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ORIGINAL RESEARCH ARTICLE

Estradiol and Spironolactone Plasma Pharmacokinetics Among Brazilian Transgender Women Using HIV Pre-Exposure Prophylaxis: Analysis of Potential Interactions

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Abstract

Background and Objective An important barrier to HIV prevention among transgender women (TGW) is the concern that oral pre-exposure prophylaxis (PrEP) negatively affects the efficacy of feminizing hormone therapy (FHT). We aimed to assess the impact of PrEP on FHT pharmacokinetics (PK) among TGW from Brazil.

Methods We performed a drug-drug interaction sub-study among TGW enrolled in a daily oral PrEP demonstration study (PrEParadas, NCT03220152). Participants had a first PK assessment (PK1) 15 days after FHT (estradiol valerate 2–6 mg plus spironolactone 100–200 mg) initiation and then started PrEP (tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg). A second PK evaluation was performed 12 weeks later (PK2). Blood samples were collected prior and after the directly observed dosing (0, 0.5, 1, 2, 4, 6, 8, and 24 hours). Pharmacokinetic parameters of estradiol, spironolactone, and metabolites were estimated by non-compartmental analysis (Monolix 2021R2, Lixoft®) and compared as geometric mean ratios (GMRs, 90% confidence interval [CI]).

Results Among 19 TGW who completed the substudy, median age was 26 years (interquartile range: 23–27.5). Estradiol area under the plasma concentration-time curve (AUC_τ) and trough concentrations did not differ between PK1 and PK2 evaluations (GMR [90% CI]: 0.89 [0.76–1.04] and 1.06 [0.94–1.20], respectively). Spironolactone and canrenone AUC_τ were statistically lower at PK2 than PK1 (0.76 [0.65–0.89] and 0.85 [0.78–0.94], respectively). Canrenone maximum concentration was also lower at PK2 than PK1 (0.82 [0.74–0.91]).

Conclusion Estradiol PK was not influenced by PrEP concomitant use. The small differences observed in some spironolactone and canrenone PK parameters should not prevent the concomitant use of estradiol-based FHT and PrEP.

Trial Registration This trial (NCT03220152) was registered on July 18, 2017.

Key Points

There is a gap in the knowledge of feminizing hormone therapy (FHT) pharmacokinetics (PK) among transgender women (TGW) and its interactions with pre-exposure prophylaxis (PrEP) drugs.

Estradiol and estrone sulfate PK were not influenced by oral PrEP drugs; spironolactone and canrenone exposure was lower when PrEP and FHT were taken concomitantly.

Our results show that oral PrEP and estradiol-based FHT may be used concomitantly.

The members of PrEParadas study team are listed in the Acknowledgments section.

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1 Introduction

Transgender women (TGW) are highly vulnerable to HIV infection, with 66-times increased odds of infection when compared to other individuals aged > 15 years [1]. Social vulnerability, high rates of unemployment and discrimination contribute to this scenario, making TGW a target population for public health policies. A study from Rio de Janeiro, Brazil, reported that 43.7% of TGW newly diagnosed with HIV had a negative test result in the previous 12 months [2]. These results underscore how crucial prevention strategies are for this specific group.

Oral pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate 300 mg and emtricitabine 200 mg (TDF/FTC) has been shown to be effective in preventing HIV [3]. It has been implemented in several countries and is available as part of the Brazilian public health system (SUS) strategy for HIV prevention since 2017 [4]. However, data to support strategies to increase PrEP acceptance among TGW are scarce [5–7].

One of the barriers to PrEP use among TGW is the potential interactions between feminizing hormone therapy (FHT) and oral PrEP drugs [7–9]. Feminizing hormone therapy usually includes estrogens, such as 17β-estradiol and estradiol valerate, and antiandrogen drugs. Spironolactone, a diuretic with antiandrogenic activity, and cyproterone acetate, a progestin with potent antiandrogenic activity, are the most used drugs for adjunctive therapy [10]. In settings with easy access to prescribed FHT, TGW usually choose their own hormones without medical supervision, increasing the risk of side effects, such as thromboembolic events, and pharmacological interactions [11–13].

Estradiol valerate is rapidly converted into 17β-estradiol by first-pass metabolism after oral administration. Estradiol is transformed into estrone, a metabolite with pharmacological activity, and into estrone sulfate and estrone glucuronide. Both cytochrome P450 (CYP) and uridine 5′-diphosphoglucuronosyltransferase (UGT1A1) enzymes are involved in estradiol and estrone metabolism [14]. Those metabolites may then enter enterohepatic recirculation, which may delay the estradiol terminal half-life ($t_{1/2}$: 13–20 h) [15]. An estradiol valerate dosage of 2 mg/day for 3 weeks in TGW resulted in an area under the plasma concentration-time curve from zero to 24 h (AUC_τ mean [coefficient of variation, CV%]) of 775.13 (26.2) pg·h/mL [16]. Spironolactone is rapidly absorbed after oral administration (time to maximum concentration [t_{max}] 2.6 h), with bioavailability greatly increased by food intake [17]. Spironolactone metabolism involves hepatic transferases and esterases [14]. One of its metabolites, canrenone, exhibits pharmacological activity [18]. After a single oral administration of 200 mg of spironolactone in a fasted state, spironolactone and canrenone AUC_{0-24} (mean [standard deviation, SD]), were, respectively, 288 (138) ng·h/mL and 2650 (482) ng·h/mL. There was no indication of non-linear pharmacokinetics (PK) after administering spironolactone 50–200 mg [19].

