PrEP (pre-exposure prophylaxis) Adherence Among East African Women

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A dissertation
submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington
2018

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Program Authorized to Offer Degree:
Public Health – Epidemiology

University of Washington

Abstract

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HIV incidence remains disproportionately high for women, particularly young women, in Sub-Saharan Africa; women are also at heightened risk while pregnant, which may account for a substantial portion of their adult lives. Although HIV pre-exposure prophylaxis (PrEP) in pill form is known to be efficacious for women, there remain unanswered questions about adherence in open-label and real-world settings, as well as regarding the effectiveness of PrEP during pregnancy. In the work presented in this dissertation, we first examined how women used PrEP in an open-label demonstration project and particularly, how adherence was related to HIV risk behaviors. Second, to better assess adherence, we evaluated the sensitivity and specificity of a biomarker among East African men and women using PrEP. Finally, we examined the effect of pregnancy on PrEP concentrations.

First, we found that women in known serodiscordant relationships were able to take PrEP effectively; more than half took PrEP during their entire risk period, with ≥6 doses for most weeks when on PrEP. HIV incidence was reduced 93% (95% CI 77%-98%) for all women and 91% (95% CI 29%-99%) among women under 25 years old. In further analysis, we found evidence of four adherence trajectories and two risk behavior trajectories over the first six months of PrEP use. Women with a declining risk behavior trajectory were more likely to have a declining adherence trajectory, while women with steady risk were more likely to have high steady adherence; this supports the idea of prevention-effective adherence, which optimizes PrEP use.

In the second aim, we found low sensitivities for the adherence biomarker tenofovir-diphosphate, using thresholds established in U.S. populations. Adherence counseling based on biomarkers should carefully consider the trade-offs between sensitivity and specificity.

Finally, we found that concentrations of PrEP are significantly lower in pregnant women compared to non-pregnant women, as well as during pregnancy compared to pre-pregnancy, after adjusting for adherence. Additional pharmacology and epidemiology studies are needed to determine if PrEP dosing should be altered to sustain systemic levels of tenofovir during pregnancy.

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Acknowledgements
This dissertation would not have been possible without the expertise, support, and advice of my committee: Renee Heffron, Elizabeth Brown, Jessica Haberer, Dr. Bukusi, and Sarah Gimbel. I am indebted to my advisor, Jared Baeten, for his mentorship, his generous time and feedback, and the many opportunities for great research and learning over the past four years.

My time at ICRC, especially on the Data Team, was invaluable to my learning. Thank you to Kathy Thomas for the many stats lessons and analysis discussions. I appreciated the support of my fellow epidemiology students at ICRC, especially my mom-mentor Kerry Thomson.

I am grateful for all the investigators I had the chance to work with and learn from: Connie Celum, Jai Lingappa, Ruanne Barnabas, Nelly Mugo, and Kenneth Mugwanya, as well as Pete Anderson for his pharmacology expertise.

Finally, thank you to the study staff, in Seattle and in Africa, who put the hard work into these studies and to the women who participated in them.

Dedication

For Tracy Irwin & Tess Pyra, my two favorite collaborators.

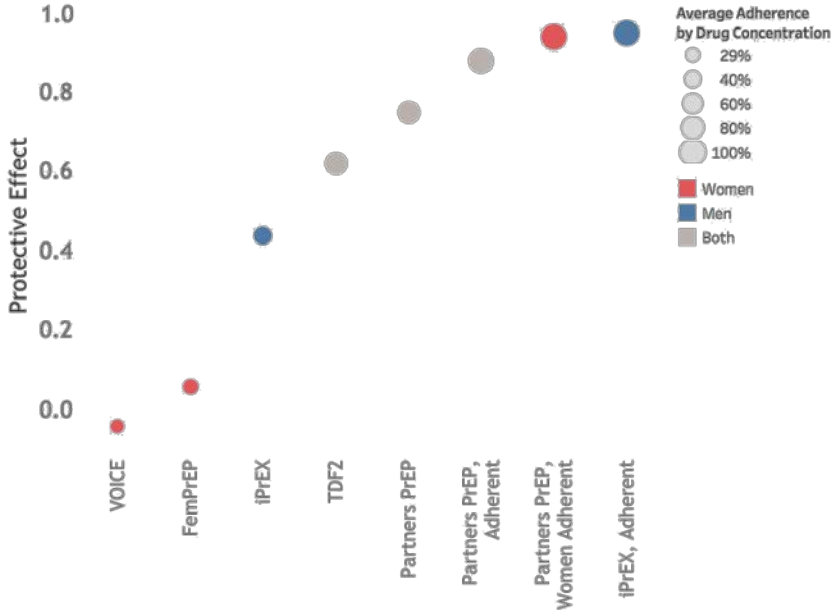
Chapter 1. Introduction

Globally, women account for 47% of new HIV infections [1]; the rate of HIV acquisition among young women, ages 15-24 years old, are double that of their male peers [2]. In Africa, the burden is even more disproportionate. Women account for 56% of new HIV infections [3]; among those age 15-19, women account for 75% of all new HIV infections [2]. During pregnancy the risk of HIV acquisition is almost three times higher, compared to non-pregnant periods [4]; women in areas of high HIV burden often experience multiple pregnancies, suggesting they are at increased risk for a significant portion of their adult lives.

Clearly, women are in need of additional HIV prevention tools and there is strong evidence that pre-exposure prophylaxis (PrEP) is efficacious for HIV prevention among women [5,6]. PrEP, in pill form, is a regimen of two antiretroviral (ARV) drugs (300mg tenofovir disproxil fumarate (TDF)/200mg emtricitabine (FTC)) taken daily to protect against HIV infection. Indeed, the WHO and other organizations identified PrEP as part of recommended HIV prevention for women at high risk, including for women who are pregnant or breastfeeding [7,8].

However, the protective effect of PrEP in women differed widely across clinical studies and early demonstration projects (Table 1.1, [5,9–16]). There is a strong relationship between PrEP adherence and HIV protection and the studies in which small or no protective effects were found also had low adherence.

Table 1.1 PrEP Protective Effect & Study Adherence, by Participant Gender



Low adherence may be due to a number of factors. Certain factors were specific to clinical research trials; women described being hesitant to take the study drug when they may have been assigned placebo or were unsure if the drug would work [17]. Other women reported pressure from fellow participants not to take the drugs and risk possible side effects [18]. There were also barriers to adherence that apply to both research and practical settings. Women were concerned about side effects

and struggled with taking medicine when they were healthy [17–19]. There is stigma around taking ARVs, as the pills are associated with having HIV and the use of ARVs as a prevention strategy is yet to be widely recognized [17,19]. The support or disapproval of partners and family members was a factor in adherence for many women [17–19]; in particular, intimate partner violence (IPV) interfered with adherence [20]. Remembering to take a daily pill, especially when traveling or using alcohol, has been a challenge for many PrEP users [21]. Finally, women who perceived themselves to be at low HIV risk had lower adherence [17,19].

Modeling work from pharmacokinetic studies suggests that women may need at least 6 doses per week to achieve protection in female genital tissue, while protection in rectal tissue may be achieved with fewer doses [22,23]. In other words, women have to achieve better execution to be protected from HIV compared to men who have sex with men (MSM) with primarily rectal exposure; protective levels from other routes of exposure have not been established.

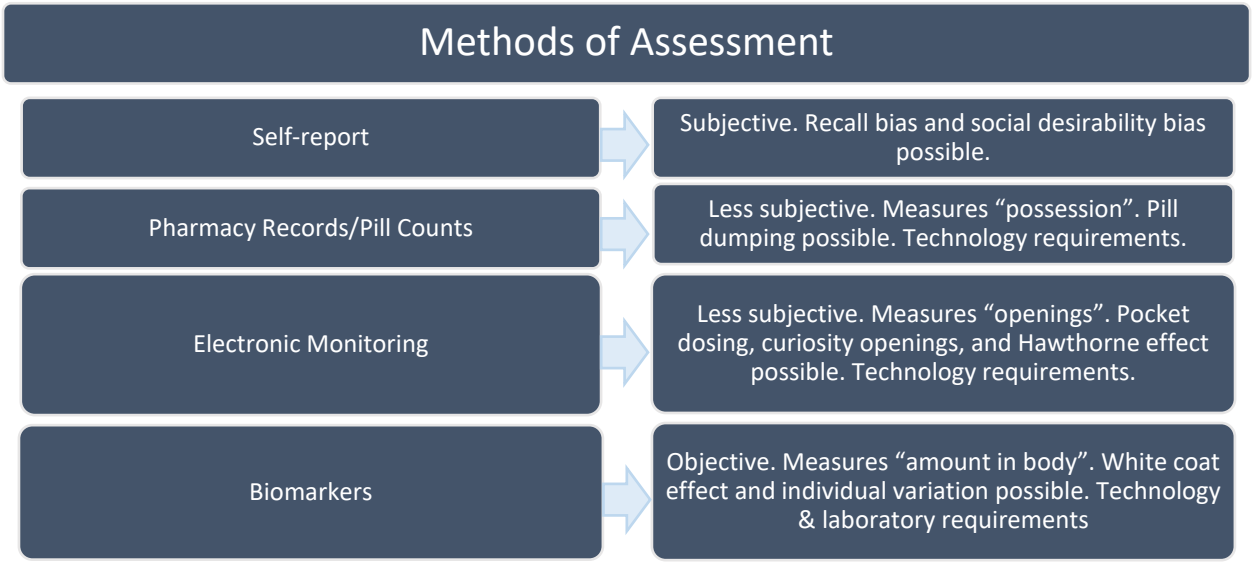
In summary, women may face unique challenges to PrEP adherence while simultaneously needing high levels of adherence to achieve protection. We developed three research aims relating to PrEP adherence and women for this dissertation. First, we described how women adhere to PrEP in an open-label setting, with particular attention to their HIV risk. Second, we evaluated and compared the use of an adherence biomarker in East African women and men. Third, we determined how PrEP concentrations change with pregnancy.

The first aim was addressed with two analyses. An important aspect of PrEP use is aligning it with periods of HIV risk, unlike ARVs for HIV treatment which is prescribed for life. Using a framework of prevention-effective adherence, lower adherence or even cessation of PrEP use during periods of low HIV risk is expected [24]. However, assessing HIV risk as it fluctuates over time is challenging for participants and providers. In these analyses, women were in relationships with a known HIV-positive partner who was able to start antiretroviral treatment (ART) during the study; this identifiable HIV exposure, along with ART initiation and sexual frequency data, allowed us to define HIV risk. In Chapter 2, we describe persistence (how long women used PrEP) and execution (how many doses per week) in relation to HIV risk; in addition, given the increased risk among younger women, we compared these aspects of adherence between older and younger women. In Chapter 3, we further explored the question of adherence among women using a novel approach to identify adherence trajectories over time and determine how they related to risk trajectories.

There are many challenges to assessing adherence (Figure 1.1, [25]) The prior two analyses used electronic monitoring, which captured daily pill bottle openings. While electronic monitoring has been validated in HIV positive individuals using ART [26] and in HIV negative individuals using PrEP [27], it can still be subject to misreporting, such as an individual opening the bottle but not removing any pills. While electronic monitoring measures bottle openings, biomarkers capture the actual amount of drug in an individual. This does not remove all measurement error [26]; there is often variation in drug metabolism between individuals and in the case of biomarkers with short half-lives, detection may only indicate a recent dose rather than sustained adherence. In the case of PrEP, several biomarkers have been used, including tenofovir (TFV) in plasma and hair and tenofovir-diphosphate (TFV-DP) in peripheral blood mononuclear cells (PBMCs) and red blood cells (RBCs). PrEP in pill form contains

tenofovir disproxil fumarate (TDF), which is rapidly converted to TFV, primarily in the liver [28]. TFV is then taken up into different cell types and converted into the active form, TFV-DP, which competes with endogenous adenosine during the synthesis of HIV DNA [28]; once TFV-DP is incorporated it inhibits further growth of the HIV DNA strand. As mentioned, TFV-DP accumulates intracellularly and, in RBCs, is a marker of cumulative use over the prior month [29]. Blood can be stored as dried blood spots (DBS) on cards and analyzed for TFV-DP concentrations [30,31]. Pharmacokinetic studies in the U.S. with directly observed dosing modeled the concentrations of TFV-DP associated with different levels of adherence [29].

Figure 1.1 Challenges of Assessing Adherence



In Chapter 4, we addressed the second aim by evaluating how the cut-offs of TFV-DP for ≥4 and ≥6 doses per week performed in an East African study population, relative to electronic monitoring data. We also compared results between men and women to determine if there were any gender differences and used bias sensitivity analysis to account for possible measurement error in electronic monitoring data.

Finally, the perinatal period is a time of increased risk for HIV acquisition for women [4]. PrEP has been approved for use during pregnancy and breastfeeding, however women stopped taking PrEP in the clinical trials once pregnancy was detected and there are little data regarding breakthrough infections among pregnant women actively taking PrEP, leaving unanswered questions about PrEP effectiveness during pregnancy. Pregnancy changes drug metabolism in many ways; most relevant to TFV, volume of distribution increases as does renal drug clearance [32,33]. In Chapter 5, we compared drug concentrations between pregnant and non-pregnant women, as well as before and during pregnancy among a subset of women; in these analyses, we adjusted for adherence as recorded by electronic monitoring, as women might alter their PrEP use once they know they are pregnant.

Taken together, these aims will help guide future research and implementation as PrEP is made available to an increasing number of women for HIV prevention.

Chapter 2. PrEP Use During Periods of HIV Risk Among East African Women in Serodiscordant Relationships

Published citation: Pyra, M., Haberer, J.E., Heffron, R., Kidoguchi, L., Brown, E.R., Bukusi, E.A., Asiimwe, S., Celum, C., Katabira, E., Mugo, N.R., Baeten, J.M., Partners Demonstration Project Team. Brief Report: PrEP Use During Periods of HIV Risk Among East African Women in Serodiscordant Relationships. J. Acquir. Immune Defic. Syndr. 2018; 77, 41–45. https://doi.org/10.1097/QAI.0000000000001561

PrEP Use During Periods of HIV Risk Among East African Women in Serodiscordant Relationships

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Data were presented at HIV Research for Prevention (HIV R4P) 2016, October 17-21, in Chicago, IL, USA.

Funding
The Partners Demonstration Project was funded by the National Institute of Mental Health of the US National Institutes of Health (R01 MH095507), the Bill & Melinda Gates Foundation (OPP1056051), and the US Agency for International Development (AID-OAA-A-12-00023). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID, NIH, or the United States Government.

Abstract:

Background: PrEP is efficacious for African women at risk for HIV, but data on adherence outside of clinical trials are sparse. We describe the persistence and execution of PrEP use among women participating in a large open-label PrEP demonstration project, particularly during periods of HIV risk.

Setting & Methods: 310 HIV-uninfected women in HIV serodiscordant couples in Kenya and Uganda were offered and accepted PrEP. Electronic monitoring caps were used to measure daily PrEP adherence. Time on PrEP while at risk for HIV (when the HIV-infected partner was on ART <6 months) and weekly adherence while on PrEP were calculated and compared among older and younger (<25 years old) women.

Results: As defined above, women were at risk for HIV for an average of 361 days; 54% took PrEP during their entire risk period and 24% stopped but re-started PrEP during their risk period. While on PrEP, women took ≥6 doses/week for 78% of weeks (67% of weeks for women <25 years old, 80% of weeks for women ≥25 years [p<0.001]), and ≥4 doses for 88% of weeks (80% for those <25, 90% for those ≥25, [p<0.001]). Compared to historical, risk-matched controls, HIV incidence was reduced 93% (95% CI 77%-98%) for all women and 91% (95% CI 29%-99%) among women <25 years old.

Conclusion: Women, including young women, in HIV-serodiscordant couples took PrEP successfully over sustained periods of risk. While young women had lower adherence than older women, they achieved strong protection, suggesting women can align PrEP use to periods of risk and imperfect adherence can still provide substantial benefit.

