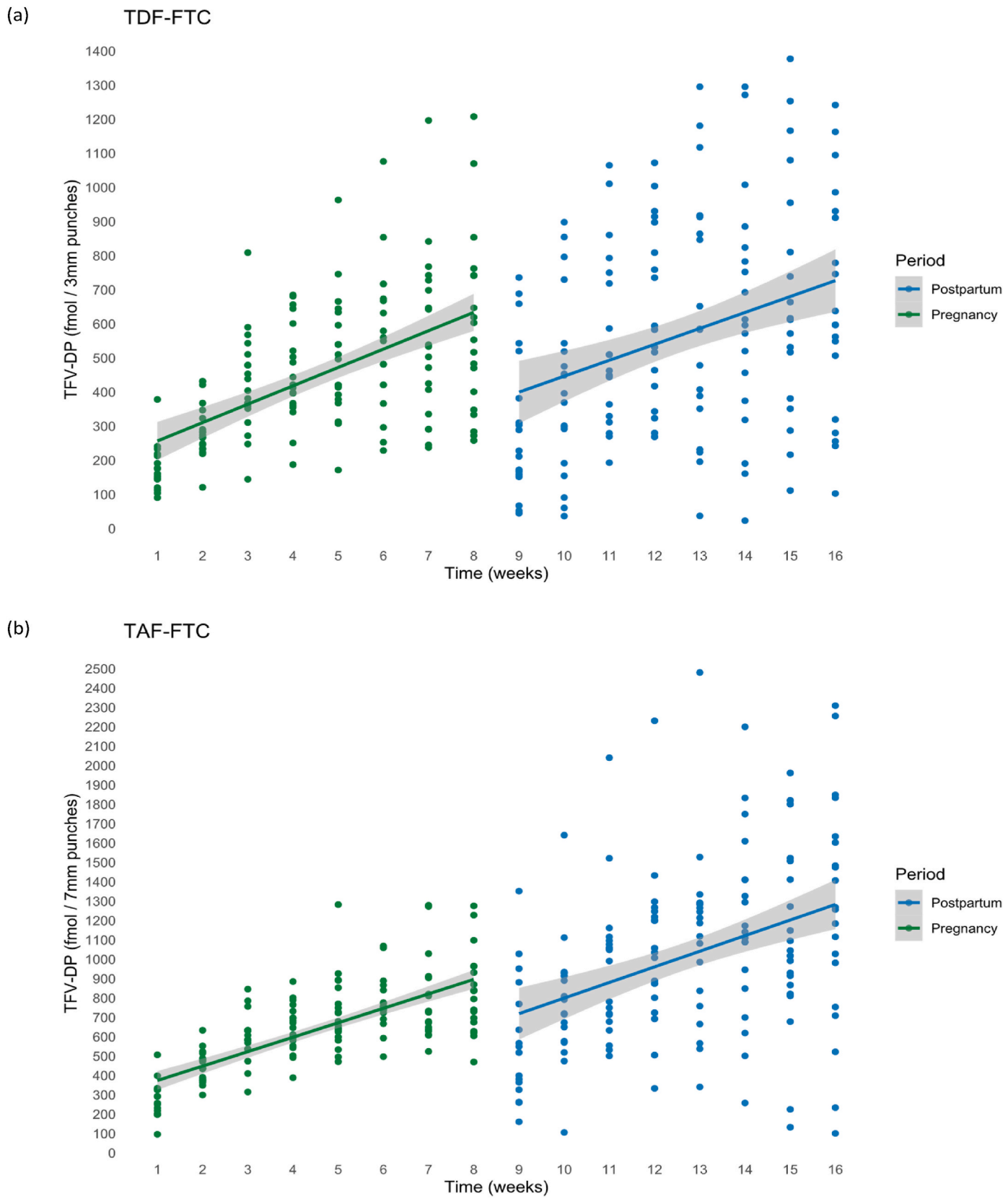

Fig. 3. Linear relationship, adjusted for age and haematocrit at baseline, of: a) TFV-DP (fmol/3 mm punch) in the TDF-FTC arm and b) TFV-DP (fmol/7 mm punch) in the TAF-FTC arm.

entirely of lower-income pregnant women in an urban setting. This limits the generalizability of results to higher-income settings and limits the ability to characterize associations between demographic characteristics and TFV-DP pharmacokinetics. We acknowledge that the high percentage of successfully observed videos (92%) is incongruous with the number of participants excluded from analysis due to BLQ. Some possible reasons include: participants taking their tablets in the video but spitting them out once they have stopped recording, some degree of drug instability as participants often decant their PrEP pills (to avoid the stigma of the packaging) resulting in sub-optimal storage conditions. Another consideration might be variation in lab equipment sensitivity as TFV-DP from the TDF/FTC and TAF/FTC arms were analysed in different laboratories which may account for the disproportional BLQs in the TDF/FTC arm. Finally, this study was not powered to assess safety or efficacy of either formulation of PrEP when used to prevent HIV in pregnant and postpartum women.

