

# Depot Medroxyprogesterone Acetate and the Vaginal Microbiome as Modifiers of Tenofovir Diphosphate and Lamivudine Triphosphate Concentrations in the Female Genital Tract of Ugandan Women: Implications for Tenofovir Disoproxil Fumarate/Lamivudine in Preexposure Prophylaxis

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## Abstract

### Background

Effective concentrations of antiretrovirals in the female genital tract (FGT) are critical for suppression of viral shedding or effective preexposure prophylaxis. The disposition of tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP) in the FGT have been previously described. Despite widespread use, however, lamivudine triphosphate (3TC-TP) exposure in the FGT is unknown. Depot medroxyprogesterone acetate (DMPA) and vaginal dysbiosis have been implicated in increased risk of human immunodeficiency virus (HIV) acquisition, but whether they alter TFV-DP or 3TC-TP exposure, and therefore compromise prevention efficacy, is unknown.

### Methods

Fifty premenopausal women living with HIV in Kampala, Uganda, and receiving daily tenofovir disoproxil fumarate/lamivudine were recruited. Ectocervical biopsies were obtained for quantification of TFV-DP and 3TC-TP using liquid chromatography–mass spectrometry. 16S ribosomal RNA gene sequencing was performed on DNA extracted from vaginal swabs. Wilcoxon rank-sum was used to test for differences between contraceptive groups.

### Results

3TC-TP concentrations were on average 17-fold greater than TFV-DP concentrations in cervical tissues. TFV-DP concentrations in cervical biopsies were 76% greater in DMPA users compared with women using nonhormonal contraception ( $n = 23$  per group). Abundance of *Lactobacillus* in vaginal swabs was correlated with 3TC-TP concentrations in cervical tissues.

### Conclusions

We found that TFV-DP concentrations were significantly greater in DMPA users compared with women using nonhormonal contraception, suggesting that prevention efficacy is unlikely to be compromised by DMPA use. Similar to reports of FTC-TP, 3TC-TP exposure was significantly greater than TFV-DP in cervical tissue and was correlated with abundance of *Lactobacillus*. These data support lamivudine as an option for preexposure prophylaxis.

### Clinical Trials Registration

[NCT03377608](#).

**Keywords:** PrEP, tenofovir, lamivudine, microbiome, HIV, DMPA

Antiretroviral exposure in the female genital tract (FGT) is critical for suppression of viral shedding or, in the case of preexposure prophylaxis (PrEP), human immunodeficiency virus (HIV) prevention. Tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP) disposition in the FGT has been previously described, with comparatively low TFV-DP and high FTC-TP concentrations following oral dosing [1, 2]. However, despite widespread use, lamivudine triphosphate (3TC-TP) exposure in FGT is unknown. Several countries have endorsed lamivudine (3TC) (in combination with tenofovir disoproxil fumarate [TDF]) for PrEP; therefore, knowledge of exposure in FGT is warranted [3].

Depot medroxyprogesterone acetate (DMPA) is the most popular contraceptive method in sub-Saharan Africa [4]. Given the high HIV prevalence in this region, coadministration with PrEP is likely. Additionally, DMPA has been associated with increased risk of HIV infection [5]. In vitro, coinubation of CD4<sup>+</sup> T cells with medroxyprogesterone acetate decreased TFV-DP concentrations and protection from HIV infection [6]. Therefore, we sought to determine whether DMPA decreased TFV-DP FGT exposure, potentially compromising PrEP efficacy.

Vaginal dysbiosis has also been associated with increased HIV risk [7]. A retrospective analysis from the Centre for the AIDS Programme of

Research in South Africa (CAPRISA) 004 study found different protection from HIV 1% TFV gel based on *Lactobacillus* status [8]. While this suggests the vaginal microbiome can affect the efficacy of topically applied medication, the effect when orally administered is less clear. A retrospective analysis of the Partners PrEP study did not find significant differences in efficacy based on Nugent score or *Gardnerella*, *Bacteroides*, or *Lactobacillus* abundance [9]. However, overall adherence to PrEP in the Partners PrEP study was high, and changes in drug exposure may not have been significant enough to put women at risk of drug failure. Donahue et al examined cervicovaginal fluid-to-plasma ratios of antiretroviral drugs and found a bimodal relationship between TFV penetration and  $\alpha$ -diversity [10]. Few studies have measured active metabolite concentrations. We therefore sought to quantify the effect of the vaginal microbiome on intracellular active metabolite concentrations following oral dosing.