Key Words: women, HIV, adherence, PrEP

Introduction

Young African women are at particularly high-risk for HIV; 25% of new infections in Sub-Saharan Africa occur in women aged 15-24, compared to 12% in the men of the same age.[1] Oral pre-exposure prophylaxis (PrEP) is an effective method of HIV prevention, including for women, when adherence is high.[9] However, concerns have been raised about the ability of women and particularly young women to take PrEP at sufficient levels to provide protection. The level of adherence necessary to achieve protection in women has been debated, with pharmacokinetic modeling analyses suggesting higher dosing is needed to achieve protective levels in cervical tissue (i.e., ≥6 doses per week), compared to ≥4 doses per week in rectal tissue.[22] Adherence to PrEP among women in clinical trials varied widely [10,11], and adherence may be higher outside of clinical trials, when PrEP is offered as a known effective prevention tool rather than an experimental drug.[24,34] We used data from a large implementation study that demonstrated a strong protective effect against HIV infection to describe the persistence and execution of PrEP use among East African women in HIV serodiscordant couples during periods of HIV acquisition risk, focusing on women <25 years of age.

Methods

Study Population

Enrollment in the Partners Demonstration Project and the counseling provided to participants has been previously described.[12,35,36] HIV serodiscordant couples who mutually disclosed their HIV status were selected, using an empiric risk score (≥5) which was associated with HIV infection rates of >3% per year in the uninfected parter.[37] Using a strategy of "PrEP as a bridge to ART," HIV uninfected partners were encouraged to take PrEP until their partners had initiated ART (not all HIV infected partners were eligible or chose to start ART at enrollment) and were on ART for six months, at which time their viral load would likely be suppressed. A total of 1,013 couples, 334 with female HIV-uninfected partners, were enrolled at two sites each in Kenya and Uganda. Women who were found to have been HIV positive at baseline (n=6), who lacked electronic monitoring data (n=7), or who did not initiate PrEP (n=11) were excluded, leaving 310 HIV uninfected women in the analysis. All participants provided written informed consent in their preferred language.

Participants were given electronic monitoring devices (MEMS caps, WestRock, Switzerland), which recorded daily bottle openings. MEMS data were downloaded and other variables collected at quarterly study visits, at which time three months of PrEP (emtricitabine/tenofovir disoproxil fumarate 200mg/300 mg) was dispensed. While initially women were not given PrEP during pregnancy, the protocol was revised midway to permit women to take PrEP during pregnancy if they chose. Study-related drug stops were implemented if a participant: acquired HIV; reported a severe, study-related adverse event; began breastfeeding; had creatinine clearance <60mL/min; or at the study physician's discretion.

Statistical analysis

Analyses focused on persistence and execution of PrEP adherence. Persistence was calculated as the time period on PrEP divided by the time period at risk, reported as a percent. By this definition, persistence can be >100% if time on PrEP exceeds the estimated time at risk.[38] Time on PrEP was

determined as the time from PrEP initiation until the earliest of: HIV seroconversion, 28 consecutive days of recorded non-use by MEMS cap, or study end. HIV risk is highest when the infected partner is not virally suppressed; however, sexual activity is also a factor. Therefore, risk was defined in two ways: any time until the HIV-infected partner had achieved ≥6 months on ART (at risk) and any time prior to ≥6 months on ART until the woman first reported no sexual activity over the previous month (at high risk). Execution was defined as the number of weekly doses while the participant had PrEP dispensed. For all analyses, participants were censored at seroconversion or study-related drug stops.

Descriptive statistics for persistence and execution were calculated overall and separately for younger women (under <25 years of age at enrollment) and older women; comparisons between these age groups were conducted, using Chi-square tests for categorical outcomes and T-tests for continuous outcomes. A counterfactual analysis was used to estimate the protective effect of PrEP against HIV acquisition, as there was no placebo group; this analysis used bootstrap resampling simulations of placebo-arm participants in an HIV-prevention clinical trial, matched on gender, risk score, time in study, and, as needed, age (< or ≥25 years old) to calculate the expected and observed HIV incidence rates, as previously described.[12] All analyses were done in SAS 9.4 (SAS Institute, Cary NC).

Results

Of the 310 women included in the analysis, 24% were <25 years at baseline. Among all the women, 97% were married to their study partner, for an average of 7.4 years, and had on average 1.6 children with their partner; couples knew their serodiscordant status for 0.7 years on average. Young women were more likely to report ≥8 years of education and shorter relationships with their study partner.

There were 10,871 weeks of follow-up time on PrEP. Overall, women recorded ≥6 weekly doses for 78% of weeks on PrEP (regardless of risk) (Figure 1). Doses recorded per week differed significantly by age, as young women took ≥6 doses for 67% of weeks and older women for 80% of weeks, p<0.001. Using a lower cut-off, 88% of weeks had ≥4 recorded doses, with younger women recording ≥4 doses for 80% of weeks, compared to 90% of weeks among older women, p<0.001.

Overall, women were at risk (i.e., HIV infected partner not yet on ART for ≥6 months) for an average of 361 days (Table 1) and time at risk did not differ between younger and older women (367 days and 360 days respectively, p=0.73). The median persistence was 100% (IQR 38%, 100%) and 54% of women recorded PrEP use for the entire risk period or longer, (i.e. ≥100% persistence). There was a significant difference by age, with 41% of younger women compared to 58% of older women recording PrEP use for their entire risk period or longer, p=0.01.

Of the 142 (46%) women who stopped PrEP while still at risk, 51% reinitiated PrEP (i.e., at least one MEMS cap opening) during their risk period; this includes 47% of younger women and 53% of older women, p=0.51. Additionally, among the 51 women whose partners initiated ART at baseline, 76% recorded PrEP use for their entire risk period, including 62% of younger women and 82% of older women, p=0.25. However, among the 259 women whose HIV-infected partners did not initiate ART at

baseline (including those whose partners never initiated ART), 50% recorded PrEP use for their entire risk period, including 37% of younger women and 54% of older women, p=0.02.

The average duration of the high-risk period (i.e., partner <6 months ART until the first report of no sexual activity) was 282 days (Table 1), and the duration was similar for younger and older women (284 and 281 days respectively, p=0.88). Among all women, 63% recorded PrEP use for their entire high-risk period (or longer), with 49% of young women recording PrEP use for the entire high risk period, compared to 67% of older women, p=0.006.

A total of three seroconversions were observed among women in the study, for an incidence rate of 0.5 per 100 person-years. The counterfactual analysis predicted an incidence rate of 7.6 per 100 person-years among all women (42.1 cases expected), for a protective effect of 93% (95% CI 77%, 98%). Similarly, among women under 25, one serconversion was observed, for a rate of 0.7 per 100 person-years, while the expected rate was 7.8 per 100 person-years with 11.4 cases expected, for a protective effect of 91% (95% CI 29%, 99%). The woman <25 years who acquired HIV infection was on PrEP for 27% of her risk period and while on PrEP, 4 out of 15 weeks recorded ≥4 doses; the six weeks prior to seroconversion recorded ≤2 doses per week. Of the two older women who seroconverted, one lacked MEMS data. The other was on PrEP until her seroconversion, with ≥4 doses reported 5 out of 12 weeks; the last four weeks had ≤2 doses.

Discussion

Women in this demonstration project were at sustained risk for HIV and the majority effectively used PrEP while at risk. More than half of women (54%) recorded PrEP use for their entire risk period or longer, 23.5% stopped and reinitiated PrEP while at risk, and only 22.5% stopped completely while at risk. While on PrEP, women recorded ≥6 doses for 78% of weeks and ≥4 doses for 88% of weeks. This degree and pattern of adherence resulted in an estimated 93% protection against HIV.

Pharmacokinetic data have been interpreted to suggest 6-7 weekly doses may be required to achieve protection from HIV in the female genital tract.[22] We found that the majority (78%) of, but not all, time on PrEP recorded ≥6 weekly doses – and, importantly, with that pattern of use HIV protection was high. Prior studies indicate that HIV uninfected individuals align their PrEP use with periods of risk[13,19,38], which may explain the strong protective effect even with imperfect adherence. Messaging about using PrEP during 'seasons' of risk may help women understand that PrEP use is short term, unlike lifelong ART use, and may increase PrEP uptake and adherence.[24,39] However, daily dosing should be emphasized during these seasons of risk to optimize efficacy and establish consistent adherence routines.[40]

Prior studies have demonstrated that unique HIV risks and adherence challenges exist for young adults.[13] In the iPrEx study of MSM and transgender women, younger men (18-24 years old) were less likely to have PrEP detected in blood samples.[41] In addition, results from a study of dapivirine ring use found no protective effect among women under 21.[42] In the present study, time at risk was similar between younger and older women. However, younger women were less likely to use PrEP during their

time at risk and while on PrEP they were less likely to have ≥6 doses per week, supporting the idea that they may face greater challenges to adherence.[43] Nevertheless, many women <25 years of age in this study took PrEP, with sustained persistence and high execution, and the HIV protection achieved in the population was high. Better understanding of how young women perceive their HIV risk and whether adherence increases during periods of perceived risk will be useful in reducing adherence barriers for this population. In addition, there may be other barriers, such as family and partner influences, that disproportionately affect young women.[17,44,18]

There are several limitations to this study. While MEMS caps provide day-level data, they are a proxy for actual doses and can be over or under-reported.[45] However, previous work has shown that electronic monitoring can detect regular PrEP users, as supported by plasma tenofovir levels.[27] Data on seroconversion, sexual activity, and ART initiation were collected quarterly and data on outside partners was insufficient to assess HIV risk, leading to imprecision in calculating risk periods and potential misclassification. Only mutually-disclosed HIV-serodiscordant couples were included, and the results may not be generalizable to all women. In particular, defining risk may be more clear-cut in a serodiscordant couple (i.e., before the HIV-infected partner is virally suppressed) and PrEP may be seen as a way for couples to maintain the relationship.[21,46]

The strengths of this analysis include large sample size, longitudinal data, use of electronic monitoring, and the inclusion of risk in assessing adherence. Our results do not provide a threshold of PrEP use for HIV protection in women, but they demonstrate that patterns of PrEP use outside of clinical trials are associated with very low HIV risk, even in a population that would otherwise be at very high risk for acquiring HIV.

In conclusion, PrEP is an effective HIV-prevention tool for East African women in serodiscordant couples. Women, including women <25 years of age, are able to adhere to PrEP over sustained periods of risk at levels that provided significant protection. While women should aim for daily PrEP use during seasons of risk, the threshold of perfect adherence should not be expected or become a barrier to PrEP delivery.

Acknowledgements
We thank the couples who participated in this study.

Partners Demonstration Project Team
Coordinating Center (University of Washington) and collaborating investigators (Harvard Medical School, Johns Hopkins University, Massachusetts General Hospital): Jared Baeten (protocol chair), Connie Celum (protocol co-chair), Renee Heffron (project director), Deborah Donnell (statistician), Ruanne Barnabas, Jessica Haberer, Harald Haugen, Craig Hendrix, Lara Kidoguchi, Mark Marzinke, Susan Morrison, Jennifer Morton, Norma Ware, Monique Wyatt

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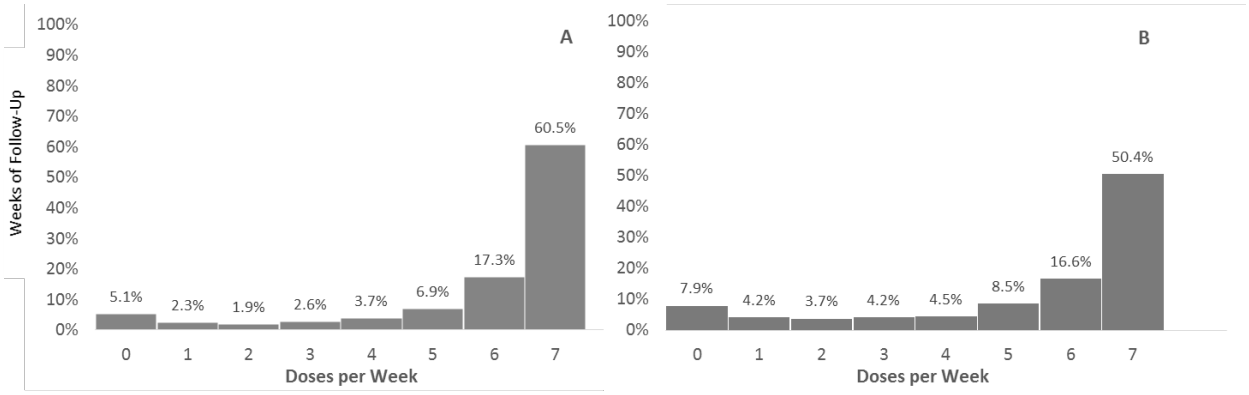
Kisumu, Kenya (Kenya Medical Research Institute): Elizabeth Bukusi, Josephine Odoyo
Thika, Kenya (Kenya Medical Research Institute, University of Washington): Nelly Rwamba Mugo, Kenneth Ngure
Data Management was provided by DF/Net Research, Inc. (Seattle, WA). PrEP medication was donated by Gilead Sciences.

Table 2.1 Persistence & Time at Risk, by Age

	At Risk				At High Risk			
	All Women (n=310)	Women <25 (n=73)	Women ≥25 (n=237)	p-value*	All Women (n=309)	Women <25 (n=73)	Women ≥25 (n=236)	p-value*
Persistence >100%	54%	41%	58%	0.01	63%	49%	67%	0.006
Mean Days at Risk (SD)	361 (170)	367 (173)	360 (169)	0.73	282 (171)	284 (160)	281 (175)	0.88

*Chi square or T-test (unequal variance)

Figure 2.1 Weekly Doses While on PrEP Among All Women (A) and Women <25 (B)



Chapter 3. Patterns of Oral PrEP Adherence And HIV Risk among Eastern African Women in HIV Serodiscordant Partnerships

Published citation: Pyra, M., Brown, E.R., Haberer, J.E., Heffron, R., Celum, C., Bukusi, E.A., Asiimwe, S., Katabira, E., Mugo, N.R., Baeten, J.M., Partners Demonstration Project Team. Patterns of Oral PrEP Adherence and HIV Risk among Eastern African Women in HIV Serodiscordant Relationships. AIDS & Behavior. (in revisions)

Patterns of Oral PrEP Adherence And HIV Risk among Eastern African Women in HIV Serodiscordant Partnerships

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Compliance with Ethical Standards:

Funding: The Partners Demonstration Project was funded by the National Institute of Mental Health of the US National Institutes of Health (R01 MH095507), the Bill & Melinda Gates Foundation (OPP1056051), and the US Agency for International Development (AID-OAA-A-12-00023). Additional funding for the present analysis came from the National Institute of Mental Health (R01 MH098744). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID, NIH, or the United States Government.

Conflict of Interest: JMB has led studies with pre-exposure prophylaxis medication donated by Gilead Sciences and served on an advisory committee.

Ethical Approval and Informed Consent: The University of Washington Human Subjects Division and ethics review committees at each site (the National HIV/AIDS Research Committee of the Uganda National Council for Science and Technology or the Ethics Review Committee of the Kenya Medical Research Institute) approved the protocol. All participants provided written informed consent in their preferred language in accordance with the Declaration of Helsinki.

Abstract

Background: Understanding how women use PrEP is important for developing successful implementation programs. We hypothesized that women have distinct patterns of adherence, related to HIV risk and other factors.

Setting & Methods: We identified patterns of PrEP adherence and HIV risk behavior over the first 6 months of PrEP use, using data from 233 HIV-uninfected women in high-risk serodiscordant couples in a demonstration project in Kenya & Uganda. We modeled PrEP adherence, assessed by daily electronic monitoring, and HIV risk behavior using group-based trajectory models. We tested baseline covariates and risk behavior group as predictors of adherence patterns.