5. Conclusion

In conclusion, we found that TFV-DP concentrations were not significantly different during pregnancy than postpartum, in both DBS as well as PBMC, and for TDF-FTC, while TAF-FTC formulations of oral PrEP were slightly higher in postpartum in DBS measures. We found high concentrations of TFV-DP in PBMC in pregnancy and postpartum on TAF, suggesting PrEP efficacy is retained. Further research is needed to explore the implications of lower PrEP levels in pregnant women on the efficacy of PrEP and levels of protection afforded during this time. Efficacy and safety studies are warranted to evaluate TAF-FTC for PrEP in pregnant and postpartum women.

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CRediT authorship contribution statement

Dvora Joseph Davey: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **Sumaya Dadan:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Conceptualization. **Kalisha Bheemraj:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Catriona Waitt:** Writing – review & editing, Validation, Supervision, Formal analysis, Conceptualization. **Saye Khoo:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Landon Myer:** Writing – review & editing, Project administration, Methodology, Investigation. **Lubbe Wiesner:** Writing – review & editing, Supervision, Resources, Formal analysis. **Laura Else:** Formal analysis, Investigation, Methodology. **Beth Thompson:** Formal analysis, Investigation, Methodology. **Sandra Castel:** Conceptualization, Investigation, Methodology, Project administration, Validation. **Nafisa Wara:** Writing – original draft, Visualization, Conceptualization. **Peter L. Anderson:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Catherine Orrell:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2024.106014.

References

Adams, J.L., Sykes, C., Menezes, P., et al., 1999. Tenofovir diphosphate and emtricitabine triphosphate concentrations in blood cells compared with isolated peripheral blood mononuclear cells: a new measure of antiretroviral adherence? J. Acquir. Immune Defic. Syndr. 62 (3), 260–266, 2013.

Anderson, P.L., Glidden, D.V., Liu, A., et al., 2012. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. Sci. Transl. Med. 4 (151), 151ra25.

Anderson, P.L., Liu, A.Y., Castillo-Mancilla, J.R., et al., 2018. Intracellular tenofovir-diphosphate and emtricitabine-triphosphate in dried blood spots following directly observed therapy. Antimicrob. Agents Chemother. 62 (1).

Anderson, P.L.S.-C.L., Huang, S., Hosek, S., Kacanek, D., 2020. Tenofovir Diphosphate in Dried Blood Spots for Pregnant/postpartum Adolescent and Young Women on PrEP in Africa. CROI. Virtual Conference.

Antoni, G., Tremblay, C., Delaugerre, C., et al., 2020. On-demand pre-exposure prophylaxis with tenofovir disoproxil fumarate plus emtricitabine among men who have sex with men with less frequent sexual intercourse: a post-hoc analysis of the ANRS IPERGAY trial. Lancet HIV 7 (2), e113–e120.

Baeten, J., Celum, C., 2013. Systemic and topical drugs for the prevention of HIV infection: antiretroviral pre-exposure prophylaxis. Annu. Rev. Med. 64, 219–232.

Baeten, J.M., Haberer, J.E., Liu, A.Y., Sista, N., 1999. Preexposure prophylaxis for HIV prevention: where have we been and where are we going? J. Acquir. Immune Defic. Syndr. 63 (Suppl. 2), S122–S129, 2013.

Baeten, J.M., Donnell, D., Ndase, P., et al., 2012. Antiretroviral prophylaxis for HIV prevention in heterosexual men and women. N. Engl. J. Med. 367 (5), 399–410.

Baeten, J.M., Heffron, R., Kidoguchi, L., et al., 2016. Integrated delivery of antiretroviral treatment and pre-exposure prophylaxis to HIV-1-Serodiscordant couples: a prospective implementation study in Kenya and Uganda. PLoS Med. 13 (8), e1002099.

Bauer, R., Netzer, E., Pintado, C., et al., 2020. Coverage of sex acts by event-driven pre-exposure prophylaxis: a sub-study of the anrs IPERGAY trial.