## METHODS

### Study Population

Procedures for this study (NCT03377608) were reviewed and approved by the Research Ethics Committee of the Uganda Virus Research Institute, the Human Research Ethics Committee of the University of Witwatersrand, and the Uganda National Council of Science and Technology. Women enrolled in the BONE: Contraception and Anti-Retroviral Effects (BONE-CARE) study (a study examining cumulative effects of HIV infection, TFV use, and DMPA use on bone health [11]) were offered participation in this substudy if they were stable on their TDF-containing regimen and virally suppressed (plasma HIV RNA <50 copies/mL) for  $\geq 6$  months. Exclusion criteria included pregnancy, breastfeeding, symptomatic vaginal infection, abnormal vaginal bleeding, history of genital dysplasia or HPV, or use of oral/vaginal antibiotics or antifungals within 30 days (exception made for sulfamethoxazole-trimethoprim). Written informed consent was obtained prior to study participation.

All sampling took place at a single visit. A brief pelvic examination was performed to assess for gross abnormalities. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were detected in urine using the Becton Dickinson ProbeTec ET CT/GC Amplified DNA assay. Syphilis was tested on blood using rapid plasma reagin. Urine was collected for a point-of-care pregnancy human chorionic gonadotropin test. Vaginal swabs were collected on polyester swabs. Two cervical biopsies were obtained using Baby Tishler Biopsy forceps (McKesson) from the outer third of the upper left and right quadrant of the cervix and snap-frozen in liquid nitrogen. Blood was collected into ethylenediaminetetraacetic acid tubes and centrifuged for plasma separation. Additional blood was collected into BD Vacutainer CPT tubes with sodium citrate, and peripheral blood mononuclear cells (PBMCs) were isolated according to protocol. Cells were lysed in 70:30 methanol:water.

### Antiretroviral Quantification

Tenofovir and 3TC were quantified in plasma using a validated assay with a 1 ng/mL lower limit of quantification. In tissue biopsies and PBMCs, TFV-DP, 3TC-TP, deoxyadenosine triphosphate (dATP), and deoxycytidine triphosphate (dCTP) were quantified. Samples were extracted by protein precipitation. Isotopically labeled internal standards were used for all analytes except for 3TC-TP, which used isotopically labeled TFV-DP as its internal standard. In PBMCs, the dynamic range of assays was 0.02–100 ng/mL for dATP and TFV-DP, 0.2–600 ng/mL for 3TC-TP, and 0.06–100 ng/mL for dCTP. In tissue homogenate, the dynamic range was 0.02–20 ng/mL. Calibration standards and quality control samples met acceptance criteria of  $\pm 15\%$  of nominal concentrations for plasma and PBMCs and  $\pm 20\%$  for tissue.

### Estradiol and Progesterone Quantification

Progesterone and estradiol were measured by a microplate enzyme immunoassay using reagents from Arbor Assays (Ann Arbor, Michigan). Prior to quantitation, hormones were extracted with hexane–ethyl acetate to remove water-soluble conjugated metabolites. Assay sensitivities for progesterone and estradiol are 0.07 ng/mL and 5 pg/mL, respectively, and interassay coefficients of variation for both assays are <10%.

### Microbiome Sequencing

DNA was extracted from vaginal swabs and fluid, approximately 450  $\mu$ L, using the DNeasy PowerSoil kit (Qiagen, Hilden, Germany) on the automated QIAcube platform. The V4 hypervariable region of the 16S ribosomal RNA (rRNA) gene was amplified and paired-end sequenced (300 nucleotides) on the Illumina MiSeq platform (Illumina, San Diego, California) using the 515F/806R primer set [12]. Sequence data were processed in “mothur” [13], as described previously [14]. In brief, sequences were pair-end joined and screened for quality. Operational taxonomic units (97% similarity) were classified against the Ribosomal Database Project version 16 release [15].

For statistical comparisons, samples were rarefied by random subsample to approximately 3000 sequence reads per sample. The  $\alpha$ - and

$\beta$ -diversity indices were calculated as Shannon index [16] and Bray-Curtis distance [17].