There is a knowledge gap on FHT PK and the interactions with oral PrEP drugs among TGW [20]. Concerns about a potential negative impact of PrEP on FHT have been hypothesized to contribute to poor PrEP adherence among TGW [7, 8]. Although estradiol and spironolactone metabolism involve CYP enzymes and hepatic esterases and transferases, which could interact with other drugs, tenofovir or emtricitabine are not known to affect CYP enzymes [14, 21]. Some studies have recently evaluated PrEP and FHT interactions in that population [16, 22–27]. However, only two studies assessed PrEP impact on estradiol intense PK among TGW: the iFact study in Thailand [16] and a study in the USA [25]. Both studies observed no difference in estradiol exposure (AUC and maximum concentration [C_{max}]) when FHT and oral PrEP were concomitantly used. Among TGW living with HIV, the Thai study reported lower estradiol AUC, C_{max} and concentration at 24 h (C_{24}) when FHT was administered with antiretroviral treatment containing TDF, FTC and efavirenz [28]. No study focused on the FHT antiandrogen component. The available data on bidirectional interactions between PrEP and FHT [16, 25, 26] are limited, thus further studies are needed in diverse settings and populations to fully evaluate potential interactions. In this context, our study aimed to evaluate the potential interactions of daily oral PrEP (TDF/FTC) on a standardized FHT (estradiol valerate and spironolactone) PK among TGW from Brazil after 12 weeks.

2 Methods

2.1 Study Participants and Procedures

This was a drug-drug interaction (DDI) study evaluating FHT and daily oral PrEP with TDF/FTC nested in the trans-specific PrEP demonstration project, *PrEParadas*, conducted in Rio de Janeiro, Brazil, from August 2017 to January 2020. *PrEParadas* study procedures and the results of DDI interactions of FHT on TDF/FTC PK are described elsewhere [7, 27]. *PrEParadas* inclusion criteria were: TGW aged ≥ 18 years, living in Rio de Janeiro or its metropolitan area, HIV negative status at screening and enrollment (baseline visit), and reporting engagement in at least one of the following: condomless anal sex in the last 6 months, sexually transmitted infection diagnosis in the last 12 months, transactional sex in the last 6 months, current sexual partner living with HIV regardless of HIV viral load. Participants who had history of pathological bone fracture, creatinine clearance (CL_{CR}) < 60 mL/min (estimated using the Cockcroft–Gault equation, using assigned sex at birth) [29], use of any medication known to interact with at least one of the study drugs,

and any previous transfeminine bottom surgery (orchiectomy and/or vaginoplasty) were not enrolled.

The Evandro Chagas National Institute of Infectious Diseases-Fiocruz Institutional Review Board approved the study. *PrEParadas* study is registered with clinicaltrials.gov (NCT03220152). All participants signed informed consent forms before any study procedure. Participants included in the DDI study were off FHT for at least 15 (oral regimens) or 45 (injectable regimens) days before screening (Fig. 1). The standardized study FHT (estradiol valerate 2–6 mg plus spironolactone 100–200 mg) was initiated at the screening visit. Throughout the 12 weeks of follow-up (enrollment and Weeks 4, 7, and 9), the participants' estradiol trough plasma concentrations (C_{trough}) were available to the study endocrinologist, who could adjust FHT dosage based on clinical evaluation and participant's self-satisfaction, as recommended by available guidelines of transgender health care [30, 31]. The main criteria for estradiol dose adjustment were participant's goals and self-satisfaction. Physiological female levels (100–200 pg/mL) served as a safety parameter for estradiol levels [10] to avoid levels above 200 pg/mL. Fifteen days after the FHT initiation, participants had the first intensive PK (PK1, only FHT) evaluation to assess the FHT PK and then initiated PrEP. A second intensive PK evaluation (PK2, FHT plus PrEP) was performed at the Week 12 visit to assess possible DDI of PrEP drugs on FHT PK.

We evaluated participants' age (years), CL_{CR} (mL/min), and race (Black, *Pardo*, White and other) at baseline; weight (kg), body mass index (BMI; kg/m^2), alanine aminotransferase (ALT; U/L), and aspartate aminotransferase (AST; U/L) at baseline and PK2. Estradiol levels were measured at screening, enrollment (PK1), follow-up visits (Weeks 4, 7, and 9), and Week 12 (PK2) at pre-dose sampling (C_{trough}).

Blood samples were collected prior and after (0, 0.5, 1, 2, 4, 6, 8, and 24 hours) the directly observed dosing administration in fasted state for both PK1 and PK2 evaluations. Thirty minutes after the drug intake, we offered a standard breakfast. One week before PK1 and PK2, all participants received reminders to adhere to FHT and PrEP. Adherence to FHT was evaluated by self-report at each PK visit. Pharmacokinetic visits were rescheduled if the participant reported missing any dose 7 days before each visit. Pre-exposure prophylaxis adherence was also evaluated by dried-blood spots (DBS) levels of tenofovir-diphosphate (tenofovir-DP) and emtricitabine-triphosphate (emtricitabine-TP) at Week 12; adherence was stratified into: low (less than 350 fmol per punch, suggestive of < 2 doses of PrEP per week), medium (350–699 fmol per punch, suggestive of two to three doses of PrEP per week), and high (700 fmol per punch or greater, suggestive of 4+ doses of PrEP per week) [32].

2.2 Laboratory Analysis

Blood samples were immediately centrifuged after collection, and plasma was stored at − 80 °C in cryotubes. Estradiol and metabolites (estrone and estrone sulfate), spironolactone and one metabolite (canrenone) were determined in plasma samples by validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods at the Fiocruz Pharmacokinetics Service (Rio de Janeiro, Brazil). Estradiol/estrone and chlorthalidone, as internal standard (IS), were extracted from plasma samples with methyl tert-butyl ether. After evaporation to dryness, the residue was reconstituted in an acetonitrile:water solution (70:30, v/v). We used a C18 column and water:acetonitrile (68:32, v/v) as mobile phase. The transitions of *m/z* 271.11 → 145.0 and *m/z* 269.114 → 145.005 were monitored for estradiol and estrone, respectively. The *m/z* 336.87 → 190.16 transition was monitored for the IS. Estradiol and estrone concentrations were linear in the range of 25–500 pg/mL and 25–1000 pg/mL, respectively. The inaccuracy and imprecision were lower than 15%. Both lower limit of quantification (LLQ) imprecision and inaccuracy were below 20%.