Results: There were four distinct adherence patterns: high steady adherence (55% of population), moderate steady (29%), late declining (8%), and early declining (9%). No baseline characteristics significantly differed between adherence patterns. Adherence patterns differed in average weekly doses (6.7 vs 5.4 vs 4.1 vs 1.5, respectively). Two risk behavior groups were identified: steady HIV risk (78% of population) and declining (22%). Compared to women with declining HIV risk behavior, women with steady risk behavior were more likely to have high steady adherence (61% vs 35%) and less likely to have early (6% vs 17%) or late (4% vs 19%) declining adherence.

Conclusions: Women's use of PrEP was associated with concurrent HIV risk behavior; higher risk was associated with higher, sustained adherence.

Introduction

Women in many areas of the world remain at high-risk for HIV acquisition. In 2015, over 50% of new HIV infections in Sub-Saharan Africa occurred in women and 25% of all new infections occurred in women ages 15-24 [47]; these young women were twice as likely to acquire HIV compared to young men [47]. UNAIDS identified young women as a gap in HIV prevention in Eastern and Southern Africa [3]. Pre-exposure prophylaxis (PrEP) has been approved for HIV prevention as one way to address that gap [3].

Adherence among women was low in two randomized controlled trials (RCT) of PrEP [10,11]; several reasons emerged as to why women were not adhering during these placebo-controlled trials, including: lack of support from family and partners; fear of side effects; uncertainty of taking placebo; doubt that PrEP worked; low perceived HIV risk; difficulty taking a daily medication when not sick; and stigma of using an ARV [17,18]. However, two RCTs with high adherence did demonstrate efficacy among women [5,6,14]. In the context of known PrEP efficacy and international and national PrEP guidelines, open-label studies have demonstrated that women can use oral PrEP effectively [12,48]. Qualitative work from open-label studies identified barriers to PrEP use, including some, like side effects, stigma, and fear of disclosure, that were also identified in the RCTs [19,49]. However, social support, belief in PrEP efficacy, and perceived HIV risk were also seen as facilitators to PrEP use [19,49]. Unlike ART, PrEP is intended to be taken when persons are at risk of HIV exposure, a framework described as prevention-effective adherence [38]. Therefore, it will be key to understand when these risk periods occur and how well women adhere during risk periods to optimize PrEP delivery.

One way to better understand PrEP adherence is to characterize adherence patterns over time. Identifying women with certain adherence patterns can help counselors tailor their approaches for those in need of additional support, assist women to better assess their HIV risk, and connect women with peers. We hypothesized that women taking PrEP would display identifiable patterns of use, and these might be related to demographic factors as well as ongoing HIV risk behavior.

Methods

Study Population

Data were drawn from the Partners Demonstration Project, an open-label PrEP demonstration study among HIV serodiscordant couples in East Africa, as previously described [12,35]. HIV serodiscordant couples were selected to be at high risk for HIV acquisition using a risk score we previously validated, which included age, cohabitation status, parity with study partner, unprotected sex, and viral load of the uninfected partner [37,50]; HIV-uninfected partners were encouraged to take PrEP until their partners had completed at least six months of antiretroviral treatment ("PrEP as a bridge to ART"). A total of 1,013 HIV serodiscordant couples, among which 334 couples had female HIV-uninfected partners, were enrolled at two sites each in Kenya and Uganda.

Participants were given electronic monitoring devices (MEMS caps, WestRock, Switzerland), which recorded daily bottle openings. MEMS data were downloaded and other variables collected at quarterly study visits, at which time PrEP (emtricitabine/tenofovir disoproxil fumarate 200mg/300 mg) was dispensed. PrEP was discontinued if a participant: acquired HIV; reported a severe, PrEP-related adverse

event; began breastfeeding; had creatinine clearance <60mL/min; or at the study physician's discretion. Midway through the study, the protocol was modified to allow women to continue PrEP during pregnancy if they chose. The study was approved by the University of Washington IRB and the ethic review committee for each site [12]. All participants provided written informed consent in their preferred language.

Statistical Analysis

For this analysis, women were excluded if they were HIV-infected at baseline (n=6), had <24 weeks of electronic monitoring data, including those with drug stops (n=94), or never initiated PrEP (n=1), leaving 233 HIV uninfected women with 24 weeks of complete data. Group-based trajectory modeling was used to identify patterns (or trajectories) of PrEP adherence and, separately, HIV risk behavior over the first 24 weeks of PrEP use. These models have been used to find adherence patterns in many health fields, including HIV [51,52]; detailed guides are available for these models as well [53,54] (Supplement). Using a specified number of trajectories and their shape (linear, quadratic, or cubic), the model estimates the proportion of the population belonging to each trajectory and then calculates the probability of an individual belonging to each trajectory based on their observed data; the individual is then assigned to the trajectory with the highest probability and the posterior probability (the chance of being in that trajectory given the observed data) is calculated [53,55–57].

Adherence was modeled on PrEP doses per week (range 0-7), using a censored normal (tobit) distribution [53], and HIV risk behavior was modeled on any sex (regardless of condom use or type of sexual partner) over the prior month, as reported at quarterly study visits, using a binomial distribution, over the first six months of PrEP use. For each outcome, models with 2-6 trajectories were compared using the Bayes test (recommended to be >10 to indicate good fit) and average posterior probabilities (recommended to be >0.7 to indicate good fit)[53,58]; when needed, content expertise was used to determine the most meaningful model. Once the number of trajectories was selected, the same criteria were used to compare linear, quadratic, and cubic terms for each trajectory. The distribution of covariates by assigned trajectory is presented.

Next, baseline covariates were added independently to the models; these are interpreted as the odds of being in each trajectory compared to a reference trajectory. Wald tests were computed to test for significance [55]. Covariates of interest as reported at enrollment were: perception of HIV risk from their study partner (dichotomized as high/moderate/unknown vs low/none); any report of problem drinking behaviors; marital status; parity; couple HIV risk score; and age (</≥25 years old). Likewise, time-varying covariates (also known as turning points) were added independently to the model; these are interpreted as the change in outcome when the covariate was present, within each trajectory [55]. Again, Wald tests were computed to test for significance. Pregnancy, partner's use of ART, and frequency of sex over the prior month (with their study partner or outside partners) were recorded at follow-up study visits and included as possible covariates. Any significant covariates were included in the final model. We compared mean adherence across groups, using the assigned group as the predictor in a mixed effects model to account for repeated observations per woman.

Finally, we modeled the joint trajectories, using the final models for adherence and risk behavior. Joint trajectories allow the calculation of both the population and individual probabilities of belonging to each adherence trajectory, given a women's risk behavior trajectory. We used chi-square tests to assess whether the population probability of each adherence trajectory differed by risk behavior trajectory. We also modeled the $\log_{10}$ individual probability of each adherence trajectory in separate linear regressions, with the individual's probability of the steady risk behavior trajectory as the predictor (rather than assigned risk behavior trajectory, to account for possible error in trajectory assignment). As a sensitivity analysis, we included women missing adherence data (n=45); women who had a drug stop were still excluded as inability to use PrEP is a separate issue from adherence. All analyses were conducted using SAS 9.4 (SAS Institute, Cary NC).

Results

Of the 233 women initiating PrEP who were included in the present analysis, 22% were <25 years old at enrollment and 98% were married to the HIV-infected partner who participated in the Partners Demonstration Project (Table I). Recent sex with any partner was reported by 98% at enrollment and 51% felt they were at high/moderate HIV risk. During the six-months of follow-up, 7% of women became pregnant and 62% of their HIV-infected partners initiated ART. Overall, the average adherence to PrEP over this period was high at 82%, with an average 5.7 doses per week taken as measured by MEMS.

All the group-based trajectory models tested fit the adherence data well when using the Bayes test. A model with 4 groups was selected to be the most meaningful, with quadratic terms as the best fit; the average posterior probabilities were ≥0.97. We described the four adherence patterns as early declining adherence (estimated as 9% of the population), late declining adherence (8% of population), moderate steady adherence (29% of population), and high steady adherence (55% of population) (Figure 1). Adherence trajectories differed by average dose per week ($p<0.0001$) when modeled in a linear regression accounting for repeated observations, with the lowest average dose (1.5) in the early declining group and the highest average dose (6.7) in the high steady group (Table II). Differences in adherence were apparent in first week of PrEP use, when the early declining group had an average 5 doses, compared to 6.5, 6.4 and 6.7 in the late declining, moderate, and high steady groups respectively. Furthermore, when comparing average adherence over the first 12 weeks to the latter 12 weeks, there was no difference for the two steady adherence groups (6.8 to 6.7 and 5.4 to 5.4 doses per week for the high and moderate steady adherence trajectories). However, there were notable differences for the two declining groups: 5.9 doses per week over the first 12 weeks compared to 2.2 over the latter 12 weeks for the late declining groups; and 2.2 doses per week then 0.7 for the early declining group.
Age, education, alcohol use, HIV risk perception, and pregnancy (Table II) were not statistically associated with adherence trajectory by Wald Test when tested individually as time-independent (or time-dependent, for pregnancy) covariates: age, $p=0.25$; education, $p=0.22$; drinking, $p=0.18$; risk perception, $p=0.75$; pregnancy, $p=0.57$. Having their HIV-infected partner start ART was statistically significant ($p<0.0001$ by Wald test) and was included in the final model (Figure 1). Specifically, late decliners took 0.5 more doses on average during weeks when their partner was on ART, compared to late decliners whose partners were not on ART ($p=0.008$, Supplemental Figure 1). On the other hand, early decliners took 0.9 fewer doses on average during weeks when their partner was on ART ($p<0.001$)

compared to early decliners whose partners were not on ART. For the moderate and high steady trajectories, the effect of having a partner on ART was not significant (-0.2 doses per week, p=0.15 and 0.02 doses per week, p=0.57, respectively).

Next, we identified HIV risk behavior trajectories, based on any reported sex in the prior month. A model with two linear trajectories was the best fit (Figure 2); the average posterior probabilities were ≥0.86. The two risk behavior trajectories were defined as declining risk (an estimated 22% of the population) and steady risk (78% of population). There did not appear to be any differences by baseline or time-varying covariates (Table III); age was tested in the model a priori and was not significant, p=0.62. There was an average of 5.9 doses per week in the steady risk group compared to 5.1 in the declining risk group, which was statistically but likely not clinically significant (p=0.002).

Finally, we modeled adherence and risk behavior trajectories jointly, using the final models for each (Figure 3). There were significant differences in the distribution of adherence trajectories by risk behavior group by Chi-square test, p<0.0001. Women with steady HIV risk were more likely to have high steady adherence, compared to those declining risk. Conversely, women with declining HIV risk were more likely to have early or late declining adherence, compared to those with steady HIV risk. This pattern was the same when examining the individual adherence probabilities by steady HIV risk probability, with p<0.01 for all comparisons. Results from the sensitivity analysis (n=278) were consistent with those already described.

One woman in this analysis acquired HIV, 12 months after enrollment. During her first 6 months of use, she was characterized as early declining adherence and steady HIV risk behavior.

Discussion

In this secondary analysis of data from East African women in HIV serodiscordant heterosexual partnerships who were taking PrEP for HIV prevention, we found evidence of different trajectories of adherence and of ongoing HIV risk behavior. Most women had high or moderate steady adherence, with average doses of 6.7 and 5.4 per week respectively over the first 6 months of PrEP use; likewise, most women remained at steady HIV risk over the first 6 months of PrEP use. However, we also identified two declining adherence trajectories. We did not find signficant differences in baseline characteristics that could differentiate between adherence trajectories. However, there were significant associations between sexual risk behavior and adherence trajectories. Specifically, women with a steady risk trajectory during follow-up were more likely to have a high steady adherence trajectory and less likely to have early or late declining adherence trajectories.

These results suggest that women align their PrEP use to times of HIV risk behavior, consistent with prevention-effective adherence [38,59]. Preventive-effective adherence is optimal both for the individual woman as well as for the health system, as it maximizes prevention while minimizing costs and side-effects when PrEP is not needed [38]. Prevention-effective adherence also distinguishes PrEP, which is only needed while at-risk, from ART use, which is taken for life [38]. However, women may benefit from additional support in assessing their HIV risk; roughly 11% of women with steady risk had

early or late declining adherence – including one woman who seroconverted to HIV – indicating a potential misalignment in adherence and risk. We found no associations between adherence and baseline risk perception, which underlies the difficulty of assessing risk perception especially with a single question [60–62].

These results are also in line with previous work that found initial adherence may be related to adherence over follow-up [10,16]. For instance, in this analysis the early declining adherence group had the lowest weekly dose from the first week of follow-up. However, not all women who start with high adherence will continue; the late declining adherence group for instance showed initially high adherence and was the least common trajectory.

Strengths of this study include use of daily electronic monitoring of adherence and the ability to compare adherence with HIV risk. MEMS caps are not without measurement error but have been shown to correspond with biomarkers of adherence better than self-report [12,27]. This analysis had a limited sample size, especially with infrequent assessments of risk behavior which led to few time points for the risk trajectory models; this may also have limited the power to detect associations with baseline characteristics. In addition, we cannot determine that HIV risk led to adherence; while the data support the idea of prevention-effective adherence, an alternative explanation is that women took more risks when adherence was high. However, previous work has not shown evidence of risk compensation in heterosexual couples using PrEP [63], and the degree of risk behavior seen on average during follow-up in this study was not greater than what was present at baseline. Finally, women in this study were members of known HIV-serodiscordant couples, whereby HIV risk may be easily identified; these results may not be generalizable to women in other situations, in which they do not clearly know their HIV risk.

In conclusion, we found evidence of distinct PrEP adherence patterns, which may be useful for implementing and understanding PrEP use in high-risk settings. Importantly, our results suggest that women may align their PrEP use with their HIV risk behavior, which would be the most efficient and cost-effective use of PrEP. Adherence counseling that improves women's assessments of their HIV risk may be important for PrEP implementation. Tools to help women better assess their HIV risk and understand when to use PrEP may motivate and enable women to effectively adhere and protect themselves from HIV.

Acknowledgements
We thank the couples who participated in this study.

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Data Management was provided by DF/Net Research, Inc. (Seattle, WA). PrEP medication was donated by Gilead Sciences.

