

# Impact of feminizing hormone therapy on tenofovir and emtricitabine plasma pharmacokinetics: a nested drug–drug interaction study in a cohort of Brazilian transgender women using HIV pre-exposure prophylaxis

Vitória Berg Cattani<sup>1\*</sup>, Emilia Moreira Jalil<sup>1</sup>, Leonardo Eksterman<sup>1</sup>, Thiago Torres<sup>1</sup>, Sandra Wagner Cardoso<sup>1</sup>, Cristiane R. V. Castro<sup>1</sup>, Laylla Monteiro<sup>1</sup>, Erin Wilson<sup>2</sup>, Lane Bushman<sup>3</sup>, Peter Anderson<sup>3</sup>, Valdilea Gonçalves Veloso<sup>1</sup>, Beatriz Grinsztejn<sup>1</sup> and Rita Estrela<sup>1,4</sup> on behalf of the PrEPParadas study team†

<sup>1</sup>Evandro Chagas National Institute of Infectious Diseases INI Fiocruz, Rio de Janeiro, Brazil; <sup>2</sup>University of California, San Francisco, USA; <sup>3</sup>University of Colorado, Denver, USA; <sup>4</sup>Faculty of Pharmacy, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

\*Corresponding author: E-mail: vibergc@gmail.com  
†Members are listed in the Acknowledgements section.

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**Objectives:** Potential interactions between feminizing hormone therapy (FHT) and pre-exposure prophylaxis (PrEP) may be a barrier to PrEP use among transgender women (TGW). We aimed to assess the impact of FHT on PrEP plasma pharmacokinetics (PK) among TGW.

**Methods:** This was a PK substudy of the effects of FHT on tenofovir disoproxil fumarate/emtricitabine nested to a trans-specific PrEP demonstration study (NCT03220152). Participants were assigned to receive PrEP only (noFHT) or standardized FHT (sFHT; oestradiol valerate 2–6 mg plus spironolactone 100–300 mg) plus PrEP for 12 weeks, after which they could start any FHT (aFHT). Short- and long-term PK assessment occurred at Weeks 12 and 30–48, respectively (plasma samples prior and 0.5, 1, 2, 4, 6, 8 and 24 h after dose). Non-compartmental PK parameters of tenofovir and emtricitabine were compared as geometric mean ratios (GMRs) between noFHT and PrEP and FHT (sFHT at short-term PK; aFHT at long-term PK) participants.

**Results:** No differences in tenofovir and emtricitabine plasma PK parameters were observed between the short-term PK of noFHT ( $n = 12$ ) and sFHT participants ( $n = 18$ ), except for emtricitabine  $C_{max}$  [GMR: 1.15 (95% CI: 1.01–1.32)], or between noFHT short-term PK and aFHT long-term PK ( $n = 13$ ). Most participants were on oestradiol valerate 2 mg at the short-term PK (56%) and 4 mg at the long-term PK (54%). Median (IQR) oestradiol levels were 56.8 (43.2–65.4) pg/mL at short-term PK (sFHT) and 44.8 (24.70–57.30) pg/mL at long-term PK (aFHT). No participants in this analysis seroconverted during the study.

**Conclusions:** Our results indicate no interaction of FHT on tenofovir levels, further supporting PrEP use among TGW using FHT.

## Introduction

HIV infection disproportionately affects transgender women (TGW), who show approximately 50-fold increased odds of having HIV globally compared with adults of reproductive age in general.<sup>1</sup> In Rio de Janeiro, the city with the second largest number of HIV/AIDS cases in Brazil, 31.2% of 345 TGW enrolled in a study during 2015–16 were living with HIV.<sup>2</sup> Moreover, 43.7% of TGW with newly diagnosed infections had a negative HIV test result in the previous 12 months,

underscoring the need for access to effective prevention strategies in this population. In a cross-sectional study conducted during 2018–20 in Rio de Janeiro, Brazil, the estimated annualized HIV incidence rate in TGW based on a limiting-antigen avidity assay was 9.16% (95% CI: 4.05–17.32).<sup>3</sup>

Pre-exposure prophylaxis (PrEP) using daily oral tenofovir disoproxil fumarate 300 mg combined with emtricitabine 200 mg has proven to be an efficacious and safe strategy to prevent HIV in people at higher risk for HIV infection.<sup>4,5</sup> However, PrEP

efficacy is based on optimal adherence, which is a challenge for many populations, including TGW.<sup>6</sup> The PrEP Brasil study showed a downward trend over time in tenofovir diphosphate (tenofovir-DP) levels measured by dried-blood spot (DBS) among TGW.<sup>4</sup> Results from the PrEPParadas study, the first trans-specific PrEP demonstration study in Latin America, which enrolled 130 TGW at high risk for HIV infection in Rio de Janeiro, Brazil, showed decreasing adherence over time up to Week 48.<sup>7</sup>