Estrone sulfate was determined after a simple acetonitrile protein precipitation, with chlorthalidone as IS, C18 column and water:acetonitrile (65:35, v/v) as mobile phase. The transitions of *m/z* 349.037 → 269.200 and *m/z* 336.936 → 189.932 were monitored for the quantification of estrone sulfate and for the IS, respectively. Estrone sulfate concentrations were linear in the range of 0.1–50 ng/mL. The

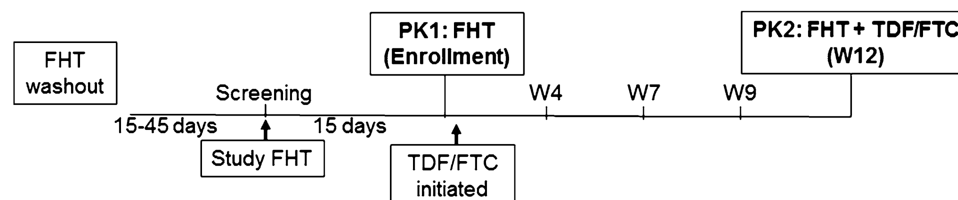

Fig. 1 Study scheme. *FHT* feminizing hormone therapy, *PK* pharmacokinetic, *PK1* first PK evaluation, participants on FHT only (enrollment) and TDF/FTC initiated by the end of the PK1 visit; PK2: second PK evaluation, participants on FHT plus PrEP (TDF/FTC). Estradiol pre-dose levels (C_{trough}) were evaluated at Weeks 4 (W4), 7 (W7), and 9 (W9)

inaccuracy was lower than 6.5%. Both LLQ imprecision and inaccuracy were below 17%. Spironolactone, canrenone, and diazepam (IS) were extracted from plasma samples with methyl tert-butyl ether and the supernatant was evaporated to dryness. The residue was reconstituted in a 65:35 (v/v) solution of methanol:formic acid (0.1% in water). We used a C8 column and ultrapure water with formic acid 0.1%:methanol (35:65, v/v) as mobile phase. The transitions of *m/z* 341.169 → 107.169 and *m/z* 341.152 → 107.041 were monitored for the quantification of spironolactone and canrenone, while the transition of *m/z* 285.320 → 193.087 was monitored for the IS. Spironolactone and canrenone concentrations were linear in the range of 1–200 ng/mL and 1–250 ng/mL, respectively. The inaccuracy and imprecision were lower than 10%. Both LLQ imprecision and inaccuracy were below 12%. All methods presented accuracy and precision according to the established in the Brazilian Health Regulatory Agency (ANVISA) guideline [33].

We used LC-MS/MS for tenofovir-DP and emtricitabine-TP quantification of DBS samples, as previously described [32, 34].

2.3 Data Analysis

Non-compartmental PK parameters, i.e., AUC_τ, maximum concentration at steady-state ($C_{max,ss}$), t_{max}, minimum concentration (C_{min}), apparent total body clearance (CL/*F*), apparent volume of distribution at steady-state (V_{ss}/F), and $t_{½}$ were estimated for estradiol and spironolactone. Estrone, estrone sulfate, and canrenone had AUC_τ, $C_{max,ss}$, t_{max}, and C_{min} estimated (Monolix Software® Suite 2021R2, Lixoft®, Antony, France). We excluded participants: (1) with low PrEP adherence (tenofovir-DP suggestive of < 2 doses/week or undetectable levels of emtricitabine-TP), (2) who did not attend successive study visits, (3) who had taken medication prohibited by the study protocol (i.e., medications that could interact with PrEP or FHT), (4) who took their FHT pills prior the pre-dose PK sampling, and (5) who had blood collection difficulties. In the descriptive analyses, we used medians and interquartile range (IQR) and absolute and relative frequencies, respectively, for continuous numerical variables and for nominal variables. Non-compartmental PK parameters were summarized as geometric means and compared between PK1 and PK2 as geometric mean ratios (GMRs, 90% CI) using a paired t-test after log transformation. Our sample size provided 80% power to detect a difference of at least 23 and 23.5% on estradiol and spironolactone plasma geometric mean AUC_τ, respectively, at a significance level of 0.05. Since our participants were using different doses of FHT, we presented AUC_τ and $C_{max,ss}$ of spironolactone, estradiol, and their metabolites normalized to a dose of 100 mg of spironolactone or 2 mg of estradiol valerate (equivalent to 1.53 mg of estradiol when accounting for the molecular weights), respectively. This means we used the ratio of the individual parameter (AUC_τ and $C_{max,ss}$) by the multiple of the lower dose (2 mg of estradiol valerate or 100 mg of spironolactone) according to the following equations:

$$AUC_\tau = \frac{AUC_{\tau_obs}}{(E2Vdose/2)} \quad \text{and} \quad C_{max,ss} = \frac{C_{max,ss_obs}}{(E2Vdose/2)} \quad \text{for estradiol}$$

$$AUC_\tau = \frac{AUC_{\tau_obs}}{(SPRdose/100)} \quad \text{and}$$

$$C_{max,ss} = \frac{C_{max,ss_obs}}{(SPRdose/100)} \quad \text{for spironolactone,}$$

where: AUC_τ and $C_{max,ss}$: individual parameter normalized, AUC_{τ_obs} and C_{max,ss_obs}: individual parameter calculated from individual plasma concentration-time curve, E2V dose: estradiol valerate dose administered, SPR dose: spironolactone dose administered.

