Evaluating PrEP use and safety among women of reproductive age in sub-Saharan Africa

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Abstract

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In sub-Saharan Africa, oral pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate and emtricitabine (TDF/FTC) has emerged as an important HIV prevention strategy for women and is being scaled up rapidly in areas with high HIV burden. New, long-acting PrEP methods are also approaching introduction, including the dapivirine vaginal ring, which women can wear continuously and discreetly for up to one month. Greater choice means women are more likely to find a method that suits their specific needs and preferences. However, questions and challenges regarding PrEP use and safety among women of reproductive age remain, which we aimed to address in this dissertation.

First, we validated a novel, urine-based tenofovir immunoassay as a potential point-of-care tool for assessing PrEP adherence using stored samples from the Partners PrEP Study (Chapter 2). Second, we estimated the per-sex-act relative HIV risk reduction among women exhibiting consistent use of the dapivirine ring in the ASPIRE trial based on an objective measure of ring exposure, the rate of dapivirine release from returned rings (Chapter 3). Third, we evaluated differences in changes in bone turnover markers associated with combined use of oral PrEP and depot medroxyprogesterone acetate (DMPA) among young women in Uganda (Chapter

4). Finally, we conducted a literature review on the safety of TDF-based PrEP use during pregnancy (Chapter 5).

We demonstrated that urine tenofovir concentrations measured by the novel immunoassay has good sensitivity (87%) and specificity (73%) for detecting tenofovir in plasma. Additionally, tenofovir concentrations over a certain threshold indicative of PrEP use in the past day were predictive of a 71% reduction in HIV risk. We established the first per-sex-act dose-response model for dapivirine ring exposure and estimated 61% per-sex-act risk reduction with ring release rates indicative of continuous ring use. We identified no substantial changes in bone turnover biomarkers after 6 months of combined DMPA and PrEP use. Finally, we found that the current body of evidence on the safety of TDF-based PrEP use during pregnancy is reassuring but that additional data are needed. Additionally, more efforts are needed to increase representation of pregnant women in studies of new HIV prevention products.

The findings from this dissertation contribute to efforts for improving women's uptake of and adherence to PrEP in the coming years by validating a novel urine-based tenofovir immunoassay and providing a better understanding the efficacy of the dapivirine ring. Additionally, this work provides reassuring data on the safety of PrEP use during periods of contraception and pregnancy which may help to facilitate integration of PrEP into family planning and antenatal care services.

Table of Contents

Dedication

For my parents, Jim and Dolly, for always encouraging me to pursue my passions and showing me the value of humility, hard work and kindness.

Chapter 1. Introduction

In June 1981, five cases of Pneumocystis pneumonia indicative of a disease later coined acquired immunodeficiency syndrome (AIDS) among men who have sex with men were first officially reported in the US [1]. Now, four decades later, the HIV/AIDS epidemic persists as a global health crisis, with an estimated 1.7 million new HIV infections and nearly 700,000 AIDS-related deaths occurring annually [2]. Currently, the areas with the highest HIV burden are in East and Southern Africa, where over 20 million individuals are living with HIV [3]. Women and adolescent girls in sub-Saharan Africa are particularly vulnerable to HIV acquisition as it is estimated that approximately six out of every ten new infections occur in this population. Additionally, younger women between the ages of 15-24 are twice as likely as men their age to acquire HIV [2]. Therefore, it is critical that women and girls have access to safe and effective interventions to prevent HIV acquisition.

The past decade has been an inflection point in the global effort to prevent the spread of the epidemic. In 2011, the HPTN 052 study was the first to show that transmission of HIV could be nearly eliminated with sustained use of antiretroviral therapy (ART) by persons living with HIV ("treatment as prevention") [4]. Shortly after this finding was reported, the use of oral tenofovir disoproxil fumarate (TDF) co-formulated with or without emtricitabine (FTC), a recommended first-line nucleoside reverse-transcriptase inhibitor (NRTI) backbone combination for treatment of individuals living with HIV, was found to be highly efficacious as pre-exposure prophylaxis (PrEP) in multiple landmark trials [5-8]. In intention-to-treat analyses, oral PrEP was found to be up to 75% effective when adherence to study drug was found to be high [5]. Subsequent analyses found that HIV protection is estimated to exceed 90% with high use [9,10] but is ineffective with poor adherence [11,12]. Since the completion of these trials, PrEP has been scaled up rapidly through demonstration and implementation projects in areas of sub-Saharan Africa [13].

In addition to daily oral pills, long-acting methods of HIV prevention are beginning to emerge from the development pipeline as additional options for women. One of these is a vaginal

ring containing 25mg of the non-nucleoside reverse transcriptase inhibitor, dapivirine. The ring can be worn continuously and discreetly for up to a month, which may help some women overcome challenges with daily oral PrEP use. The dapivirine vaginal ring has been evaluated in two major randomized placebo-controlled efficacy trials among African women, MTN-020 (ASPIRE) and IPM 027 (The Ring Study), and has been shown to be approximately 30% effective at preventing HIV acquisition in intention-to-treat analyses [14,15]. Lower adherence to the ring, however, was also observed among certain groups of women, notably younger women. Since these initial studies, the ring has been evaluated in two open label extension studies [16,17], and promising collective data on the product's safety and effectiveness from these four evaluations has led to receipt of a positive regulatory opinion from the European Medical Agency and prequalification from the World Health Organization, bringing the product closer to introduction in individual countries [18].

While oral PrEP and the dapivirine ring are both promising prevention options for women, questions remain both about how to improve adherence to these products as well as their safety, which will be important to address as access and uptake increase in sub-Saharan Africa. With this work, we utilized data collected from three clinical studies involving women in East and Southern Africa to address critical issues related to PrEP use and safety, including: 1) validating a point-of-care (POC) urine-based tenofovir assay for potential use as a tool to provide adherence feedback in real-time, 2) gaining a better understanding of the efficacy of the dapivirine vaginal ring by assessing per-sex-act risk reduction with consistent product use, 3) assessing changes in bone turnover among young women using PrEP and the hormonal contraceptive, depot medroxyprogesterone acetate (DMPA), and 4) synthesizing the current evidence on the safety of oral TDF/FTC PrEP use during pregnancy.

Chapter 2: Validating a novel urine-based, point-of-care assay to assess PrEP adherence

Given the challenges women experienced with PrEP adherence in past studies, novel approaches to supporting adherence, including methods of providing POC drug-level feedback, are needed. Currently, pharmacologic measures are available to measure tenofovir (TFV), FTC and their metabolites in various biological matrices, including plasma [10,19], serum [20], peripheral blood mononuclear cells (PBMCs) [21] and hair [22], and have been shown to be superior indicators of user adherence than self-report. However, as they are currently performed, these approaches are not amenable to real-time drug monitoring and feedback given that they require shipment of samples to a laboratory for processing by skilled technicians and involve expensive and time-consuming assays that are not feasible for implementation in low-resource settings. To address potential limitations with existing objective adherence measures, scientists from the University of California, San Francisco and Abbott Laboratories have developed the first PrEP immunoassay, which can measure TFV concentrations in urine with high sensitivity (96%) and specificity (100%) relative to the current gold standard mass spectrometry assay [23]. The goal is to eventually develop the assay into a low-cost, lateral flow, rapid strip test for point-of-care use. Additional validation of the assay is needed prior to implementation, however, including comparison of the immunoassay to gold-standard measures of adherence, such as plasma TFV concentrations.

Objective: Validate a novel urine immunoassay for assessing PrEP use and evaluate the assay's ability to predict protection from HIV infection

Hypothesis: (1) TFV concentrations measured with the immunoassay will be highly correlated with plasma TFV; (2) Detectable urine TFV levels will be significantly associated with protection from HIV.

Chapter 3: Per-sex-act HIV risk reduction associated with consistent dapivirine ring use

In MTN-020 (ASPIRE) and IPM 027 (The Ring Study), women assigned to use of the dapivirine ring experienced approximately 30% lower risk of HIV acquisition compared to users of a placebo ring [14,15]. Greater reductions in HIV risk (39-61%) were estimated in open label extension studies, MTN-025 (HOPE) and IPM 032 (DREAM), when HIV incidence among participants was compared to simulated placebo groups [16,17]. In each of these studies, effectiveness of the ring was largely dependent on product adherence. A better understanding of the efficacy of the dapivirine ring by considering objective measures of exposure to the ring will help to inform ring use as the product is registered and scaled up in settings with high HIV burden. In HIV prevention studies, incomplete control for sexual behaviors can result in residual confounding leading to biased risk reduction estimates. While a previous analysis adjusted for visit-level frequencies of total sex and condomless sex [24], this may not fully account for differences in women's HIV risk due to sexual behaviors with a more detailed accounting being of greater utility. Assessing risk reduction on the sex act scale may help to more completely account for women's sexual behaviors across follow-up.

Objective: Estimate the relative HIV risk reduction associated with consistent use of the dapivirine ring compared to placebo ring use based on an objective measure of ring exposure, the rate of dapivirine release from returned rings

Hypothesis: Due to better accounting for longitudinal sexual behavior, per-sex-act relative risk reduction estimates will be higher than those previously estimated in an analysis adjusting for visit-level sex acts (approximately 43%)

Chapter 4: Evaluating changes in bone turnover associated with PrEP and DMPA use

The most commonly used modern contraceptive method in sub-Saharan Africa is the 150mg intramuscular DMPA (DMPA-IM or Depo-Provera) product [25]. DMPA-IM is typically

administered in the upper arm or buttock, which allows for discreet use, and provides up to three months of contraceptive protection. Recently, a lower dose 104mg subcutaneous formulation (DMPA-SC or Sayana Press) was introduced in several African countries that exhibits similar contraceptive effectiveness, duration and side effect profiles as DMPA-IM [26-28]. DMPA-SC, however, provides additional convenience as it can be administered in the thigh or abdomen by the provider or self-injected by the user. DMPA use is high in areas of sub-Saharan Africa with high HIV prevalence. Therefore, HIV-negative women who currently opt to use DMPA for contraception will likely have more opportunities to also use PrEP for HIV prevention, adding HIV prevention to their existing tool to prevent unintended pregnancy. Numerous clinical studies have identified an association between DMPA use, in either the intramuscular or subcutaneous form, and reduced bone mineral density (BMD) [29-35]. These data raise concerns about DMPA use potentially limiting women from achieving normal peak bone mass. Few studies have directly compared bone health outcomes of DMPA-IM and DMPA-SC users. TDF use has also been linked to BMD loss, although only two studies have been identified that assess this association in women [36,37]. Despite these studies, it is not known whether a synergistic effect exists between DMPA and PrEP use on bone health outcomes and if women concurrently using both products will experience reduced bone growth or density. Addressing this question is especially pertinent for young women who are still acquiring bone mass and could miss the chance to reach their full potential bone mass.

Objective: Assess changes in the concentrations of bone turnover biomarkers among subcutaneous and intramuscular DMPA users compared to women not using DMPA and test for effect modification by PrEP use

Hypothesis: Women using DMPA will have greater increases in bone turnover biomarkers compared to non-users. Additionally, women using a higher dose of DMPA (150 mg) will have greater increases in concentrations of biomarkers of bone turnover compared to

women using a lower dose of DMPA (104 mg) and these differences will be higher with PrEP use.

Chapter 5: Synthesizing the evidence on the safety of oral PrEP use during pregnancy

Evidence has shown that pregnant women have a two-fold higher risk of HIV infection compared to non-pregnant women and the probability of acquiring HIV rises steadily throughout pregnancy and the post-partum period [38,39]. With pregnancy rates in Africa being among the highest in the world [40], oral PrEP use during pregnancy is a welcome strategy during this period with heightened susceptibility. However, the safety and effectiveness of PrEP among pregnant women in high HIV-prevalence settings must be fully characterized, a challenging feat once product efficacy is established in non-pregnant women. Currently, based on data that are somewhat limited, oral PrEP containing TDF is recommended as an HIV prevention strategy by the World Health Organization [41], including for pregnant and postpartum women at substantial risk of HIV infection. Although TDF use during pregnancy appears generally safe, data on PrEP use during pregnancy remain limited.

Objective: Review the current literature on the safety of TDF use during pregnancy for HIV prevention. We also summarize corollary data from relevant studies assessing pregnancy outcomes among women who are living with HIV (WLHIV) and using TDF-based therapy. Finally, we make comparison to the safety profiles of other emerging HIV prevention options.

Thus, with this collective work, we aimed to address key issues pertaining to the use and safety of PrEP by women of reproductive age in sub-Saharan Africa. The implications of this work include informing and supporting efforts to improve PrEP adherence, expand HIV prevention options for all women of reproductive age and integrate PrEP into family planning and antenatal care services.

Chapter 2. Urine tenofovir levels measured by a novel immunoassay predict HIV protection

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URINE TENOFOVIR LEVELS MEASURED BY A NOVEL IMMUNOASSAY PREDICT HIV PROTECTION

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Abstract

New tools are needed to support PrEP adherence for individuals at risk for HIV, including those that enable provision of real-time feedback. In a large, completed PrEP trial, adequate urine tenofovir levels measured by a novel immunoassay predicted HIV protection and showed good sensitivity and specificity for detectable plasma tenofovir.

Introduction

Use of oral pre-exposure prophylaxis (PrEP) is a highly efficacious strategy for preventing HIV when taken daily.[42] However, adherence to PrEP has proven challenging among many populations.[43] Due to the limited accuracy of self-reported adherence, objective biological adherence metrics, such as measurement of PrEP drug concentrations in plasma, dried blood spots (DBS) and hair, have become valuable tools for appraising recent or cumulative PrEP adherence.[44] However, these methods can be costly and often require shipment of samples to an external laboratory, skilled laboratory personnel and specialized equipment, making them impractical options for routine use, particularly in resource-limited settings.

Innovative approaches are needed to support PrEP adherence, including those that enable counseling at the point of care (POC). Recently, we reported on the development of a novel antibody with high selectivity for tenofovir (TFV),[45] with the resultant immunoassay demonstrating high sensitivity and specificity relative to the standard liquid chromatography/tandem mass spectrometry (LC-MS/MS) assay. Here we further evaluate the immunoassay by comparing urine TFV measurements to TFV concentrations measured in plasma, which served as the gold standard metric for short-term adherence in most PrEP trials. Further, we assess the novel urine assay's ability to predict protection from HIV in a large completed PrEP trial.

Methods

We used a randomly-sampled, nested cohort of women and men, as well as cases of those who HIV seroconverted, assigned to the use of tenofovir disoproxil fumarate (TDF) or TDF/emtricitabine (FTC) in the Partners PrEP Study (NCT00557245), a randomized, placebo-controlled PrEP efficacy trial conducted among HIV serodiscordant couples in Kenya and

Uganda.[5] During study follow-up, urine samples were archived at 3-, 12-, 24-, and 36-month visits. TFV concentrations were measured in all available archived urine samples using a quantitative enzyme-linked immunosorbent assay (ELISA) (lower limit of quantification [LLOQ]=1000 ng/mL) with this novel antibody.[45] TFV concentrations in date-matched plasma specimens were previously measured among the cohort using a validated LC-MS/MS assay (LLOQ=0.31 ng/mL). TFV concentrations below the LLOQ were assigned a value of half the LLOQ for each respective assay. Urine and plasma samples were stored at -80^{o}C upon collection and shipped on dry ice.

Correlation between paired urine and plasma concentrations was assessed and the sensitivity and specificity of detectable TFV in urine for determining detectable TFV concentrations in plasma were calculated. Additionally, we determined the sensitivity and specificity of urine TFV levels ≥1500 ng/mL, a concentration indicative of PrEP dosing in the past day among Thai men and women,[46] for determining plasma TFV concentrations >40 ng/mL, levels consistent with daily PrEP dosing.[10]

To assess the association between urine TFV concentrations ≥1500 ng/mL and protection from HIV acquisition, we conducted a nested case-control analysis. Case samples collected on the date of the first evidence of HIV infection (i.e., first positive test for HIV-1 RNA) were matched with control samples collected at the same study visit month. If the case's first evidence of HIV was observed between regular urine sample archiving, controls from the nearest archive visit were selected. Control samples were matched 35:1, the ratio where estimates began to stabilize, and randomly sampled from the risk set of participants who were HIV-negative at the case's date of HIV detection, including future seroconverters. Controls could be matched to multiple cases. Conditional logistic regression, adjusted for matched sets, estimated the odds ratio of HIV acquisition given a urine TFV concentration ≥1500 ng/mL, which approximates a rate ratio (RR) given our time-matched risk set sampling approach. Adjusted models controlled for participant

sex, age, and report of any condomless sex with their study partner in the prior month at enrollment. All models were replicated to also assess the association of plasma TFV >40 ng/mL with HIV protection. Case samples were too few to conduct adequately powered sex-based subgroup analyses.

The protocol for the parent study received ethical approval from the Institutional Review Board at the University of Washington and ethics review committees at each study site. All participants provided written informed consent.

Results

Of 4,432 individuals randomized to use of TDF or TDF/FTC in the Partners PrEP Study, 292 were included in the nested cohort. Among these participants, 39% were female and the median age was 33 (interquartile range [IQR]=28-39). Participants in the cohort contributed 722 paired urine and plasma samples. Of 52 individuals who seroconverted to HIV while using PrEP in the study, 22 had urine samples available from the visit where HIV was first detected and were included as cases. An additional 69 seroconverter samples collected prior to HIV infection were included as possible controls. Among cases, 55% were female and the median age was 33 (IQR=27-39).