## Statistical Analysis

The primary study question was the effect of DMPA on TFV-DP and 3TC-TP cervical concentrations. Secondary analyses explored associations between the vaginal microbiome and drug concentrations. Data are presented as median (interquartile range [IQR]) unless otherwise noted. Statistical analyses were performed using XLSTAT version 17.06 (Addinsoft, Belmont, Massachusetts) or SAS version 9.4 (SAS Institute, Cary, North Carolina) software. Bivariate analyses were performed using Wilcoxon rank-sum test or Spearman correlation test. Differences in community composition were evaluated using analysis of similarity (ANOSIM) of Bray-Curtis distances [18], and taxa that showed significant differences in relative abundance between groups were determined by linear discriminant analysis of effect size [19]. All statistics were evaluated at  $\alpha = .05$  with Bonferroni correction for multiple comparisons when appropriate.

To identify significant predictors of drug concentrations, multivariable linear regression was performed on log-transformed data using stepwise regression. Significance criteria for entry into the model and stay criteria were set at  $\alpha = .15$ . Model criterion was evaluated on best adjusted  $r^2$  value.

## RESULTS

### Subject Characteristics

Fifty women were enrolled between November 2017 and March 2018. Twenty-five were receiving DMPA, 12 were using a copper intrauterine device (Cu-IUD), and 13 were using condoms as their primary contraceptive method. Characteristics of study participants at the time of sampling are reported in Table 1. Women <25 years old comprised 36% and 32% of DMPA and nonhormonal groups, respectively. All participants had been receiving TDF/3TC 300 mg/300 mg daily for at least 30 weeks. One participant on DMPA was receiving boosted 300 mg atazanavir, whereas the other 49 women were receiving 600 mg efavirenz. All participants were receiving daily sulfamethoxazole-trimethoprim 800 mg/160 mg.

Table 1.  
Subject Characteristics

| Characteristic               | Nonhormonal <sup>a</sup> (n = 25) | DMPA (n = 25)  |
|------------------------------|-----------------------------------|----------------|
| Age, y                       | 26.0 (23.8–30.7)                  | 26.4 (24.0–29) |
| Weight, kg                   | 59.1 (52.2–66.0)                  | 61.0 (53.8–68) |
| BMI, kg/m <sup>2</sup>       | 20.0 (17.5–22.6)                  | 24.0 (21.2–27) |
| Years since HIV diagnosis    | 12.0 (3.4–17)                     | 14.0 (2–20)    |
| Years on current ART regimen | 13.0 (5.1–17)                     | 14.0 (6.1–17)  |

Table 1.

### Subject Characteristics

The majority (94%) of participants reported being sexually active. Thirty percent reported being married or in a monogamous relationship, while the remainder reported casual partner (60%), separated (6%), or single (4%).

### Adherence

Three subjects, 1 on DMPA and 2 in the nonhormonal group, had plasma concentrations <1 ng/mL for both TFV and 3TC, suggesting no dose within the past 3–5 days. These subjects were excluded from further antiretroviral quantification. In the remaining women, TFV and 3TC plasma exposures were 66.4 (IQR, 51.3–83.5) ng/mL and 190.0 (IQR, 116–267) ng/mL, respectively, consistent with steady-state daily dosing [20, 21], and were not significantly different by contraceptive group.

### Intracellular Metabolites and Endogenous Nucleotides in PBMCs

In PBMCs, TFV-DP, 3TC-TP, dATP, and dCTP did not differ by contraceptive group (Supplementary Table 1). Correlation coefficients and  $P$  values of correlation tests between analytes are reported in Supplementary Table 2. All 4 PBMC analytes were significantly correlated with each other, and TFV-DP and 3TC-TP were significantly correlated with respective plasma concentrations. There was no correlation between TFV-DP or 3TC-TP concentrations in PBMCs vs tissue. PBMC concentrations were not correlated with age (TFV-DP:  $P = .316$ ; 3TC-TP:  $P = .611$ ) or body mass index (BMI) (TFV-DP:  $P = .469$ ; 3TC-TP:  $P = .544$ ).

Endogenous hormones were not correlated with drug analytes or endogenous nucleotides; however, progesterone was positively correlated with the 3TC-TP to dCTP ratio in cells ( $r = 0.33$ ,  $P = .027$ ).