A linear regression model with random effect for individuals was used to evaluate the association between estradiol C_{trough} and time (study week) of PrEP use (enrollment [PK1], weeks 4, 7, 9, and 12 [PK2]). We considered $p < 0.05$ as statistically significant. All statistical analyses were performed in R v.4.0.5 software, utilizing the 'nlme' library to develop statistical models.

3 Results

3.1 Study Population

From August 2017 to January 2020, 33 participants were enrolled and underwent PK1 evaluations (Fig. 2). Of these, 27 underwent PK2 evaluations, with 8 participants excluded afterwards due to use of prohibited medication ($n = 1$), FHT intake before directly observed dosing ($n = 2$), and low adherence (self-reported or based on tenofovir-DP DBS levels) ($n = 5$). As such, PK2 assessment analysis included 19 participants and a total of 304 observations.

All participants presented high PrEP adherence. At baseline, median age was 26 years (23–27.5), and BMI was 22.4 kg/m^2 (20.2–27.4) (Table 1). Estradiol valerate doses ranged from 2 to 4 mg at PK1 and from 2 to 6 mg at PK2. Most participants were on daily estradiol valerate 2 mg throughout the 12 weeks of follow-up: 16/19 (84%) participants at PK1 and 10/19 (53%) at PK2 (Table 2). Spironolactone doses ranged from 100 to 200 mg during the study; 18/19 (95%) and 10/19 (53%) participants were taking 100 mg at PK1 and PK2, respectively.

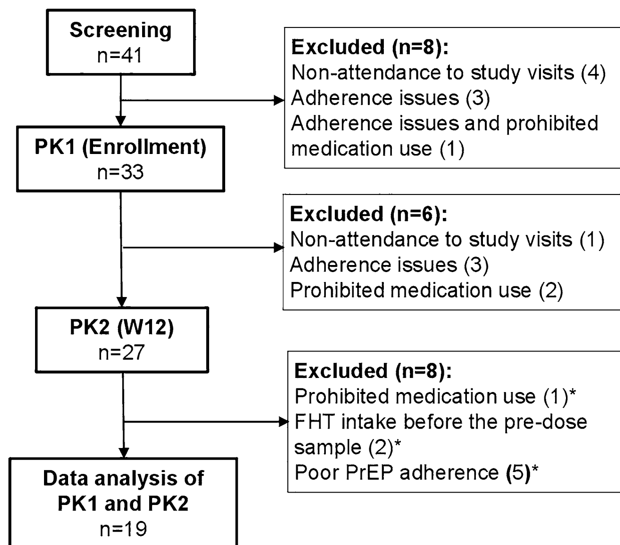

Fig. 2 Study flow chart of study participants. *FHT* feminizing hormone therapy; *PK* pharmacokinetic; *PK1* first PK evaluation, participants on FHT only (enrollment) and TDF/FTC initiated by the end of the PK1 visit; PK2: second PK evaluation, participants on FHT plus PrEP (TDF/FTC). *Excluded during data analysis

3.2 Estradiol and Metabolites PK

Concentration-time plasma profiles of estradiol, estrone and estrone sulfate are presented in Fig. 3 and Supplementary Figure 1 (one participant on 6 mg of estradiol valerate at PK2). No differences were observed between estradiol PK parameters at PK1 (only FHT) and PK2 (FHT plus PrEP) evaluations (Table 3).

Considering estradiol metabolites, estrone AUC_{τ} and $C_{max,ss}$ were 20% and 16% lower at PK2 (FHT plus PrEP) than PK1 (only FHT) (GMR [90%CI] AUC_{τ}: 0.80 [0.71–0.91]; $C_{max,ss}$: 0.84 [0.73–0.95]). No other differences on estradiol metabolites PK parameters were detected. Furthermore, there were no differences in estradiol C_{trough} values after FHT initiation (p = 0.54) throughout the 12 weeks of follow-up (Suppl. Fig. 2).

3.3 Spironolactone and Canrenone

Spironolactone and canrenone plasma concentration versus time at PK1 and PK2 are presented in Fig. 4. Spironolactone

Table 1 Characteristics of study participants

Characteristics Median (IQR)	PK1[a]	PK2[b]	p value[c]
Age (years)	26 (23–27.5)		–
Weight (kg)	67.1 (57.9–81.1)	64.7 (57.7–83.2)	0.68
BMI (kg/m^2)	22.4 (20.2–27.4)	21.9 (20.2–28.1)	0.76
Race, *n* (%)			
Black	6 (26)		–
Pardo	12 (52)		–
White	4 (18)		–
Other	1 (4)		–
Condomless anal sex in last 6 mo, *n* (%)	17 (89)		–
HIV-positive partner, *n* (%)	1 (5)		–
Transactional sex, *n* (%)	6 (32)		–
AST (U/L)	23 (18.3–27.8)	22.5 (19.3–25.8)	0.97
ALT (U/L)	29 (24.5–44)	31 (26–41.5)	0.98
CL_{CR} (mL/min)[d]	132.9 (120.7–163.1)		–

ALT alanine aminotransferase (data from screening and Week 12 [PK2])

AST aspartate aminotransferase (data from screening and Week 12 [PK2]), *BMI* body mass index, CL_{CR} estimated creatinine clearance (date from screening), *IQR* interquartile range, *TDF/FTC* tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg

[a]PK1: first pharmacokinetic (PK) evaluation (n = 19), participants on FHT only (enrollment) and TDF/FTC initiated by the end of the PK1 visit

[b]PK2: second PK evaluation, participants on FHT plus PrEP (TDF/FTC) (n=19)