The median duration from collection to assay of plasma and urine samples was 20 months and 103 months, respectively. In the cohort, the median TFV concentration was 37,500 ng/mL (IQR=500-90,000 ng/mL) in urine via ELISA and 65.4 ng/mL (IQR=1.6-103.0 ng/mL) in plasma via LC-MS/MS. Spearman's rank correlation coefficient (ρ) for the two measures was 0.46 ($p<0.001$). Of 558 plasma samples with detectable TFV (≥LLOQ of 0.31 ng/mL), 486 had a paired urine sample with detectable TFV (≥LLOQ of 1000 ng/mL) for a sensitivity of 87% (95% CI=84-90%). There were 164 plasma samples with undetectable TFV, of which 119 had a paired urine

sample with undetectable TFV for a specificity of 73% (95% CI=65-79%). Of 468 individuals with plasma TFV >40 ng/mL, 420 had a paired urine sample with TFV ≥1500 ng/mL, for a sensitivity of 90% (95% CI=87-92%). Finally, 254 plasma samples had TFV levels ≤40 ng/mL, of which 146 had a paired urine sample with TFV <1500 ng/mL for a specificity of 57% (95% CI=51-64%).

In total, 770 control samples from 280 individuals were matched to the 22 case samples in our case-control study. Among participants in both active PrEP study arms, urine TFV ≥1500 ng/mL was associated with a 71% (95% CI=30-88%) reduction in HIV risk in the adjusted model (Table 1). By contrast, plasma TFV >40 ng/mL was associated with an 87% (95% CI=54-96%) reduction in HIV risk (Supplemental Table 1).

Discussion

The development of a low-cost POC assay to evaluate PrEP adherence would facilitate the implementation of real-time, drug-level feedback in current PrEP programs in resource-limited settings. Here we demonstrate that urine TFV concentrations, measured by a novel immunoassay, predict protection from HIV acquisition among PrEP users in sub-Saharan Africa. The concentrations measured by the urine assay also had good sensitivity and specificity for plasma levels, the gold-standard metric of adherence in placebo-controlled PrEP trials.

Previous studies have highlighted the advantages of using a urine-based assay to evaluate PrEP use. TARGET, a pharmacokinetic study that randomized Thai adults to directly-observed TDF in arms simulating low, medium and high adherence patterns, demonstrated that paired urine and plasma TFV concentrations measured by LC-MS/MS were highly correlated.[47] The study also showed that urine TFV concentrations can evaluate time since dosing, further demonstrated in other U.S.-based studies.[48,49] Data from the U.S. have also suggested that urine collection is highly acceptable, which may result in high uptake compared to other biological

measures.[50] Additional evidence regarding the feasibility and acceptability of urine-based measures of PrEP use in other settings, including sub-Saharan Africa, is needed.

We previously demonstrated that urine levels via this immunoassay were correlated with other biomarkers, including TFV and FTC levels in hair and TFV-diphosphate and FTC-triphosphate concentrations in DBS, among men who have sex with men (MSM) and transwomen in the iPrEx open-label extension (OLE) study. Moreover, low urine concentrations among participants in iPrEx OLE were associated with subsequent HIV seroconversion.[51] The data presented here extend these prior results to heterosexual men and women on PrEP in sub-Saharan Africa.

There are several potential limitations to our results. First, we were unable to account for specific gravity of the urine samples or normalize to creatinine levels.[48] Second, longer storage of urine compared to plasma samples prior to analysis may have resulted in differential rates of sample degradation. Moderate correlation observed between urine and plasma TFV concentrations may be partially explained by these factors. Use of a specified TFV threshold for other analyses, however, may have mitigated the influence of measurement variability. Finally, most seroconverters did not have urine samples available from the visit of first HIV detection, limiting study power, and incomplete availability of control samples at urine archive months may have limited our control matching. However, our overall risk reduction estimates with plasma TFV >40 were nearly identical to those previously identified in this cohort,[10] suggesting bias may have been minimal.

In summary, we show the high predictive utility of an adequate urine tenofovir level for HIV protection among men and women using PrEP in sub-Saharan Africa. The urine immunoassay has been developed into a lateral flow assay (LFA), which is low-cost, easy to perform, can be administered at the POC, and provides results within minutes.[45] The LFA is portable and requires no reagents so it may also be administered in the field by non-medical personnel to help

reach stigmatized or hidden populations. The assay should be evaluated in a variety of populations for adherence monitoring and feedback.

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Table 1. Percent HIV risk reduction associated with urine TFV concentrations >1500 ng/mL as measured by a novel immunoassay

n (%) with urine TFV ≥1500 ng/mL					
Case samples: First evidence of HIV	**Control samples**	**% HIV risk reduction[a] (95% CI)**	**p-value**	**Adjusted % HIV risk reduction[a,b] (95% CI)**	**Adjusted p-value[b]**
8/22 (36%)	527/770 (68%)	73% (36 to 89%)	0.003	71% (30 to 88%)	0.006

TFV: tenofovir, CI: confidence interval
Analyses include individuals assigned to TDF/FTC or TDF-only PrEP. Estimates were generated using conditional logistic regression.
[a]% risk reduction calculated as follows: (1-RR)*100
[b]Adjusted for sex, age at enrollment, and report of any condomless sex with study partner in the month prior to enrollment

Supplemental Table 1. Percent HIV risk reduction associated with plasma concentrations >40 ng/mL as measured by a liquid chromatography-tandem mass spectrometry method

n (%) with plasma TFV >40 ng/mL					
Case samples: First evidence of HIV	**Control samples**	**% HIV risk reduction[a] (95% CI)**	**p-value**	**Adjusted % HIV risk reduction[a,b] (95% CI)**	**Adjusted p-value[b]**
3/22 (14%)	461/770 (60%)	89% (64 to 97%)	<0.001	87% (54 to 96%)	0.002

TFV: tenofovir, CI: confidence interval
Analyses include individuals assigned to TDF/FTC or TDF-only PrEP. Estimates were generated using conditional logistic regression.
[a]% risk reduction calculated as follows: (1-RR)*100
[b]Adjusted for sex, age at enrollment, and report of any condomless sex with study partner in the month prior to enrollment

Chapter 3. Assessing per-sex-act HIV risk reduction among women using the dapivirine vaginal ring

ASSESSING PER-SEX-ACT HIV RISK REDUCTION AMONG WOMEN USING THE DAPIVIRINE VAGINAL RING

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Abbreviations

BV: bacterial vaginosis

CI: confidence interval

DMPA: depot medroxyprogesterone acetate

EMA: European Medicines Agency

FTC: emtricitabine

HIV: human immunodeficiency virus

IPCW: inverse probability of censoring weights

IQR: interquartile range

IUD: intrauterine device

LLOQ: lower limit of quantitation

MCMC: Markov chain Monte Carlo

NET-En: norethisterone enanthate

PrEP: pre-exposure prophylaxis

RNA: ribonucleic acid

RRR: relative risk reduction

STI: sexually transmitted infection

TDF: tenofovir disoproxil fumarate

WHO: World Health Organization

ABSTRACT

In HIV prevention studies, confounding by sexual behavior is potentially a significant source of bias. To more completely account for sexual behaviors, we estimated per-sex-act risk reduction associated with use of the dapivirine vaginal ring, a new longer-acting HIV prevention option for women. Data came from ASPIRE, a Phase III, randomized, placebo-controlled efficacy trial of the dapivirine ring that recruited HIV-uninfected African women between the ages of 18-45. Using cumulative sex acts as the time scale, we used multivariable Cox regression with inverse probability of censoring weights to estimate risk reduction associated dapivirine ring use, including analyses accounting for dapivirine release from returned rings indicative of consistent use. Women in the dapivirine ring group (n=1188) had an expected incidence rate of 2.3 (95% CI: 1.8-3.1) HIV acquisition events per 10,000 sex acts versus 3.6 (2.9-4.4) per 10,000 acts in the placebo group (n=1189). A dose-response relationship was demonstrated, with dapivirine release indicating consistent use associated with a 61% (95% CI: 30-78%) per-sex-act HIV risk reduction. These results support the efficacy of the dapivirine vaginal ring for HIV prevention and help informed decision-making for women, providers, and policymakers pending public introduction of this novel product.

Key Words: dapivirine, vaginal ring, pre-exposure prophylaxis, HIV, per-sex-act

INTRODUCTION

In sub-Saharan Africa, women and girls account for nearly six out of every ten new HIV infections, underscoring the need for novel, safe and effective biomedical prevention interventions in this population [2]. Oral pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate and emtricitabine (TDF/FTC) has emerged as an important antiretroviral-based prevention strategy and is being scaled up rapidly in areas of sub-Saharan Africa with high HIV burden [13]. However, challenges with daily PrEP adherence have been observed in past studies [11,12], indicating additional options are needed to fit women's diverse needs and preferences. One such option is the vaginal ring containing the non-nucleoside reverse transcriptase inhibitor dapivirine, a product with demonstrated HIV prevention efficacy that women can wear continuously and discreetly for up to one month [14,15]. The ring has recently received a positive opinion from the European Medicines Agency (EMA) and prequalification from the World Health Organization (WHO), bringing it closer to public introduction [18,52].

As the dapivirine ring becomes an available PrEP option for women, it is critical that its efficacy be well understood so that women's decisions are well-informed. The dapivirine ring was evaluated in two Phase III efficacy trials in sub-Saharan Africa, MTN-020 (ASPIRE) and IPM 027 (The Ring Study) [14,15], and was found to be effective, reducing HIV acquisition risk by 27-31% in intention-to-treat analyses. As seen in studies of other PrEP agents [5,8], HIV prevention effects were associated with adherence, and thus the intention-to-treat findings underestimate the product's efficacy. For the ring, disproportionate low adherence was seen among certain groups, particularly younger women. Additional work has been conducted to better understand the relationship between product exposure, as determined by objective measures of adherence, and HIV risk reduction, with one analysis finding at least 48% reduction in HIV risk among women demonstrating ideal use of the ring based on measured rates of dapivirine released from the ring [24].

In addition to adherence, another key factor to consider in interpreting results of trials of efficacy of novel HIV prevention products is the potential for confounding by sexual behavior. HIV acquisition is dependent on exposure to the virus through sex, specifically with a partner who is living with HIV. However, analytic approaches to evaluating HIV prevention methods that use a standard time scale assume that individuals who are not having sex accumulate risk at the same rate as individuals who are having frequent sex, which can lead to biased efficacy estimates if sexual behaviors systematically differ between product use groups. By assessing risk on a sex act scale, periods of time with no sexual activity, and therefore without any chance of exposure, would not inform relative risk estimates. In this analysis, we performed detailed accounting for both sexual behavior and adherence to the dapivirine vaginal ring, estimating per-sex-act risk reduction associated with rates of dapivirine release from returned rings indicative of continuous use. We hypothesized that by more thoroughly accounting for women's sexual behaviors and objective markers of ring use, risk reduction estimates will be higher than previously estimated with dapivirine ring use.

METHODS

Study population and procedures

We conducted a secondary longitudinal analysis of data collected from ASPIRE, a Phase III randomized placebo-controlled trial to assess the efficacy of the dapivirine vaginal ring for prevention of HIV acquisition. Study procedures have been previously described in detail elsewhere [14]. Briefly, cisgender women between the ages of 18 and 45 who were living without HIV, sexually active and using an effective contraceptive method were recruited from 15 sites in Malawi, South Africa, Uganda and Zimbabwe. Women were randomized 1:1 to use a silicone vaginal ring formulated with 25 mg of dapivirine or an inactive placebo ring and instructed to use

the product continuously for 28 days. At monthly visits, women received a replacement ring as well as serologic HIV testing and product adherence counseling. Safety of ring use was evaluated at each follow-up visit and study product was held for one or more of the following criteria: pregnancy or breastfeeding, positive HIV rapid test, experience of adverse event or allergic reaction related to the study product, or report of HIV postexposure prophylaxis. Ethics review committees at each site approved the study protocol and all participants provided written informed consent.

Survey and laboratory measures

Information on women's socio-demographic characteristics, family planning use, menstrual bleeding and partner HIV status and knowledge of ring use were collected by interviewer-administered surveys.

HIV testing followed a standardized algorithm. At monthly study visits, women received two separate serological HIV-1 tests. If either test yielded a positive result, confirmatory Western Blot and HIV-1 RNA testing were carried out on serum specimens collected at the most recent quarterly visit. Archived serum samples from previous quarterly visits were also analyzed for HIV-1 RNA if needed to identify the earliest evidence of HIV infection.

Testing for STIs and bacterial vaginosis (BV) occurred at the screening visit and then semi-annually and if indicated. Urine samples were tested for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using a nucleic acid amplification test. Vaginal fluid samples were assessed for BV by Gram stain using the Nugent score.

Adherence measures

Ring adherence was evaluated using multiple objective measures. First, stored plasma specimens from quarterly study visits were analyzed for dapivirine concentrations using a validated ultra-performance liquid chromatography-tandem mass spectrometry assay. Second, approximately one year after study enrollment began, used rings were collected at monthly visits and analyzed for quantities of residual dapivirine to assess cumulative use. Residual dapivirine in rings was collected using acetone extraction and then quantified using high-pressure liquid chromatography. The amount of drug released during intravaginal use was estimated by subtracting the amount of residual dapivirine measured in the ring from the loading mass (25 mg). Given that women may not have returned to the clinic exactly four weeks after their previous visit, we standardized the amount of dapivirine released to 28 days of use. In a previous Phase 1 study, approximately 4-5 mg of drug was release from the ring among women who reported perfect product use over a 28-day period [53]. Therefore, in this analysis, we used the point estimate at the midpoint of these values (4.5 mg/month) to assess risk reduction associated with consistent ring use.

Multiple issues related to missingness and measurement error in exposure and outcome variables were addressed by sampling posterior samples from a Bayesian joint model of ring adherence and HIV risk using Markov chain Monte Carlo (MCMC) methods. First, as measurement of residual dapivirine in returned rings began a year after the study commenced, most women assigned to the active ring were missing measurements at early study visits. Without imputation of these data, women who acquired HIV or completed follow-up during these early visits would be excluded. Second, as tests for HIV-1 RNA were only conducted quarterly, the exact month when HIV acquisition occurred could not be determined from the testing data. To address these issues, we constructed a joint hidden Markov model to estimate the conditional probability distributions of: 1) monthly binary adherence status, 2) monthly dapivirine release rates and 3) monthly HIV-1 infection status as defined by a positive RNA result. The model included

baseline and time-varying covariates and allowed for random intercepts for each site to account for site-specific baseline HIV-1 risk. HIV seroconversion was modeled using both the monthly Western Blot and quarterly HIV-1 RNA laboratory testing results based on the stages of acute HIV infection outlined by Fiebig, et al. [54] Twenty imputations of the three variables were generated, each the mean of 50 randomly selected posterior samples from the posterior probability distributions.

Estimating per-sex-act risk reduction

a. *Modeling the sex act time scale*

At quarterly study visits, women were asked to provide information about their sexual behaviors, including the number of sexual partners they had in the past 3 months, whether they had a consistent primary partner over the past 3 months, the number of vaginal sex acts with their primary partner in the past week and number of times a condom was used. To carry out our analysis on the per-sex-act scale, we required complete monthly sexual behavior data for participants across follow-up. To predict numbers of monthly sex acts, we modeled reported sex acts in the past week using a mixed effects Poisson model that allowed for random intercepts and slopes. The model included baseline and time-varying covariates thought to be correlated with sexual behaviors based on our own hypotheses and the published literature, including study site, age, marital status, number of live births, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection, alcohol use, smoking status, having a primary or new partner sexual partner, number of sexual partners, number of sex acts at baseline, family planning use and having any vaginal cleaning practices. The fitted model was then used to predict numbers of sex acts since the last monthly visit by adjusting the model offset to the number of days since the previous visit. Distributions of sex acts were plotted across follow-up.

b. Regression models

Models of per-sex-act risk reduction included women who were confirmed to be without HIV at enrollment and had imputed sex acts and dapivirine release data across follow-up. Additionally, we excluded women from two study sites with lower adherence to study product and protocol. Baseline participant characteristics were summarized and stratified by study group and, for women assigned to the dapivirine ring group, expected ring use during follow-up based on our MCMC imputations (any or no expected release rate of 4.5 mg/month). To model per-sex-act HIV risk reduction associated with ring release rates of 4.5 mg/month (i.e., consistent ring use), we fit Cox regression models using the cumulative number of predicted sex acts between enrollment and first HIV detection as the outcome. Primary exposure variables for study group (dapivirine or placebo ring) and continuous ring release rate were included; the exponentiated linear combination of the coefficients of these two terms was used to obtain a parameter estimate analogous to a hazard ratio (HR), comparing the probability of HIV infection at a particular number of cumulative sex acts between women in the dapivirine ring group with 4.5 mg/month drug release and women in the placebo ring group (for whom dapivirine release rate was 0 mg/month), assuming no HIV infection up to that point. Percent relative risk reduction (RRR) was calculated using the following formula: %RRR=(1-HR)*100. Women were censored at first pregnancy, first product hold >3 days and loss-to-follow-up given likely changes in HIV risk after these events. All models were stratified by study site. Multivariable models were adjusted for time-varying condomless sex in the past 7 days and the following baseline variables: age, education, marital status, bacterial vaginosis status, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* diagnosis, any new sexual partners, multiple sexual partners, number of sex acts in last 3 months, any condomless sex in last seven days, alcohol use, partner HIV status and knowledge of ring use, family planning method and age-specific local male HIV prevalence (assuming that women had

partners who were in their age group). Model parameter estimates were calculated separately for each of the twenty imputed datasets and averaged; standard errors were pooled using Rubin's rules to obtain 95% confidence intervals and p-values.

c. *Adjustment with inverse probability of censoring weights*

Given that women in our analysis were censored for reasons that may be related to the study outcome of HIV acquisition, we further adjusted our models using inverse probability of censoring weights (IPCWs) to account for potential selection bias due to informative censoring. IPCWs are calculated as the inverse of the probability of remaining uncensored at time t given that the participant was uncensored at t-1, conditional on a set of baseline covariates and time-dependent confounders that are associated with time to censoring and time to failure (i.e., HIV acquisition). Women who remain uncensored and have similar characteristics to women who were censored receive greater weights. In this analysis, IPCWs were estimated by Cox regression using the 'ipcwswitch' package in R, which generates weights using the Kaplan-Meier limit estimator and is well suited for longitudinal data that are collected at different times (cumulative sex acts here) for different participants [55]. Baseline variables used in the weight denominator included site, age, any condomless sex in the past seven days, marital status, number of live births and *Chlamydia trachomatis* and *Neisseria gonorrhoeae* diagnoses. Time-dependent confounders included continuous ring release rate per month, family planning method, having menstrual period since last visit, previous missed visit, multiple sex partners, and condomless sex in last 7 days. Weights were stabilized by including the same set of baseline covariates in the numerator. Models included a term for follow-up time (i.e., cumulative sex acts) to allow for changes in weighting with time accrual. Weights were generated separately for South African versus non-South African sites to account for differences in frequencies in type of censoring and

predictors of censoring between locations. Distributions of the weights were summarized and plotted to assess for excess variation and the need for truncation.

d. Subgroup and sensitivity analyses

Subgroup analyses were carried out based on baseline age due to known differences in adherence between younger and older women as well as *Neisseria gonorrhoeae/Chlamydia trachomatis* diagnoses and bacterial vaginosis status, which were hypothesized to be effect modifiers of the relationship between ring use and HIV protection. We also stratified results by whether women reported bleeding in the prior month given that women may have less HIV exposure while experiencing bleeding. All statistical analyses were carried out in R version 3.6.1 (R Core Team, Vienna, Austria).