## Intracellular Metabolites and Endogenous Nucleotides in Cervical Tissue

Measured concentrations of intracellular metabolites and their endogenous nucleotide analogues in cervical tissue are shown in [Figure 1](#) and summarized in [Supplementary Table 1](#). All tissue analytes were significantly correlated with each other ([Supplementary Table 2](#)). Median 3TC-TP concentrations were 17-fold higher than TFV-DP concentrations. Median TFV-DP was 76% higher in DMPA users compared with the nonhormonal group ( $P = .035$ ). No differences were found in 3TC-TP, dATP, or dCTP between groups ([Supplementary Table 1](#)). Notably, TFV-DP and 3TC-TP in cervical tissue were more closely correlated with respective plasma concentrations of TFV or 3TC than with TFV-DP or 3TC-TP in PBMCs ([Supplementary Table 2](#)).

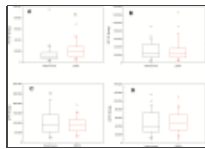


Figure 1.

Concentrations of intracellular metabolites and endogenous nucleotides in cervical tissue. A–D, Box plots showing distribution of analytes measured in cervical homogenates by contraceptive group: tenofovir diphosphate (A), lamivudine triphosphate ...

Age was inversely correlated with 3TC-TP and dCTP in cervical tissues (3TC-TP:  $r = -0.41$ ,  $P = .005$ ; dCTP:  $r = -0.35$ ,  $P = .014$ ). Neither TFV-DP nor dATP in tissue was correlated with age (TFV-DP:  $r = -0.23$ ,  $P = .13$ ; dATP:  $r = -0.23$ ,  $P = .11$ ). However, there was a moderate inverse correlation between age and tissue TFV-DP within the nonhormonal group ( $r = -0.56$ ,  $P = .006$ ). BMI was not correlated with any analyte in tissue (TFV-DP:  $P = .608$ ; 3TC-TP:  $P = .506$ ; dATP:  $P = .105$ ; dCTP:  $P = .142$ ). Endogenous estradiol and progesterone at time of sample collection was not correlated with any tissue analyte ([Supplementary Table 1](#)); however, estradiol was negatively correlated with the TFV-DP to dATP ratio in cervical tissue ( $r = -0.33$ ,  $P = .024$ ). There was no correlation between the TFV-DP to dATP ratio and estradiol within contraceptive groups, suggesting that estradiol concentrations did not explain any greater variability than DMPA use.

No women reported history of any sexually transmitted infection; however, 6 women tested positive for gonorrhea and/or syphilis on the day of sampling ([Table 1](#)). Sensitivity analyses performed excluding these 6 subjects with sexually transmitted infections did not affect overall results or significance.

## Microbiome and Effect on Drug Concentrations

Women receiving DMPA had lower bacterial  $\alpha$ -diversity compared to the nonhormonal group (mean Shannon diversity: 1.36 vs 2.00,  $P = .035$ ). The relative abundance of *Lactobacillus* was inversely correlated with age ( $r = -0.31$ ,  $P = .027$ ) but did not differ between groups. The  $\alpha$ -diversity in the vaginal microbiome was negatively correlated with the TFV-DP to dATP ratio in cervical tissue ( $r = -0.40$ ,  $P = .006$ ) but not with TFV-DP concentrations ( $r = -0.22$ ,  $P = .13$ ). 3TC-TP and dCTP concentrations in cervical tissue were correlated with relative abundance of *Lactobacillus* ( $r = 0.32$ ,  $P = .033$  and  $r = 0.30$ ,  $P = .044$ , respectively). No vaginal genera were significantly correlated with cervical TFV-DP or dATP. TFV-DP was inversely correlated with *Shuttleworthia* (or bacterial vaginosis-associated bacteria) abundances within DMPA users ( $r = -0.43$ ,  $P = .040$ ) but not within nonhormonal contraceptive users ( $r = -0.14$ ,  $P = .5$ ). Endogenous hormones were not correlated with  $\alpha$ -diversity or abundance of any genera.

Linear discriminant analysis of effect size identified 12 genera differentially abundant between groups ([Figure 2A](#)). Of those, only *Prevotella*, *Atopobium*, *Dialister*, *Parvimonas*, and *Saccharofermentans* comprised  $>1\%$  of the relative abundance, and all were  $>1000$ -fold greater in the nonhormonal group compared with the DMPA group. Among the top 5 most prevalent genera ([Figure 2B](#)), TFV-DP and 3TC-TP concentrations were consistently highest when *Lactobacillus* was the most dominant genera ([Figure 2C](#) and [2D](#)).