Part of the standard of care for TGW, the goal of feminizing hormone therapy (FHT) is to induce secondary female sex characteristics while reducing male sex characteristics by using a combination of an oestrogen (e.g. oestradiol valerate) and an anti-androgen (e.g. spironolactone).<sup>8,9</sup> Some studies suggest that FHT reduces the efficacy of PrEP among TGW, which would limit the benefit of PrEP for this population.<sup>10,11</sup> Other studies indicated that seroconversions among TGW were probably related to adherence issues and different baseline characteristics rather than to potential interactions between FHT and tenofovir disoproxil fumarate/emtricitabine.<sup>12,13</sup> Given the mixed results shown by studies to date, the clinical significance of drug interactions between tenofovir disoproxil fumarate/emtricitabine and FHT among TGW remains unclear.<sup>14</sup> We aimed to evaluate the impact of FHT on tenofovir disoproxil fumarate/emtricitabine pharmacokinetics (PK) among TGW in the context of the PrEPParadas demonstration study.<sup>7</sup>

## Methods

### Study design and participants

This was a drug–drug interaction (DDI) substudy on daily oral PrEP with tenofovir disoproxil fumarate/emtricitabine (300/200 mg) and FHT nested to PrEPParadas, a 48 week PrEP trans-specific demonstration study conducted in Rio de Janeiro, Brazil between August 2017 and January 2020. PrEPParadas study procedures have been described elsewhere.<sup>7</sup> Briefly, eligibility criteria were: (1) male sex assigned at birth; (2) self-identification as TGW or any gender identity of the feminine spectrum; (3) age  $\geq 18$  years; (4) living in Rio de Janeiro or its metropolitan area; (5) HIV-negative status at screening and enrolment (baseline visit); and (6) engaging in HIV high-risk behaviour [at least one of the following: condomless anal sex in the last 6 months; sexually transmitted infection (STI) diagnosis in the last 12 months; transactional sex in the last 6 months; current sexual partner known to be living with HIV, regardless of HIV viral load]. During the screening visit, a subset of PrEPParadas participants were invited to participate in the DDI substudy. Participants using any medication known to interact with at least one of the study drugs, whose estimated creatinine clearance ( $CL_{CR}$ ) was  $< 60$  mL/min, or who had previous transfeminine bottom surgery were not enrolled.

### Study procedures

The Evandro Chagas National Institute of Infectious Diseases (INI)-FIOCRUZ Institutional Review Board reviewed and approved this project. The PrEPParadas study is registered with Clinicaltrials.gov (NCT03220152). All participants provided written consent before any study procedures. All participants had to be off FHT for at least 15 (oral regimens) or 45 (injectable regimens) days before screening. Participants were assigned to receive PrEP only (noFHT), or PrEP plus standardized FHT (sFHT: oestradiol valerate 2–6 mg plus spironolactone 100–300 mg) at the screening visit, according to the participant's willingness to use FHT for 12 weeks. All participants initiated PrEP at the baseline visit and those willing to use hormones initiated FHT at screening. The study

endocrinologist prescribed FHT and could adjust doses according to each participant's goals and self-satisfaction, according to international guidelines.<sup>15,16</sup> After 12 weeks, participants from both groups could use any oestradiol-containing FHT. Participants underwent an intensive PK evaluation at the Week 12 visit to assess short-term DDI of FHT on PrEP. Subsequently, participants who decided to start or maintain any oestradiol-containing FHT (aFHT) were invited for an intensive PK evaluation between Weeks 30 and 48 to assess long-term DDI.

We evaluated participants' weight, BMI, ALT and AST at baseline and both intensive PK evaluation visits: short-term (Week 12) and long-term (between Weeks 30 and 48). Serum creatinine (SCr), estimated  $CL_{CR}$  and estimated glomerular filtration rate (eGFR) were measured at baseline, Week 4 and the long-term intensive PK visit. eGFR was calculated using the modification of diet in renal disease study (MDRD) equation. Serum total testosterone, prolactin, sex hormone-binding globulin (SHBG) and oestradiol levels were measured at baseline and at the short-term PK visit (oestradiol was also measured at the Week 4 visit). At the long-term PK visit, testosterone, oestradiol and prolactin were also evaluated. All hormonal levels were evaluated at pre-dose sampling ( $C_{24}$ ). We used the physiological female levels (100–200 pg/mL) as reference for oestradiol levels.<sup>8</sup>