RESULTS

In ASPIRE, 2629 women were randomized to use of either the dapivirine (n=1313) or placebo ring (n=1316). Of those women, 3 were retrospectively discovered to have had acute HIV infection at baseline and were excluded. An additional 12 women did not have any follow up after the enrollment visit. For this analysis, 6 women who had a product hold before their first follow-up visit, 17 with missing data for variables used in prediction and MCMC models and an additional 214 women from two sites with lower protocol and product adherence were also excluded. Therefore, we included a total of 2377 women (1188 in dapivirine group and 1189 in placebo group) in our analysis.

Women in the placebo group and women in the active group (regardless of expected ring release rates) had comparable socio-demographic characteristics (Table 1). The median age was 26 (IQR: 22-31), nearly half (45.1%) of women completed secondary schooling, and four out of

ten were married. Approximately two-thirds of women's partners knew about their ring use. Less than 2% of women stated that their primary partner was living with HIV but nearly half (44.6%) of women did not know their partner's HIV status. Women who were expected to demonstrate consistent ring use at least once during the trial (≥4.5 mg/month) tended to report fewer sex acts in the last three months (median 13.5; IQR: 6.0, 36.0) and a smaller percentage reported recent condomless sex (32.7%) relative to placebo users and dapivirine ring users not expected to demonstrate ideal adherence. Depot medroxyprogesterone acetate (DMPA) was the most commonly used contraceptive method (used by 40.1% of women), and most women experienced regular bleeding (>50%) and had vaginal cleaning practices (>60%).

Predicted numbers of sex acts had good correlation with the reported numbers of sex acts in the past week at quarterly visits (rho=0.73). The median number of predicted sex acts per 28 days across follow-up was similar between women in the dapivirine ring (median: 8, IQR: 5-12) and placebo ring groups (median: 8, IQR 5-13) and gradually declined over time (Figure 1a). Among women in the dapivirine ring group, the median expected dapivirine release rate across the twenty posterior samples was 3.1 mg per 28 days (IQR: 2.0-3.8). The median expected dapivirine release rate was similar across categories of sex acts (Figure 1c).

Overall, women in the placebo group experienced 83 HIV acquisition events over 231,911 predicted sex acts, for an expected incidence rate of 3.6 events per 10,000 acts (95% CI: 2.9-4.4) (Table 2). Women in the dapivirine group experienced 54 HIV acquisition events over 230,658 predicted sex acts, for an expected incidence rate of 2.3 events per 10,000 acts (95% CI: 1.8-3.1).

Women in the dapivirine ring group expected to have consistent ring use (dapivirine release of 4.5 mg/28 days) had a 54% reduction in HIV risk (95% CI: 20-74%) relative to placebo in unadjusted models. In adjusted models, release rates associated with consistent ring use were associated with a 60% HIV relative risk reduction (RRR) (95% CI: 28-78%) compared to placebo.

In the adjusted model incorporating IPCWs, dapivirine release indicative of consistent ring use was associated with a 61% relative risk reduction (95% CI: 30-78%). The dose-response of relative risk reduction estimates across rates of dapivirine release is depicted in Figure 2.

In subgroup analyses, expected per-sex-act HIV incidence was highest among women who were diagnosed with *Neisseria gonorrhoeae* (11.0 events per 10,000 acts in placebo group and 7.2 events per 10,000 acts in dapivirine group) and *Chlamydia trachomatis* (7.3 events per 10,000 acts in placebo group versus 6.2 events per 10,000 acts in dapivirine group) at enrollment. However, RRR estimates did not differ between women with or without *Neisseria gonorrhoeae* or *Chlamydia trachomatis* diagnoses according to interaction models (p=0.810 and p=0.319, respectively). Additionally, no significant difference in RRR was identified between age and bacterial vaginosis status subgroups in interaction models based on baseline characteristics. In our interaction model based on time-varying experience of bleeding in the past month, risk reduction estimates were higher for months when bleeding was reported verses when not reported (Supplemental Figure 1), but this difference was not statistically significant (p=0.209).

DISCUSSION

In this secondary analysis of data from the ASPIRE trial, we conducted a rigorous evaluation of HIV risk reduction associated with use of the dapivirine vaginal ring with a more complete accounting for women's sexual behaviors. Unlike other analyses that evaluate HIV risk reduction on a time scale, assessing risk reduction on the sex act scale may better control for the heterogeneity in women's sexual behaviors and ensure that accumulation of HIV risk is directly tied to potential exposure. We demonstrated a dose-response relationship between rate of drug release from the dapivirine vaginal ring and HIV protection. Further, expected dapivirine release rates indicative of consistent ring use were associated with an estimated 61% reduction in per-

sex-act HIV risk after adjusting for other potential confounders and accounting for informative censoring using inverse probability of censoring weights.

Dapivirine release rates were imputed in our analysis using MCMC methods to account for missingness and measurement error in observed ring release measures. In our previous analysis using all available measured ring release data, which excluded 46 women with HIV endpoints that occurred prior to collection of used rings for dapivirine quantification, we demonstrated a 43% risk reduction associated with dapivirine release rates consistent with ideal use [24]. The same levels of dapivirine release were associated with a 71% HIV risk reduction when starting follow-up at 12 months to create a common start time; however, as this secondary analysis excluded an additional 54 HIV endpoints and 1,436 woman-years of follow-up, it is unclear whether this estimate was biased due to missing data from early study visits. Our ability to use the full range of study follow-up data in the current analysis due to our imputation methods allow us to correct for selection bias due to previous exclusion of these endpoints.

Studies of oral PrEP users have similarly identified correlations between drug exposures consistent with regular use and HIV protection. Within the Partners PrEP Study, a case-cohort analysis of HIV protection associated with various plasma tenofovir concentration thresholds demonstrated a 91% relative risk reduction associated with plasma TFV concentrations consistent with daily use among TDF/FTC users [10], in contrast to the 75% effectiveness of TDF/FTC use intention-to-treat analysis [5]. Similarly, a substudy of data collected from the iPrEx trial found that intracellular tenofovir metabolite concentrations that were indicative of four or more oral doses per week was predictive of 90% HIV risk reduction relative to placebo pill users compared to 44% in intention-to-treat analysis [9,56].

We saw very little change in HIV risk reduction estimates when models were weighted using IPCWs. This was also observed in previous work utilizing IPCWs to evaluate the efficacy of oral PrEP, which censored participants at first missed visit and detection of poor adherence [56].

Estimating unbiased IPCWs is dependent on several assumptions, including correct model specification [57,58] and inclusion of all variables associated with time to censoring and time to failure. Therefore, this small change could be due to poor prediction of the weights model and may suggest our models exclude other unmeasured variables that may be strong predictors of censoring. It may also suggest minimal selection bias in our analysis due to informative censoring.

In subgroup analyses, a notable finding was that the relative risk estimates between women <25 and ≥25 years of age did not significantly differ based on our interaction model. The primary ASPIRE intention-to-treat analysis indicated a lack of efficacy among women <25 years of age and that this result was statistically different than women ≥25 years of age. Therefore, our approach in this analysis may have eliminated this difference by more thoroughly accounting for sexual behaviors and adherence within these subgroups.

Potential limitations of our analysis should be considered. First, predicted numbers of monthly sex acts were based on women's self-reported sex acts in the prior week. Such data are subject to recall bias (particularly among women who have frequent sex) and social desirability bias as questions could potentially be embarrassing or stigmatizing to answer. Second, per-sex-act risk depends largely on the HIV status of their partner. However, <2% of women reported that their primary partner was living with HIV at baseline while nearly half of women did not know their partner's status. We also adjusted for local HIV prevalence among males to further account for partner HIV status and variation in women's HIV exposure, but this is an imperfect proxy for confirmation of women's partner status. As a result, expected per-act incidence estimated in our analysis is likely an underestimate of the per-act incidence of women who are HIV-exposed. Finally, our use of 4.5 mg/month as an indicator of consistent use is based on self-reported adherence from a small sample of women (n=8) in a Phase 1 study. Therefore, it may not be the most appropriate value for our study population. Future work may identify pharmacokinetic thresholds for HIV protection for the dapivirine ring.

In conclusion, our analysis furthers the evidence to support the use of the dapivirine ring as an efficacious HIV prevention product for women. While efficacy of the ring is not as high as oral PrEP based on our estimates, the dapivirine ring has important characteristics (e.g., longer duration, allows for more discreet use, potentially fewer side effects) that may better suit women's needs compared to oral PrEP. Therefore, communication of risk associated with the ring relative to other PrEP options should be part of a person-centered approach, acknowledging that women are in the best position to decide what is best for them [59]. As additional HIV prevention products emerge from the development pipeline, including multipurpose prevention technologies, we encourage similar analyses to evaluate risk reduction associated with product exposure as a complement to intention-to-treat analysis as an approach to best understand product efficacy.

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Study Product: The vaginal rings used in this study were developed and supplied by the International Partnership for Microbicides (IPM).

Tables and Figures

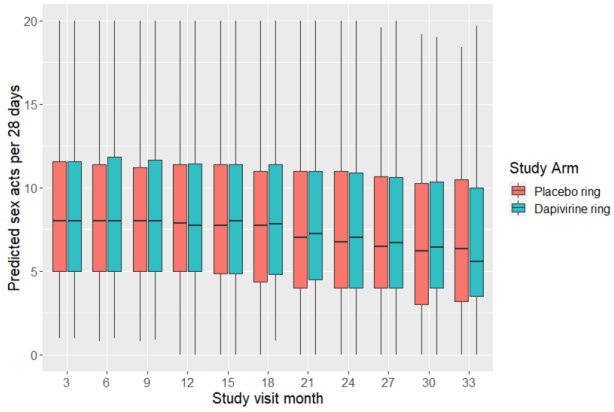
Table 1. Baseline participant characteristics

	Placebo group (N=1189)	Dapivirine group: No expected release rate ≥4.5mg/month (N=813)	Dapivirine group: At least one expected release rate ≥4.5mg/month (N=375)
Age, years	26.0 (23.0, 31.0)	27.0 (23.0, 32.0)	26.0 (22.0, 31.0)
Country			
Malawi	136 (11.4%)	103 (12.7%)	29 (7.7%)
South Africa	594 (50.0%)	373 (45.9%)	221 (58.9%)
Uganda	126 (10.6%)	100 (12.3%)	27 (7.2%)
Zimbabwe	333 (28.0%)	237 (29.2%)	98 (26.1%)
Highest level of education			
None	11 (0.9%)	10 (1.2%)	1 (0.3%)
Primary, not complete	111 (9.3%)	98 (12.1%)	26 (6.9%)
Primary, complete	65 (5.5%)	50 (6.2%)	18 (4.8%)
Secondary, not complete	466 (39.2%)	313 (38.5%)	136 (36.3%)
Secondary, complete	464 (39.0%)	311 (38.3%)	156 (41.6%)
College or university	72 (6.1%)	31 (3.8%)	38 (10.1%)
Married	537 (45.2%)	383 (47.1%)	135 (36.0%)
Number of live births	2.0 (1.0, 3.0)	2.0 (1.0, 3.0)	2.0 (1.0, 2.0)
Has primary partner	1183 (99.5%)	811 (99.8%)	374 (99.7%)
Partner knows about ring use	779 (65.8%)	530 (65.4%)	239 (63.9%)
Partner is circumcised	509 (43.0%)	331 (40.8%)	162 (43.3%)
Partner HIV status			
Positive	12 (1.0%)	15 (1.8%)	7 (1.9%)
Negative	650 (54.9%)	429 (52.9%)	199 (53.2%)
Don’t know	521 (44.0%)	367 (45.3%)	168 (44.9%)
Multiple sexual partners in the past 3 months	211 (17.7%)	135 (16.6%)	58 (15.5%)
Any new partner in past 3 months	34 (2.9%)	28 (3.4%)	16 (4.3%)
Number of sex acts in last 3 months	21.0 (8.0, 36.0)	24.0 (10.0, 40.0)	15.0 (6.0, 36.0)
Number of sex acts in last 7 days	2.0 (1.0, 3.0)	2.0 (1.0, 4.0)	1.0 (0.0, 3.0)

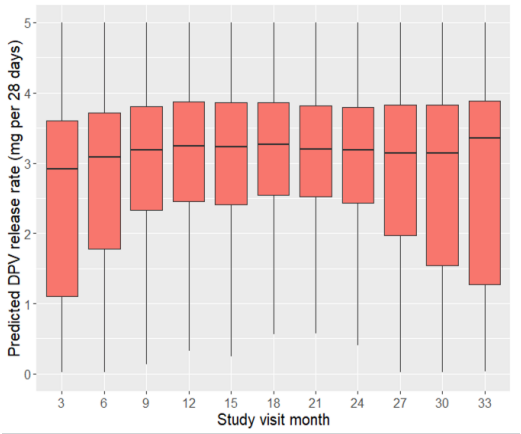
Any condomless sex with partner in last 7 days	524 (44.1%)	359 (44.2%)	125 (33.3%)
Intra-uterine device (IUD)	159 (13.4%)	128 (15.7%)	32 (8.5%)
Oral pills	119 (10.0%)	88 (10.8%)	33 (8.8%)
Hormonal implant	238 (20.0%)	194 (23.9%)	62 (16.5%)
DMPA	501 (42.1%)	279 (34.3%)	173 (46.1%)
Norethisterone enanthate (NET-En)	149 (12.5%)	96 (11.8%)	75 (20.0%)
Any menstrual bleeding in past 3 months	928 (78.0%)	656 (80.8%)	277 (74.1%)
Any vaginal practices	772 (64.9%)	533 (65.6%)	220 (58.7%)
Usual bleeding pattern			
Amenorrheic	254 (21.4%)	147 (18.1%)	99 (26.4%)
Irregular	301 (25.3%)	194 (23.9%)	95 (25.3%)
Regular	634 (53.3%)	472 (58.1%)	181 (48.3%)
Neisseria gonorrhoeae diagnosis	48 (4.0%)	39 (4.8%)	14 (3.7%)
Chlamydia trachomatis diagnosis	126 (10.6%)	96 (11.8%)	51 (13.6%)
Syphilis diagnosis	82 (6.9%)	60 (7.4%)	21 (5.6%)
Bacterial vaginosis	473 (39.8%)	379 (46.6%)	120 (32.0%)

Figure 1. Distributions of imputed sex acts and dapivirine release rates

a. Predicted sex acts across study follow-up, by study group



b. Expected dapivirine release rates among women in active ring group across follow-up



c. Expected dapivirine release rates by sex act category

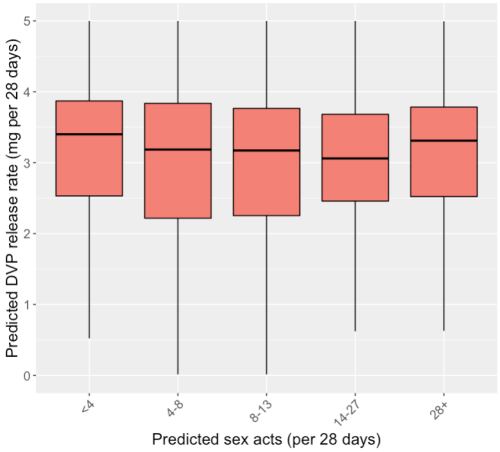


Figure 2. Dose-response curve of per-sex-act risk reduction with incremental dapivirine release rates. Solid line indicates % relative risk reduction estimate. Shaded area indicates 95% confidence interval.