Table 3.1 Participant Characteristics

Baseline Characteristics	**% (N women)**
N	233
<25 years old	21.9% (51)
>8 years education	33.5% (78)
HIV Risk Score, Mean (SD)	6.7 (1.4)
Married to Partner	98.3% (229)
Parity with Partner, Median (IQR)	1 (0,3)
Any sex, past month	94.9% (221)
Any condomless sex, past month	60.1% (140)
Problem drinking	16.3% (38)
High/moderate/unknown perception of HIV risk	50.6% (118)
During 24 week follow-up	
Weekly Doses, Mean (SD)	5.7 (2.2)
Pregnant	6.9% (16)
Partner Starts ART	61.8% (144)
Any sex	99.6% (232)

Table 3.2 Participant Characteristics by Adherence Trajectories

Baseline Characteristics	Group 4 (High Steady) (n=130)	Group 3 (Moderate Steady) (n=66)	Group 2 (Late Decline) (n=17)	Group 1 (Early Decline) (n=20)
<25 years old	14.6% (19)	25.8% (17)	35.3% (6)	45.0% (9)
>8 years education	30% (39)	34.9% (23)	52.9% (9)	35.0% (7)
HIV Risk Score, Mean (SD)	6.7 (1.4)	6.8 (1.5)	6.6 (1.0)	6.7 (1.7)
Married to Partner	99.2% (129)	97.0% (64)	94.1% (16)	100.0% (20)
Parity with Partner, Median (IQR)	1.5 (0, 3)	1 (0,2)	1 (1,3)	1 (0,2)
Any sex, past month	95.4% (124)	93.9% (62)	100.0% (17)	90.0% (18)
Any condomless sex, past month	61.5% (80)	60.6% (40)	52.9% (9)	55.0% (11)
Problem drinking	13.9% (18)	24.2% (16)	17.7% (3)	5.0% (1)
High/moderate/unknown perception of HIV risk	50% (65)	54.5% (36)	47.1% (8)	45.0% (9)
Site				
Thika	23.1% (30)	16.7% (11)	17.7% (3)	20% (4)
Kampala	33.1% (43)	48.5% (32)	47.1% (8)	30% (6)
Kabwohe	21.5% (28)	12.1% (8)	5.9% (1)	15% (3)
Kisumu	22.3% (29)	22.7% (15)	29.4% (5)	35% (7)
During 24 week follow-up				
Weekly Doses, Mean (SD)	6.7 (0.8)	5.4 (2.0)	4.1 (2.9)	1.5 (2.2)
Pregnant	5.4% (7)	10.6% (7)	11.8% (2)	0.0% (0)
Partner Starts ART	65.4% (85)	59.1% (39)	58.8% (10)	50.0% (10)
Any sex	99% (129)	100% (66)	100% (17)	100% (20)

Association between charactersitics and adherence trajectories: age (p=0.25); education (p=0.22); problem drinking (p=0.18); HIV risk perception (p=0.75); pregnancy (time-varying, p=0.57); partner's ART use (time-varying, p<0.0001)

Table 3.3 Participant Characteristics by Risk Behavior Trajectories

Baseline Characteristics	Group 2 (Steady Risk) (n=186)	Group 1 (Declining Risk) (n=47)
<25 years old	22.0% (41)	21.3% (10)
>8 years education	32.3% (60)	38.3% (18)
HIV Risk Score, Mean (SD)	6.7 (1.4)	6.7 (1.4)
Married to Partner	98.4% (183)	97.9% (46)
Parity with Partner, Median (IQR)	1 (0,3)	1 (0,2)
Any sex, past month	97.3% (181)	85.1% (40)
Any condomless sex, past month	60.8% (113)	57.5% (27)
Problem drinking	17.2% (32)	12.8% (6)
High/moderate/unknown perception of HIV risk	50.0% (93)	53.2% (25)
Site		
Thika	21.5% (40)	17.0% (53)
Kampala	38.2% (71)	38.3% (18)
Kabwohe	19.4% (36)	8.5% (4)
Kisumu	21.0% (39)	36.2% (17)
During 24 week follow-up		
Weekly Doses, Mean (SD)	5.9 (2.0)	5.1 (2.6)
Pregnant	7.5% (14)	4.3% (2)
Partner Starts ART	61.3% (114)	63.8% (30)
Any sex	100.0% (186)	97.9% (46)

Association between charactersitics and risk behavior trajectories: age (p=0.62).

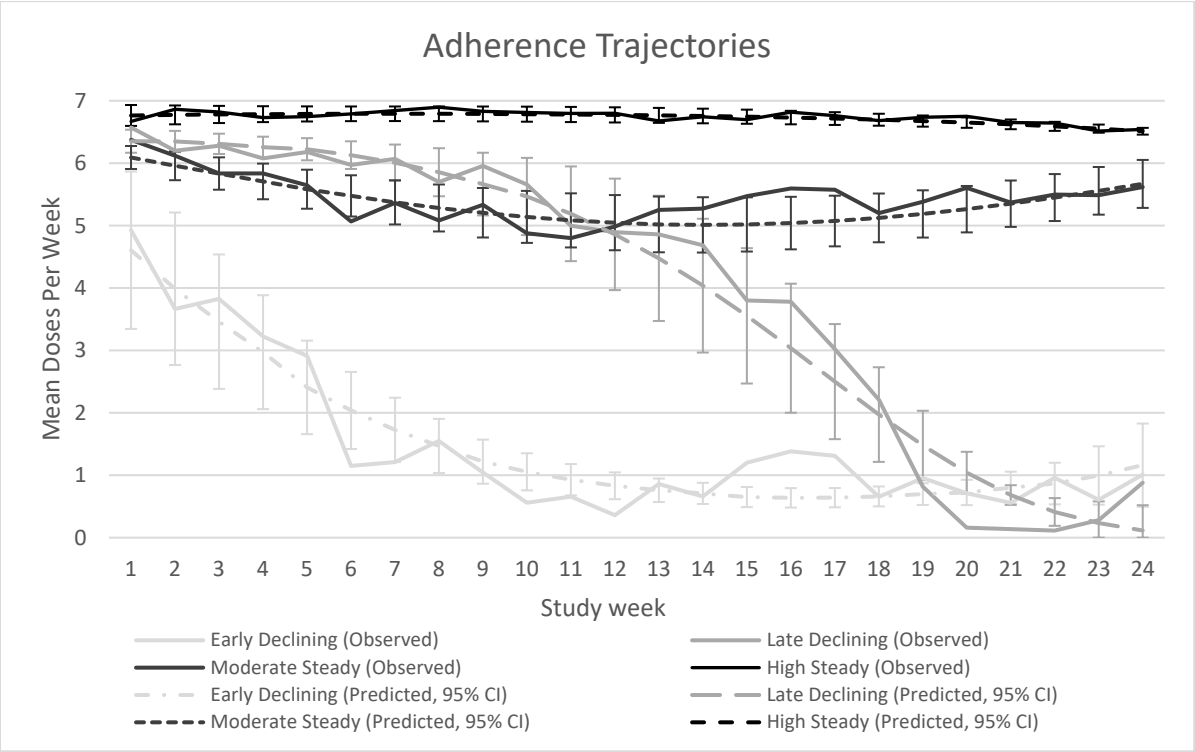


Figure 3.1: Group-based Trajectories of adherence. Over the first six months of PrEP use, four adherence trajectories were identified. Dotted lines represent the predicted values from the models, with 95% confidence intervals; solid lines represent the observed data.

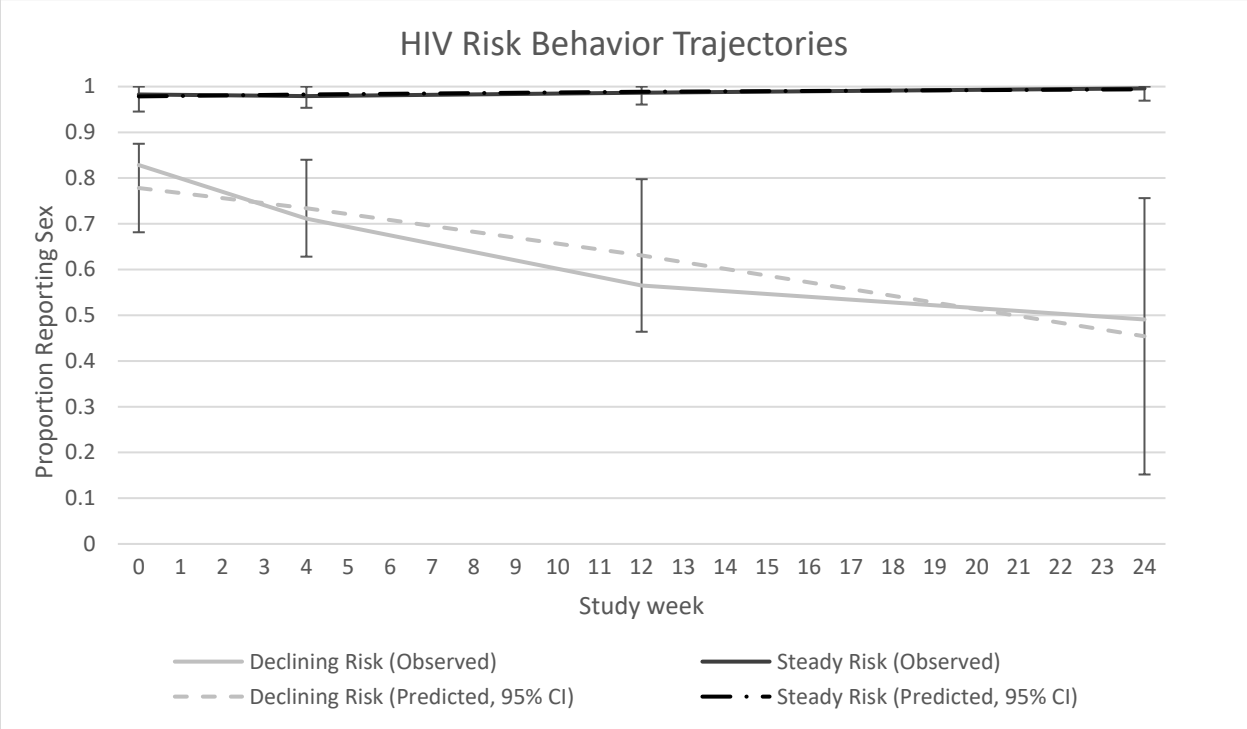


Figure 3.2: Group-based Trajectories of Risk Behavior. Over the first six months of PrEP use two risk behavior trajectories were identified based on reported sexual activity. Dotted lines represent the predicted values from the models; solid lines represent the observed data.

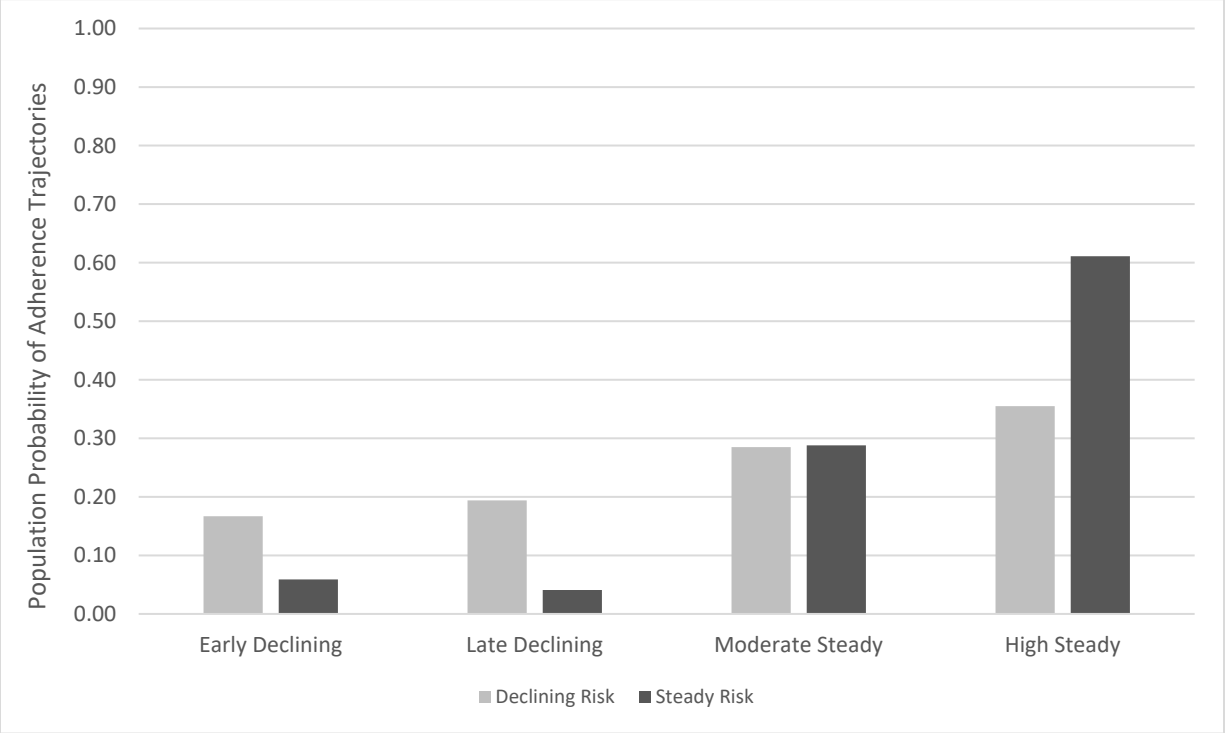
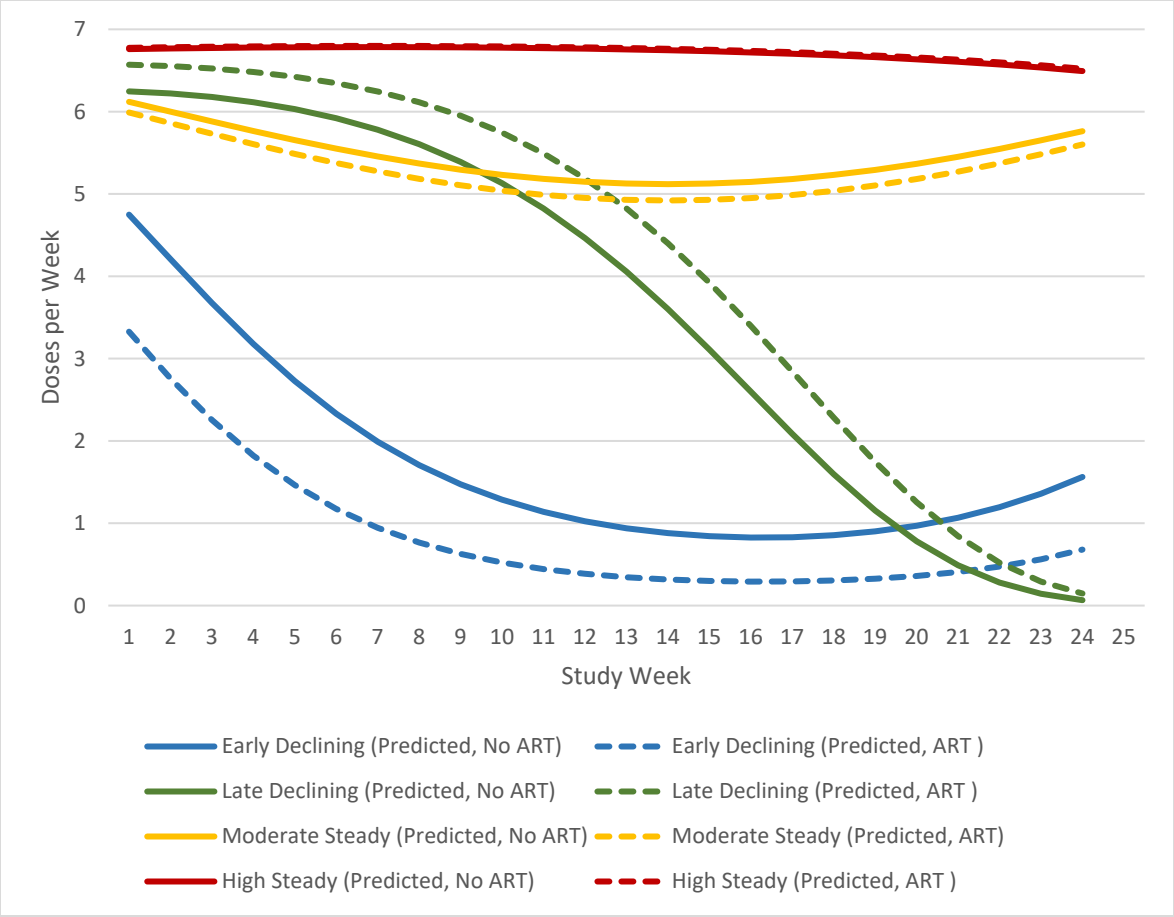


Figure 3.3: Population Probability of Adherence Trajectory by Risk Group. The probability of being in the high steady, early declining, or late declining adherence trajectory differed according to risk trajectory, $p<0.0001$ (Chi-square test).



Supplemental Figure 3.4: Changes in Average Doses per Week when Partner on ART, by Adherence Trajectory

The solid lines are the predicted average doses per week when the HIV-infected partner is not on ART and the dotted lines represent the predicted average doses per week when the HIV-infected partner is on ART. The changes were not significant for the moderate steady (-0.2 doses per week, p=0.15) or the high steady (0.02 doses per week, p=0.57) trajectories. In the late declining trajectory, the difference was 0.5 doses per week on average (p=0.008) and in the early declining trajectory, the difference was -0.9 doses per week on average (p<0.001).