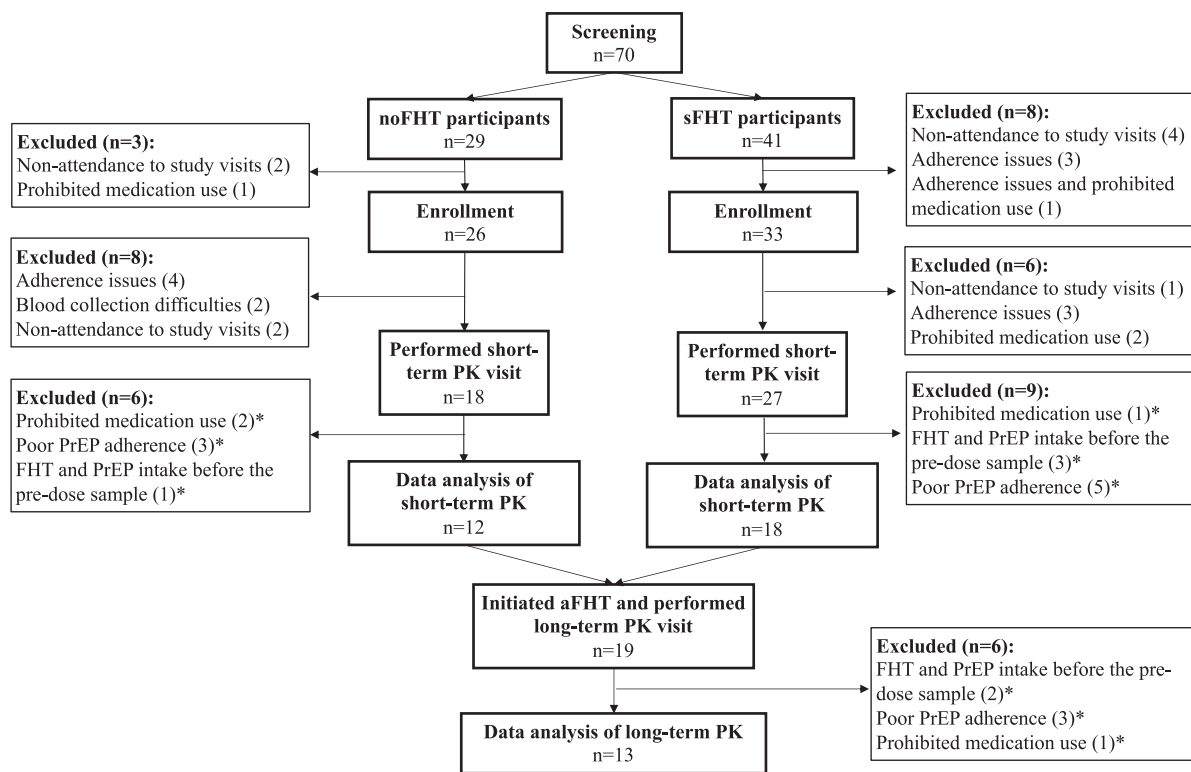
During intensive PK visits, blood samples were collected in a fasted state prior to directly observed dosing administration of PrEP and FHT, and after 0.5, 1, 2, 4, 6, 8 and 24 h. Thirty minutes after the drug intake, we offered a standard breakfast. All participants were contacted by a study team member the week prior to each intensive PK visit. In addition, they received text-message reminders 7 days and the day before the PK visit to reinforce adherence. We rescheduled the PK visit in case of any self-reported missing dose in the previous 7 days. We evaluated adherence by DBS levels [tenofovir-DP and emtricitabine triphosphate (emtricitabine-TP)] at PK visits. We estimated adherence using tenofovir-DP levels as previously described: low ( $< 350$  fmol/punch, suggestive of  $< 2$  doses of PrEP per week), medium (350–699 fmol/punch, suggestive of 2–3 doses of PrEP per week) and high ( $\geq 700$  fmol/punch, suggestive of  $\geq 4$  doses of PrEP per week).<sup>17</sup>

### Laboratory analysis

After collection, blood samples were immediately centrifuged to perform plasma separation and stored at  $-80^{\circ}\text{C}$  in cryotubes. We determined tenofovir and emtricitabine concentrations in plasma samples by LC-MS/MS at the University of Colorado Antiviral Pharmacology Laboratory (Denver, CO, USA) with standard procedures.<sup>18</sup> The lower limit of quantification of tenofovir and emtricitabine was 10 ng/mL. We also used an LC-MS/MS assay for tenofovir-DP and emtricitabine-TP quantification of DBS samples.<sup>17,19</sup> Serum total testosterone, prolactin, SHBG and oestradiol levels were determined by chemiluminescence immunoassay (ADVIA Centauro; Siemens).

### Data analysis

We used non-compartmental analysis (Pharsight WinNonlin version 7.6, Certara) to estimate tenofovir and emtricitabine PK parameters, such as  $AUC_{0-24}$ ,  $C_{max}$ ,  $C_{min}$ ,  $CL/F$ ,  $V/F$  and  $t_{1/2}$ . We excluded participants: (1) with low adherence (undetectable emtricitabine-TP DBS levels or tenofovir-DP DBS levels consistent with  $< 2$  doses per week); (2) who did not attend successive study visits; (3) who had blood collection difficulties; (4) who had taken medication prohibited by the study protocol; or (5) who had taken PrEP or FHT before the direct observed dosing at the PK visit. We used median (IQR) or frequency (percentage) to describe participants' characteristics. Non-compartmental PK parameters were summarized as geometric means with 95% CI. We initially compared PK parameters (tenofovir and emtricitabine) at the short-term PK visit using independent two-sample  $t$ -test after log transformation. In addition, we compared PK parameters (tenofovir and emtricitabine) of noFHT participants at the short-term PK visit versus aFHT participants at the long-term PK visit. Wilcoxon rank sum test was used to compare hormonal levels between noFHT versus sFHT participants, and also between noFHT