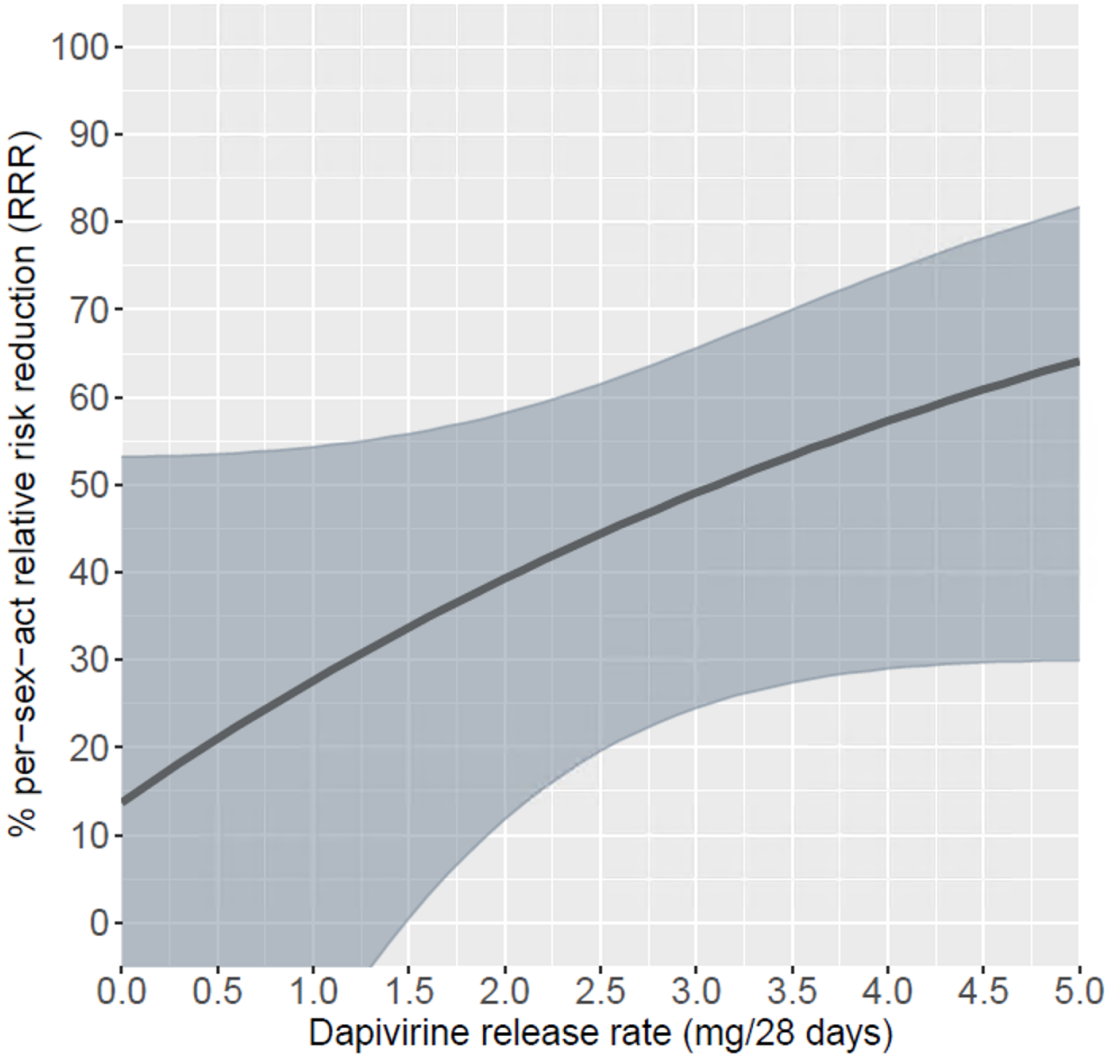


Table 2. Per-sex-act relative risk reduction with consistent dapivirine ring use (4.5 mg/month)

	Dapivirine group: events per 10,000 acts (95% CI)	Placebo group: events per 10,000 acts (95% CI)	Unadjusted		Adjusted[a]		Weighted[a,b]		
			Per-sex-act RRR (95% CI)	*P*	Per-sex-act RRR (95% CI)	*P*	Per-sex-act RRR (95% CI)	*P*	Interaction *P*
Overall	2.3 (1.8, 3.1)	3.6 (2.9, 4.4)	54% (20, 74%)	0.006	60% (28, 78%)	0.002	61% (30, 78%)	0.002	
Baseline age									
<25 years (n=887)	5.0 (3.6, 6.9)	5.3 (3.9, 7.3)	49% (-6, 75%)	0.071	57% (9, 80%)	0.026	57% (9, 80%)	0.027	0.964
≥25 years (n=1490)	1.1 (0.7, 1.8)	2.7 (2.1, 3.7)	47% (-25, 77%)	0.148	54% (-16, 82%)	0.100	56% (-12, 83%)	0.084	
Baseline STIs/BV									
N. gonorrhoeae not detected (n=2276)	2.2 (1.6, 2.8)	3.3 (2.6, 4.2)	56% (19, 76%)	0.008	61% (27, 79%)	0.003	62% (29, 80%)	0.003	0.810
N. gonorrhoeae detected (n=101)	7.2 (3.4, 15.2)	11.0 (5.7, 21.1)	33% (-175, 84%)	0.580	51% (-133, 90%)	0.373	53% (-122, 90%)	0.339	
C. trachomatis not detected (n=2104)	1.9 (1.4, 2.6)	3.2 (2.5, 4.1)	50% (40, 73%)	0.037	54% (10, 76%)	0.023	54% (11, 77%)	0.021	0.319
C. trachomatis detected (n=273)	6.2 (3.7, 10.3)	7.3 (4.5, 11.9)	67% (30, 89%)	0.045	74% (21, 92%)	0.018	77% (28, 92%)	0.012	
BV not detected (n=1405)	2.0 (1.4, 3.0)	2.7 (2.0, 3.7)	46% (-13, 74%)	0.101	49% (-4, 75%)	0.062	51% (-1, 76%)	0.053	0.441

BV detected (n=972)	2.8 (1.9, 4.1)	4.9 (3.7, 6.6)	62% (9, 84%)	0.031	69% (22, 88%)	0.013	69% (22, 88%)	0.014	
Menses/bleeding in past month									
No	2.9 (2.1, 3.9)	4.0 (3.1, 5.2)	43% (-6, 69%)	0.074	51% (50, 75%)	0.034	52% (7, 75%)	0.030	0.209
Yes	1.6 (1.0, 2.7)	2.9 (2.0, 4.2)	77% (27, 93%)	0.013	79% (30, 94%)	0.011	80% (33, 94%)	0.009	

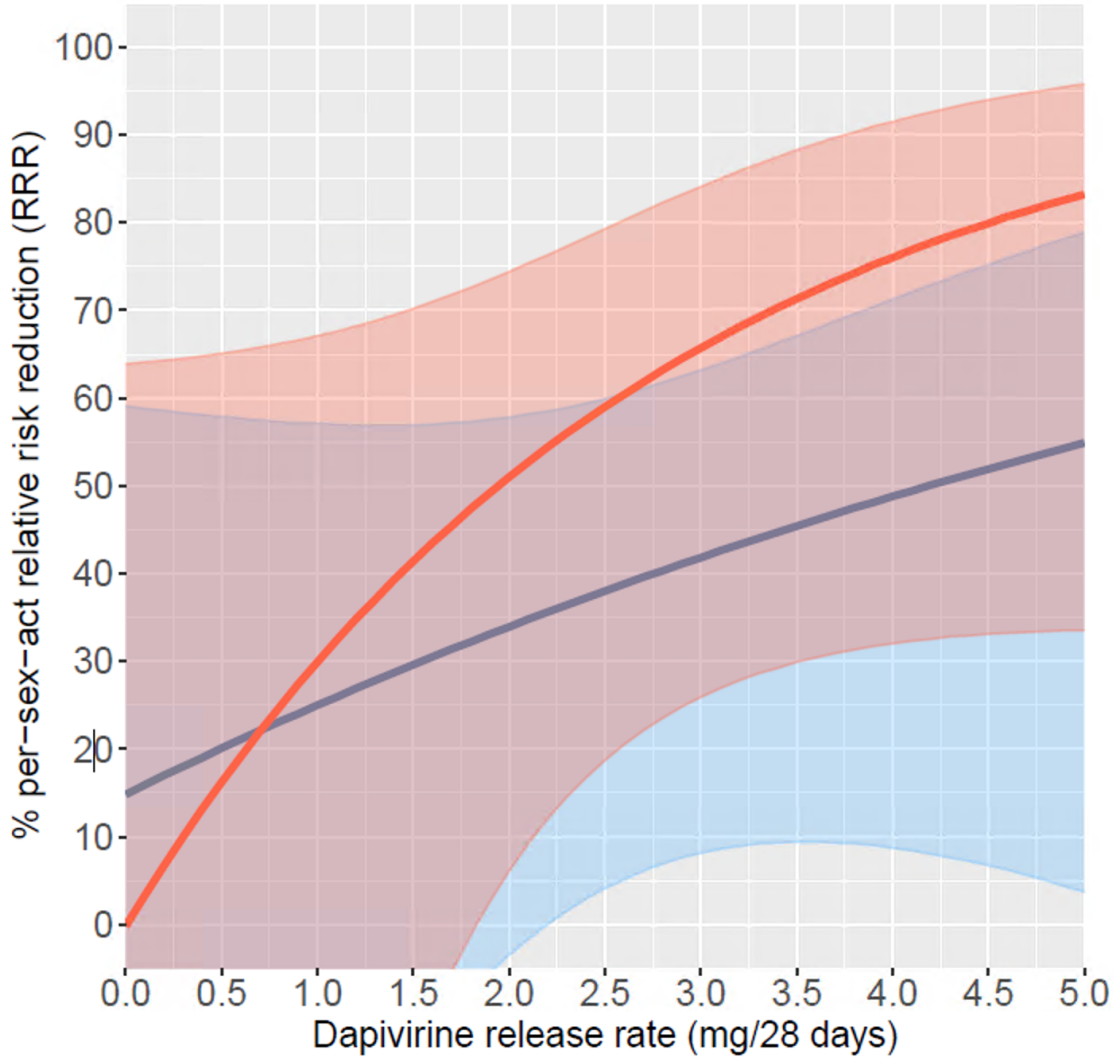
STI: sexually transmitted infection; BV: bacterial vaginosis; RRR: relative risk reduction calculated as (1-hazard ratio)*100; CI: confidence interval

All models were stratified by study site

[a]Multivariable Cox regression models were adjusted for report of any condomless sex and the following baseline variables: age, education, marital status, BV status, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* diagnosis, any new sexual partners, multiple sexual partners, number of sex acts in last 3 months, any condomless sex in last seven days, alcohol use, partner HIV status and knowledge of ring use, family planning method and local HIV prevalence for males within the same age group

[b]Adjusted using inverse probability of censoring weights (ICPWs) to account for selection bias due to participant censoring at first pregnancy, first product hold >3 days and loss-to-follow-up given likely changes in HIV risk after these events

Supplemental Figure 1. Dose-response curve of per-sex-act risk reduction with incremental dapivirine release rates, by experience of menses/bleeding in the past month. Solid line indicates % relative risk reduction estimate. Shaded area indicates 95% confidence interval. Red=menses/bleeding in the past month; Blue=no menses/bleeding in the past month.



Chapter 4. Evaluating short-term changes in bone turnover among young women initiating subcutaneous and intramuscular depot medroxyprogesterone acetate and oral PrEP

Short-term changes in bone turnover among young women initiating subcutaneous and intramuscular depot medroxyprogesterone acetate and oral PrEP

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Abstract

Objectives: Despite being independently linked to reductions in bone mineral density in women, combined use of depot medroxyprogesterone acetate (DMPA) for contraception and oral HIV pre-exposure prophylaxis (PrEP) containing tenofovir disoproxil fumarate (TDF) has not been evaluated. We assessed for changes in short-term bone turnover among women initiating PrEP and either intramuscular DMPA (DMPA-IM) or subcutaneous DMPA (DMPA-SC) and assess for synergistic effects.

Study Design: Prospective cohort among women between the ages of 16-25 from Kampala, Uganda, who were using DMPA-IM or DMPA-SC and/or daily oral PrEP, or neither.

Results: Among 166 women with data available at baseline and 6 months (33% of the 501 women in the total cohort), concentrations of five serum biomarkers indicative of bone resorption (CTX), bone formation (P1NP), and calcium availability and regulation (intact PTH, calcium and phosphorus), were comparable across DMPA and PrEP use groups at baseline. DMPA-IM, but not DMPA-SC users, experienced a positive, small (0.3%) mean change in concentrations of P1NP that significantly differed from non-users (p=0.0095). No differences in 6-month changes in markers of bone resorption or calcium availability were observed among users of DMPA-SC or DMPA-IM or users exhibiting high levels of PrEP use. Additionally, no clear synergistic relationship between PrEP and DMPA use on bone turnover were observed.

Conclusions: Disruptions of bone turnover within the first six months of concurrent DMPA and PrEP use were limited. This points to the safety of women's decision to use TDF-based PrEP and DMPA together for preventing HIV and unintended pregnancy in high HIV burden settings.

Keywords: PrEP, HIV, DMPA, bone turnover, biomarkers

Implications: Data from our analysis suggest that concurrent use of PrEP and DMPA to prevent HIV and unintended pregnancy results in little impact on bone turnover for young women in the short-term. More data are needed to evaluate the implications of longer concomitant PrEP and DMPA use.

1. Introduction

Nearly half of women who use modern contraception to space or limit pregnancies in sub-Saharan Africa use an injectable method, particularly intramuscular DMPA 150 mg (DMPA-IM or Depo-Provera) [25]. DMPA-IM is typically administered in the upper arm or buttock, which allows for discreet use, and provides up to three months of contraceptive protection. Recently, a lower dose 104mg subcutaneous formulation (DMPA-SC or Sayana Press) was introduced in several sub-Saharan African countries that exhibits similar contraceptive effectiveness, duration and side effect profiles as DMPA-IM [26-28]. DMPA-SC, however, provides additional convenience as it can be self-injected by the user into the thigh or abdomen, eliminating the need for women to travel to the clinic for re-injection. Data have shown that self-injection of DMPA-SC is feasible [60], acceptable [61], and is associated with better method continuation compared to receiving the injection at a clinic [62].

DMPA use is particularly high in areas of sub-Saharan Africa with high HIV prevalence [63]. Given the rapid rollout of oral pre-exposure prophylaxis (PrEP) for HIV in the region over the past decade, more women will likely be concurrently using DMPA and PrEP in the years to come. Therefore, it is important to consider the unintended effects that concomitant use of these medications has on women's health. Numerous clinical studies have identified an association between DMPA use, in either the intramuscular or subcutaneous form, and reduced bone mineral density (BMD) [29-35]. Evidence also suggests that the effect of DMPA on BMD is more pronounced in adolescent populations, raising concerns that DMPA use could prevent young women from achieving their maximum normal peak bone mass [31,32,64-66]. Use of tenofovir disoproxil fumarate (TDF) has also been independently linked to subclinical loss of BMD, including among women in sub-Saharan Africa using TDF-based PrEP[36,37].

Despite independent associations of DMPA and PrEP with BMD reduction, it is not known whether a synergistic relationship exists between DMPA and PrEP exposure and bone health outcomes, which could exacerbate the detrimental impact of either, resulting in the need for

modified counseling and/or different product combinations. Addressing this question will be especially pertinent for young women who are still acquiring bone mass and could be prevented from attaining their full potential bone mass. In this prospective analysis, we evaluated changes in short-term bone turnover among women initiating PrEP and either DMPA-IM and DMPA-SC and evaluated synergistic effects related to concurrent use of DMPA and PrEP

2. Material and methods

2.1. Study procedures

We conducted a secondary analysis of data collected within the Kampala Women's Bone Study, a two-year prospective cohort study of bone health outcomes among young women in Uganda offered DMPA and PrEP. From May 2018 to March 2020, women between the ages of 16-25 years from Kampala, Uganda, who were eligible for PrEP under Uganda national guidelines (including HIV-uninfected women who are in serodiscordant relationships, have had unprotected sex with an individual with unknown HIV status, have had transactional sex or a recent STI diagnosis[67]), had initiated DMPA within the past 90 days or were using condoms for contraception, were sexually active and had no intentions of getting pregnant or moving away from the study area in the following 24 months were enrolled. Women who opted to use DMPA either received their choice of the DMPA-IM or DMPA-SC injection from a trained provider at their enrollment visit or from another family planning provider prior to enrollment. All women in the study were offered PrEP at enrollment and all follow-up study visits in alignment with their potential exposure to HIV and discussions with study counselors. Those who accepted PrEP received counseling on medication adherence and were given enough pills to allow for once daily use until the following study visit. All women enrolled in the study received risk reduction and contraception counseling as well as HIV testing with pre- and post-test counseling at each visit. Scheduled study follow-up visits took place one and three months after enrollment, and quarterly thereafter for up to 24 months.

2.2. Data collection

At study enrollment, women were asked by study staff about their socio-demographic characteristics. Additional information was collected through interviewer-administered standardized questionnaires at enrollment and follow-up visits about women's medical histories (last menstrual period, history of bone fracture), dates and types of past contraceptive use, sexual behaviors, PrEP use (for women who were dispensed PrEP at their last visit), diet, physical activity, and alcohol, cigarette and drug use. Physical examinations were conducted to collect biometric data, including height and weight and body site circumferences. At enrollment and annual study visits women underwent dual-energy x-ray absorptiometry (DXA) scanning to collect BMD measurements for the lumbar spine, total hip and neck of the hip in addition to fat and lean muscle mass and other anthropometric data.

The dates of women's DMPA injections were recorded in study case report forms. Dates of first DMPA injections that occurred prior to study enrollment visit were retrieved either through medical chart review or, if unavailable, by participant self-report. PrEP adherence was evaluated through self-report (number of daily doses missed in the past month and general patterns of pill usage) and by an electronic adherence monitoring pill box that recorded a date-time stamp every time the box was closed (Wisepill, Wisepill Technologies, South Africa). Women also self-reported the number of times they took multiple pills out of the device or opened the device but did not remove any pills.

2.3. Laboratory procedures

To assess bone turnover, blood and urine specimens were collected at baseline and each quarterly visit; serum and urine were archived at -80^{o}C and then shipped to the University of Washington for processing through the Department of Laboratory Medicine Research Testing Service. Serum concentrations of CTX-I (CrossLaps) and the amino terminal peptide from

procollagen type 1 (P1NP), measures of bone resorption and formation, respectively, were determined using the iSYS automated immunoassay platform from Immunodiagnostic Systems (IDS; Tyne and Wear, UK). Serum concentrations of parathyroid hormone (PTH), a marker of calcium availability, as well as estradiol were determined using the DxI 800 automated immunoassay platform from Beckman Coulter (Brea, CA). Serum concentrations of calcium and phosphate, and total protein and urine concentrations of creatinine, glucose, and albumin were determined using the AU automated chemistry platform from Beckman Coulter.