[c]Wilcoxon rank sum test comparing PK1 and PK2

[d]Estimated using the Cockcroft–Gault equation (using assigned sex at birth)

Table 2 Feminizing hormone therapy doses at PK1 and PK2 among study participants

Drug	Dose (mg/day)	PK1[a]	PK2[a]
Estradiol valerate	2	16 (84%)	10 (53%)
	4	3 (16%)	8 (42%)
	6	0 (0%)	1 (5%)
Spironolactone	100	18 (95%)	10 (53%)
	200	1 (5%)	9 (47%)

PK1: first pharmacokinetic (PK) evaluation (n= 19), participants on FHT only (enrollment) and TDF/FTC initiated by the end of the PK1 visit; PK2: second PK evaluation, participants on FHT plus PrEP (TDF/FTC) (n=19). TDF/FTC: tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg

FHT feminizing hormone therapy, *PK* pharmacokinetics, *PrEP* pre-exposure prophylaxis, *TDF/FTC* tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg

[a] n (%)

AUC_τ was 24% lower at PK2 (FHT plus PrEP) than PK1 (only FHT) (GMR [90% CI]: 0.76 [0.65–0.89]) while CL/F was 32% higher (1.32 [1.13–1.54]) (Table 4). Similarly, canrenone AUC_τ and $C_{max,ss}$ were lower at PK2 (0.85 [0.78–0.94] and 0.82 [0.74–0.91], respectively).

4 Discussion

The concomitant use of FHT (estradiol valerate plus spironolactone) and daily oral PrEP (TDF/FTC) for 12 weeks did not influence estradiol or estrone sulfate PK. We observed differences of low magnitude (lower than 25% for AUC_τ and $C_{max,ss}$) for estrone PK, but no differences on estradiol exposure (AUC_τ and $C_{max,ss}$) or trough levels. We observed an AUC_τ 25% lower for spironolactone and canrenone, and a $C_{max,ss}$ 28% lower for canrenone at PK2, when FHT was combined with PrEP. To our knowledge, this is the first study to evaluate not only the estradiol component (estradiol