Chapter 4. Tenofovir-diphosphate as a Marker of HIV Pre-Exposure Prophylaxis Use among East African Men and Women

Citation: Pyra, M., Anderson P.L., Haberer, J.E., Heffron, R., Celum, C., Asiimwe, S., Katabira, E., Mugo, N.R., Bukusi, E.A., Baeten, J.M., Partners Demonstration Project Team. Tenofovir-diphosphate as a Marker of HIV Pre-Exposure Prophylaxis Use among East African Men and Women. Frontiers in Pharmacology. (under review)

Tenofovir-diphosphate as a Marker of HIV Pre-Exposure Prophylaxis Use among East African Men and Women

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Funding:
The Partners Demonstration Project was funded by the National Institute of Mental Health of the US National Institutes of Health (R01 MH095507), the Bill & Melinda Gates Foundation (OPP1056051), and the US Agency for International Development (AID-OAA-A-12-00023). Research reported in this publication was supported by the UW/Fred Hutch Center for AIDS Research, funded by NIAID, NCI, NIMH, NIDA, NICHD, NHLBI, NIA, NIGMS, NIDDK of the National Institutes of Health under award number P30 A1027757 and the HIV Prevention Trials Network (UM1 AI068613). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID, NIH, or the United States Government.

Keywords: tenofovir-diphosphate, adherence, pre-exposure prophylaxis, women, Africa, HIV

Abstract

Background: Controlled pharmacokinetic (PK) studies in U.S. populations have defined categories of tenofovir-diphosphate (TFV-DP) quantified in dried blood spots (DBS) for various pre-exposure prophylaxis (PrEP) adherence targets. We evaluated the sensitivity and specificity of DBS TFV-DP categories compared to daily medication electronic adherence monitoring (MEMS) data among East African men and women using daily PrEP.

Methods: Participants were enrolled as members of HIV serodiscordant couples as part of an open-label PrEP study in Kenya and Uganda. Blood samples were taken at quarterly visits and stored as DBS, which were analyzed for TFV-DP concentrations.

Results: Among 150 samples from 103 participants, MEMs data indicated that 87 (58%) took ≥4 doses and 62 (41%) took ≥6 per week consistently over the four weeks prior to sample collection. Sensitivities of DBS TFV-DP levels were 62% for the ≥4 doses/week category (≥700 fmol/punch TFV-DP) and 44% for the ≥6 doses/week category (≥1050 fmol/punch TFV-DP); specificities were 86% and 94%, respectively. There were no statistically significant differences in these sensitivities and specificities by gender.

Conclusions: In this sample of East African PrEP users, categories of TFV-DP concentrations developed from directly observed PrEP use among U.S. populations had high specificity but modest sensitivity. Sensitivity was lowest when MEMS data indicated high adherence (i.e., ≥6 doses/week). PrEP studies and implementation programs using TFV-DP as a marker of adherence should consider the sensitivity and specificity of DBS TFV-DP categories in the population they are evaluating.

Introduction

Clinical trials have shown that pre-exposure prophylaxis (PrEP) is highly effective for preventing HIV [5,14,15]. However, effectiveness depends strongly on adherence [9]. Clinical studies and open-label implementation programs have used many methods to assess PrEP adherence. As participants may misreport PrEP use, biomarkers are of particular interest as an objective marker of adherence. Some biomarkers, including concentrations of tenofovir in plasma or emtricitabine-triphosphate in blood cells, detect only recent use and are susceptible to white-coat effects, when individuals take a dose before a visit to appear adherent.

In contrast, the active metabolite tenofovir-diphosphate (TFV-DP) accumulates in blood cells in a dose-proportional manner [29] and is a marker of cumulative use over the prior month. As a biomarker, TFV-DP is increasingly being used to assess adherence in research and implementation projects, with adherence counseling sometimes tailored based on these values [64]. Intensive, controlled pharmacokinetic studies in U.S. populations were conducted with directly observed treatment (DOT) to estimate the expected levels of TFV-DP for specific adherence targets (i.e., ≥2 or ≥4 doses/week) [29]; these levels have been associated with HIV protection among men who have sex with men in the iPrEx placebo-controlled trial conducted in the U.S., South America, Thailand and South Africa [41,65].

However, these categories require assessment in African populations, where PrEP implementation is underway in many countries. Therefore, our goal was to evaluate the sensitivity and specificity of these categories in African men and women, using electronic adherence monitoring data for comparison.

Materials and Methods

Study Sample

These data come from the Partners Demonstration Project, an open-label PrEP demonstration study among HIV serodiscordant couples in East Africa, as previously described [12,35]. A total of 1,013 couples were enrolled in Kenya and Uganda. Participants were given electronic monitoring devices (MEMS caps, WestRock, Switzerland), which recorded daily bottle openings. MEMS data were downloaded and other variables collected at quarterly study visits, when PrEP (emtricitabine/tenofovir disoproxil fumarate 200mg/300 mg, prescribed for daily use) was dispensed. Participants received PrEP adherence counseling at study visits, but neither MEMS data nor TFV-DP concentrations were shared. Blood samples were prepared into dried blood spots (DBS) at quarterly visits and stored at -20°C. The University of Washington Human Subjects Division as well as ethics review committees at each site (either the National HIV/AIDS Research Committee of the Uganda National Council for Science and Technology or the Ethics Review Committee of the Kenya Medical Research Institute approved the protocol). All participants provided written informed consent in their preferred language in accordance with the Declaration of Helsinki.

To ensure variation in adherence patterns, we selected 120 random DBS, stratified by gender and evenly distributed with 0-2, 3-5, or 6-7 recorded openings by MEMS in the week prior to collection. In addition, any samples from the same participant at both the first and third month study visit were included (n=76 additional samples), to assess changes over time. To optimize accuracy of MEMS data, we excluded any

DBS when the participant reported curiosity openings (opening without removing pills, n=8) or pocket dosing (removing multiple pills, n=17); we also limited the data to one bottle opening per day. Finally, we excluded any visits during pregnancy (n=21) [66]. TFV-DP was analyzed from DBS by liquid chromatography tandem mass spectrometry (LC-MS/MS) at the University of Colorado [30,31,67]; values below the lower limit of quantitation of 31.25 fmol/punch were set to half the lower limit.

Statistical Analysis

We considered three adherence targets as recorded by MEMS consistently for the four weeks prior to sample collection, and corresponding TFV-DP categories based on previous DOT analyses [29,68]: $\geq$700 fmol/punch for $\geq$4 doses, $\geq$1050 fmol/punch for $\geq$6 doses, and $\geq$1250 fmol/punch for 7 doses/week. The PK thresholds were established at 25th percentiles from prior studies such that 75% of adherent PrEP users were captured by the category.

We reported concentrations of TFV-DP among consistent users by gender and duration of PrEP use (early use, defined as the first month of PrEP, or later use). We tested gender, duration of PrEP use, total doses over the prior month, and study site in a generalized estimating equation model predicting TFV-DP concentration. We reported sensitivity and specificity, using MEMS data as the standard, with Wald 95% confidence intervals. In a sensitivity analysis, we used average (vs consistent) doses. We also compared sensitivities and specificities by gender, using an interaction term in generalized estimating equations with a binomial distribution to account for repeated observations. In sensitivity analyses, we excluded samples with unquantifiable TFV-DP and repeated the analyses using doses over only the prior week. To assess the effect of misclassification in the MEMS data, we used a multidimensional bias analysis [69]. Finally, we calculated positive and negative predictive values of the TFV-DP categories. Analyses were conducted in SAS 9.4, with bias and predictive value analyses in Excel.

Results

Our analysis includes 150 DBS from 103 participants with 55 samples from 35 women and 95 samples from 68 men. The median age of men was 32.9 years and women was 29.5 years at baseline. Men recorded an average of 4.6 doses/week over the prior 4 weeks and women recorded 4.1 doses/week on average (Supplemental). Twenty-two samples were classified as early PrEP use. Overall, 58% (87) of samples consistently had $\geq$4 doses, 41% (62) had $\geq$6 doses, and 14% (21) had 7 doses/week for all of the prior 4 weeks as recorded by MEMS.

The average concentration among samples with $\geq$4 doses/week by MEMS was 925 fmol/punch (standard deviation [SD] 509) (Table 1). For those with $\geq$6 doses/week, the average was 994 fmol/punch (SD 517) and for those with 7 doses/week, it was 928 fmol/punch (SD 390). After controlling for site, duration of PrEP use, and total doses by MEMS, the concentration of TFV-DP was 12% higher among women compared to men, which was not statistically significant (adjusted risk difference [aRD] 80.8 fmol/punch [95% CI -78.7, 242.0]). In the same model, early use (first month of PrEP) was significantly associated with 20% lower concentrations, aRD -129.1 fmol/punch (95% CI -227.8, -30.3).

The sensitivity of the ≥700 fmol/punch category was 62% for the ≥4 doses/week dosing and the specificity was 86% (Table 2, Supplemental). The sensitivity of the ≥1050 fmol/punch category was lower, 44%, and the specificity was higher, 93%, for ≥6 doses/week dosing. The ≥1250 fmol/punch category had a sensitivity of 19% and a specificity of 90% for the 7 doses/week dosing. There were no statistical differences by gender, though we were unable to test the difference for the ≥1250 category due to small sample size. When excluding 18 samples with unquantifiable TFV-DP, results were similar (Table 2). In addition, results based on doses over the prior 1 week and on average doses were similar to those described (Supplemental).

We recognized potential for misclassification of adherence from MEMS data. Both over and under-reporting have been observed with electronic monitoring, but over-reporting would explain the low sensitivities we observed. Therefore, we tested a hypothetical scenario where 98% of those truly meeting the adherence targets were captured by MEMS and between 10-30% of low-adherers were misclassified by MEMs as adherent (Supplemental). We found there would have to be >20% misclassification among low-adherers for sensitivities to reach the expected level of 75%.

Finally, using the observed sensitivity and specificity for the ≥700 fmol/punch and ≥1050 fmol/punch categories, we calculated positive and negative predictive values over a range of adherence levels (Supplemental). These values indicate how likely an individual's test result correctly predicts adherence and depend not only on sensitivity and specificity but also on the ratio of true positives to false positives, i.e. the prevalence of the outcome. If ≥50% of PrEP users are meeting the ≥4 doses/week target, for instance, there is a >80% probability that a TFV-DP result >700 fmol/punch is correctly identifying an adherent user; however, there is <70% probability that a result <700 fmol/punch is correctly identifying a low-adherent user (Supplemental).

Discussion

In this analysis, we assessed categories of TFV-DP derived from controlled PK studies in the U.S. to TFV-DP concentrations measured among PrEP-taking populations in Africa. We found that these categories have high specificity but relatively low sensitivities, compared to MEMS data from African participants using PrEP. The sensitivity for the ≥700 fmol/punch category for the ≥4 doses/week (62% [95% CI 52%, 72%]) was closest to the expected 75%. The sensitivity declined to 44% and 19% for ≥ 6 and 7 doses/week, indicating unexpectedly low TFV-DP with high MEMS openings. However, the high specificity for all the categories tested (generally >85%) means that most low-adherent users would have TFV-DP concentrations below the cut-offs.

In planning to use adherence monitoring, especially for counseling, it is important to consider the relative value of correctly identifying adherent versus low-adherent users, as sensitivity and specificity are trade-offs. While identifying low-adherent users (i.e., true negatives) may be useful to improve adherence, misidentifying participants who are achieving good adherence (i.e., false negatives) may have undesirable consequences including demotivation. Studies are needed about how best to frame adherence counseling messages using biomarker feedback

Our results are in line with the existing pharmacokinetic literature. Previous work has found higher TFV-DP levels in women compared to men with similar patterns of pill-taking [29]; we found a similar trend by gender, though it did not reach significance. In addition, TFV-DP is not expected to reach steady state within the first month of use [29]; in adjusted analyses, we found significantly lower concentrations from early use compared to later use samples.

The low sensitivities we observed were unexpected; several explanations are possible. TFV-DP concentrations have previously been reported to be approximately 14% lower in African-American participants compared to Caucasians, though this finding was not statistically significant in a small study from the US [29]. Potential mechanisms for this difference are not known, but may include differential expression or function of transporters or enzymes that influence TFV and/or TFV-DP cellular pharmacology (including esterases, P-glycoprotein (ABCB1), breast cancer resistance protein (BCRP), adenylate kinase I, pyruvate kinase, nucleoside diphosphate kinase, or factors influencing red blood cell turnover) [70–72]. It may be important to conduct additional controlled PK studies in an African population.

However, over-reporting of adherence by MEMS would also bias the results in the direction we observed. To address this, we conducted a sensitivity bias analysis and found that a large fraction (>20%) of low-adherent users would have to be misclassified in order to achieve the expected 75% sensitivity. Finally, while sensitivity and specificity are not dependent on prevalence, in this situation, one group having exactly 4 doses per week and another group having exactly 7 doses per week would both meet the dichotomized $\geq$4 doses/week target – but likely have different proportions $\geq$700 fmol/punch and thereby different sensitivities. However, this would not explain the very low sensitivity for the 7 doses/week target, where there should be no variation.

The major limitation of this analysis, as already discussed, is the use of MEMS data, which may be subject to misclassification. However, MEMS has been shown to be valid of measure of PrEP use and used as the standard in other studies comparing adherence measures [27,73,74]. Finally, we had small sample sizes for some categories that limited analyses.

These results provide important information regarding the sensitivity and specificity of TFV-DP categories in an African population. In addition, even in U.S. populations, the PK-derived cut-offs were designed to have only 75% sensitivity, which should be taken into account for studies assessing PrEP adherence. Determining categories of TFV-DP as an objective measure of adherence is important for clinical trials evaluating adherence and for counselors and clinicians using drug levels to provide feedback to patients. Indeed, patients have indicated that biomarker feedback is acceptable and even desired [75,76]. However, it is important to consider the desired sensitivity and specificity in different populations, and to design adherence messaging accordingly.

Acknowledgements
We thank the couples who participated in this study and the members of the Colorado Antiviral Pharmacology Laboratory.

Data available upon request.

We declare no conflicts of interest.

Author contributions: MP and JMB designed the research question. PA analyzed the samples; SA, EK, NM & EB collected data. MP analyzed the data and drafted the manuscript; all authors contributed to the manuscript.

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Data Management was provided by DF/Net Research, Inc. (Seattle, WA). PrEP medication was donated by Gilead Sciences.