2.4. Statistical analysis

For this analysis, we included all women who had bone turnover biomarkers measured at both their enrollment and month 6 study visits. Baseline participant characteristics were summarized using medians and interquartile ranges (IQR) for continuous variables and frequencies and percentages for categorical variables. Women who were included in the analysis were compared to women who were excluded due to missing laboratory results using a chi-square test for categorical variables and the Wilcoxon rank-sum test for continuous variables. We defined our primary outcome as the percent change in biomarker concentrations between enrollment and month 6 and we used t-tests to assess differences in mean percent change in biomarker concentrations between enrollment DMPA group (DMPA-IM, DMPA-SC, no DMPA) and PrEP group (declined or accepted PrEP). To assess potential interactions between DMPA and PrEP use, we used linear regression to assess differences in biomarkers across DMPA use categories among women who exhibited no or low PrEP use versus high PrEP use, according to Wisepill opening data. To categorize adherence, daily Wisepill opening data were summarized by calculating the total number of expected days of pill use (equal to the number of the days where the Wisepill device was in women's possession and recorded an opening event or signal) and the total number of Wisepill opening events. Women were considered to have high adherence if they had Wisepill data collected at each visit between enrollment and month 6 and had an opening

event on >80% of expected use days across the six-month period. Women with incomplete Wisepill collection or opening events on ≤80% days of expected use were classified as having lower PrEP adherence. Models were adjusted for potential confounders known to be associated with DMPA, PrEP, and/or bone health, including age, number of servings of foods containing calcium or vitamin D in the past week and baseline serum estradiol concentrations. Due to small sample sizes, formal tests for interaction were not conducted. Given that baseline biomarker concentrations could be influenced by DMPA injections received prior to the enrollment date, and women could switch their contraceptive method over the course of the study, we conducted a sensitivity analysis that limited DMPA-IM and DMPA-SC groups to women who began their respective method at enrollment and had continuous use of the method throughout the six-month period without any delay in the subsequent injection (i.e., subsequent injection occurred within 119 days after the prior injection). Statistical analyses were carried out in R version 3.6.1 (R Core Team, Vienna, Austria) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) statistical software.

2.5. Ethics Statement

The protocol for the parent study received ethical approval from the Human Subjects Division at the University of Washington as well as the institutional review boards at the National Council of Science and Technology, National HIV/AIDS Research Committee and the National Drug Association in Uganda. All participants provided written informed consent.

3. Results

Of 501 women enrolled in the Kampala Women's Bone Study, 166 who had baseline and 6-month data quantifying biomarker concentrations were included in this secondary analysis. At enrollment, 94 (57%) of these women chose to use DMPA-IM, 8 (5%) chose to use DMPA-SC

and 64 (39%) chose to use condoms only as their contraceptive method. PrEP was initiated by 151 women (91%) at enrollment. Women in the DMPA-IM, DMPA-SC and no DMPA groups had similar baseline characteristics, including age, body composition, level of physical activity, diet, alcohol and cigarette use, and PrEP initiation (Table 1). Approximately 5% of women who did not receive a DMPA injection at enrollment had a prior bone fracture compared to 0% and 1% of women who received DMPA-SC and DMPA-IM injections, respectively. Compared to women who were excluded from the analysis due to missing biomarker data, women who were included in the analysis were more likely to have a partner who is living with HIV, have had a previous bone fracture, have lower lumbar spine BMD z-score, have had more servings of calcium and have consumed alcohol in the past month (Supplemental Table 1). Included women were also more likely to have had multiple sexual partners and any new partner in the last three months.

At month 6, serum estradiol concentrations were significantly lower for DMPA-IM (median 40.4, IQR: 27.0-60.6; Wilcoxon rank sum test $p<0.001$) users but not DMPA-SC users (median 58.5, IQR: 36.5-71.7; $p=0.10$) relative to women who did not use DMPA (median 112.1, IQR: 58.8-232.3) (Supplemental Figure 1). Concentrations of the five biomarkers of interest, CTX, P1NP, Intact PTH, calcium and phosphorus were similar among women in the three DMPA user groups at baseline (Table 2). The greatest changes in biomarker concentrations between baseline and month 6 visits were observed for CTX, the marker of bone resorption, where mean concentration changes range from -18.1% (DMPA-SC) to 32.6% (no DMPA) and 36.5% (DMPA-IM). Differences in CTX between women who opted for DMPA-IM and DMPA-SC versus no DMPA at enrollment were not statistically significant. DMPA-IM users were the only group that experienced a positive, albeit small (0.3%), mean change in concentrations of P1NP, the marker of bone formation. This change was significantly different compared to non-users ($p=0.0095$). Changes in intact PTH, calcium and phosphorus did not significantly differ between DMPA groups.

Between women who accepted and declined PrEP at enrollment, mean changes in each of the measured biomarker between enrollment and month six did not significantly differ (Table 3). Mean changes were highest for CTX, which ranged from 25.7% among women who declined PrEP to 33.0% among women who accepted PrEP. The mean percent change in eGFR was comparable between women who accepted and declined PrEP at enrollment (-1.29% $\pm$ 5.1% versus -1.28% $\pm$ 4.5%, respectively; p=0.99) (Supplemental Figure 2).

Mean percentage change in biomarker concentrations by DMPA use and PrEP acceptance are illustrated in Figure 1; the largest mean percentage changes were observed in CTX for women who were using only PrEP and women using DMPA-IM with or without PrEP at enrollment. In adjusted subgroup analyses designed to evaluate interactions between PrEP and DMPA use, we did not observe significant differences in mean percent change between DMPA users and non-users among groups of women who exhibited high PrEP use or no/low PrEP use for any of the measured biomarkers (Table 4). Qualitatively, mean differences in percent changes between DMPA-IM users and non-DMPA users were higher among women also exhibiting higher levels of PrEP use for CTX-1, P1NP, intact PTH and phosphorus.

Of the 94 women who opted to use DMPA-IM at enrollment, 40 received their first injection prior to the enrollment visit. An additional 19 women included in the analysis switched their family planning method between enrollment and the month 6 visit. In a sensitivity analysis excluding these women and focusing on women who continuously used their enrollment method until month 6, we similarly found no significant differences between DMPA users and non-users within high and no/low PrEP user groups (Supplemental Table 1). Trends in directionality and magnitude of the mean percent change in biomarker concentrations between DMPA groups within PrEP use subgroups were similar to the primary analytic cohort, except that DMPA-IM users had a negative mean percent change in CTX relative to non-users among women with high PrEP use (mean

difference in % change of -24.6% [95% CI: -114.1-64.9] versus 8.8 [95% CI: -128.6-146.1] in the primary sample).

4. Discussion

In this secondary analysis of biological specimens collected from women age <25 years in Kampala, Uganda, we identified minimal differences in the rate of change in bone turnover markers within the first six months of DMPA or PrEP use, including with concurrent use. Of five markers of bone turnover measured, women who used the higher dose, intramuscular formulation of DMPA experienced a significantly higher increase in P1NP concentrations, indicative of increased bone formation. However, no differences in 6-month changes in markers of bone resorption or calcium availability were observed among users of DMPA-SC or DMPA-IM or users exhibiting high levels of PrEP use. Additionally, we did not observe any clear synergistic relationship between PrEP and DMPA use on bone turnover.

Despite clear data demonstrating a detrimental impact of DMPA on bone density, studies assessing biomarkers of bone turnover among DMPA users are limited and have presented mixed findings. Multiple studies have found DMPA use to be associated with higher concentrations of biomarkers of bone resorption, including urine N-terminal telopeptide of type I collagen (NTX) and deoxypyridinoline [68,69], as well as higher concentrations of markers of bone formation, including serum P1NP and osteocalcin [69,70]. Others did not observe changes in osteocalcin and other markers of bone resorption [68]. Additionally, changes in PTH or calcium have not been linked with DMPA use[68]. Therefore, our finding of a greater increase in concentrations of P1NP but no significant differences in changes in calcium or PTH among DMPA-IM users agree in part with these studies. Reasons for differences across studies can be related to differing lengths of DMPA exposure among participants and inconsistent control for individual-level factors that may influence biomarker concentrations such as age, diet, endogenous hormone concentrations, and specific gravity for urine samples.

Among women who have used TDF/FTC for PrEP, two studies have noted bone loss with medication use [36,37], including a finding of 1.4% and 0.9% lower BMD in the lumbar spine and total hip among highly adherent PrEP users in the VOICE trial compared to placebo pill users [37]. Only one study to date has assessed changes in biomarker concentrations among individuals using TDF/FTC for PrEP. Among a random sample of individuals randomized to the active PrEP arm in the Partners PrEP Study, concentrations of CTX were found to be significantly higher after 24 months of medication use relative to a random sample of participants from the placebo arm[71].

Observed bone loss with PrEP use is thought to be mediated through subclinical kidney injury given the key role that the kidneys play in regulating systemic calcium and phosphorus levels through bone turnover. This is supported by data from the Partners PrEP Study that showed a small decline in estimated glomerular filtration rate (eGFR) among male and females in Africa after 24 months of TDF and TDF/FTC user[72]. However, in contrast to the findings from the Partners PrEP Study [71], we did not observe a significant difference in percent change of CTX or any other biomarker of bone turnover among adherent PrEP users. While this could simply be explained by our limited sample size, another possible reason could be that more than 6 months of PrEP exposure is needed to induce the levels of kidney function injury needed to impact bone turnover. This is supported by the finding that percent change in eGFR did not significantly differ at month 6 between women who accepted and declined PrEP. More research on the relationship between kidney function and bone health among PrEP users is needed and planned within this ongoing study.

Some potential limitations of our analysis should be considered. First, given that only a portion of laboratory data were available at the time of analysis, our sample is small and may not be representative of the full cohort. This limits the power to compare biomarker changes between study groups, particularly comparisons involving DMPA-SC users, as well as our ability to conduct formal tests of interaction between PrEP and DMPA. We plan to re-run our analysis with complete

data from all participants when available. Second, biomarker concentrations may have been influenced by individual and environmental factors that may have not been measured or were measured inadequately in our study, such as circadian and seasonal variation, menstrual cycle, and underlying medical conditions. Third, use of Wisepill devices to assess PrEP adherence is limited in that it is a measure of device usage and not actual pill consumption. Additionally, use of the devices was not mandatory and thus some women may not have used them consistently. Finally, these results may not be generalizable to women in older age groups or women in other settings.

The results from our analysis indicate that disruptions of bone turnover with DMPA and PrEP use are limited within the first six months of medication use. This points to the safety of young women's decisions to use a combination of PrEP and DMPA for concurrent prevention of HIV and unintended pregnancy in high HIV burden settings. More research is needed however to further explore bone turnover with longer PrEP and DMPA exposure as well as the biological mechanisms by which PrEP use acts on bone turnover.

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Table 1. Baseline participant characteristics

	No DMPA (N=64)	DMPA-SC (N=8)	DMPA-IM (N=94)
	N (%) or median (IQR)		
Socio-demographics			
Age (years)	20.0 (19.0, 22.0)	20.5 (18.8, 22.5)	19.5 (18.0, 21.0)
Married			
Single with no steady partner	10 (15.6%)	1 (12.5%)	14 (14.9%)
Single with a steady partner	53 (82.8%)	7 (87.5%)	80 (85.1%)
Married	1 (1.6%)	0 (0.0%)	0 (0.0%)
Widowed, divorced or separated	0 (0.0%)	0 (0.0%)	0 (0.0%)
Lives with partner			
Yes	3 (4.7%)	0 (0.0%)	6 (6.4%)
No	56 (87.5%)	8 (100.0%)	78 (83.0%)
No Partner	5 (7.8%)	0 (0.0%)	10 (10.6%)
Years of education	11.0 (10.0, 12.0)	7.5 (6.8, 11.5)	9.0 (7.0, 11.0)
Body composition and bone health			
BMI (kg/m^2)	22.4 (20.5, 25.0)	22.1 (20.7, 24.3)	22.7 (20.6, 24.7)
% body fat	36.4 (30.8, 38.7)	35.8 (31.4, 40.2)	34.1 (30.3, 37.5)
Waist circumference (cm)	81.8 (74.4, 88.7)	78.1 (75.2, 81.4)	80.4 (74.1, 86.2)
Fat-to-lean mass ratio	0.6 (0.4, 0.6)	0.6 (0.5, 0.7)	0.5 (0.4, 0.6)
Previous broken bone			
Yes	3 (4.7%)	0 (0.0%)	1 (1.1%)
No	60 (93.8%)	8 (100.0%)	93 (98.9%)
Not Sure	1 (1.6%)	0 (0.0%)	0 (0.0%)
BMD total hip (g/cm^2)	1.0 (0.9, 1.0)	0.9 (0.9, 1.0)	0.9 (0.9, 1.0)
Z-score total hip	0.1 (-0.7, 0.7)	0.1 (-0.1, 0.3)	-0.2 (-0.8, 0.7)
BMD lumbar spine (g/cm^2)	0.9 (0.9, 1.0)	0.9 (0.9, 1.0)	0.9 (0.9, 1.0)
Z-score lumbar spine	-0.8 (-1.4, -0.2)	-0.8 (-1.5, -0.2)	-1.0 (-1.5, -0.3)
TBS spine L1-L4	1.4 (1.3, 1.4)	NA	1.3 (1.3, 1.4)
BMD neck of the hip (g/cm^2)	0.9 (0.8, 0.9)	0.9 (0.8, 0.9)	0.8 (0.8, 0.9)
Z-score neck of the hip	0.2 (-0.4, 0.9)	0.0 (-0.3, 0.2)	-0.1 (-0.6, 0.6)
PrEP and sexual behaviors			
PrEP accepted	57 (89.1%)	8 (100.0%)	86 (91.5%)
Number of sex acts in past 3 months	12.0 (8.0, 36.0)	26.0 (18.8, 39.5)	35.0 (12.0, 97.5)
Any condomless sex in past 3 months	41 (64.1%)	4 (50.0%)	66 (70.2%)
Condom used at last sex	42 (65.6%)	7 (87.5%)	51 (54.3%)

Number of sexual partners in last 3 months	2.0 (1.0, 3.0)	2.5 (2.0, 5.0)	2.0 (1.0, 9.8)
Any new partners in last 3 months	18 (28.1%)	5 (62.5%)	44 (46.8%)
Partner HIV status			
Positive	3 (4.7%)	0 (0.0%)	4 (4.3%)
Negative	30 (46.9%)	1 (12.5%)	23 (24.5%)
Not Sure	31 (48.4%)	7 (87.5%)	67 (71.3%)
Diet and lifestyle			
Time spent in the sun each day			
<1 hour	50 (78.1%)	7 (87.5%)	70 (74.5%)
1-3 hours	12 (18.8%)	1 (12.5%)	22 (23.4%)
>3 hours	2 (3.1%)	0 (0.0%)	2 (2.1%)
Regular physical activity (>3 hours/week)	63 (98.4%)	8 (100.0%)	93 (98.9%)
Weekly servings of foods high in Vitamin D	7.0 (3.0, 12.5)	3.0 (2.0, 10.2)	7.0 (4.0, 10.2)
Weekly servings of foods high in calcium	10.0 (5.8, 15.0)	11.0 (8.0, 16.8)	11.0 (6.2, 14.0)
Any alcohol in past month	19 (29.7%)	2 (25.0%)	29 (30.9%)
Any cigarette smoking in past month	1 (1.6%)	0 (0.0%)	0 (0.0%)
Any recreational drugs in past month	4 (6.2%)	0 (0.0%)	3 (3.2%)

Table 2. Changes in biomarker concentrations among baseline DMPA use groups

	Baseline		Month 6			
	N	Mean (SD)	N	Mean (SD)	Mean % difference from baseline (SD)	p-value
CTX (ng/mL)						
No DMPA	64	0.3 (0.3)	64	0.3 (0.3)	32.6% (130.6%)	Ref
DMPA-SC	8	0.2 (0.1)	8	0.2 (0.1)	-18.1% (67.7%)	0.1005
DMPA-IM	94	0.4 (0.4)	94	0.4 (0.3)	36.5% (117.5%)	0.8515
P1NP (ng/mL)						
No DMPA	64	112.9 (51.3)	64	100.2 (50.6)	-10.1% (19.9%)	Ref
DMPA-SC	8	107.7 (61.0)	8	95.9 (57.7)	-8.9% (23.7%)	0.8982
DMPA-IM	94	122.6 (59.4)	94	115.6 (51.7)	0.3% (29.6%)	0.0095
Intact PTH (pg/mL)						
No DMPA	64	39.4 (14.1)	64	36.6 (13.0)	0.0% (38.0%)	Ref
DMPA-SC	8	43.0 (13.0)	8	34.0 (11.1)	-17.7% (27.3%)	0.1285
DMPA-IM	94	41.1 (20.1)	94	36.1 (16.5)	0.0% (59.8%)	0.9958
Calcium (mg/dL)						
No DMPA	63	9.3 (0.3)	63	9.3 (0.4)	-0.2% (3.9%)	Ref
DMPA-SC	8	9.6 (0.4)	8	9.6 (0.5)	0.7% (4.1%)	0.5841
DMPA-IM	92	9.3 (0.3)	92	9.3 (0.3)	0.1% (3.8%)	0.7121
Phosphorus (mg/dL)						
No DMPA	64	3.4 (0.5)	64	3.5 (0.6)	4.4% (17.7%)	Ref
DMPA-SC	8	3.4 (0.3)	8	3.4 (0.6)	1.6% (19.6%)	0.7076
DMPA-IM	94	3.6 (0.6)	94	3.6 (0.5)	2.8% (20.2%)	0.6013