**Figure 1.** Study flow chart of participants of the PrEPParadas DDI substudy. \* excluded during data analysis.

participants at the short-term PK visit versus aFHT participants at the long-term PK visit. We initially aimed to include 24 participants in each group to observe a difference of at least 20% between PrEP and PrEP + FHT, with 80% power, considering a non-paired analysis and 5% two-sided alpha error. Sample size at short-term PK evaluation provided 80% power to detect a difference of at least 26% and 17% on tenofovir and emtricitabine plasma geometric mean  $AUC_{0-24}$ , respectively, at a significance level of 0.05. Sample size at long-term PK evaluation enabled detection of a difference of at least 31% and 26% on tenofovir and emtricitabine plasma geometric mean  $AUC_{0-24}$ , respectively. We used Spearman rank correlation to evaluate whether tenofovir or emtricitabine  $AUC_{0-24}$  had potential correlations with hormone levels, BMI, AST, ALT and age at both PK assessments, and  $CL_{CR}$  at the long-term PK assessment. We used R software version 4.0.5 to perform all statistical analyses, which considered a two-tailed 5% significance level.

## Results

### Study population

Overall, 59 participants were enrolled in the DDI study: 45 (76.3%) underwent the short-term PK assessment (noFHT: 18; sFHT: 27) and 30 (50.8%) were included in the short-term PK analysis (noFHT: 12; sFHT: 18) (Figure 1). At the short-term PK assessment, noFHT participants were older than sFHT participants [median (IQR) 30.5 years (26.8–39.3) and 26.0 years (23.0–27.8), respectively;  $P=0.03$ ] (Table 1). Among the 30 participants included in the short-term PK analysis, 27 (90%) had DBS levels consistent with high adherence [noFHT participants: 9/12 (75%); sFHT participants: 18/18 (100%)]. The levels of three noFHT participants [3/12 (25%)] were consistent with medium

adherence. Among the 30 participants included in the short-term PK analysis, 19 (63.3%) initiated any oestradiol-based FHT and performed the long-term PK visit; 13 of them were included in the long-term PK analysis. All of them had DBS levels suggestive of high adherence. After 48 weeks of follow-up, no DDI substudy participant had seroconverted to HIV.

### FHT use and hormone levels

At the short-term PK visit, most sFHT participants were on daily oestradiol valerate 2 mg (10/18; 56%); oestradiol valerate dosages of the remaining participants were 4 mg (7/18; 39%) and 6 mg (1/18; 6%). Median (IQR) oestradiol dosage was 2 (2–4) mg. Spironolactone dosages were, respectively, 100 and 200 mg among 56% (10/18) and 44% (8/18) of sFHT participants at the short-term PK visit. In the long-term PK visit, all aFHT participants ( $n=13$ ) were on oestradiol valerate at the following dosages: 2 mg (5/13; 38%), 4 mg (7/13; 54%) and 6 mg (1/13; 8%). Median (IQR) oestradiol dosage was 4 (2–4) mg. Most aFHT participants (10/13; 79%) maintained spironolactone at the long-term PK visit at the following dosages: 100 mg (5/13; 38%), 200 mg (4/13; 31%) and 300 mg (1/13; 8%); 46% of participants (6/13) were using cyproterone acetate (25 mg: 3/6; 50 mg: 3/6).

At the Week 4 visit, oestradiol levels were significantly higher among sFHT participants compared with noFHT participants (Table 2). Only 11% (2/18) of sFHT participants at the short-term PK visit and 7% (1/13) of aFHT participants at the long-term PK evaluation had oestradiol levels in the physiological female range. aFHT participants at the long-term PK evaluation had significantly lower testosterone levels compared with noFHT

**Table 1.** Characteristics of TGW on PrEP only (noFHT) and on PrEP plus standardized FHT (sFHT) of the PrEPParadas DDI substudy