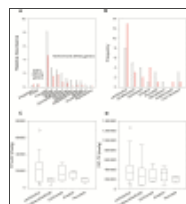


Figure 2.

Vaginal microbiota and cervical drug concentrations. A, Relative abundance of the 12 genera that separated the depot medroxyprogesterone acetate (DMPA) users (hashed bars) from the nonhormonal (clear bars) group in linear discriminant analysis of effect ...

## Drug Exposure in Copper IUD Users

When comparing tissue analytes between condom users, Cu-IUD users, and DMPA users, the 3 groups had different TFV-DP concentrations ( $P =$

.029, Kruskal-Wallis test). In pairwise comparisons, TFV-DP was not significantly different between condom users and DMPA users (median, 14 570 vs 18 477 fmol/g, respectively;  $P = .418$ ) but was significantly lower in Cu-IUD users (median, 9375; vs DMPA,  $P = .007$ ; vs condom,  $P = .094$ ). 3TC-TP, dATP, and dCTP were not significantly different in 3-way or pairwise comparisons based on contraceptive use.

Vaginal microbiomes did not significantly differ between condom and Cu-IUD users based on  $\alpha$ -diversity (1.92 vs 2.08,  $P = .424$ ) or ANOSIM ( $P = .737$ ). However, *Fusobacterium* and *Prevotella* were significantly more abundant in Cu-IUD users in linear discriminant analysis, whereas condom users were defined by *Arcanobacterium*, *Peptoniphilus*, and *Saccharofermentans*.

## Multivariable Regression to Identify Predictors of Drug Exposure in Cervical Tissue

In multivariable analysis, DMPA use, age, and TFV plasma concentrations were identified as significant in the best model to predict cervical TFV-DP concentrations. Age, 3TC plasma concentrations, and progesterone concentrations were significant predictors of cervical 3TC-TP. Other parameters tested in models included BMI, estradiol concentrations,  $\alpha$ -diversity, and *Lactobacillus* abundance.

## DISCUSSION

DMPA use has been associated with increased risk of HIV acquisition [5]; therefore, understanding PrEP efficacy in DMPA users is warranted. This study compared intracellular metabolites of TFV and 3TC following oral dosing, and their endogenous nucleotide analogues, in PBMCs and cervical tissue in women receiving DMPA compared to women using nonhormonal contraceptive methods. TFV-DP concentrations in cervical tissues were 76% greater in DMPA users, whereas 3TC-TP, dATP, and dCTP were comparable between groups. Our findings suggest that DMPA is unlikely to compromise efficacy of oral TFV-based PrEP. This is consistent with a retrospective analysis of the Partners PrEP study, which found similar HIV protection in DMPA users compared with users of nonhormonal contraception [22]. Our findings are also consistent with a recent report of increased vaginal TFV-DP with DMPA use in women using 1% TFV gel [23]. In our study, TFV-DP exposure in PBMCs was lower in DMPA users, suggesting a mechanism unique to the FGT. In one study, estradiol and progesterone increased TFV-DP formation in CD4<sup>+</sup> cells isolated from the FGT, but had no effect on CD4<sup>+</sup> cells isolated from blood [24]. As DMPA use dramatically reduces endogenous levels of estrogen and progesterone, these in vitro findings are consistent with our observations reported here. Our findings on DMPA were specific to TFV-DP and not 3TC-TP, which may be related to different transport and phosphorylation pathways of TFV vs 3TC [25, 26].

Overall, TFV-DP cervical concentrations in our study were about 2-fold higher than concentrations after a single dose of TDF, a difference consistent with accumulation from first dose to steady state [27]. Compared to concentrations previously reported in HIV-negative US women, cervical dATP in our study were slightly higher but within range of previous reports, whereas dCTP concentrations in our study were consistently approximately 10-fold lower [27], perhaps due to population differences.