DMPA: depot medroxyprogesterone acetate; DMPA-IM: intramuscular DMPA 150 mg; DMPA-SC: subcutaneous DMPA 104 mg; CTX-1: C-terminal telopeptide 1; P1NP: N-terminal propeptide of type I procollagen; PTH: parathyroid hormone

p-values generated with t-test

Table 3. Changes in biomarker concentrations among baseline PrEP use groups

	Baseline		Month 6			
	N	Mean (SD)	N	Mean (SD)	Mean % difference from baseline (SD)	p-value
CTX (ng/mL)						
PrEP accepted	151	0.4 (0.3)	151	0.4 (0.3)	33.0% (121.9%)	0.8165
PrEP declined	15	0.3 (0.2)	15	0.3 (0.2)	25.7% (114.2%)	Ref
P1NP (ng/mL)						
PrEP accepted	151	119.7 (57.2)	151	110.5 (53.2)	-3.9% (26.8%)	0.6804
PrEP declined	15	102.7 (45.4)	15	91.0 (32.5)	-6.5% (21.9%)	Ref
Intact PTH (pg/mL)						
PrEP accepted	151	40.8 (17.3)	151	36.8 (15.1)	0.2% (52.0%)	0.3299
PrEP declined	15	38.4 (21.6)	15	29.9 (11.9)	-11.2% (40.8%)	Ref
Calcium (mg/dL)						
PrEP accepted	148	9.3 (0.3)	148	9.3 (0.4)	0.2% (3.9%)	0.1230
PrEP declined	15	9.5 (0.3)	15	9.3 (0.3)	-1.3% (3.4%)	Ref
Phosphorus (mg/dL)						
PrEP accepted	151	3.5 (0.5)	151	3.5 (0.5)	3.1% (19.1%)	0.6265
PrEP declined	15	3.4 (0.4)	15	3.5 (0.5)	5.9% (20.3%)	Ref

SD: standard deviation; PrEP: pre-exposure prophylaxis; CTX-1: C-terminal telopeptide 1; P1NP: N-terminal propeptide of type I procollagen; PTH: parathyroid hormone

p-values generated with t-test

Figure 1. Mean percent change in biomarker concentrations by DMPA use and PrEP acceptance at enrollment

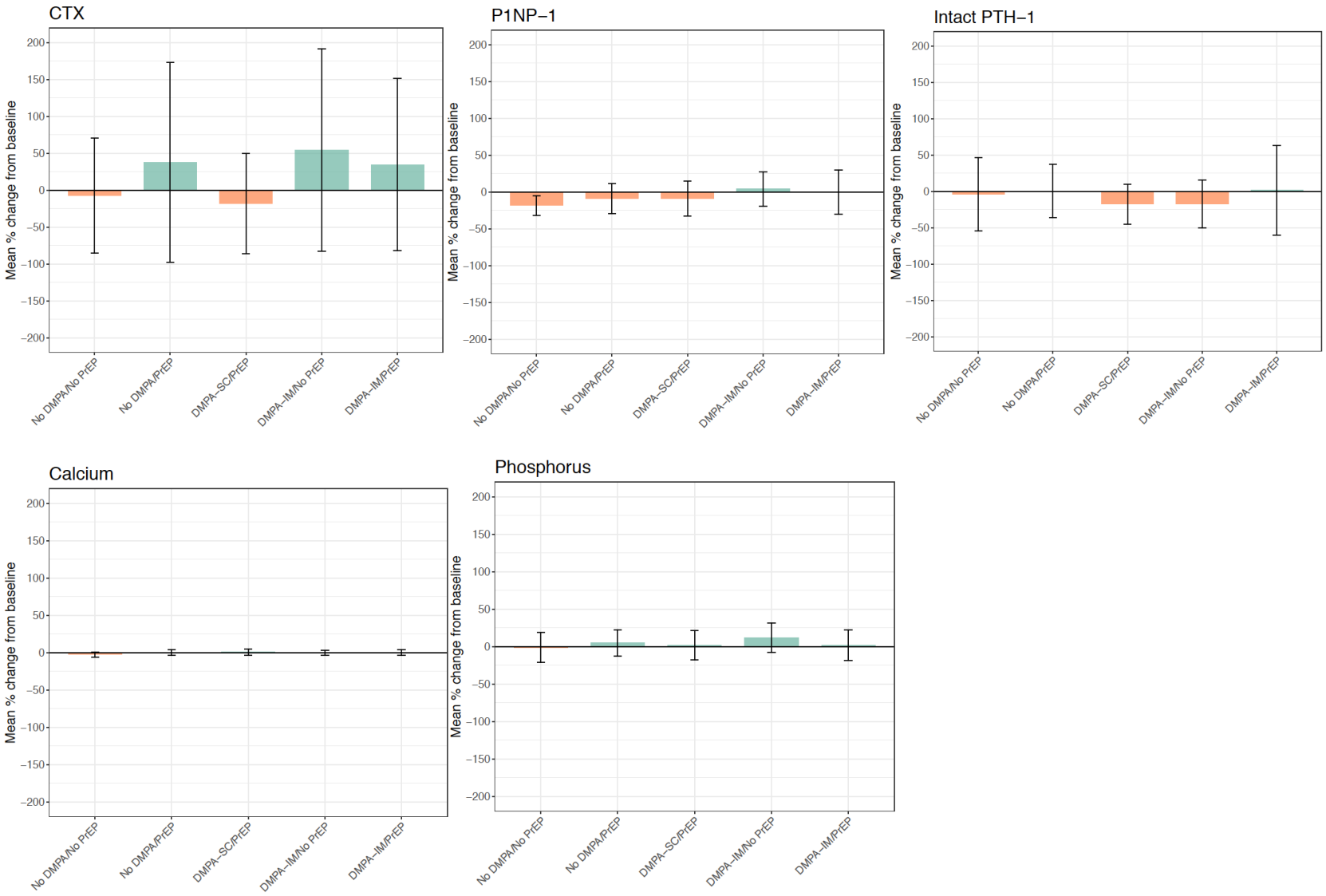


Table 4. Assessing Interactions between PrEP and DMPA on changes in bone turnover biomarkers

	No DMPA		DMPA-SC		DMPA-IM		DMPA-SC vs. No DMPA		DMPA-IM vs. No DMPA	
	N	Mean % change from baseline (SD)	N	Mean % change from baseline (SD)	N	Mean % change from baseline (SD)	Mean difference (95% CI)	p-value	Mean difference (95% CI)	p-value
CTX (ng/mL)	.		.		.					
High PrEP use	10	14.8 (77.9)	3	24.2 (98.2)	14	44.6 (148.3)	-3.4 (-191.8-185.1)	0.97	8.8 (-128.6-146.1)	0.90
No/Low PrEP use	54	36.0 (138.5)	5	-43.5 (32.5)	80	35.0 (112.4)	-87.9 (-205.3-29.5)	0.14	-7.8 (-54.2-38.6)	0.74
P1NP (ng/mL)	.		.		.					
High PrEP use	10	-12.8 (14.6)	3	-12.6 (40.3)	14	0.9 (39.3)	-4.8 (-56.2-46.7)	0.85	10.8 (-26.6-48.3)	0.55
No/Low PrEP use	54	-9.6 (20.8)	5	-6.7 (12.4)	80	0.2 (27.8)	2.6 (-20.3-25.5)	0.82	7.8 (-1.3-16.8)	0.09
Intact PTH (pg/mL)	.		.		.					
High PrEP use	10	-15.9 (28.3)	3	-24.0 (37.2)	14	5.6 (124.8)	33.4 (-102.6-169.4)	0.61	33.5 (-65.6-132.7)	0.49
No/Low PrEP use	54	2.9 (39.0)	5	-13.9 (23.8)	80	-0.9 (40.5)	-15.4 (-52.8-22.0)	0.42	-3.2 (-18.0-11.6)	0.67
Calcium (mg/dL)	.		.		.					
High PrEP use	9	-0.6 (3.4)	3	0.5 (7.0)	14	0.3 (4.2)	0.2 (-6.3-6.7)	0.96	-0.4 (-5.1-4.3)	0.86
No/Low PrEP use	54	-0.1 (4.0)	5	0.9 (2.2)	78	0.0 (3.8)	0.1 (-3.5-3.8)	0.95	-0.5 (-2.0-0.9)	0.46
Phosphorus (mg/dL)	.		.		.					
High PrEP use	10	-2.1 (10.5)	3	4.3 (30.0)	14	3.2 (32.5)	0.5 (-40.3-41.4)	0.98	10.6 (-19.1-40.4)	0.47
No/Low PrEP use	54	5.6 (18.6)	5	-0.0 (14.6)	80	2.8 (17.5)	-1.1 (-16.8-14.6)	0.89	-1.2 (-7.4-5.0)	0.70

Supplemental Table 1. Assessing Interactions between PrEP and DMPA on changes in bone turnover biomarkers among women with continuous method use

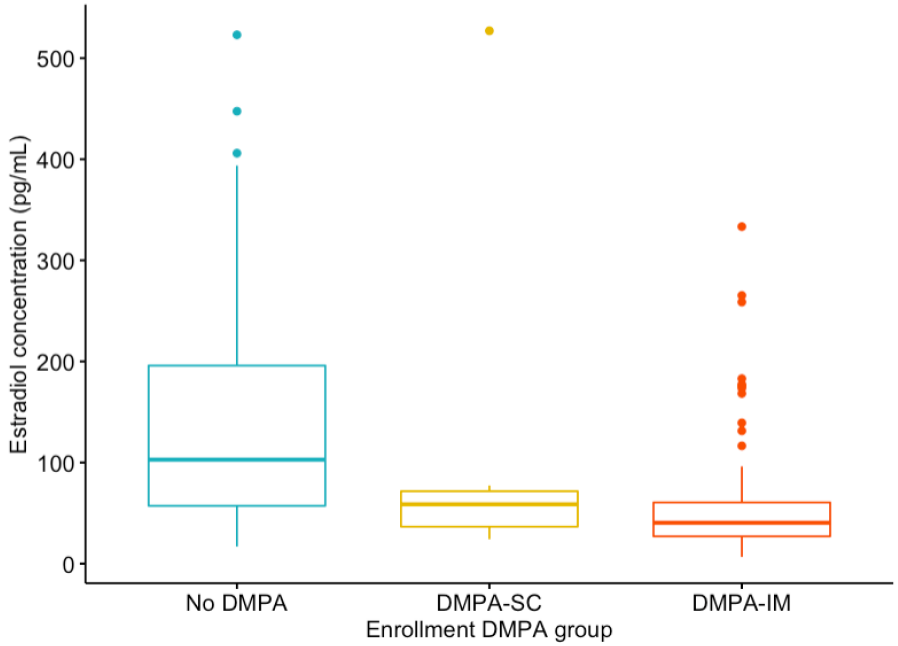
	No DMPA		DMPA-SC		DMPA-IM		DMPA-SC vs. No DMPA		DMPA-IM vs. No DMPA	
	N	Mean % change from baseline (SD)	N	Mean % change from baseline (SD)	N	Mean % change from baseline (SD)	Mean difference in % change (95% CI)	p-value	Mean difference in % change (95% CI)	p-value
CTX (ng/mL)										
High use	10	14.8 (77.9)	2	59.5 (108.6)	10	-7.4 (72.7)	26.0 (-123.8-175.7)	0.72	-24.6 (-114.1-64.9)	0.57
No/Low use	52	34.7 (138.4)	4	-38.5 (35.3)	29	55.6 (149.0)	-92.9 (-245.0-59.2)	0.23	6.3 (-63.9-76.4)	0.86
P1NP (ng/mL)										
High use	10	-12.8 (14.6)	2	-33.9 (23.1)	10	-8.6 (38.2)	-27.1 (-86.8-32.5)	0.35	3.9 (-31.8-39.5)	0.82
No/Low use	52	-9.4 (20.9)	4	-3.8 (12.1)	29	-7.5 (22.0)	4.1 (-18.1-26.2)	0.72	1.2 (-9.0-11.5)	0.81
Intact PTH (pg/mL)										
High use	10	-15.9 (28.3)	2	-45.2 (8.5)	10	8.1 (149.0)	2.8 (-191.6-197.2)	0.98	31.6 (-84.5-147.8)	0.57
No/Low use	52	4.3 (38.9)	4	-12.7 (27.3)	29	-12.2 (37.0)	-12.2 (-52.7-28.3)	0.55	-14.0 (-32.7-4.7)	0.14
Calcium (mg/dL)										
High use	9	-0.6 (3.4)	2	4.3 (2.9)	10	1.1 (4.5)	4.3 (-3.1-11.7)	0.23	0.2 (-4.3-4.6)	0.93
No/Low use	52	-0.2 (4.0)	4	1.6 (1.8)	29	-0.3 (4.1)	-0.04 (-4.1-4.0)	0.98	-1.2 (-3.1-0.6)	0.19
Phosphorus (mg/dL)										
High use	10	-2.1 (10.5)	2	21.0 (11.4)	10	-0.5 (36.6)	23.2 (-29.7-76.2)	0.94	9.0 (-22.7-40.7)	0.55
No/Low use	52	5.1 (18.7)	4	3.3 (14.5)	29	3.0 (18.5)	0.3 (-17.1-17.7)	0.97	-1.8 (-9.9-6.2)	0.65

Supplemental Table 2. Comparison of baseline characteristics of women who were included and excluded from the analysis based on availability of biomarker data

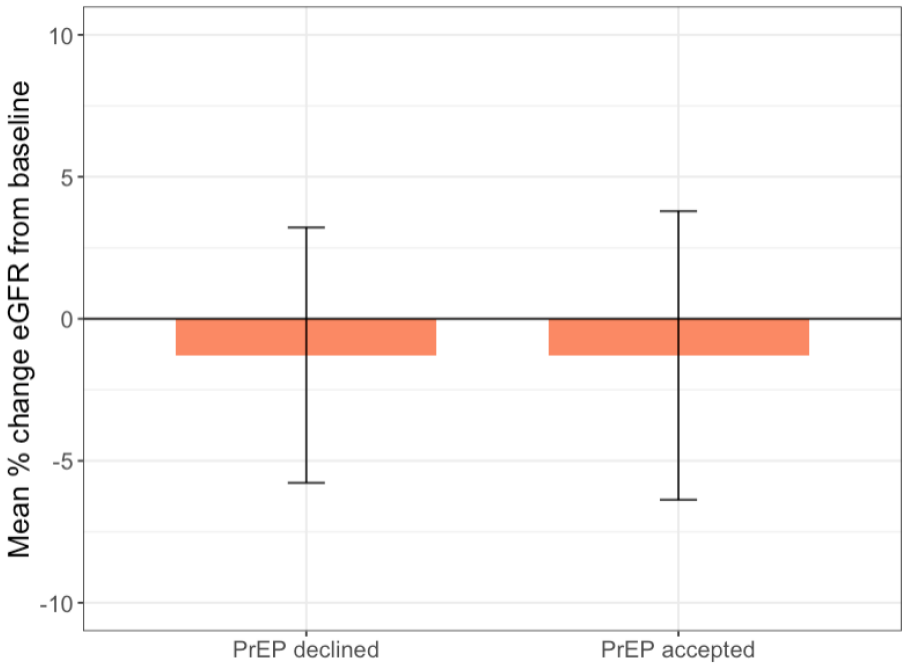
	Included (N=166)	Excluded (N=333)	p value
	N (%) or median (IQR)		
Age (years)	20.0 (19.0, 22.0)	20.0 (18.0, 21.0)	0.069
Married			0.525
Single with no steady partner	25 (15.1%)	37 (11.1%)	
Single with a steady partner	140 (84.3%)	290 (87.1%)	
Married	1 (0.6%)	4 (1.2%)	
Widowed, divorced or separated	0 (0.0%)	2 (0.6%)	
Lives with partner			<0.001
Yes	9 (5.4%)	15 (4.5%)	
No	142 (85.5%)	312 (93.7%)	
No Partner	15 (9.0%)	6 (1.8%)	
Years of education	11.0 (7.0, 11.0)	11.0 (9.0, 13.0)	0.008
BMI (kg/m2)	22.5 (20.5, 24.9)	22.6 (20.7, 25.0)	0.823
% body fat	35.2 (30.6, 38.4)	35.7 (31.6, 39.5)	0.157
Waist circumference (cm)	81.0 (74.1, 86.8)	78.9 (73.9, 85.6)	0.199
PrEP accepted	151 (91.0%)	280 (84.1%)	0.050
Previous broken bone			0.004
Yes	4 (2.4%)	0 (0.0%)	
No	161 (97.0%)	333 (100.0%)	
Not Sure	1 (0.6%)	0 (0.0%)	
BMD total hip	0.9 (0.9, 1.0)	0.9 (0.9, 1.0)	0.623
Z-score total hip	-0.1 (-0.7, 0.7)	0.0 (-0.6, 0.6)	0.436
BMD lumbar spine	0.9 (0.9, 1.0)	0.9 (0.9, 1.0)	0.073
Z-score lumbar spine	-0.9 (-1.5, -0.2)	-0.7 (-1.2, -0.1)	0.035
TBS spine L1-L4	1.4 (1.3, 1.4)	1.3 (1.3, 1.4)	0.527
BMD neck of the hip (g/cm2)	0.9 (0.8, 0.9)	0.9 (0.8, 0.9)	0.989
Z-score neck of the hip	0.1 (-0.5, 0.8)	0.1 (-0.5, 0.7)	0.804
Number of times had sex in past 3 months	24.0 (9.0, 71.5)	18.0 (8.0, 60.0)	0.099
Any condomless sex in past 3 months	111 (66.9%)	226 (67.9%)	0.822
Condom used at last sex	100 (60.2%)	190 (57.1%)	0.497
Number of sexual partners in last 3 months	2.0 (1.0, 4.8)	2.0 (1.0, 3.0)	< 0.001
Any new partners in last 3 months	67 (40.4%)	92 (27.7%)	0.006
Any partner living with HIV			0.016
Positive	7 (4.2%)	2 (0.6%)	

Negative	54 (32.5%)	123 (37.0%)	
Not Sure	105 (63.3%)	207 (62.3%)	
Time spent in the sun each day			0.011
<1 hour	127 (76.5%)	277 (83.2%)	
1-3 hours	35 (21.1%)	39 (11.7%)	
>3 hours	4 (2.4%)	17 (5.1%)	
Regular physical activity (>3 hours/week)	164 (98.8%)	333 (100.0%)	0.110
Weekly servings of foods high in Vitamin D	7.0 (3.5, 11.0)	7.0 (3.0, 10.2)	0.573
Weekly servings of foods high in calcium	11.0 (6.0, 15.0)	8.0 (4.0, 12.0)	< 0.001
Any alcohol in past month	50 (30.1%)	67 (20.1%)	0.018
Any smoking in past month	1 (0.6%)	4 (1.2%)	1.000
Any recreational drugs in past month	7 (4.2%)	9 (2.7%)	0.526

Supplemental Figure 1. Estradiol concentrations at month 6 visit by enrollment DMPA use



Supplemental Figure 2. Percent change in eGFR between enrollment and month 6 visit of women who accepted and declined PrEP



Chapter 5: Safety review of tenofovir disoproxil fumarate/emtricitabine pre-exposure prophylaxis for pregnant women at risk of HIV infection

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Safety review of tenofovir disoproxil fumarate/emtricitabine pre-exposure prophylaxis for pregnant women at risk of HIV infection

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Abstract

Introduction: Pregnancy is a period of elevated HIV risk in high-burden settings, motivating the need for prevention tools that are both safe for use and effective during pregnancy. Oral pre-exposure prophylaxis (PrEP) containing tenofovir disoproxil fumarate (TDF) is recommended by the World Health Organization, including for pregnant and postpartum women at substantial risk of HIV infection. Although TDF use during pregnancy appears generally safe, data on PrEP use during pregnancy remain limited.