Characteristics	noFHT <sup>a</sup> (n=12)	sFHT <sup>b</sup> (n=18)	P value <sup>c</sup>
Age (years), median (IQR)	30.5 (26.8–39.3)	26.0 (23.0–27.8)	<b>0.03</b>
Weight (kg), median (IQR)			
Baseline	71.9 (69.5–87.0)	67.2 (56.2–82.3)	0.13
Short-term PK	71.6 (68.5–86.7)	66.0 (55.9–83.7)	0.14
BMI (kg/m <sup>2</sup> ), median (IQR)			
Baseline	25.7 (25.0–28.6)	22.7 (20.1–27.7)	0.15
Short-term PK	25.4 (24.8–28.8)	22.5 (20.0–28.2)	0.18
Race, n (%)			
Black	1 (8)	4 (22)	—
Pardo	9 (75)	9 (50)	—
White	2 (17)	4 (22)	—
Other	0 (0)	1 (6)	—
Condomless anal sex in last 6 months	10 (83)	16 (89)	—
HIV-positive partner	1 (8)	1 (6)	—
Transactional sex	6 (50)	6 (33)	—
AST (U/L), median (IQR)			
Baseline	23.5 (21.8–25.3)	23.0 (19.0–28.0)	0.93
Short-term PK	23.0 (22.0–28.5)	21.5 (19.0–25.8)	0.26
ALT (U/L), median (IQR)			
Baseline	26.5 (22.6–36.8)	30.0 (25.0–45.5)	0.40
Short-term PK	28.5 (26.8–31.8)	231.5 (26.0–41.8)	0.31
SCr (mg/dL), median (IQR)			
Baseline	0.86 (0.79–0.93)	0.76 (0.70–0.88)	0.08
Short-term PK	0.85 (0.82–1.00)	0.85 (0.75–0.95)	0.45
eGFR <sup>d</sup> (mL/min/1.73 m <sup>2</sup> ), median (IQR)			
Baseline	102.0 (94.5–108.5)	122.0 (105.0–134.0)	<b>0.01</b>
W4	97.0 (90.3–109.0)	107.0 (96.0–129.0)	0.15
CL <sub>CR</sub> <sup>e</sup> (mL/min), median (IQR)			
Baseline	122.3 (119.6–168.5)	138.5 (119.2–163.7)	0.63
W4	123.3 (110.6–151.1)	129.2 (110.5–146.9)	0.98

Bold type indicates statistical significance.

<sup>a</sup>noFHT, only on PrEP at baseline and short-term PK; n=12 (baseline and short-term PK).

<sup>b</sup>sFHT, on standardized hormones at baseline and on PrEP+standardized hormones at short-term PK; n=18 (baseline and short-term PK).

<sup>c</sup>Wilcoxon rank sum test comparing noFHT versus sFHT participants.

<sup>d</sup>eGFR using MDRD equation (using assigned sex at birth).

<sup>e</sup>Estimated using the Cockcroft–Gault equation (using assigned sex at birth).

participants at the short-term PK visit [median (IQR) 61.1 (25.2–145.9) and 424.1 (315.4–682.1) ng/dL, respectively; *P* value=0.001]. Higher prolactin levels were observed among aFHT participants at the long-term PK evaluation compared with noFHT participants at the short-term PK visit [median (IQR) 13.0 (8.6–18.6) versus 7.6 (6.7–10.0) ng/mL, respectively; *P* value=0.03]. Oestradiol levels of aFHT participants did not differ from levels observed among noFHT participants at the short-term PK visit [median (IQR) 44.8 (24.70–57.30) versus 44.3 (32.8–48.6) pg/mL, respectively; *P* value=0.89].

### Tenofovir and emtricitabine PK

Tenofovir and emtricitabine mean concentration–time profiles measured at the short-term PK assessment are presented in

Figure 2. There were no differences between non-compartmental tenofovir and emtricitabine PK parameters when PrEP and standardized FHT were co-administered (sFHT, short-term PK; Table 3), except for emtricitabine *C*<sub>max</sub> (*P*=0.04).

Tenofovir and emtricitabine PK parameters of aFHT participants (long-term PK evaluation) did not differ from the short-term assessment of noFHT participants (Table 3). Tenofovir and emtricitabine AUC<sub>0–24</sub> were not correlated to hormonal levels, age or CL<sub>CR</sub> (*P*>0.05). BMI had a negative correlation with tenofovir AUC<sub>0–24</sub> (rho=−0.49; *P*<0.001).

### Discussion

Our results suggest that PK parameters of tenofovir and emtricitabine for daily oral PrEP are not significantly affected by

**Table 2.** Hormonal levels of participants of the PrEPParadas DDI substudy during the short-term assessment according to FHT use

Hormone	noFHT <sup>a</sup> (n=12)	sFHT <sup>b</sup> (n=18)	P value <sup>c</sup>
Baseline <sup>d</sup>			
Oestradiol (pg/mL)	43.0 (37.7–47.2)	39.4 (33.8–56.8)	1
Testosterone (ng/dL)	421.6 (260.5–543.5)	507.6 (84.4–594.5)	1
Prolactin (ng/mL)	6.25 (5.12–8.07)	7.85 (5.55–12.33)	0.09
SHBG (nmol/L)	48.5 (41.91–57.0)	65.8 (51.1–92.4)	<b>0.02</b>
Week 4			
Oestradiol (pg/mL)	38.9 (34.8–44.5)	46.0 (39.8–69.6)	<b>0.03</b>
Week 12			
Oestradiol (pg/mL)	44.3 (32.8–48.6)	56.8 (43.2–65.4)	0.05
Testosterone (ng/dL)	424.1 (315.4–682.1)	363.1 (152.5–617.7)	0.42
Prolactin (ng/mL)	7.55 (6.68–10.00)	9.40 (6.98–11.78)	0.32
SHBG (nmol/L)	41.6 (31.1–65.6)	85.4 (54.8–140.0)	<b>0.003</b>

Bold type indicates statistical significance.