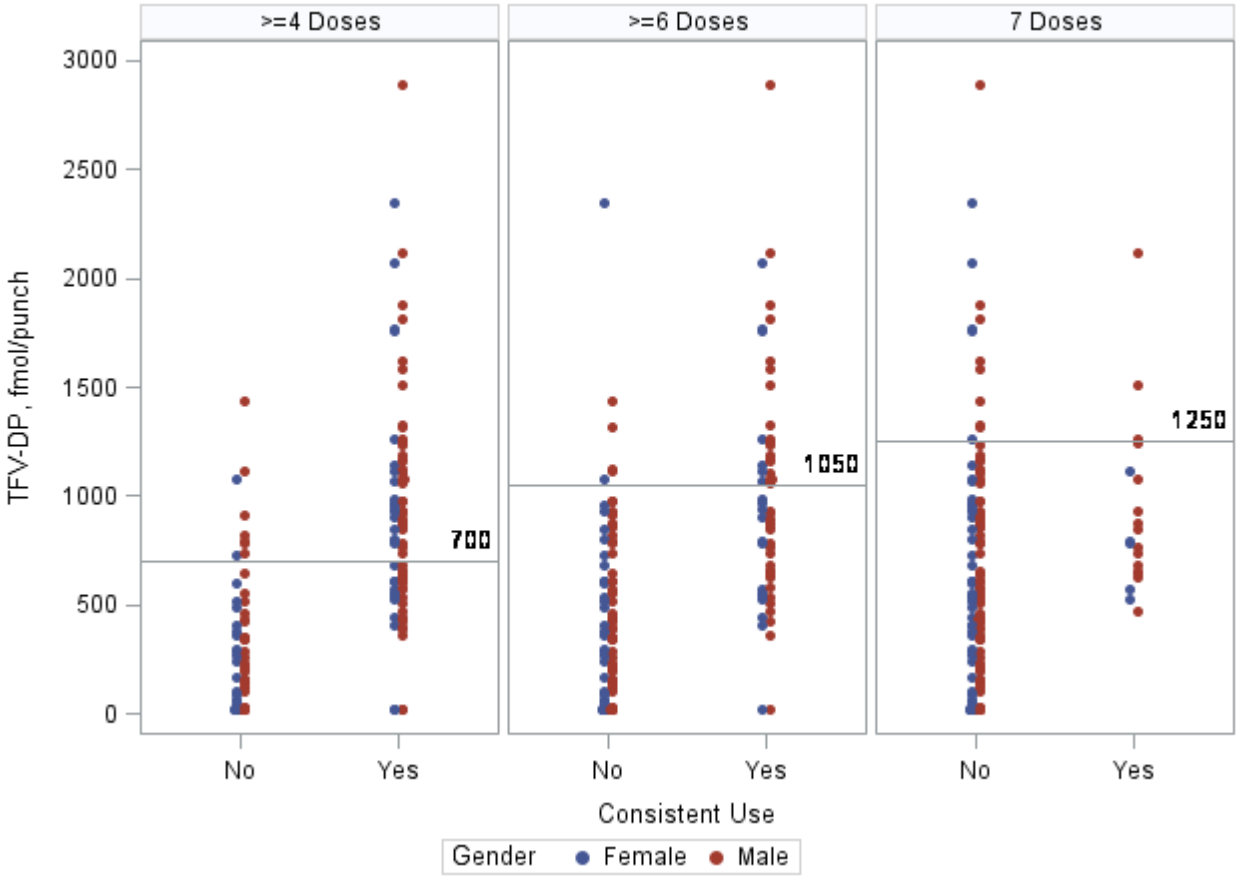
Table 4.1 TFV-DP Concentrations & Detection Among Consistent Users, by Gender and by Use

	Consistent ≥4 Weekly Doses			Consistent ≥6 Weekly Doses			Consistent 7 Weekly Doses		
	Women (n=30)	Men (n=57)	Total (n=87)	Women (n=20)	Men (n=42)	Total (n=62)	Women (n=5)	Men (n=16)	Total (n=21)
Mean TFV-DP fmol/punch (SD)	898 (530)	939 (502)	925 (509)	931 (507)	1024 (525)	994 (517)	759 (233)	981 (420)	928 (390)
Unquantifiable TFV-DP, % (n)	7% (2)	4% (2)	5% (4)	5% (1)	2.4% (1)	3% (2)	0% (0)	0% (0)	0% (0)
	Early Use (n=22)	**Late Use (n=65)**	**Total (n=87)**	**Early Use (n=18)**	**Late Use (n=44)**	**Total (n=62)**	**Early Use (n=6)**	**Late Use (n=15)**	**Total (n=21)**
Mean TFV-DP fmol/punch (SD)	942 (460)	920 (528)	925 (509)	1011 (447)	988 (548)	994 (517)	1097 (537)	860 (311)	928 (390)
Unquantifiable TFV-DP, % (n)	5% (1)	5% (3)	5% (4)	0% (0)	5% (2)	3% (2)	0% (0)	0% (0)	0% (0)

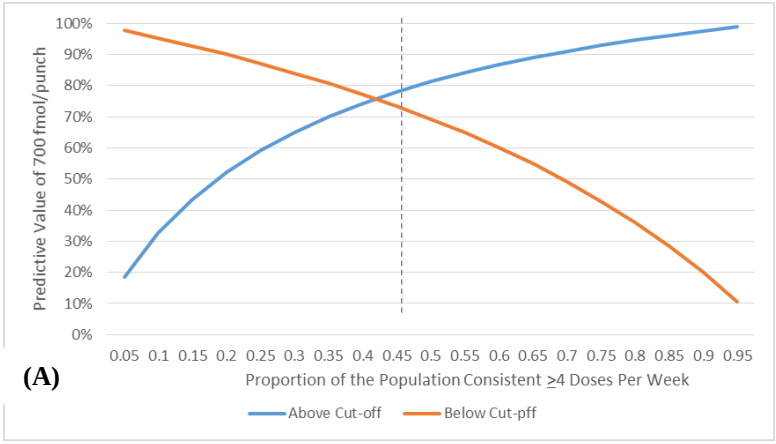
Table 4.2 Sensitivity & Specificity of TFV-DP Categories by Consistent Doses over Prior 4 Weeks

	≥700 fmol/punch (≥4 Doses per Week)		≥1050 fmol/punch (≥6 Doses per Week)		≥1250 fmol/punch (7 Doses per Week)	
	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
All Samples	62% (52%, 72%)	86% (77%, 94%)	44% (31%, 56%)	93% (88%, 98%)	19% (2%, 36%)	90% (85%, 95%)
Women	60% (42%, 78%)	92% (81%, 100%)	35% (14%, 56%)	94% (87%, 100%)	0% (na)	90% (82%, 98%)
Men	63% (51%, 76%)	82% (69%, 94%)	48% (33%, 63%)	92% (85%, 100%)	25% (4%, 46%)	90% (83%, 97%)
Early Use	73% (54%, 91%)	83% (62%, 100%)	33% (12%, 55%)	94% (82%, 100%)	17% (0%, 46%)	89% (78%, 100%)
Quantifiable TFV-DP	65% (55%, 75%)	82% (71%, 92%)	45% (32%, 58%)	92% (85%, 98%)	19% (2%, 36%)	88% (82%, 94%)

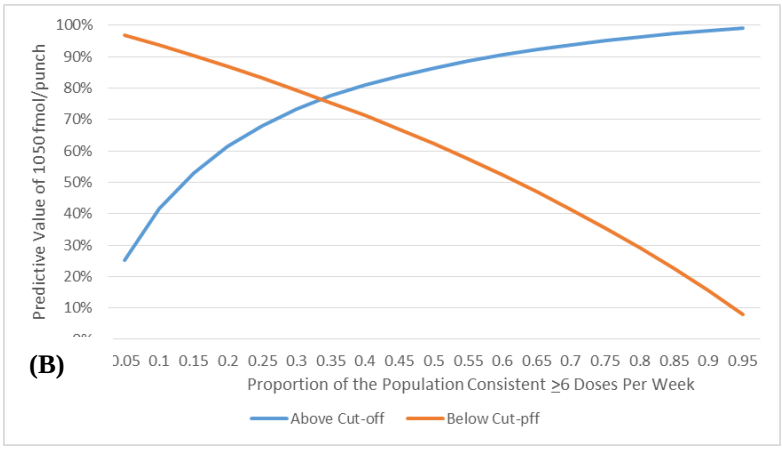
Supplemental Figure 4.1 TFV-DP Concentrations by Consistent Use Categories & Gender TFV-DP levels are presented using three adherence targets according to electronic adherence monitoring (≥4 doses, ≥6 doses, and 7 doses per week, over each of the prior 4 weeks); the reference lines indicate the established PK-derived categories based on 25th percentiles.



Supplemental Figure 4.2 Predictive Values by Adherence in the Population The probability that an individual with a TFV-DP result above the cut-off is actually adherent (blue lines) and the probability than an individual with a TFV-DP result below the cut-off is actually non-adherent (orange lines) are presented for two adherence targets (≥4 and ≥6 doses/week) and their associated PK-cutoffs. These probabilities vary according to the proportion of the overall population who are adherent, shown on the x-axis. As an example, the dotted line indicates that if 50% of the population is adherent at ≥4/week, a positive test means an 80% chance that the individual is adherent, while a negative test means a 70% that the individual is non-adherent.



(A)



(B)

Supplemental Table 4.3 Characteristics of Samples

	Women (n=55)	Men (n=95)	Total (n=150)
Mean Age (SD) at Baseline	29.5 (6.0)	32.9 (10.5)	31.7 (9.2)
Mean BMI (SD) at Baseline	23.9 (4.0)	22 (2.4)	23.7 (3.2)
Median creatinine clearance (IQR)	100 (87, 114)	122 (105, 145)	110.5 (96,136)
Consistent ≥4 Doses per week by electronic monitoring*	55% (30)	60% (57)	58% (87)
Consistent ≥6 Doses per week by electronic monitoring*	36% (20)	44% (42)	41% (62)
Consistent 7 Doses per week by electronic monitoring*	9% (5)	17% (16)	14% (21)
Average Doses per Week*(SD)	4.1 (2.8)	4.6 (2.6)	4.5 (2.6)
Average Doses over Prior Week* (SD)	3.6 (2.8)	4.5 (2.7)	4.1 (2.7)

* over prior 4 weeks

Supplemental Table 4.4 Sensitivity & Specificity of TFV-DP Categories by Average Doses over Prior 4 Weeks

	≥700 fmol/punch (≥4 Doses per Week)		≥1050 fmol/punch (≥6 Doses per Week)	
	Sensitivity (95% CI)	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
All Samples	60% (50%, 70%)	89% (81%, 97%)	38% (27%, 50%)	94% (88%, 99%)
Women	56% (39%, 73%)	91% (80%, 100%)	27% (10%, 44%)	93% (84%, 100%)
Men	62% (50%, 74%)	88% (76%, 99%)	45% (30%, 59%)	94% (87%, 100%)

Supplemental Table 4.5 Bias Sensitivity Analysis of Misclassification in Electronic Adherence Data

Electronic Adherence Data*	Sensitivity of 700 fmol/punch for ≥4 doses per week	Sensitivity of 1050 fmol/punch for ≥6 doses per week
Observed	0.62	0.44
10% Misclassified	0.66	0.50
20% Misclassified	0.73	0.64
30% Misclassified	0.84	1.00

*Electronic adherence data with a sensitivity of 98% and 90% specificity (10% misclassified as adherent); 80% specificity (20% misclassified as adherent); and 70% specificity (30% misclassified as adherent).

Chapter 5. Tenofovir and tenofovir-diphosphate concentrations during pregnancy among HIV-uninfected women using oral pre-exposure prophylaxis

Published citation: Pyra, M., Anderson P.L., Hendrix, C.W., Haberer, Heffron, R., Mugwanya, K., J.E., Thomas, K.K., Celum, C., Donnell, D., Marzinke, M.A., Bukusi, E.A., Mugo, N.R., Asiimwe, S., Katabira, E., Baeten, J.M., Partners Demonstration Project Team. Tenofovir and tenofovir-diphosphate concentrations during pregnancy among HIV-uninfected women using oral pre-exposure prophylaxis. AIDS. Published Online First: June 12, 2018.

Tenofovir and tenofovir-diphosphate concentrations during pregnancy among HIV-uninfected women using oral pre-exposure prophylaxis

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Funding:
The Partners Demonstration Project was funded by the National Institute of Mental Health of the US National Institutes of Health (R01 MH095507), the Bill & Melinda Gates Foundation (OPP1056051), and the US Agency for International Development (AID-OAA-A-12-00023). This analysis was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health (TL1 TR002318). Research reported in this publication was supported by the UW/Fred Hutch Center for AIDS Research, funded by NIAID, NCI, NIMH, NIDA, NICHD, NHLBI, NIA, NIGMS, NIDDK of the National Institutes of Health under award number P30 A1027757 and the HIV Prevention Trials Network (UM1 AI068613). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID, NIH, or the United States Government.

Abstract:
Objectives: Pregnancy is a time of increased HIV acquisition risk and pregnancy reduces concentrations of antiretrovirals used for treatment. We assessed whether pregnancy lowers concentrations of tenofovir (TFV) and tenofovir-diphosphate (TFV-DP) among HIV-uninfected women using oral pre-exposure prophylaxis (PrEP).

Methods: We analyzed data from an open-label PrEP study, comparing concentrations of TFV in plasma and TFV-DP in dried blood spots (DBS) among 37 pregnant women and 97 non-pregnant women. Analyses controlled for adherence from daily electronic monitoring.

Results: The average plasma concentration of TFV among pregnant women was 34.7 ng/mL with 22.2 average recorded doses over the prior month versus 86.5 ng/mL with 23.1 doses among non-pregnant women. After controlling for adherence, TFV concentrations were 58% lower among pregnant women, a statistically significant difference of -50.4 ng/mL (95%CI -68.3 to -32.5). The average TFV-DP concentration was 450.3 fmol/punch among pregnant women and 636.7 fmol/punch among non-pregnant women. This difference was not statistically significant after adjusting for adherence; however, among those with quantifiable TFV-DP, concentrations were 27% lower during pregnancy (-202 fmol/punch [95%CI -384 to -19]). Among participants with samples before and during pregnancy, there were significant decreases during pregnancy, controlling for adherence: -28.1 ng/mL TFV (95%CI -52.3 to -4.0) and -289.2 fmol/punch TFV-DP (95%CI -439.0 to -139.3).

Conclusions: Consistent with studies among HIV-infected women on ART, we found TFV and TFV-DP concentrations were lower during pregnancy. There is no established TFV concentration threshold to achieve HIV prevention. Additional pharmacokinetic studies and studies of PrEP efficacy in pregnancy are needed.

Introduction

Pregnancy is a period of increased risk for HIV acquisition [4,77–80] which may last for a substantial portion of women's reproductive years in settings with a high burden of HIV. Oral tenofovir-based PrEP is safe for pregnant women and their infants, and breastfed infants are exposed to very low tenofovir levels in breastmilk [81–83], leading the World Health Organization to provide guidance recommending that PrEP can be safely used during pregnancy and breastfeeding for women at high HIV risk [7]. As PrEP becomes widely used in heterosexual populations, an increasing number of women who are pregnant or trying to become pregnant may use PrEP for HIV prevention [84,85].

However, data on the effectiveness of PrEP for HIV prevention during pregnancy are relatively limited. In the clinical trials of PrEP that established its safety and efficacy, women stopped study drug once pregnancy was detected. Pregnancy can affect drug pharmacokinetics in several ways [32]. Most relevant to oral PrEP, pregnancy increases blood volume, leading to a higher volume of distribution, and increases kidney flow rate, leading to higher clearance, with the greatest changes in the 3rd trimester [33,86]. Increases in volume of distribution can result in lower peak drug concentrations and longer half-lives, while increases in clearance can result in lower steady-state drug concentrations and shorter half-lives [87].

In this analysis, we used stored plasma and dried blood spots (DBS) from an open-label study that included women using PrEP during pregnancy to examine potential differences in blood concentrations of tenofovir when compared to non-pregnant women, as well as to compare pre-pregnant and pregnant periods within women. As pregnancy may result in women changing their use of PrEP [88], we controlled for adherence as measured by electronic monitoring data.

Methods

Women in this analysis were members of mutually-disclosed HIV serodiscordant couples who participated in an open-label PrEP demonstration project in Uganda and Kenya (the Partners Demonstration Project), as previously described [12]. Briefly, participants were seen quarterly for up to 24 months. Initially, the study protocol required that women stop using PrEP once pregnant. However, during the study the protocol was updated at each site and women could continue PrEP (coformulated emtricitabine/tenofovir disoproxil fumarate 200mg/300 mg) if they chose to during pregnancy; if they continued PrEP, follow-up became monthly during pregnancy. PrEP adherence was assessed by electronic monitoring (MEMS caps, WestRock, Switzerland), which recorded daily pill bottle openings; for this analysis, openings were limited to one per day. Blood samples were also taken at quarterly study visits and at monthly visits when pregnant; samples were stored as plasma at -80C and, partway through the study, as dried blood spots (DBS) stored at -20C. Age, BMI, and creatinine clearance were assessed at baseline. All participants provided written informed consent in their preferred language.