Areas covered: We provide an overview of the clinical pharmacology and efficacy of daily TDF-based PrEP and summarize current evidence on the safety of PrEP use by pregnant HIV-uninfected women. We synthesize relevant studies assessing pregnancy outcomes among pregnant women who are living with HIV (WLHIV) and using TDF-based therapy. Finally, we make comparison to the safety profiles of other emerging HIV prevention options.

Expert opinion: The current evidence indicates that TDF/FTC PrEP use is not associated with increased risk of adverse pregnancy and early infant growth outcomes. While safety data are generally reassuring, there is need for continued accrual of data on growth and pregnancy outcomes in PrEP research, implementation projects, and controlled pharmacokinetic studies to support current evidence and to understand concentration-efficacy relationship in pregnant women.

Keywords: tenofovir disoproxil fumarate, PrEP, HIV prevention, safety, pregnancy, women

Article highlights:

- Safety of TDF/FTC use during pregnancy has been evaluated in multiple completed PrEP efficacy trials and real-world PrEP implementation projects
- Women who have been exposed to TDF/FTC experience similar frequencies of pregnancy, perinatal and early childhood outcomes compared to unexposed women
- Studies of women living with HIV who have been given treatment regimens containing TDF/FTC support these findings
- Safety data among pregnant women are limited for other PrEP products nearing the market, including longer-acting vaginal ring and injectable options
- Active inclusion of pregnant women in future evaluations of oral TDF/TDF use and other PrEP products is critical to addressing evidence gaps and promoting equity

Abbreviations

ART: antiretroviral therapy

CDC: Centers for Disease Control and Prevention

DTG: dolutegravir

EFV: efavirenz

eGFR: estimate glomerular filtration rate

EMA: European Medicines Agency

FTC: emtricitabine

HBV: hepatitis B virus

HIV: human immunodeficiency virus

NRTI: nucleoside reverse-transcriptase inhibitor

PrEP: Pre-exposure prophylaxis

TAF: tenofovir alafenamide

TDF: tenofovir disoproxil fumarate

TFV: tenofovir

TFV-DP: tenofovir diphosphate

WHO: World Health Organization

WLHIV: women living with HIV

ZDV: zidovudine

1. Introduction

For women living in high HIV prevalence settings, pregnancy is a period of elevated HIV risk with estimated average HIV incidence of 3.6 infections per 100 person-years among pregnant and postpartum women in sub-Saharan Africa [73]. Longitudinal data demonstrate a nearly two-fold increase in HIV risk for pregnant women in HIV-serodiscordant relationships and the per-condomless-sex-act probability of acquiring HIV rises steadily throughout pregnancy and into the post-partum period [38,39]. These findings underscore the critical need for safe and effective HIV prevention options for pregnant women.

In 2012, a fixed-dose combination oral tablet of co-formulated emtricitabine (FTC) 200 mg and tenofovir disoproxil fumarate (TDF) 300 mg (Truvada, Gilead Sciences) became the first medication approved for HIV prevention by the US Food and Drug Administration after efficacy was demonstrated in multiple clinical trials [5,7]. Global scale-up of oral TDF/FTC for HIV pre-exposure prophylaxis (PrEP) has expanded rapidly. By the end of 2020, Truvada had been registered in over 40 countries and nearly 800,000 individuals were enrolled in PrEP programs around the world [74]. Though some countries include recommendations for PrEP use during pregnancy in their national HIV/AIDs guidelines, the paucity of safety data is still an impediment to PrEP integration into national antenatal and postnatal care in many settings [75]. In this review, we summarize the current safety data on TDF-based PrEP among pregnant women and highlight key evidence gaps.

2. Mechanism of action and clinical pharmacology

TDF is a prodrug of tenofovir (TFV), an antiviral agent with potent anti-HIV and anti-hepatitis B virus (HBV) activity (Box 1) [76,77]. As an acyclic nucleoside phosphonate analogue of adenosine 5'-monophosphate, TFV inhibits HIV reverse transcriptase activity by competing with the endogenous nucleotide deoxyadenosine 5'-triphosphoate for incorporation into viral

DNA. Once incorporated into the viral DNA sequence, TFV then halts further DNA chain elongation due to its lack of a ribose ring [77]. The prodrug TDF, with two additional methyl carbonate esters, was formulated to overcome TFV's poor oral bioavailability and limited intestinal absorption [78,79]. Upon oral administration, TDF is converted to TFV by removal of the additional ester groups through esterase hydrolysis and cellular phosphorylation to its active metabolite, TFV diphosphate (TFV-DP) [80]. TFV reaches maximum concentrations within one hour after oral dosing and the terminal elimination half-life of ~17 hours and intracellular half-life >60 hours make it conducive to once-daily dosing [81]. Studies among pregnant women living with HIV (WLHIV) show that TFV readily accumulates in amniotic fluid and crosses the placenta [82-84] with umbilical cord TFV concentrations approximately 70-100% of maternal plasma concentrations [6,85,86]. However, excretion of TFV into breastmilk and subsequent transfer to infants has been shown to be minimal among lactating HIV-uninfected women using TDF/FTC PrEP [6]. Pharmacokinetics studies of TDF-based PrEP among pregnant women have observed significantly lower (~30-40%) plasma TFV and intracellular TFV-DP concentrations during pregnancy versus postpartum or non-pregnancy periods [87,88].

Oral TDF can be coformulated with FTC, a nucleotide reverse transcriptase inhibitor that is also an effective anti-HIV and anti-HBV agent. FTC has a shorter terminal elimination half-life in plasma (10 hours) compared to TFV [81] and is readily transferred across placenta and into breastmilk [82,89]. Both TDF and FTC are eliminated by glomerular filtration and tubular secretion [81]. Use of both TDF and FTC for PrEP are well tolerated, with the most common side effects being diarrhea, nausea, headache and fatigue. TDF/FTC PrEP is associated with small declines in estimated glomerular filtration rate (eGFR) but the risk of clinically relevant declines in eGFR (≥25%) is very rare (<2%) and quickly resolves within weeks of product discontinuation [90]. Small reductions in bone mineral density (BMD) have also been observed with TDF-based PrEP [36,37,91], with recovery of BMD observed months after PrEP discontinuation [37,92].

3. Clinical application of TDF-based PrEP for HIV prevention among women

The US Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend PrEP for individuals at substantial risk for HIV acquisition [41,93], including at-risk pregnant and lactating women. PrEP efficacy was demonstrated in three clinical trials involving women: the Partners PrEP Study, the TDF2 Study, and the Bangkok Tenofovir Study (BTS) [5,6,8]. However, two trials among sub-Saharan Africa women, the Preexposure Prophylaxis Trial for HIV Prevention among African Women (FEM-PrEP) and the Vaginal and Oral Interventions to Control the Epidemic (VOICE) trial, failed to demonstrate PrEP efficacy due to low adherence to the study product [11,12]. The key results from these five trials are summarized in Table 1. Study protocols for these trials required women to discontinue PrEP upon detection of pregnancy. Therefore, relative efficacy of PrEP during periods of pregnancy compared to periods of non-pregnancy could not be evaluated.

4. Safety evaluation

4.1 HIV uninfected women using TDF-based PrEP during pregnancy

Safety of PrEP in pregnant women was evaluated in four of the major PrEP efficacy trials: the Partners PrEP Study, the TDF2 Study, FEM-PrEP, and VOICE [94-96]. Of 288 pregnancies that occurred during the Partners PrEP Study, the frequencies of pregnancy loss, preterm birth or pregnancy-related complications did not significantly differ between the active PrEP and placebo arms [96]. There were no significant differences in weight, length or head circumference between infants born to women in either arm.

Frequencies of fetal loss was similar between pregnant women assigned to TDF/FTC and placebo pill use in the TDF2 Study [8]. A total of 107 pregnancies from 101 women were reported in the trial.

In the FEM-PrEP trial, 119 total pregnancies from 115 women were observed [95]. Complete data on pregnancy complications were not reported but the authors note that vaginal bleeding was the most common complication and frequency of complications did not differ between active and placebo arms.

The VOICE trial observed 452 pregnancies from 428 women [11]. Report of adverse pregnancy outcomes, including premature birth, stillbirth, spontaneous abortion and ectopic pregnancy, did not differ between women in the active and placebo arms [94]. Subsequent follow-up of infants born to enrolled mothers found no difference in growth indicators in the first year of life between active and placebo arms [97]. However, the VOICE and FEM-PrEP data are limited due to poor adherence to trial product.

Safety data from women who used PrEP throughout pregnancy came from the Partners Demonstration Project, a prospective, open-label evaluation of a combined strategy of PrEP and antiretroviral therapy (ART) use for HIV prevention in serodiscordant couples [98]. Among 30 women who become pregnant and who chose to continue on PrEP during pregnancy, the frequency of live births and adjusted infant weight, length and head circumference z-scores across the first year post-delivery were similar between PrEP users and non-users. No preterm deliveries or congenital abnormalities were observed among women using PrEP [99].

The PrEP Implementation for Young Women and Adolescents (PrIYA) program in Kenya integrated PrEP into maternal and child health and family planning services [100,101]. The frequency of experiencing pre-term birth or low birthweight did not differ and 6-weeks postpartum, growth measures were similar between PrEP-exposed and PrEP-unexposed infants. There was

no evidence of difference in frequency of underweight, stunted, or wasting. Among 721 women exposed to PrEP in a sister cluster randomized trial called PrEP Implementation for Mothers in Antenatal Care (PrIMA) [102,103], the frequencies of miscarriage, stillbirth, preterm birth, congenital malformations, low birth weight, or small for gestational age were no different from the 3520 PrEP-unexposed women. Ongoing extended follow-up in this study will provide additional important evidence.

Finally, a recent pharmacokinetic study (IMPAACT 2009) of daily directly observed doses of TDF/FTC for 12 weeks among pregnant and post-partum women in sub-Saharan Africa found that PrEP use was well tolerated and none of the maternal or infant adverse events reported were found to be related to women's PrEP use [88].

4.2 Safety data from WLHIV using TDF-based HIV treatment during pregnancy

TDF/FTC is indicated for treatment of individuals living with HIV and is commonly used as a nucleoside reverse-transcriptase inhibitor (NRTI) 'backbone' in combination antiretroviral therapy (ART) regimens. For pregnant and breastfeeding women, the WHO recommends daily TDF/FTC with efavirenz (EFV) as a first-line regimen [104]. While evaluations of the safety of TDF in studies of pregnant WLHIV may be confounded by concurrent use of other classes of antiretroviral medications and exposure to HIV infection, they can provide useful corollary data to supplement the limited PrEP data.

Multiple reviews have synthesized the studies of TDF use among pregnant WLHIV extensively [105,106]. A meta-analysis that estimated pooled risk ratios using data from 17 prospective studies and randomized controlled trials found that rates of preterm delivery and stillbirth were significantly lower among women who received TDF-based ART compared to women who did not receive TDF-based ART. No differences in risk of Grade 2, 3, or 4 adverse

events, small for gestational age, miscarriage, congenital abnormalities or low birth weight were observed between groups. In the PROMISE trial among pregnant WLHIV ≥14 weeks gestation and CD4≥350 cells/mm^3, women randomized to TDF-based ART were more likely to experience preterm delivery (<34 weeks) and neonatal mortality relative to those assigned zidovudine (ZDV)-based ART, but not when compared to women using ZDV alone [107], hypothesized to potentially be due to interactions between TDF and lopinavir/ritonavir. Most studies report normal infant growth for children of women who have used TDF-based ART, including z-scores for weight-for-age, length-for-age and head circumference at birth [108-110]. In a study of HIV-exposed, uninfected infants in the US, adjusted mean length-for-age z-score and head circumference z-score at 1-year were smaller among children whose mothers used TDF during pregnancy [110]. Studies that have assessed bone and kidney outcomes among infants generally did not find significant changes with *in utero* TDF exposure [108,111-113]. In two studies, bone mineral content was found to be lower among neonates exposed to TDF compared to TDF unexposed infants [109,114].

4.3 Comparison with safety of potential alternative PrEP drugs

While TDF/FTC is currently the only medication approved for use by HIV-uninfected cisgender women for HIV prevention, including pregnant women, there are several promising HIV prevention products in late stages of development or regulatory approval. As potential alternative HIV prevention options, it is important to consider how their safety profiles compare to oral TDF/FTC.

4.3.1 Oral tenofovir alafenamide with emtricitabine

Tenofovir alafenamide (TAF) is a newer TFV prodrug that has been shown to achieve 4.4-7 times higher intracellular concentrations of active TVF-DP with oral dosing compared to TDF despite ~90% lower drug exposure with 25 mg dosing [115-117] with potential to result in better renal and bone health outcomes [118]. For PrEP, current FDA approval for F/TAF (Discovy) oral PrEP only covers at-risk men and transgender women; cisgender women were excluded from the indication due to lack of trial data [119]. Clinical trials and pharmacokinetic studies of Discovy in women are ongoing in Uganda and South Africa [119]. Similar to how safety data on prenatal TDF use for ART among WLHIV helped inform guidelines in support of using oral TDF-based PrEP in pregnancy [105], accruing data on prenatal TAF use among WLHIV may help advance the evidence base for eventual TAF use as PrEP in pregnancy. Currently, data on F/TAF's safety during pregnancy comes from WLHIV. The IMPAACT P1026s study, a pharmacokinetic study conducted among pregnant women using TAF 10mg with cobicistat or TAF 25 mg, identified two preterm deliveries and hepatic steatosis that were potentially associated with treatment [120]. Five infant abnormalities were observed among women using TAF 10 mg with cobicistat versus two among women using 25 mg TAF alone. In the IMPAACT 2010 study, dolutegravir (DTG)+FTC/TAF was associated with significantly fewer adverse pregnancy outcomes (driven by lower preterm and small-for-gestational-age rates) and less neonatal deaths than either DTG/TDF/FTC or EFV/FTC/TDF [121]. Mechanisms for these observations are unknown but higher exposure to TFV in the TDF-containing arms (vs. TAF) could be one possible mechanism. TAF is not currently recommended in pregnancy due to limited safety and pharmacokinetic data but additional evidence from ongoing phase 3 trials will become available soon [122].

4.3.2 Dapivirine vaginal ring

Use of a vaginal ring containing the non-nucleoside reverse transcriptase inhibitor, dapivirine, has been shown to reduce the risk of HIV acquisition among cisgender women by 27-

31% in two major trials [14,15]. In July 2020, the European Medicines Agency (EMA) provided positive benefit-risk opinion of the dapivirine vaginal ring which, with recent prequalification by WHO, will bring this new HIV preventive modality closer to public introduction and approval by individual countries. Women who became pregnant had similar frequencies of pregnancy outcomes (preterm birth, stillbirth, spontaneous abortion, ectopic pregnancy) and early childhood outcomes (congenital abnormality and infant growth metrics) when assigned to the dapivirine ring relative to women assigned to a placebo ring [123]. Two phase IIIb Microbicide Trials Network studies to evaluate the safety of dapivirine ring and oral PrEP in pregnant and breastfeeding women are underway in Malawi, South Africa, Uganda and Zimbabwe [124].

4.3.3 Injectable cabotegravir

Recently, the HPTN 084 study of long-acting injectable cabotegravir (CAB-LA), an HIV integrase strand transfer inhibitor, demonstrated that CAB-LA given once every eight weeks was safe and superior to daily oral TDF/FTC for HIV prevention among cisgender women [125]. CAB-LA, when it gets regulatory approval, could be instrumental to overcoming the challenges observed with daily adherence to TDF/FTC. Limited data from 13 women who became pregnant after exposure to oral or injectable cabotegravir during Phase 2/3 studies show that four resulted in live births, two resulted in spontaneous abortion, two in medical or induced abortion and one possible early miscarriage [126]. It is unclear how these reported frequencies compare to those in the underlying population.

5. Conclusions

The data collected to date on TDF-based PrEP use during pregnancy are reassuring, indicating that the frequency of adverse pregnancy, birth and infant growth outcomes among

mother infant exposed to TDF-based PrEP in pregnancy is no more common than that experienced by mothers or infants without TDF exposure. This evidence is further supported by extensive corollary data on TDF use for treatment among pregnant WLHIV. While current data are reassuring on safety in pregnancy, additional robust data from prospective studies and implementation projects that actively recruit women prior to conception or during pregnancy are still essential to better understand the pharmacokinetics, safety, and efficacy of TDF-based PrEP in pregnancy.