<sup>a</sup>On PrEP only, no FHT at baseline and short-term PK.

<sup>b</sup>On PrEP plus FHT on short-term PK.

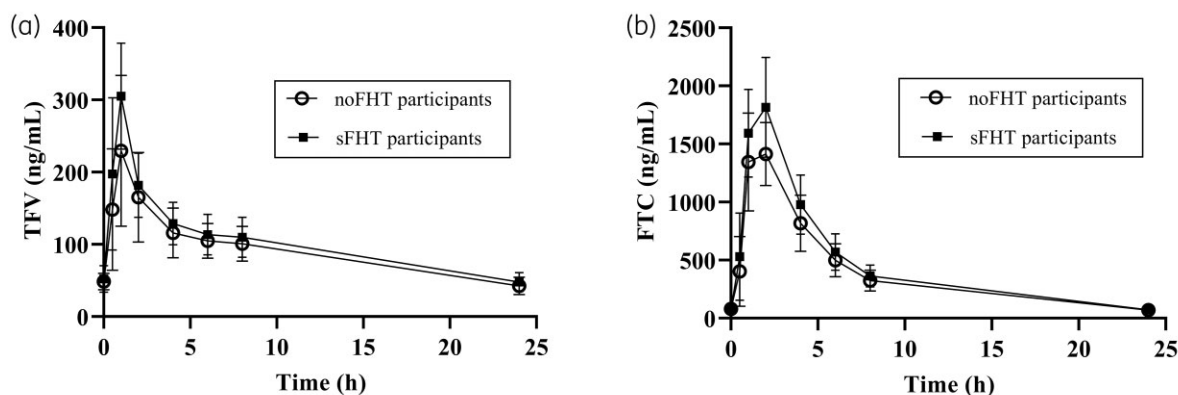
<sup>c</sup>Wilcoxon rank sum test comparing noFHT versus sFHT participants.

<sup>d</sup>noFHT and sFHT participants evaluated at enrolment and screening visits, respectively.

oestradiol-based FHT. Previous studies had assessed this interaction via PK evaluations; however, results were based on short-term follow-up: one study from Thailand evaluated after 8 weeks (iFact, n=20);<sup>10</sup> two studies from the USA evaluated after 7 days (n=8)<sup>20</sup> and 14 days (n=15).<sup>21</sup> To our knowledge, this is the first trans-specific PK study to evaluate the impact of both standardized and aFHT on PrEP among participants at a higher risk for HIV that includes longer-term follow-up (i.e. beyond 12 weeks). The PrEPParadas study showed a high retention rate and decreasing adherence levels over time, particularly among those with higher social vulnerability.<sup>7</sup>

Our analysis did not detect differences between tenofovir and emtricitabine PK parameters between sFHT participants and noFHT participants, except for emtricitabine C<sub>max</sub>. Moreover, no differences were detected between the short-term assessment of noFHT participants and aFHT participants in a long-term follow-up.

Recent short-term PK studies evaluated DDI between FHT and tenofovir disoproxil fumarate/emtricitabine. A study from Thailand revealed decreased tenofovir AUC<sub>0–24</sub> and concentration at 24 h after the dose (C<sub>24</sub>) (12% and 18%, respectively) among TGW on tenofovir disoproxil fumarate/emtricitabine and FHT (oestradiol valerate and cyproterone acetate) after 8 weeks.<sup>10</sup> The authors suggested an association between lower tenofovir exposure and potentially increased oral clearance related to FHT. A recent PK study conducted in Baltimore, USA reported lower tenofovir C<sub>24</sub> and AUC<sub>0–24</sub> [32% (P=0.01) and 27% (P=0.07), respectively] and 38% higher (P=0.07) tenofovir CL/F among TGW (n=8) on tenofovir disoproxil fumarate/emtricitabine and aFHT as compared with cisgender men (n=8) on tenofovir disoproxil fumarate/emtricitabine after 7 days.<sup>20</sup> The same study also showed that TGW had 32% lower emtricitabine C<sub>24</sub> (P=0.04), 24% lower emtricitabine AUC<sub>0–24</sub> (P=0.03) and 31% higher emtricitabine



**Figure 2.** Concentration versus time at short-term PK assessment of noFHT and sFHT participants. Plasma tenofovir (TFV) and emtricitabine (FTC) at pre-dose and 0.5, 1, 2, 4, 6, 8 and 24 h in TGW are shown in (a) and (b), respectively, for noFHT participants (no FHT, PrEP only; circles, n=12) and sFHT participants (PrEP plus standardized FHT; squares, n=18). Data are means with error bars indicating SDs.