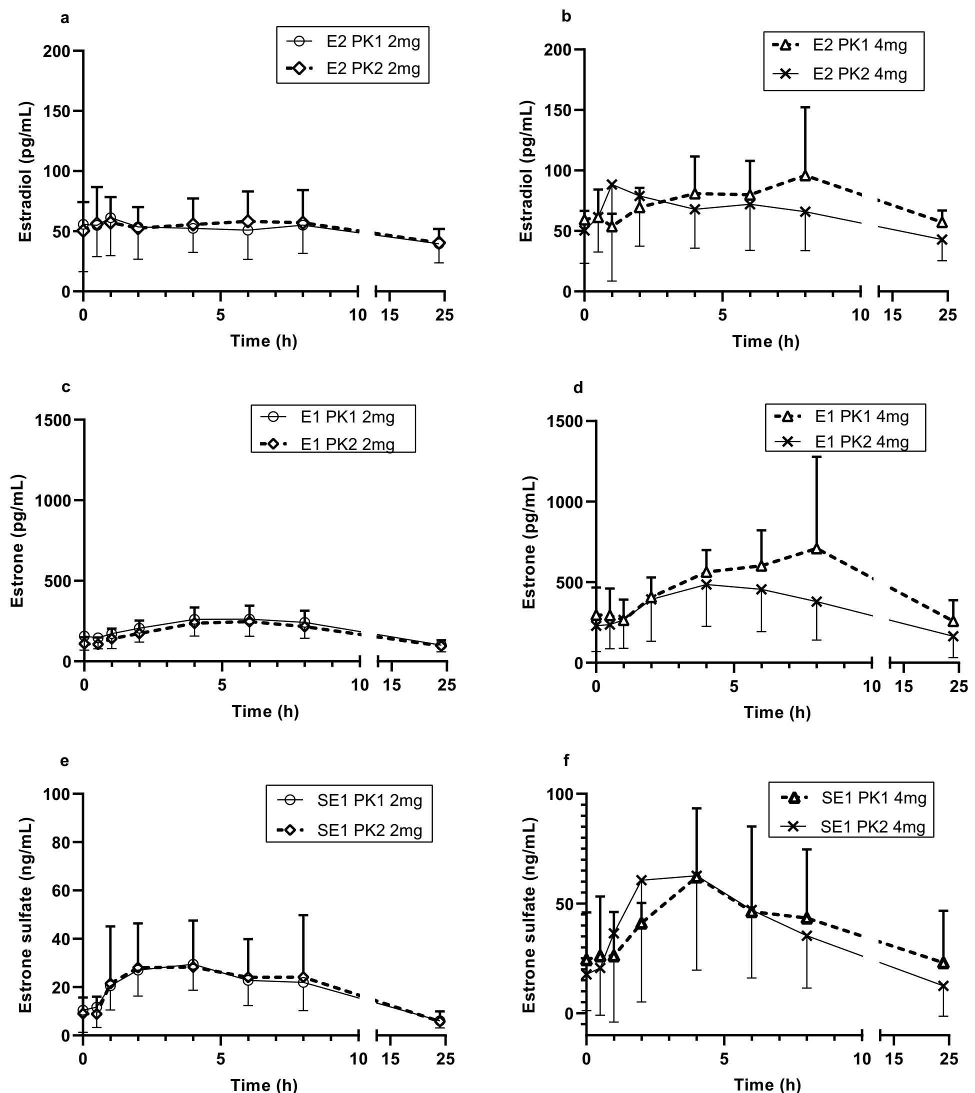

Fig. 3 Plasma estradiol, estrone and estrone sulfate concentration over 24 h in transgender women study participants. Plasma estradiol (**a**, **b**), estrone (**c**, **d**), and estrone sulfate (**e**, **f**) concentration versus time curves (sample times: pre-dose and 0.5, 1, 2, 4, 6, 8, and 24 hours) are shown for the indicated doses at PK1 and PK2. Data are shown as means with error bars indicating standard deviations. E2V doses at PK1: 2 mg (n = 16); 4 mg (n = 3); 6 mg (n = 0); E2V doses at PK2: 2 mg (n = 10); 4 mg (n = 8); 6 mg (n = 1—Suppl. Fig. 1). E2: estradiol; E2V: estradiol valerate; E1: estrone; SE1: estrone sulfate; *FHT* feminizing hormone therapy, *PK* pharmacokinetic, *PK1* first PK evaluation (n = 19), participants on FHT only (enrollment) and TDF/FTC initiated by the end of the PK1 visit; PK2: second PK evaluation, participants on FHT plus PrEP (TDF/FTC) (n = 19)

Table 3 Estradiol, estrone and estrone sulfate non-compartmental pharmacokinetic parameters at PK1 and PK2 among transgender women

PK parameter	PK1 (FHT only)[a] GM (90% CI)	PK2 (FHT + PrEP)[b] GM (90% CI)	PK1/PK2 GMR (90% CI)	p value[c]
E2 AUC_{τ} [d] (pg·h/mL)	1002.55 (821.99–1222.79)	893.15 (735.99–1083.86)	0.89 (0.76–1.04)	0.22
E2 $C_{max,ss}$ [d] (pg/mL)	61.95 (51.16–75.01)	54.81 (43.79–68.62)	0.88 (0.72–1.08)	0.31
E2 $t_{max,ss}$ (h)	1.64 (0.90–2.98)	2.04 (1.22–3.41)	1.25 (0.56–2.77)	0.64
E2 CL/*F* (L/h)	1994.91 (1635.61–2433.13)	2239.28 (1845.27–2717.42)	1.12 (0.96–1.31)	0.22
E2 V_{ss}/F (L)[e]	102,707.1 (64,143.2–164,456.0)	81,090.7 (52,150.5–126,090.9)	0.79 (0.44–1.40)	0.47
E2 C_{min} (pg/mL)	35.00 (30.28–40.45)	37.18 (32.13–43.03)	1.06 (0.94–1.20)	0.39
E2 $t_{1/2}$ (h)[e]	36.73 (26.94–50.07)	29.61 (21.55–40.69)	0.81 (0.52–1.24)	0.39
E1 AUC_{τ}[d] (pg·h/mL)	4384.72 (3699.46–5196.91)	3525.24 (2895.34–4292.19)	0.80 (0.71–0.91)	**0.01**
E1 $C_{max,ss}$[d] (pg/mL)	275.06 (232.98–324.74)	230.23 (193.46–273.99)	0.84 (0.73–0.95)	**0.03**
E1 $t_{max,ss}$ (h)	3.67 (2.38–5.63)	4.48 (3.87–5.17)	1.22 (0.78–1.92)	0.45
E1 C_{min} (pg/mL)	103.84 (83.16–129.67)	104.31 (80.35–135.40)	1.00 (0.85–1.18)	0.96
SE1 AUC_{τ}[d] (ng·h/mL)	378.34 (313.79–456.16)	312.83 (237.80–411.53)	0.83 (0.69–1.00)	0.08
SE1 $C_{max,ss}$[d] (ng/mL)	29.75 (25.59–34.58)	26.88 (20.80–34.73)	0.90 (0.76–1.08)	0.34
SE1 $t_{max,ss}$ (h)	3.17 (2.50–4.01)	3.23 (2.69–3.88)	1.02 (0.72–1.45)	0.92
SE1 C_{min} (ng/mL)	4.73 (2.88–7.75)	5.38 (3.54–8.20)	1.14 (0.73–1.79)	0.62

Bold type indicates statistical significance

CI confidence interval, *E1* estrone, *E2* estradiol, *FHT* feminizing hormone therapy, *GM* geometric mean, *GMR* geometric mean ratio, *PK* pharmacokinetics, *PrEP* pre-exposure prophylaxis, *SE1* estrone sulfate, *TDF/FTC* tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg

[a]PK1: first PK evaluation ($n = 19$), participants on FHT only (enrollment) and TDF/FTC initiated by the end of the PK1 visit

[b]PK2: second PK evaluation, participants on FHT plus PrEP (TDF/FTC) ($n = 19$)

[c]PK1 and PK2 compared as geometric mean ratios (GMRs, 90% CI) using a paired *t* test after log transformation

[d]Parameters normalized by the lower dose of estradiol valerate (2 mg)

[e]Estimated for 11/19 participants

valerate) but also the antiandrogen component (spironolactone) of a standardized FHT. Our analysis provides a detailed description of FHT drugs and active metabolites levels when administered alone or concomitantly with PrEP, in a Brazilian population.

Our estradiol PK results agreed with previous studies. The iFact study conducted in Thailand in 2018 did not detect differences in estradiol AUC_{τ} (GMR [95% CI] 1.01 [0.89–1.15], $p = 0.88$), $C_{max,ss}$ (1.08 [0.94–1.24], $p = 0.25$), and C_{24h} (0.95 [0.75–1.19], $p = 0.63$) among TGW (n = 20) using only FHT (estradiol valerate 2 mg plus cyproterone acetate 25 mg) and FHT plus TDF/FTC [16]. Similarly, a study from Colorado, USA, evaluating trans adolescent girls ($n = 25$) only on non-standardized FHT (administered by different routes of administration and dosages) and after PrEP initiation did not observe impact of TDF/FTC on estradiol AUC_{last} (GMR [95% CI] 0.87 [0.73–1.03], $p = 0.1$) and C_{max} (0.85 [0.65–1.11], $p = 0.2$) [25]. However, the second part of the iFact study that evaluated TGW living with HIV on FHT only (estradiol valerate 2 mg plus cyproterone acetate 25 mg) and after started on ARV (TDF/FTC/efavirenz) showed lower estradiol AUC_{τ} (GMR [90% CI]: 0.72 [0.64–0.81]), C_{max} (0.81 [0.72–0.92]) and C_{24} (0.64 [0.50–0.83]) for participants taking FHT plus ARV [28].