These data are the first to describe 3TC-TP exposure in the FGT. Although efficacy of 3TC for PrEP has never been formally tested, several countries have recommended TDF/3TC for PrEP due to its wider availability and cost-benefit compared to TDF/emtricitabine [3]. Similar to previous reports for FTC-TP [1, 2], 3TC-TP exposure in cervical tissues was more than a log higher than TFV-DP when comparing molar concentrations. Although 3TC-TP concentrations did not differ by contraceptive method, our multivariable regression did identify an inverse relationship between 3TC-TP in cervical tissues and progesterone concentrations in plasma. Although we did not observe an effect on PBMCs, Anderson et al previously reported that in vitro, progesterone decreases 3TC-TP formation in PBMC by 22% [28]. Overall, the data generated here provide a pharmacologic rationale for 3TC as a PrEP agent.

Increasing age was associated with decreased exposure of drug metabolites and endogenous nucleotides, an effect specific to FGT as there was no correlation between age and PBMC drug concentrations. Our group previously found significantly lower ex vivo conversion of TFV and emtricitabine to their phosphorylated metabolites in cervical tissue from postmenopausal women [29]. The mechanism is unknown but is unlikely to be hormone related as the women in our current study were all premenopausal, and age remained a significant predictor of drug concentrations even after adjusting for hormone use as a covariate. Future studies with an expanded age range that account for other physiologic variables associated with aging, such as inflammation response and cervical ectopy, will be required to elucidate this mechanism.

We observed decreased microbial diversity in DMPA users. This is consistent with reports that DMPA either has a neutral or protective effect against bacterial vaginosis [30, 31]. Whether this is due to progestin regulation or to decreased menses associated with DMPA use is not clear. Regardless, as diversity in the vaginal microbiome increases, so does the abundance of potentially proinflammatory taxa. In vitro, Klatt et al found that the anaerobic genera *Gardnerella*, *Prevotella*, and *Mobiluncus* metabolize TFV to an inactive metabolite, adenine, whereas *Lactobacillus* has minimal effect [8]. Although our observational study can only identify correlations and not causation, decreased diversity in the vaginal microbiome and reduced abundance of proinflammatory taxa could be a potential mechanism of greater FGT drug exposure with DMPA

use.

Cu-IUD users had the lowest TFV-DP concentrations compared with DMPA or condom users. Overall, vaginal microbial diversity in Cu-IUD users was not significantly different than condom users; however, there was increased *Prevotella* and *Fusobacterium* abundance in the Cu-IUD users. This is consistent with a recent study that followed 48 women initiating Cu-IUD contraception and found increased bacterial vaginosis prevalence as well as increased *Gardnerella vaginalis* and *Atopobium vaginae* concentrations [30]. These data further support the rationale of vaginal dysbiosis regulating drug exposure in the FGT.

There are limitations to the interpretation of our study. First, we could not control for menstrual cycle in menstruating women. However, previous studies found that menstrual cycle did not affect drug exposure in cervicovaginal fluid [32]. Furthermore, Thurman et al found with topical dosing that while vaginal TFV was increased in the luteal phase, TFV-DP was similar across the menstrual cycle [23]. Menses can affect the vaginal microbiome, but changes are most predominant during active bleeding, which none of the women in our study were experiencing at time of sample collection. In addition, we did not find any differences in the vaginal microbiome between women reporting amenorrhea and those with normal cycles. Second, use of 16S rRNA gene sequencing limited our interpretation of the vaginal microbiome. Using this approach, we could only determine relative and not absolute abundance, which may have limited our ability to identify correlations between drug concentrations and specific bacterial taxa. Due to short sequence reads, we were only able to reliably identify bacteria at the genus and not species level [33], and we could not account for fungal species, which may also have an impact on drug metabolism and may also have been associated with Cu-IUD use. Last, to avoid confounding results, we excluded women with factors that may also influence the vaginal microbiome (eg, antibiotic use or symptomatic vaginal infection). This may have led to vaginal microbiota profiles that are not generalizable to the general population of female PrEP users.

## CONCLUSIONS

These data provide pharmacokinetic support that DMPA is unlikely to compromise TFV-based PrEP efficacy. These data fill a critical gap by describing exposure of the active metabolite of 3TC in the FGT, providing reassurance in 3TC use for PrEP. We also present a plausible mechanism by which DMPA increases concentrations of genital metabolites in mucosal tissues via decreased diversity in the vaginal microbiome.

## Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

ciz443\_Suppl\_Supplementary\_Material

[Click here for additional data file.](#) (21K, docx)

## Notes

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