**Table 3.** Tenofovir (TFV) and emtricitabine (FTC) non-compartmental PK parameters of participants of the PrEPParadas DDI substudy according to FHT use and period of evaluation

Parameter	Short-term PK				Long-term PK		
	noFHT <sup>a</sup> (n=12) GM (95% CI)	sFHT <sup>b</sup> (n=18) GM (95% CI)	GMR <sup>c</sup> (95% CI)	P value <sup>d</sup>	aFHT <sup>e</sup> (n=13) GM (95% CI)	GMR <sup>f</sup> (95% CI)	P value <sup>g</sup>
TFV AUC <sub>0-24</sub> (ng·h/mL)	2136.27 (1831.41–2491.87)	2392.38 (2109.75–2712.87)	1.12 (0.92–1.37)	0.25	2160.80 (1858.14–2512.74)	1.01 (0.82–1.24)	0.91
TFV C <sub>max</sub> (ng/mL)	247.86 (208.92–294.05)	299.60 (260.58–344.46)	1.21 (0.97–1.51)	0.09	269.15 (229.50–315.65)	1.09 (0.85–1.38)	0.49
TFV T <sub>max</sub> (h)	0.94 (0.76–1.17)	0.89 (0.75–1.06)	0.94 (0.72–1.24)	0.67	1.00 (0.74–1.34)	1.06 (0.71–1.57)	0.76
TFV CL/F (L/h)	140.43 (120.39–163.81)	125.40 (110.58–142.20)	0.89 (0.73–1.09)	0.25	138.84 (119.39–161.45)	0.99 (0.80–1.22)	0.91
TFV V/F (L)	2778.20 (2309.96–3341.36)	2503.86 (2153.58–2911.12)	0.90 (0.71–1.14)	0.38	2872.17 (2398.88–3438.85)	1.03 (0.78–1.36)	0.80
TFV C <sub>min</sub> (ng/mL)	39.41 (33.44–46.44)	43.13 (37.72–49.32)	1.09 (0.89–1.35)	0.39	40.70 (34.08–48.60)	1.03 (0.84–1.27)	0.75
TFV lambda (h <sup>-1</sup> )	0.05 (0.045–0.057)	0.05 (0.046–0.056)	1.00 (0.86–1.17)	0.97	0.049 (0.044–0.055)	0.98 (0.82–1.17)	0.82
FTC AUC <sub>0-24</sub> (ng·h/mL)	9693.51 (8761.14–10725.11)	10980.78 (10110.49–11925.99)	1.13 (0.99–1.29)	0.06	9512.94 (8200.43–11035.53)	0.98 (0.82–1.17)	0.83
FTC C <sub>max</sub> (ng/mL)	1656.00 (1489.67–1840.90)	1911.02 (1752.79–2083.54)	1.15 (1.01–1.32)	<b>0.04</b>	1848.62 (1642.59–2080.49)	1.12 (0.96–1.30)	0.15
FTC T <sub>max</sub> (h)	1.50 (1.22–1.84)	1.59 (1.34–1.87)	1.06 (0.81–1.38)	0.66	1.30 (1.06–1.61)	0.87 (0.65–1.17)	0.34
FTC CL/F (L/h)	20.63 (18.23–22.83)	18.21 (16.77–19.78)	0.88 (0.77–1.01)	0.06	21.02 (18.12–24.39)	1.02 (0.86–1.21)	0.83
FTC V/F (L)	192.30 (163.26–226.51)	167.55 (146.58–191.51)	0.87 (0.71–1.08)	0.19	205.78 (163.45–259.07)	1.07 (0.81–1.42)	0.63
FTC C <sub>min</sub> (ng/mL)	63.39 (53.20–75.52)	66.41 (57.56–76.62)	1.05 (0.84–1.31)	0.68	60.97 (49.66–74.85)	0.96 (0.76–1.22)	0.74
FTC lambda (h <sup>-1</sup> )	0.11 (0.10–0.12)	0.11 (0.10–0.12)	1.01 (0.89–1.14)	0.86	0.10 (0.09–0.12)	0.96 (0.82–1.10)	0.44

GM, geometric mean; GMR, geometric mean ratio. Bold type indicates statistical significance.

<sup>a</sup>On PrEP only (n=12).

<sup>b</sup>On PrEP plus standardized FHT (n=18).

<sup>c</sup>sFHT (short-term PK)/noFHT (short-term PK) GMR.

<sup>d</sup>t-test comparing noFHT versus sFHT participants.

<sup>e</sup>On PrEP plus any oestradiol-based FHT (n=13).

<sup>f</sup>aFHT (long-term PK)/noFHT (short-term PK) GMR.

<sup>g</sup>t-test comparing noFHT (short-term PK) versus aFHT (long-term PK) participants.