Lower exposure of estrone (around 20% of AUC_{τ} and $C_{max,ss}$) was observed when FHT and PrEP were taken concomitantly (PK2) in our study. Recently, a study with TGW on FHT observed no association between estrone levels and the feminization process when assessed by breast development or change in body fat in 12 months of FHT [35]. These results support our rationale that the lower estrone exposure among our participants after PrEP introduction did not have a relevant impact on the feminizing therapy. As for the possible mechanisms of interaction, it is not clear how TDF/FTC could affect estrone levels due to the complex metabolism pathway of estradiol metabolites. Estrone, formed by the 17β-hydroxysteroid dehydrogenase in the first-pass metabolism of estradiol, is conjugated before biliary excretion and then undergoes enterohepatic recirculation mediated by intestinal microbiota [36]. CYP3A4 and CYP1A2, as well as UGT1A1 enzymes, are involved in estradiol and estrone metabolism. Estradiol and estrone conjugates can be subject to interactions at the level of hepatic transport mediated by organic-anion transporting polypeptide (OATP) 1B1/1B3 [14]. There are no reports of influence of PrEP components on the activity of any of the aforementioned enzymes. An impact of PrEP on intestinal microbiota has been reported

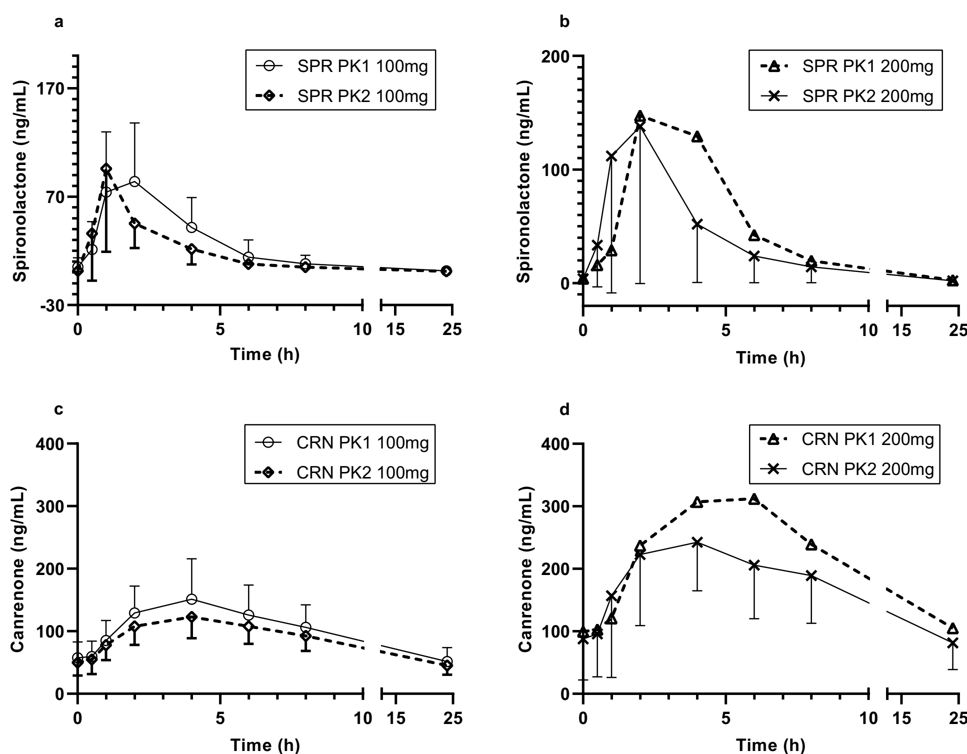

Fig. 4 Plasma spironolactone and canrenone concentration over 24 hours in transgender women study participants. Plasma spironolactone (**a**, **b**) and canrenone (**c**, **d**) concentration versus time curves (sample times: pre-dose and 0.5, 1, 2, 4, 6, 8, and 24 hours) are shown for the indicated doses at PK1 and PK2. Data are shown as means with error bars indicating standard deviations. SPR doses at PK1: 100 mg (n = 18); 200 mg (n = 1); SPR doses at PK2: 100 mg (n = 10); 200 mg (n = 9). SPR: spironolactone; CRN: canrenone; *FHT* feminizing hormone therapy, *PK* pharmacokinetic, *PK1* first PK evaluation, (n = 19), participants on FHT only (enrollment) and TDF/FTC initiated by the end of the PK1 visit; PK2: second PK evaluation, participants on FHT plus PrEP (TDF/FTC) (n = 19)

[37]; however, it is not clear if it can affect the enterohepatic recirculation of estrone conjugates.