For this analysis, non-pregnant plasma samples came from a selection of HIV-uninfected women (who never became pregnant or were sampled before their first pregnancy during follow-up): either a random sample tested serially at each study visit or women enrolled in a pharmacokinetic substudy tested at a single study visit. Plasma samples were tested from all pregnant HIV-uninfected women who took part

in monthly follow-up, as well as any women in the substudy who became pregnant. In addition, we selected one DBS sample from each trimester of pregnancy, as well as a pre-pregnancy sample when available; one non-pregnant sample was also chosen from a random sample of women. Throughout, samples from women who had <1 recorded PrEP dose in the month prior were excluded as non-users. When multiple samples were available for the same time period (non-pregnant or trimester), we selected the one with the most recorded doses.

Plasma was tested for tenofovir (TFV) and DBS were tested for tenofovir-diphosphate (TFV-DP), the active metabolite, using ultra-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) [12,31]. Values below the lower limit of 0.31 ng/mL for plasma TFV and 31.25 fmol/punch for TFV-DP were set to half the lower limit (0.15 ng/mL and 15.6 fmol/punch respectively). With a half-life of 14 hours in plasma, TFV is a marker of recent use [28], while TFV-DP has a half-life of 17 days in red blood cells and DBS, and provides a marker of cumulative adherence over the prior month [29].

We compared the mean concentrations of each analyte between pregnant and non-pregnant women using linear models with concentration as the outcome and pregnancy status as the primary predictor; women were not included as both pregnant and non-pregnant in these models (only pregnant samples were included if both were available). As pregnant women could contribute multiple samples while pregnant, generalized estimating equations with an unstructured correlation matrix were used to account for repeated observations. The analysis was then repeated comparing each trimester to non-pregnant women. We next compared the mean concentration of each analyte from pre-pregnancy to pregnancy among the subset of women who had samples from both time periods; this comparison of pregnancy within woman is conditional on a random intercept for each woman and mixed effects models were used. We conducted two sensitivity analyses: restricting to those samples with tenofovir above the lower limit of quantitation and restricting to samples with 100% adherence over the prior month, according to MEMS. For all analyses (except the 100% adherence sensitivity analysis), models were adjusted for adherence (number of days with a MEMS opening over the prior month (for TFV-DP), or prior 2 days (for TFV)). Baseline age, BMI, creatinine clearance, and time in study were tested and only included if they changed the estimated pregnancy effect; we did not adjust for BMI during pregnancy, as changes in weight may be part of the causal pathway. All analyses were conducted in SAS 9.4.

Results

Participant characteristics. Overall, thirty-seven (37) women contributed either plasma or DBS concentrations while pregnant and 97 women contributed only non-pregnant samples. All women were 48 years or younger, with a mean age of 29 at baseline. Overall, 97% were married to their study partner and had an average of 2.4 children (Table 1).

Plasma TFV concentrations. There were 389 plasma samples (163 from pregnant and 226 from non-pregnant visits) from 116 women (33 pregnant and 83 non-pregnant) available for the comparison between pregnant and non-pregnant women. The average TFV concentration among pregnant women was 34.7 ng/mL, with an average of 22.2 recorded PrEP doses over the prior month; the average TFV

concentration among non-pregnant women was 86.5 ng/mL, with an average 23.1 recorded PrEP doses (Table 2). After adjusting for adherence, average TFV concentrations were significantly lower among pregnant women, -50.4 ng/mL (95%CI -68.3, -32.5) (Table 3); this finding translates into a mean concentration of TFV in pregnant women 58% lower compared to non-pregnant women. TFV concentrations were also significantly lower in each trimester ($p<0.001$) relative to non-pregnant women: -40.0 ng/mL for the 1st trimester; -49.4 ng/mL for the 2nd trimester; and -59.2 ng/mL for the 3rd trimester.

In a sensitivity analysis of the 322 samples from 103 women with quantifiable TFV, the results were similar and remained statistically significant, with an adjusted difference of -50.8 ng/mL for pregnant compared to non-pregnant women. Restricting to the 105 samples from 44 women with 100% MEMS adherence over the prior month, the results remained consistent: -51.8 ng/mL for pregnancy overall, -53.9 ng/mL for the 1st trimester, -48.8 ng/mL for the 2nd trimester, and -52.3 ng/mL for the 3rd trimester compared to non-pregnant women (Figure 1).

DBS TFV-DP concentrations. In the corresponding analysis using DBS, there were 70 samples contributed by 31 women while pregnant and 32 samples contributed by 32 non-pregnant women. The average TFV-DP concentration was 450.3 fmol/punch among pregnant women and 636.7 fmol/punch among non-pregnant women; the average number of doses recorded by MEMS was 21.4 for pregnant women and 20.7 for non-pregnant women (Table 2). In the fully adjusted model (including adherence, age, and BMI), there were no statistically significant differences between pregnant and non-pregnant women, although the point estimate was lower for pregnant periods: -136.6 fmol/punch (95% CI -318, 44.8); in addition, there was a significant difference between the 2nd trimester and non-pregnant periods in adjusted analysis (Table 3). In the sensitivity analysis restricted to those with quantifiable TFV-DP, the differences between pregnant (-201.9 fmol/punch), 2nd trimester (-278.4 fmol/punch), and 3rd trimester (-259.6 fmol/punch) samples were all statistically significantly lower than non-pregnant samples. There were only 30 samples with 100% adherence over the prior month; differences were larger compared to those with quantifiable TFV-DP, but only remained significant in the 3rd trimester (Table 3, Figure 1).

Pre- and during pregnancy comparisons. When comparing levels of TFV among the 9 women with plasma samples before and during pregnancy (Table 4), there was a statistically significant decrease during pregnancy of -28.1 ng/mL after controlling for adherence (Supplemental Table 1); by trimester, only the 3rd trimester was statistically different from pre-pregnancy. Among 12 women with DBS samples prior to and during pregnancy (Table 4), there was also a statistically significant decrease during pregnancy, by -289.2 fmol/punch overall and with significant differences in each trimester as well (Supplemental Table 1). These results indicated that concentrations of TFV were 45% lower and concentrations of TFV-DP were 49% lower during pregnancy compared to pre-pregnancy, controlling for adherence. In both analyses, results were similar when restricted to women with any quantifiable tenofovir; samples sizes were too small to conduct a sensitivity analysis restricted to 100% adherence over the prior month.

Discussion

Among HIV-uninfected women using oral PrEP, we found that plasma TFV and intracellular TFV-DP in DBS were 45%-58% lower during pregnancy compared to non-pregnant periods after adjusting for adherence as measured by MEMS openings. Differences in concentrations were generally larger in the second and third trimester than in early pregnancy. These results suggest that pregnancy alters the pharmacokinetics of oral PrEP among HIV-uninfected women.

Prior studies of tenofovir used as antiretroviral treatment in pregnant women living with HIV have shown similar results to those we found for HIV-uninfected women using PrEP, though most have focused on US and European populations. In particular, three studies reported higher TFV clearance during pregnancy (compared to either non-pregnant or post-partum periods) [89–91]. In addition, two of the studies found lower TFV concentrations in the 3rd trimester compared to post-partum (23% lower C_{24} and 20% lower AUC) [90,91]. In addition, two studies of TFV-based regimens in HIV-infected pregnant women included viral suppression endpoints and found 83% and 94% of women on TFV were virally suppressed in the 3rd trimester, compared to 82% and 85%, respectively in the post-partum period [90,91]. One study had a target AUC based on the 10th percentile of non-pregnant levels and found that 73% of 3rd trimester samples met the cut-off [90]; the other study reported no cases of vertical transmission among the 34 women studied, indicating sufficient suppression [91]. Some studies have used higher doses (600mg TDF/400mg FTC) at delivery for prevention of mother to child transmission and found it was possible to achieve drug exposures similar to non-pregnant populations [92–95]. Altogether, these findings of similar viral suppression rates in spite of modestly lower tenofovir levels during pregnancy has led to recommendations in the US that TDF/FTC dosing in HIV infected women does not need to be altered during pregnancy [96].

For PrEP, the clinical significance of the differences in blood concentrations of tenofovir that we observed is not clear. Clinical trials of PrEP efficacy stopped women from taking PrEP once pregnancy was detected and the number of pregnant users in reported implementation trials to date is too small to conduct efficacy analyses. There is no established protective concentration of tenofovir for women and protective levels needed for PrEP may differ from suppressive levels when used as ART [28]. Furthermore, it is unclear how blood concentrations of tenofovir correlate with tissue concentrations at the site of infection or in regional lymph nodes, where the protective action presumably occurs [97]. Prior studies, including a directly-observed dosing study, have used 35 ng/mL (or sometimes 40 ng/mL) of serum or plasma TFV as the lower cut-off to indicate consistent, daily PrEP use [97,16]. In our sample, most participants with 100% adherence by MEMS were above this cut-off, regardless of trimester, although the proportion falling below this threshold increases in pregnancy (Figure 1); the appropriate threshold for TFV-DP is not yet clear.

Pharmacokinetic modeling (in men and women) has suggested that 230 fmol/punch of TFV-DP is equivalent to one dose of PrEP per week [68] and our results suggested that pregnancy has an effect on TFV-DP concentrations of a similar magnitude; in other words, a pregnant woman taking seven doses of PrEP per week would have TFV-DP concentrations similar to a non-pregnant woman taking six doses per

week. These results, as well as other work indicating women may need ≥6 doses per week to achieve protection [22], suggest that adherence could be even more crucial for women during pregnancy.

Our study has some limitations. This was not a pharmacokinetic study and the exact timing of doses is unknown, making direct comparisons with previous ART studies difficult. MEMS data can be subject to both over and underreporting and there may be residual adherence effects that were not controlled for. However, MEMS data have been shown to correlate well with plasma TFV in prior work in this population [12,27]. The concentrations among women are lower than expected from direct observation studies. For instance, the mean TFV-DP in non-pregnant women was 636.7 fmol/punch for 20.7 doses/month, or approximately 67% adherence to daily dosing. This value was lower than the mean steady-state TFV-DP observed for 67% adherence in a directly observed dosing study conducted in the U.S., 997 fmol/punch [29], suggesting controlled studies are needed to define TFV-DP in African participants.

Strengths of this study include comparing the effect of pregnancy on plasma tenofovir and intracellular levels of tenofovir diphosphate in DBS between pregnant and non-pregnant women, as well as within the same woman over the course of her pregnancy. We accounted for changes in PrEP adherence during pregnancy not only by adjusting for adherence, using electronic monitoring, but with sensitivity analyses of those with complete adherence over the prior month and of those with biomarker evidence of PrEP use.

In conclusion, concentrations of tenofovir and its active metabolite tenofovir-diphosphate appear to be lower during pregnancy in the setting of PrEP; however, most women with high adherence still achieved 35 ng/mL of plasma TFV during pregnancy, which correlated with daily dosing in prior observational studies in this population. Epidemiologic studies within ongoing PrEP implementation projects are needed to determine if the effectiveness of PrEP is altered in pregnancy. Additional pharmacokinetic studies[98] are also warranted to understand the protective threshold of tenofovir among women. This work also highlights the importance of including pregnant women in research related to the development of novel prevention products [99].

Acknowledgements
We thank the couples who participated in this study and Director of the Kenya Medical Research Institute (KEMRI).

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MP, JB, RH, KM, & KT contributed to the analysis plan and MP conducted the analyses. PA, CW, and MM analyzed the specimens. EB, NM, SA, & EK coordinated data and specimen collection. MP & JB drafted and all authors contributed to the manuscript.

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Data Management was provided by DF/Net Research, Inc. (Seattle, WA). PrEP medication was donated by Gilead Sciences.

Table 5.1 Participant Characteristics at Enrollment

	Non-Pregnant	Pregnant
N women	97	37
Mean age (SD)	30.6 (7.4)	25.1 (4.8)
<25 years old	26.8% (26)	46.0% (17)
Married to partner	99.0% (96)	91.9% (34)
Mean parity (SD)	2.8 (2.0)	1.4 (1.0)
Mean BMI (SD)	24.5 (4.3)	24.6 (4.7)
Mean creatinine clearance (SD)	101.8 (18.4)	111.7 (27.5)
% with Plasma Sample	85.6% (83)	89.2% (33)
Median # Plasma Samples (IQR)	1 (1,4)	4 (3,7)
% with DBS Sample	33.0% (32)	86.1% (31)
Median # DBS Samples (IQR)	1 (1,1)	2 (2,3)

Table 5.2 PrEP Use & Analyte Concentrations among Pregnant vs Non-Pregnant Women

TFV ng/mL					
	Non-Pregnant (n=83 women, 226 samples)	**Pregnant (n=33 women, 163 samples)**	**1st Trimester (n=23 women, 42 samples)**	**2nd Trimester (n=23 women, 59 samples)**	**3rd Trimester (n= 23 women, 62 samples)**
Mean doses (SD)*	23.1 (9.4)	22.2 (10.1)	23.6 (9.4)	22.0 (10.2)	21.5 (10.4)
Recent dose**	93.4% (211)	89.0% (145)	90.5% (38)	88.1% (52)	88.7% (55)
Mean TFV ng/mL (SD)	86.5 (90.6)	34.7 (44.5)	45.5 (65.8)	36.6 (36.8)	25.5 (30.0)
% TFV below limit of quantitation (n)	11.5% (26)	25.0% (41)	17.7% (7)	23.7% (14)	32.3% (20)
TFV-DP fmol/punch					
	Non-Pregnant (n=32 women, 32 samples)	**Pregnant (n=31 women, 70 samples)**	**1st Trimester (n= 25 women, 25 samples)**	**2nd Trimester (n=24 women, 24 samples)**	**3rd Trimester (n=21 women, 21 samples)**
Mean doses (SD)*	20.7 (10.6)	21.4 (10.5)	22.5 (9.4)	19.3 (11.7)	22.4 (10.4)
Mean TFV-DP fmol/punch (SD)	636.7 (523.0)	450.3 (388.1)	561.6 (423.1)	368.1 (340.7)	411.9 (382.2)
% TFV-DP below limit of quantitation (n)	15.6% (5)	20.0% (14)	12.0% (3)	25.0% (6)	23.8% (5)

*Over past month according to MEMS caps **Over past 2 days according to MEMS cap

Table 5.3 Differences in Tenofovir Concentrations between Pregnant and Non-Pregnant Women

TFV ng/mL				
	Pregnant	**1st Trimester**	**2nd Trimester**	**3rd Trimester**
Unadjusted Difference (95%CI)	-52.3 (-70.4, -34.2) (p<0.001)	-41.2 (-68.8, -13.7) (p=0.004)	-51.4 (-72.4, -30.3) (p<0.001)	-61.5 (-80.6, -42.3) (p<0.001)
Adjusted Difference * (95%CI)	-50.4 (-68.3, -32.5) (p<0.001)	-40.0 (-66.8, -13.3) (p=0.004)	-49.4 (-69.5, -29.2) (p<0.001)	-59.2 (-77.7, -40.7) (p<0.001)
Adjusted Difference * (95%CI), Among Detectable TFV	-50.8 (-69.7, -31.9) (p<0.001)	-41.8 (-71.2, -12.5) (p=0.007)	-49.0 (-70.5, -27.5) (p<0.001)	-60.6 (-80.3, -40.9) (p<0.001)
Unadjusted Difference (95%CI), Among 100% Adherent	-51.8 (-85.3, -18.4) (p=0.003)	-53.9 (-91.7, -16.2) (p=0.009)	-48.8 (-88.7, -9.0) (p=0.02)	-52.3 (-90.5, -14.2) (p=0.01)
TFV-DP fmol/punch				
	Pregnant	**1st Trimester**	**2nd Trimester**	**3rd Trimester**
Unadjusted Difference (95%CI)	-196.1 (-420.2, 28.0) (p=0.08)	-83.4 (-321.6, 154.8) (p=0.48)	-262.8 (-486.1, -39.5) (p=0.02)	-264.0 (-498.0, -29.9) (p=0.02)
Adjusted Difference ** (95%CI)	-136.6 (-318.0, 44.8) (p=0.13)	-52.0 (-249.6, 145.7) (p=0.59)	-187.1 (-367.9, -6.3) (p=0.04)	-178.7 (-373.2, 15.7) (p=0.07)
Adjusted Difference ** (95%CI), Among Detectable TFV-DP	-201.9 (-384.4, -19.4) (p=0.03)	-104.3 (-296.8, 88.3) (p=0.27)	-278.4 (-464.0, -92.8) (p=0.004)	-259.6 (-463.4, -55.8) (p=0.01)
Adjusted Difference * (95%CI), Among 100% Adherent**	-383.3 (-807.7, 41.4) (p=0.07)	-138.8 (-638.9, 361.3) (p=0.52)	-490.1 (-982.2, 2.0) (p=0.05)	-503.8 (-999.7, -7.9) (p=0.04)

GEE models with unstructured correlation matrix and non-pregnant as reference.