CL/F ( $P=0.03$ ). Tenofovir-DP and emtricitabine-TP levels in PBMCs and colon tissue were similar among cisgender men and TGW, although the authors noted that this result may be due to the small sample size and assay variability. A study from Nebraska, USA reported 24% and 14% lower tenofovir and emtricitabine AUC<sub>0-24</sub>, respectively, among TGW (n=15) on 17 $\beta$ -oestradiol plus spironolactone after 14 days of PrEP when compared with historical controls (cisgender men and women).<sup>21</sup> Tenofovir exposure decrease was lower when restricting the analysis to participants with a BMI of <30 kg/m<sup>2</sup>. The Discover study reported that cisgender men and TGW on FHT plus PrEP had clinically comparable levels of tenofovir-DP and emtricitabine-TP in PBMC samples.<sup>22</sup> Among people living with HIV, TGW using tenofovir disoproxil fumarate/

emtricitabine-based ART and FHT presented rectal levels of tenofovir-DP:deoxyATP ratio 7-fold lower than cisgender participants. The rectal levels of tenofovir-DP:deoxyATP ratio were also inversely correlated with female sex hormones, suggesting a negative impact of FHT on PrEP efficacy, independently of tenofovir plasma levels.<sup>11</sup>

Interestingly, tenofovir and emtricitabine exposures (AUC<sub>0-24</sub>) were lower in our study population, regardless of FHT use, than in controls from previous studies from the USA. Median (IQR) tenofovir and emtricitabine were, respectively, 3430 ng·h/mL (2720–3650) and 12 700 ng·h/mL (11 110–13 640) among cisgender men from Baltimore, USA,<sup>20</sup> and historical controls of cisgender men from Denver and San Francisco, USA were 2650 ng·h/mL

(1900–3800) and 11 000 ng·h/mL (8800–13 100).<sup>23</sup> Previous PK studies had evaluated US<sup>20,21</sup> and Thai<sup>10</sup> populations. Tenofovir and emtricitabine PK present high variability (10%–30%) and may be influenced by several factors, including race.<sup>24</sup> Brazil is a multi-ethnic country with the sixth largest population globally and its diversity limits the evaluation of possible correlations between race and PrEP exposure, as well as the comparison between current results and other studies enrolling populations of different ancestry.<sup>25</sup> However, it is noteworthy that median emtricitabine and tenofovir AUC<sub>0–24</sub> in short- and long-term assessments were within the range described in previous controlled studies (8000–16 484 ng·h/mL for emtricitabine and 2000–3000 ng·h/mL for tenofovir).<sup>24,26</sup>

Our study has some important strengths toward understanding DDIs between PrEP and FHT among TGW. We enrolled TGW at high risk of HIV infection who would also most benefit from PrEP. Furthermore, we closely monitored FHT use until Week 12 to avoid interference of concomitant drugs with PrEP PK. In addition, we evaluated any oestradiol-based FHT in a long-term follow-up.

This study has several limitations. As we did not evaluate tenofovir-DP and emtricitabine-TP in PBMCs or rectal tissue CD4+ cells, the evaluation of tenofovir disoproxil fumarate/emtricitabine efficacy is beyond the scope of the current analysis. We did not perform directly observed therapy throughout the study follow-up. However, we indirectly estimated adherence based on tenofovir-DP and emtricitabine-TP in DBS and excluded participants with levels consistent with low adherence. The parallel study design without randomization could contribute to data variability regarding tenofovir and emtricitabine PK. We did not have long-term data on TGW on PrEP only. As many TGW consider FHT an important component of their medical transition,<sup>27</sup> it would be neither ethical nor feasible to ask them to remain off FHT for a long period of time. FHT dosages were adjusted according to participants' goals. Despite the option of increasing the oestradiol valerate dosage, the majority of participants remained on their initially prescribed dosage along the study, though only a small percentage of them had achieved oestradiol levels within the adult female range. This was also observed in other DDI studies on FHT and PrEP.<sup>10,21</sup> Our study evaluated only one oestrogen-based FHT regimen in a short period and diverse regimens in a long period. As such, current results may not be extrapolated to other FHT regimens and/or dosages. Finally, PK studies usually comprise small samples among specific groups, and results cannot be generalized to the whole population.

PrEP and FHT are important biomedical approaches that may be lifesaving for TGW. Our results contribute to a growing body of evidence supporting the concomitant use of FHT and PrEP among TGW. PrEP may and should be offered to all TGW engaging in HIV high-risk behaviour, regardless of their hormone use. Future studies should focus on examining factors that impact PrEP retention, adherence and persistence in order to improve the benefit of PrEP for TGW around the world.

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## Members of the PrEPParadas study team

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## Author contributions

Emilia Moreira Jalil, Valdilea Gonçalves Veloso, Beatriz Grinsztejn and Rita Estrela conceived the study and interpreted the findings. Emilia Moreira Jalil, Beatriz Grinsztejn, Rita Estrela and Vitória Berg Cattani drafted the manuscript. Vitória Berg Cattani did the statistical analyses with aid from Emilia Moreira Jalil and Rita Estrela. Thiago Torres, Sandra Wagner Cardoso, Leonardo Eksterman, Cristiane R.V. Castro and Laylla Monteiro helped with data acquisition, interpretation of the findings, and drafting the manuscript. Valdilea Gonçalves Veloso, Peter Anderson, Lane Bushman and Erin Wilson were involved in revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

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