Differences in spironolactone and canrenone AUC_τ and in canrenone $C_{max,ss}$ were observed between PK1 and PK2. Lower bioavailability of spironolactone could explain our

Table 4 Spironolactone and canrenone non-compartmental pharmacokinetic parameters at PK1 and PK2 among study participants

PK parameter	PK1 (FHT only)[a] GM (90% CI)	PK2 (FHT plus PrEP)[b] GM (90% CI)	PK1/PK2 GMR (90% CI)	p value[c]
SPR AUC_τ[d] (ng·h/mL)	329.74 (258.14–421.20)	250.20 (198.98–314.61)	0.76 (0.65–0.89)	**0.01**
SPR $C_{max,ss}$[d] (ng/mL)	85.05 (66.53–108.74)	75.36 (57.49–98.77)	0.89 (0.64–1.22)	0.52
SPR $t_{max,ss}$ (h)	1.61 (1.37–1.89)	1.29 (1.07–1.59)	0.80 (0.62–1.04)	0.16
SPR CL/F (L/h)	303.27 (237.42–387.39)	399.68 (317.85–502.58)	1.32 (1.13–1.54)	**0.01**
SPR V_{ss}/F (L)	2684.80 (1961.44–3674.92)	3517.59 (2607.38–4745.54)	1.31 (0.99–1.74)	0.12
SPR C_{min} (ng/mL)	1.06 (0.66–1.70)	1.03 (0.70–1.50)	0.97 (0.62–1.49)	0.89
SPR $t_{1/2}$ (h)	6.17 (5.36–7.11)	6.24 (5.64–6.91)	1.01 (0.86–1.19)	0.89
CRN AUC_τ[d] (ng·h/mL)	2135.41 (1904.33–2394.53)	1820.57 (1619.23–2046.94)	0.85 (0.78–0.94)	**0.01**
CRN $C_{max,ss}$[d] (ng/mL)	147.86 (128.98–169.50)	121.21 (107.45–136.72)	0.82 (0.74–0.91)	**0.01**
CRN $t_{max,ss}$ (h)	3.17 (2.73–3.70)	3.10 (2.63–3.65)	0.98 (0.78–1.23)	0.87
CRN C_{min} (ng/mL)	48.41 (41.07–57.06)	36.72 (19.90–67.79)	0.76 (0.41–1.40)	0.45

Bold type indicates statistical significance

CI confidence interval, *CRN* canrenone, *E1* estrone, *E2* estradiol, *FHT* feminizing hormone therapy, *GM* geometric mean, *GMR* geometric mean ratio, *PK* pharmacokinetics, *PrEP* pre-exposure prophylaxis, *SPR* spironolactone, *TDF/FTC* tenofovir disoproxil fumarate 300 mg/emtricitabine 200 mg

[a]PK1: first PK evaluation (n = 19), participants on FHT only (enrollment) and TDF/FTC initiated by the end of the PK1 visit

[b]PK2: second PK evaluation, participants on FHT plus PrEP (TDF/FTC) (n = 19)

[c]PK1 and PK2 compared as geometric mean ratios (GMRs, 90% CI) using a paired t-test after log transformation

[d]Parameters normalized by the lower dose of spironolactone (100 mg)

results showing higher CL/*F* (32%) at PK2 as well as higher V_{ss}/F (31%, $p = 0.12$) at PK2. As spironolactone bioavailability is highly influenced by food (by 95% on AUC) [17], we instructed our participants to fast for 8 hours before attending intensive PK visits and offered a standard breakfast 30 minutes after the drug intake. Although these instructions may have not been followed by all participants, we would not expect interactions of TDF/FTC or estradiol with spironolactone PK [21]. Spironolactone is extensively metabolized by hepatic transferases and esterases, and no influence of CYP inducers or inhibitors on its metabolism has been reported [14]. Spironolactone is reported as an inducer of hepatic microsomal drug metabolizing enzymes [18, 38]. Nevertheless, only specific DDI are considered clinically significant [17]. Despite scarce information about the mechanisms of possible interactions, our findings indicate minor changes of PrEP drugs on spironolactone and canrenone PK.

Our study had some limitations. First, it was not possible to implement daily directly observed therapy, so we may have overestimated participants' adherence. However, we indirectly estimated adherence based on tenofovir-DP in DBS and only participants with high adherence were included in data analysis. Among estradiol PK parameters, we could not estimate V_{ss}/F for 8/19 (42%) participants in one of the PK evaluations, possibly due to the dosing interval (24 h) and insufficient data (sample points between 8 to 24 h) to provide a better characterization of the estradiol elimination process, a limitation previously reported [39]. In addition, we normalized AUC_{τ} and $C_{max,ss}$ from different FHT doses to make them comparable. Despite no indication of non-linear PK of estradiol valerate and spironolactone in the range of FHT doses prescribed to our participants, the normalization could be a limitation of our analysis. Our study evaluated only one estrogen-based FHT regimen. As such, the current results may not be extrapolated to other FHT regimens and/or dosages. Finally, as in other PK studies, our results derive from a specific sample and may not be generalized to the whole population.

In conclusion, despite small magnitude differences observed in spironolactone and canrenone PK, our results indicate no meaningful influence of PrEP with TDF/FTC on estradiol PK. Current findings support the concomitant use of PrEP and estradiol-based FHT and may be used to build confidence and trust among TGW communities, consequently increasing PrEP uptake, adherence and persistence.

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Declarations

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Conflict of interest Peter Anderson has received consulting fees from Gilead, Merck, and ViiV, and research funding paid to his institution from Gilead. All other authors declare no competing interests.

Ethics approval and consent to participate This study was performed in line with the principles of the Declaration of Helsinki. It was approved by the Evandro Chagas National Institute of Infectious Diseases-FIOCRUZ Institutional Review Board. The PrEParadas study is registered with clinicaltrials.gov (NCT03220152). Informed consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

Availability of data and material Data are available from the corresponding author upon request.

Code availability Not applicable.

Author contributions EMJ, VGV, BG and RE conceived the study and interpreted the findings. EMJ, BG, RE and VBC drafted the manuscript. VBC did the statistical analyses with aid from EMJ and RE. TT, SWC, LE, CRVC and LM helped with data acquisition, interpretation of the findings, and drafting the manuscript. VGV, PA, LB and EW were involved in revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

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