*Adjusted for MEMS cap over prior 2 days.

**Adjusted for MEMS cap over prior month, age and BMI at baseline

***Adjusted for age and BMI at baseline

Table 5.4 PrEP Use & Analyte Concentrations Before and During Pregnancy

	TFV ng/mL				
	Pre-Pregnancy (n=9 women, 7 samples)	**Pregnancy (n=49 samples)**	**1st Trimester (n=7 women, 15 samples)**	**2nd Trimester (n=7 women, 20 samples)**	**3rd Trimester (n=5 women, 14 samples)**
Mean doses (SD)*	15.7 (11.5)	23.3 (10.3)	23.9 (9.8)	22.5 (11.3)	23.8 (10.1)
Mean TFV ng/mL (SD)	63.0 (65.0)	43.2 (50.8)	53.5 (55.3)	42.4 (52.1)	33.4 (45.3)
% TFV below limit of quantitation (n)	23.5% (4)	30.6% (4)	13.3% (2)	40% (8)	35.7 % (5)
Recent dose**	82.4% (14)	87.8% (43)	93.3% (14)	85% (17)	85.7% (12)
	TFV-DP fmol/punch				
	Pre-Pregnancy (n=12 women, 12 samples)	**Pregnancy (n=12 women, 27 samples)**	**1st Trimester (n=11 women, 11 samples)**	**2nd Trimester (n=10 women, 10 samples)**	**3rd Trimester (n=6 women, 6 samples)**
Mean doses (SD)*	18.8 (12.1)	15.6 (10.1)	17.3 (10.8)	13.3 (10.0)	16.2 (10.3)
Mean TFV-DP fmol/punch (SD)	591.7 (464.9)	232.1 (233.9)	257.7(270.5)	231.2 (232.1)	186.6 (193.3)
% TFV-DP below limit of quantitation (n)	8.3% (1)	25.9% (7)	18.2% (2)	30.0% (3)	33.3% (2)

*Over past month according to MEMS caps **Over past 2 days according to MEMS caps

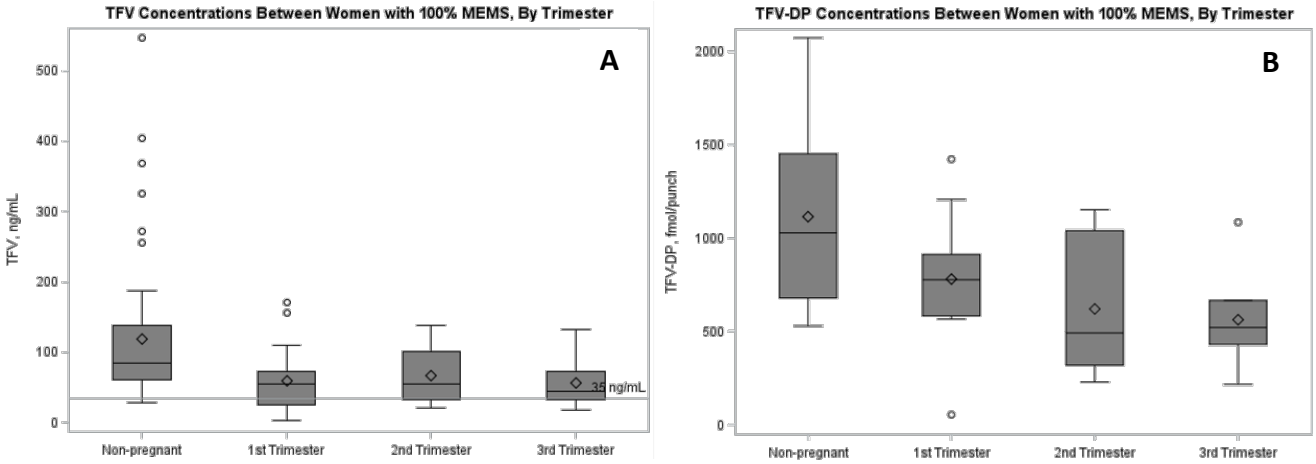

Figure 5.1 Analyte Concentrations Among Women With 100% Adherence by MEMS. Samples with 100% adherence over the prior month, by MEMS cap. Lines represent 25th, 50th and 75th percentiles and diamonds indicate means. **A.** TFV concentrations among the 55 non-pregnant, 18 1st trimester, 17 2nd trimester, and 15 3rd trimester samples. The reference line of 35 ng/mL represents the 10th percentile of steady-state TFV concentrations [97]. **B.** TFV-DP concentrations among 8 non-pregnant, 9 1st trimester, 6 2nd trimester, and 7 3rd trimester samples.

Supplemental Table 5.5 Changes in Tenofovir Concentrations Before and During Pregnancy

TFV ng/mL				
	Pregnant	**1st Trimester**	**2nd Trimester**	**3rd Trimester**
Unadjusted Difference (95%CI)	-27.8 (-52.9, -2.7) (p=0.03)	-22.7 (-52.9, 7.5) (p=0.13)	-27.5 (-57.8, 2.8) (p=0.07)	-36.4 (-69.5, -3.2) (p=0.03)
Adjusted Difference * (95%CI)	-28.1 (-52.3, -4.0) (p=0.02)	-24.5 (-53.7, 4.7) (p=0.09)	-26.8 (-56.0, 2.4) (p=0.07)	-35.8 (-67.7, -3.8) (p=0.02)
Adjusted Difference * (95%CI) Among Detectable TFV	-33.0 (-64.5, -1.6) (p=0.04)	-30.4 (-67.0, 6.3) (p=0.10)	-27.8 (-67.2, 11.7) (p=0.16)	-44.8 (-87.5, -2.1) (p=0.04)
TFV-DP fmol/punch				
	Pregnant	**1st Trimester**	**2nd Trimester**	**3rd Trimester**
Unadjusted Difference (95%CI)	-357.1 (-547.4, 166.7) (p<0.001)	-336.7 (-575.0, -98.3) (p=0.007)	-361.0 (-606.8, -115.2) (p=0.005)	-391.4 (-685.0, -97.9) (p=0.01)
Adjusted Difference ** (95%CI)	-289.2 (-439, -139.3) (p<0.001)	-302.4 (-487.0, -117.9) (p=0.002)	-256.0 (-451.3, -60.8) (p=0.01)	-319.8 (-549.1, -90.5) (p=0.008)
Adjusted Difference (95%CI) Among Detectable TFV-DP**	-315.4 (-476.3, -154.6) (p<0.001)	-332.7 (-535.4, -129.9) (p=0.003)	-302.4 (-525.8, -79.1) (p=0.01)	-298.8 (-575.2, -22.5) (p=0.03)

Mixed effects models with random intercept and pre-pregnant as reference.

*Adjusted for MEMS cap over prior 2 days. **Adjusted for MEMS cap over prior month

Chapter 6. Discussion

Aim 1 Summary & Implications

Our work in this dissertation focused on addressing questions that would support PrEP implementation among women at risk for HIV. The first aim was to understand how women use PrEP in pill form in an open-label setting. We found that women in serodiscordant couples were able to use PrEP effectively. As the results from Chapter 2 show, most women were able to take PrEP for their entire risk period and while on PrEP, most achieved 6 or more doses per week, reaching the level of adherence needed to provide protection. However, we also found evidence, seen in other studies as well [13,41,42], that younger women had poorer adherence, in terms of lower persistence and lower execution. Even with imperfect adherence, PrEP demonstrated a strong protective effect, 93% overall and 91% among young women; importantly, there were no observed seroconversions among women taking ≥6 doses per week.

The results from Chapter 3 further examined PrEP use among this cohort to understand the relationship between adherence and risk patterns over time. While we had previously seen that adherence was high overall, using group-based trajectory models we were able to identify four unique adherence trajectories. In addition, we identified two risk trajectories. While no baseline covariates were associated with any of these trajectories, we did find that membership in the steady risk trajectory was associated with high steady adherence; conversely, membership in the declining risk trajectory was associated with early and late declining adherence. This suggests that women align their PrEP use with HIV risk behaviors to a significant extent, benefiting from prevention-effective adherence.

Aim 2 Summary & Implications

The second aim focused on how to assess PrEP adherence, specifically with the biomarker TFV-DP. In chapter 4, we found that the sensitivity of TFV-DP in an East African population (ranging from 19% to 62%) was lower than the expected 75%, using thresholds developed in a U.S. population; in contrast, specificities were 86% or higher. Furthermore, there was no difference in sensitivity or specificity by gender. These results call for additional work to establish thresholds in an African population, particularly as some programs are using biomarkers in adherence counseling. We also highlighted the role of the overall adherence rate in a specific population when determining appropriate thresholds; in a highly adherent population, even a threshold with a low sensitivity will have a high positive predictive value. However, counseling programs should carefully consider the balance between sensitivity and specificity. While accurately identifying true non-users may be useful to refer them for additional adherence counseling, misidentifying true users (i.e., labeling adherent users as non-adherent) may discourage individuals from an effective prevention strategy.

Chapter 4 also relates to questions of how adherence monitoring should be integrated into PrEP delivery and implementation. There is evidence that some PrEP users would welcome adherence feedback from biomarkers [75,76]. Use of biomarkers and electronic monitoring will continue to play a role in research studies, where exposure ascertainment is important for interpreting results. However, few implementation programs will have the resources for regular individual adherence testing. One model, out of a health system in the U.S., uses a stratified approach, relying initially on electronic medical records and then using more intensive adherence monitoring and telehealth for individuals struggling

with adherence [100,101]. Programs that do not have access to electronic medical records may be able to use pharmacy refill data to screen for adherence.

Aim 3 Summary & Implications

Finally, in Chapter 5, we found both TFV and TFV-DP concentrations were lower during pregnancy, after adjusting for adherence. This result was consistent when comparing pregnant and non-pregnant women, as well as within the same women before and during her pregnancy. The results, especially in light of recent work indicating that there are increased biological risks for HIV acquisition during pregnancy [4], suggest that we need enhanced follow-up of demonstration and implementation projects providing PrEP to pregnant women; the current number of observations are too small to determine whether the efficacy of PrEP is altered during pregnancy. Such observational studies will face challenges as adherence may also change during pregnancy, with limited adherence assessments available in implementation settings. Therefore pharmacokinetic studies can provide insights in determining the protective level of PrEP for women, as well as clarifying the role of emtricitabine (or the related anti-retroviral lamivudine) in protection [22,102,103], which was not included in this analysis. Given that PrEP efficacy has remained strong in sub-analyses of individuals at increased risk of HIV acquisition due to other factors, including co-occuring STIs or partners with high viral load [6], it is arguably unlikely that HIV risk due to pregnancy alters the protective threshold of PrEP. Additional epidemiologic and pharmacokinetic work aligned together will be important to determine whether dosing changes are necessary during pregnancy to achieve the protective level of PrEP and to prevent HIV infections; even if dosing changes are not needed, adherence may be even more critical during pregnancy to maintain protection. This work is also relevant to the growing concerns about how to appropriately include pregnant women in clinical research, including future formulations of PrEP, so that perinatal safety and effectiveness data are available when implementation programs begin [99,104,105].

Next Steps for Implementation

Better assessment of HIV risk for women is needed for PrEP implementation. Understanding risk perception, which may influence an individual's motivation, and risk behavior, which relates to actual HIV risk, are important; current measures of risk perception, especially in the setting of PrEP use, are inadequate, failing to disentangle underlying risk from risk in the presence of PrEP [60–62]. We were able to define risk relatively clearly in these analyses, as the male partner was known to be HIV-infected and not yet virally suppressed; further work is needed in other populations where risk may be more difficult to discern. Some prevention programs are developing risk scores or clinical criteria to help identify individuals at risk for HIV who would benefit from PrEP; factors may include age, partnership characteristics, education, alcohol use, and history of sexually transmitted infections [37,106–111]. Alternatively, some prevention programs are taking a geographic approach, where the highest prevalence areas are targeted [112,113]. While identifying appropriate risk factors is important, individuals should also be allowed to self-select for PrEP use. There is evidence that early PrEP users are self-selecting appropriately [114,115] and that self-selection, in one study, lead to earlier PrEP use compared to individuals identified by a risk score [107]. In addition, the broader availability of PrEP to those who seek it out will help avoid stigmatizing PrEP [116,117].

Identifying at-risk individuals to initiate PrEP is only one piece of the implementation puzzle. As suggested by Chapters 2 and 3, achieving prevention-effective adherence over time, particularly among young women, may require additional tools. Ongoing assessments of HIV risk are part of any PrEP program but may need to be more frequent for younger users; for instance, a clinical trial of 15-17 year old men suggested that quarterly visits were not sufficient to maintain adherence in this age group [118]. mHealth strategies for adherence support may be attractive to younger users [119], both for adherence support and on-going risk assessment. Messaging beyond HIV risk may also play an important role [43]. For instance, couples often find that PrEP improves their relationship [21]. Likewise, some PrEP campaigns have explicitly focused on PrEP as a method of empowerment and attaining "peace of mind" [113,120]. While some have argued that PrEP should be integrated into, and perhaps will increase interest in existing HIV prevention programs [121], making PrEP easily accessible will also be important for women to use PrEP effectively [43]. This can include integrating PrEP with antenatal care and family planning clinics, or using pharmacies and mobile sites to increase access and convenience.

Finally, the results from the Partners Demonstration Project [12] and the analyses of adherence presented here illustrate that the possibility of imperfect adherence should not be a barrier to PrEP initiation and use. Daily adherence should be the goal for women on PrEP; this is supported by pharmacokinetic studies and direct comparisons of daily versus event-driven regimens [22,48,122]. However, women can still benefit greatly from PrEP with imperfect use. Young women in particular may benefit as additional formulations of PrEP, including vaginal rings and longer acting injectables, become available [42,123,124]. Parallels are often made between and contraception, another prevention program targeted at sexually active women; the contraceptive literature shows that women often switch between methods, suggesting that other PrEP options will also help women find a method that works for them [125–127]. Importantly, the contraceptive literature shows that methods that rely less on the user, such as implants, show higher rates of real-world effectiveness, compared to user-dependent methods like oral pills and condoms [128].

Conclusion

In summary, we have found evidence that women, including young women, can use PrEP in pill form effectively and should be central to PrEP implementation programs. While understanding HIV risk behaviors and risk perception among women will be important, PrEP messaging and implementation should go beyond HIV risk and focus on empowerment and improved relationships to increase uptake and adherence among women. Adherence monitoring in implementation programs will be challenging; additional work on biomarkers is needed for African populations, but most implementation programs will rely on lower-cost options for adherence assessment. Among the many factors that increase the risk of HIV for women, pregnancy may be an important period and PrEP adherence during this time may be even more crucial; more work is needed to determine if pregnancy requires a change in PrEP dosing to prevent HIV.

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