Bekker, L.G., Das, M., Abdool Karim, Q., et al., 2024. Twice-yearly lenacapavir or daily F/TAF for HIV prevention in cisgender women. The New England Journal of Medicine, 10.1056/NEJMoa2407001. Advance online publication. https://doi.org/10.1056/NEJMoa2407001.

Brooks, K.M., Ibrahim, M.E., Castillo-Mancilla, J.R., et al., 2019. Pharmacokinetics of tenofovir monoester and association with intracellular tenofovir diphosphate following single-dose tenofovir disoproxil fumarate. J. Antimicrob. Chemother. 74 (8), 2352–2359.

Callahan, R., Nanda, K., Kapiga, S., et al., 1999. Pregnancy and contraceptive use among women participating in the FEM-PrEP trial. J. Acquir. Immune Defic. Syndr. 68 (2), 196–203, 2015.

Castillo-Mancilla, J.R., Zheng, J.H., Rower, J.E., et al., 2013. Tenofovir, emtricitabine, and tenofovir diphosphate in dried blood spots for determining recent and cumulative drug exposure. AIDS Res. Hum. Retrovir. 29 (2), 384–390.

Celum, C., Baeten, J.M., 2012a. Antiretroviral-based HIV-1 prevention: antiretroviral treatment and pre-exposure prophylaxis. Antivir. Ther. 17 (8), 1483–1493.

Celum, C., Baeten, J.M., 2012b. Tenofovir-based pre-exposure prophylaxis for HIV prevention: evolving evidence. Curr. Opin. Infect. Dis. 25 (1), 51–57.

Gandhi, M., Glidden, D.V., Liu, A., et al., 2015. Strong correlation between concentrations of tenofovir (TFV) emtricitabine (FTC) in hair and TFV diphosphate and FTC triphosphate in dried blood spots in the iPrEx open label extension: implications for pre-exposure prophylaxis adherence monitoring. J. Infect. Dis. 212 (9), 1402–1406.

Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., Conde, J.G., 2009. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. J. Biomed. Inf. 42 (2), 377–381.

Joseph Davey, Bekker, L.G., Gomba, Y., Coates, T., Myer, L., Johnson, L.F., 2019. Modelling the potential impact of providing preexposure prophylaxis in pregnant

and breastfeeding women in South Africa. AIDS (London, England) 33 (8), 1391–1395. https://doi.org/10.1097/QAD.0000000000002221.
Joseph Davey, D., Nyemba, D.C., Castillo-Mancilla, J., et al., 2022. Adherence challenges with daily oral pre-exposure prophylaxis during pregnancy and the postpartum period in South African women: a cohort study. J. Int. AIDS Soc. 25 (12), e26044.
Moran, A., Mashele, N., Mvududu, R., et al., 2022. Maternal PrEP use in HIV-uninfected pregnant women in South Africa: role of stigma in PrEP initiation, retention and adherence. AIDS Behav. 26 (1), 205–217.
Stranix-Chibanda, L., Anderson, P.L., Kacanek, D., et al., 2021a. Tenofovir diphosphate concentrations in dried blood spots from pregnant and postpartum adolescent and young women receiving daily observed pre-exposure prophylaxis in sub-saharan Africa. Clin. Infect. Dis. 73 (7), e1893–e1900.
Stranix-Chibanda, L., Tierney, C., Pinilla, M., et al., 2021b. Effect on growth of exposure to maternal antiretroviral therapy in breastmilk versus extended infant nevirapine prophylaxis among HIV-exposed perinatally uninfected infants in the PROMISE randomized trial. PLoS One 16 (8), e0255250.
Stranix-Chibanda, L., Tierney, C., Sebikari, D., et al., 2021c. Impact of postpartum tenofovir-based antiretroviral therapy on bone mineral density in breastfeeding women with HIV enrolled in a randomized clinical trial. PLoS One 16 (2), e0246272.
Trang, T.P., Dong, B.J., Kojima, N., Klausner, J.D., 2016. Drug safety evaluation of oral tenofovir disoproxil fumarate-emtricitabine for pre-exposure prophylaxis for human immunodeficiency virus infection. Expet Opin. Drug Saf. 15 (9), 1287–1294.
Van Damme, L., Corneli, A., Ahmed, K., et al., 2012. Preexposure prophylaxis for HIV infection among African women. N. Engl. J. Med. 367 (5), 411–422.
Yager, J., Castillo-Mancilla, J., Ibrahim, M.E., et al., 1999. Intracellular tenofovir-diphosphate and emtricitabine-triphosphate in dried blood spots following tenofovir alafenamide: the TAF-DBS study. J. Acquir. Immune Defic. Syndr. 84 (3), 323–330, 2020.