6. Expert opinion

In settings with high HIV burden, cisgender women face elevated risk of acquiring HIV in pregnancy and postpartum with the risk of HIV acquisition per-condomless-sex-act as high as 3- and 4-fold during late pregnancy and in the postpartum, respectively, compared to non-pregnant periods [39]. Oral TDF/FTC PrEP is recommended and is an attractive strategy for HIV prevention during periods of elevated HIV risk including during pregnancy and lactation.

In first-generation PrEP clinical trials, women discontinued PrEP when pregnancy was diagnosed resulting in limited pregnancy time on PrEP. However, there is a growing body of reassuring evidence from women with brief *in utero* TDF exposure from these trials as well as from real-world PrEP implementation projects. In trials among women, those who became pregnant and exposed to PrEP before protocol-mandated discontinuation, pregnancy outcomes were similar between placebo and TDF-based PrEP arms. Similarly, no reported discernable differences in weight or height z-scores or head circumference between infants with *in utero* PrEP exposure vs. placebo. Additional data from the Partners Demonstration Project among 30 women who become pregnancy and elected to continue PrEP antenatally are also reassuring – with no increases in pregnancy loss, preterm birth, or congenital anomalies [99]. Similar data from real-world PrEP implementation project in Kenyan women (PrIYA and PrIMA), lend credence to these

findings [100,101,103]. While safety data are generally reassuring, there is need for continued accrual of data on growth and pregnancy outcomes in PrEP research studies and implementation projects.

The pharmacokinetics of TDF/FTC PrEP in pregnancy is a critical knowledge gap for the HIV prevention field. Recent clinical studies in African pregnant women using TDF/FTC PrEP suggest that blood (TFV) and cellular (TFV-DP) concentrations may be as low as 45-58% during pregnancy than non-pregnant periods even when adjusted for adherence, with the reduction more pronounced in the third trimester [87,88]. In the IMPAACT 2009 directly observed therapy study of TDF/FTC PrEP in pregnancy and postpartum, DBS TFV-DP levels in pregnancy were nearly 30-40% lower than in postpartum [88]. These data have raised important questions about the appropriate TDF/FTC PrEP dosing strategy in pregnancy. Whether these reductions in TFV-DP concentrations translate into sub-optimal protection against HIV during pregnancy is not clearly known. At the root of this confusion is the lack of clinical data that link cumulative dosing thresholds and concentrations with PrEP efficacy in women.

New PrEP options are nearing the market, which will allow women to select a method that best suits their needs and preferences. Longer-acting methods, such as the dapivirine vaginal ring and CAB-LA, hold potential to increase PrEP adherence and persistence by reducing the frequency that women will need to return to the clinic. Additionally, these methods can be used more discreetly than pills, which may help women to cope with stigma associated with PrEP in their communities [127]. However, to provide women with a well-informed choice, more evidence on the safety and efficacy of these products during pregnancy is needed.

The paucity of research on the use and safety of PrEP during pregnancy highlights wider barriers and disincentives to involving pregnant women in clinical studies of investigational biomedical products. While restrictions on enrollment of pregnant women are taken to protect the mother and fetus, this creates harm by perpetuating existing evidence gaps. As new PrEP agents

come through the development pipeline, it will be important to actively recruit pregnant women in pivotal studies and provide women who experience incident pregnancies during follow-up with an informed option to remain on study product to address these gaps and promote equity [128].

In summary, TDF/FTC PrEP is a potent and recommended HIV prevention option, with a demonstrably good safety profile, including in pregnancy. While safety data are generally reassuring, there is need for continued accrual of data on growth and pregnancy outcomes in PrEP research studies and implementation projects. Lastly, pharmacologic studies that link TFV-concentrations to efficacy in cisgender women are needed to definitively understand the clinical relevance of observed reduction in cellular TFV-DP concentrations during pregnancy.

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Disclosure statement

The authors have no conflicts to declare.

Box 1. Drug Summary

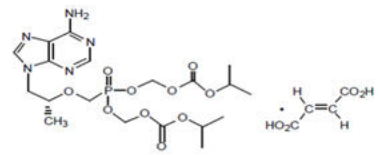
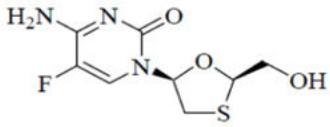
Drug name	Tenofovir Disoproxil Fumarate (TDF), alone or formulated with emtricitabine (FTC)
Phase	US FDA approval of emtricitabine FTC/-TDF for prevention of HIV infection: 2012
Indication	Prevention and Treatment of HIV infection
Pharmacology description	TDF is an oral prodrug of tenofovir, an acyclic nucleotide (nucleoside monophosphate) analogue with activity against retroviruses, including HIV, and hepadnaviruses. FTC is a synthetic nucleoside analogue of cytidine with activity against HIV reverse transcriptase.
Route of administration	Oral
Chemical structure	TDF  FTC 
Pivotal trial for safety and efficacy against HIV acquisition in heterosexual women	The Partners PrEP Study was a randomized, double-blind, placebo-controlled 3 arm trial of daily TDF alone or in combination with FTC in 4747 serodiscordant heterosexual couples in Kenya and Uganda [Reference 5].

Table 1. Summary of completed PrEP efficacy studies involving cisgender women living without HIV

Study	Location	Participants	No. of pregnancies Reported (no. women)	Main Findings
Partners PrEP Study[5]	Kenya and Uganda	4747 HIV serodiscordant, heterosexual couples randomized 1:1:1 to TDF/FTC, TDF, placebo pill use (included 1785 couples with women living without HIV)	288 (267 women)	• Overall, TDF/FTC was shown to be 75% (95% CI: 55-87%) effective and TDF was 67% (95% CI: 44-81%) effective relative to placebo • Effectiveness of 49% (95% CI: -22-81%) among couples where HIV-uninfected partners are women
TDF2 Study [8]	Botswana	1219 heterosexual men and women randomized 1:1 to TDF/FTC or placebo pill use (included 557 women)	107 (101 women)	• The overall efficacy of TDF/FTC demonstrated in the trial was 62% (95% CI: 22-83%). • Among women, TDF/FTC was 49% (95% CI: -22-81%) efficacious relative to placebo
Bangkok Tenofovir Study (BTS) [6]	Thailand	2413 men and women who inject drugs randomized to TDF or placebo pill use (included 489 women)	58 (58 women)	• TDF was 49% (95% CI: 10-72%) effective at preventing HIV overall • TDF effectiveness of 79% (95% CI: 17-97%) among women (p=0.03)
Vaginal and Oral Interventions to Control the Epidemic (VOICE) [11]	South Africa, Uganda and Zimbabwe	5029 women randomized to use of oral TDF/FTC, oral TDF, vaginal 1% TFV gel, oral placebo or vaginal placebo	452 (428 women)	• TDF was -49% (95% CI: -129-3%) effective and TDF/FTC was -4% (95% CI: -49-27%) effective • Adherence to study product was low: plasma TFV was detected in 29-30% of random sample of oral PrEP users
Preexposure Prophylaxis Trial for HIV Prevention among African Women (FEM-PrEP) [12]	Kenya, South Africa, Tanzania	2120 women randomized to TDF/FTC or placebo pill use	117 (115 women)	• Overall TDF effectiveness of 6% (95% CI: -52 to 41%) • Adherence to study product was low: plasma TFV concentrations were above target concentrations in <40% of women at seroconverters' infection windows

TDF: tenofovir disoproxil fumarate, FTC: emtricitabine; TFV: tenofovir; CI: confidence interval

Chapter 6: Discussion

This dissertation addressed key issues related to PrEP use and safety among women of reproductive age in sub-Saharan Africa. In Chapter 2, we demonstrated that a novel urine-based point-of-care tenofovir immunoassay has good sensitivity and specificity for detecting plasma tenofovir and can predict protection from HIV. Chapter 3 contributed additional information towards the understanding of the efficacy of the dapivirine vaginal ring by establishing the first per-sex-act dose-response model for ring exposure; we estimated 61% per-sex-act risk reduction with ring release rates indicative of continuous ring use. In Chapter 4, we addressed concerns surrounding the effect of the hormonal contraceptive DMPA and PrEP on young women's bone health and identify no substantial increases in bone turnover biomarkers after 6 months with combined medication use. Finally, in Chapter 5, we found that the body of evidence on the safety of TDF-based PrEP use during pregnancy is reassuring but that additional data are needed. The collective evidence from this dissertation will help to inform implementation and policy surrounding the integration of PrEP into family planning and antenatal services, future research on the safety of PrEP and novel HIV prevention products, and most importantly, women's decision-making about their own reproductive health.

New tools to support women's PrEP use

Novel approaches to supporting women's adherence to PrEP in sub-Saharan Africa are urgently needed. In the VOICE and FEM-PrEP trials, >90% of women reported that they had adhered to the study product, but tenofovir was detectable in <30% of plasma samples collected from women in the active product arms [11,12]. These findings not only highlight the challenges that women face in adhering to daily PrEP use (although low pill use in these studies was partly due to concerns given the investigational nature of the product at the time) but also point to the value of point-of-care drug-level testing to inform counseling in real-time as there can be disconnect between what users report and do. Qualitative interviews from the VOICE trial reinforced this notion, with some women reporting that drug monitoring during the study motivated

their pill use [129]. To date, only one study has evaluated the impact of drug-level feedback on women's subsequent PrEP use. HPTN 082 tested an intervention of providing drug-level feedback to young African women two and three months after initiating PrEP based on prior DBS TFV-DP levels. The study assessed whether this intervention influenced whether women achieved TFV-DP concentrations indicative of high PrEP adherence at three- and six-month follow-up visits [130]. Results of the trial suggested that the intervention did not alter user adherence. However, given that drug-level feedback was delayed, the intended effect of the intervention may have been attenuated. Therefore, it is worth evaluating to determine whether point-of-care drug-level testing has more success in improving adherence.

Our work in Chapter 2 highlights the value that a novel, urine-based immunoassay could have as a point-of-care test for monitoring PrEP adherence. We found that tenofovir concentrations over a threshold indicative of PrEP use in the past day were predictive of a 71% reduction in HIV risk. Additionally, urine TFV concentrations measured by the assay had sensitivity of 87% and specificity of 73% for detection of tenofovir in plasma. Within the same cohort, we found that plasma concentrations indicative of daily use were predictive of 87% reduction in risk. Therefore, while it could be an effective tool, our results suggest a tradeoff between the convenience of sample collection and real-time result availability that comes with the urine immunoassay and accuracy of identifying recent PrEP use. It will be important for study investigators and program staff to consider the implications of this tradeoff considering potentially 27% of individuals who have not used PrEP recently will receive a false-positive result and 13% of individuals have used PrEP recently will receive a false-negative result. Misclassification of adherence with the assay could result in misinformed counseling messages as well as mistrust between the user and provider.

Some potential disadvantages of using urine-based assays for PrEP adherence monitoring should also be considered prior to implementation into existing counseling services. First, TFV can be detected in urine shortly after oral dosing, which makes the method susceptible

to “white-coat dosing”, where users may take a dose immediately prior to meeting with a provider knowing their pill use is being monitored. An analysis of urine samples from 24 men who have sex with men (MSM) using two different ARV regimens (TDF/FTC and TAF/FTC/COBI/EVG) found that urine tenofovir concentrations 4 and 24 hours after dosing with TDF/FTC were a mean 146,875 ng/mL and 10,045 ng/mL, well above the concentration detected by the POC assay [48]. Therefore, comparison of the results of the POC assay with measures of longer-term adherence, such as hair and intracellular TFV-DP levels, will be useful to determine the extent of white coat dosing in different populations. Another disadvantage of urine-based assays relative to other biological matrices is that results can vary based on the specific gravity of the sample, which can be influenced by various individual-level factors such as hydration, medication use and medical conditions. In the above-mentioned study involving MSM, higher specific gravity of the sample was shown to be associated with higher TFV and FTC concentrations among TDF/FTC users [48]. Therefore, theoretically, individuals may have recently used PrEP but tenofovir may be undetectable due to urine dilution. The relatively low concentration threshold of the assay is designed to account for this limitation, but this limitation should be kept in mind when interpreting and communicating test results.

The first pilot study to evaluate the clinical performance of the novel POC urine assay among women, the Point-of-Care Urine Monitoring of Adherence (PUMA) Study, is underway in sub-Saharan Africa [131]. Within this study, and in future evaluations of the test, it will be critical to assess the acceptability of the assay among African women. As shown by studies of hair collection for antiretroviral adherence monitoring, acceptance of sample collection methods can vary depending on the population and context of use [132-134]. Urine collection is less invasive than a blood draw and can be conducted by the participant in private, which could make it a more acceptable option as has been suggested in one US cohort [50]. However, one concern by younger participants that their urine samples would be tested for recreational drugs. Evidence has shown that views on the concept of real-time testing to assess adherence in general may be

more mixed. In a qualitative study that asked MSM living with HIV in the US about their perceptions towards a point-of-care assay to monitor adherence, some participants saw utility in the test while others said it could make them feel untrusted and voiced concerns about their privacy, confidentiality and autonomy over their own care [135]. Some respondents suggested it may be more appropriate to use the test for other purposes, including self-testing prior to sex to ensure PrEP protection; providers saw it as a potential tool to confirm adequacy of non-daily dosing regimens and check drug levels against viral loads to detect resistance. It will be important to ask women and providers targeted questions about their perspectives on real-time testing and feedback as well in African settings.

After client acceptability of the test is evaluated within a particular setting, careful consideration about how to implement the test into routine clinical practice will be needed. First, a decision should be made about whether the test should be given to all PrEP clients or implemented as an opt-in intervention. Given the qualitative data discussed above, some clients may be discouraged by having their pill use monitored which may negatively impact their and PrEP use and/or retention in care. Therefore, it may be more effective to only use the assay with women who accept it and see it as useful tool to support their adherence behaviors. Second, it will be important to develop appropriate messaging for individuals who test positive and negative for PrEP use with the assay. The counseling approach used in HPTN 082 could serve as a good model, which used positive re-enforcement messaging for participants who had blood TFV levels indicative of regular use [130]. If low or no use was observed, counselors engaged women in a conversation about their PrEP use patterns and explored barriers to use while avoiding punitive language. However, specific messaging should be piloted and refined in each setting prior to widescale rollout. Messaging should also take into consideration the imperfect sensitivity and specificity of the assay. Third, the additional cost and time burden of implementing such an intervention will need to be evaluated at the clinic and health system level. For clinics that already have limited financial and human resources and high patient flow, use of the assay with all clients

may be impractical. Therefore, more targeted use of the assay, for example with individuals who exhibit higher HIV risk, may be required.

Finally, as PrEP access expands and new HIV prevention methods become available for women, it will be important to also to evaluate the performance of the POC assay among pregnant women as well as with new tenofovir-based PrEP modalities. Studies that have assessed the pharmacokinetics of TDF-based PrEP among pregnant women have observed significantly lower plasma TFV and intracellular TFV-DP concentrations during pregnancy overall versus postpartum or non-pregnancy periods [87,88]; urine tenofovir benchmarks among pregnant PrEP users have not been established, however. Therefore, the POC test will need additional validation to assess if the current tenofovir concentration threshold is sufficient for detecting recent PrEP use among pregnant women. Additionally, while F/TAF has not yet been approved for use by cisgender women, future studies should evaluate that performance of the POC with F/TAF use. Systemic exposure is lower with F/TAF dosing and therefore applicability of the current concentration threshold is uncertain [115-117].

Like other objective measures of PrEP adherence, the novel POC has important strengths but also key limitations and therefore should be used as one of many tools to address women's adherence. Given imperfect sensitivity and specificity for detecting systemic tenofovir, the test should not be relied on as a gold-standard measure of pill use, and more as a means for providers to generate an open dialogue with users about their PrEP use.

Increasing PrEP use through informed choices

While supporting women's use of currently available PrEP methods is important, creating new HIV prevention options is also needed to allow women to find a method that best suits their needs and preferences. Providing women with more method options has been linked to uptake and continuation of family planning in numerous settings [136-138], giving hope that the same trends will be seen by expanding the HIV prevention method mix. This includes introduction of long-

acting, non-oral medications that help women overcome challenges faced with oral PrEP use. In Chapter 3, we further examined the efficacy of the dapivirine intravaginal ring to inform women's decisions when the product reaches the market. We found that women with dapivirine release rates from returned rings consistent with continuous ring use over 28 days was associated with a 55% per-sex-act reduction in HIV risk compared to users of the placebo ring; risk reduction reached >60% with consistent use among certain subgroups of women.

Our analysis is unique in that it assesses risk reduction on the per-sex-act scale, providing more complete accounting for women's sexual behaviors over time. In longitudinal analyses, simple visit-level adjustment of frequencies of sexual behavior do not fully account for sexual activity prior to a given study visit, which could result in residual confounding in studies of HIV acquisition. Additionally, this approach continues to treat individuals who are not sexually active at a certain visit as at-risk for infection. When time-to-event analyses are not used, adjustment for time-varying sexual behavior variables can also be complicated as sexual behaviors may mediate the relationship between previous product exposure and HIV acquisition; in this case, standard adjustment techniques would bias estimates of risk reduction. More complex analytic techniques such as marginal structural models would be needed to safely account for time-varying confounding [139]. Future studies of the efficacy of HIV prevention products should similarly consider assessing risk on the per-sex-act scale; to do so, collection of frequent and comprehensive data on the numbers of total sexual acts and condomless sex acts is recommended. If possible, confirmation of partners' HIV status, antiretroviral use and/or viral load would be helpful to best estimate per-sex-act risk [140].

Based on our results and those from a previous analysis assessing risk reduction associated with ideal ring use based on dapivirine release rates [24], consistent use of the dapivirine ring results in a 45-61% reduction in HIV risk; estimates between 75-91% efficacy were obtained in exploratory analyses but are likely overestimates. By contrast, risk reductions >90% have been consistently estimated among users of oral TDF/FTC who have plasma TFV and

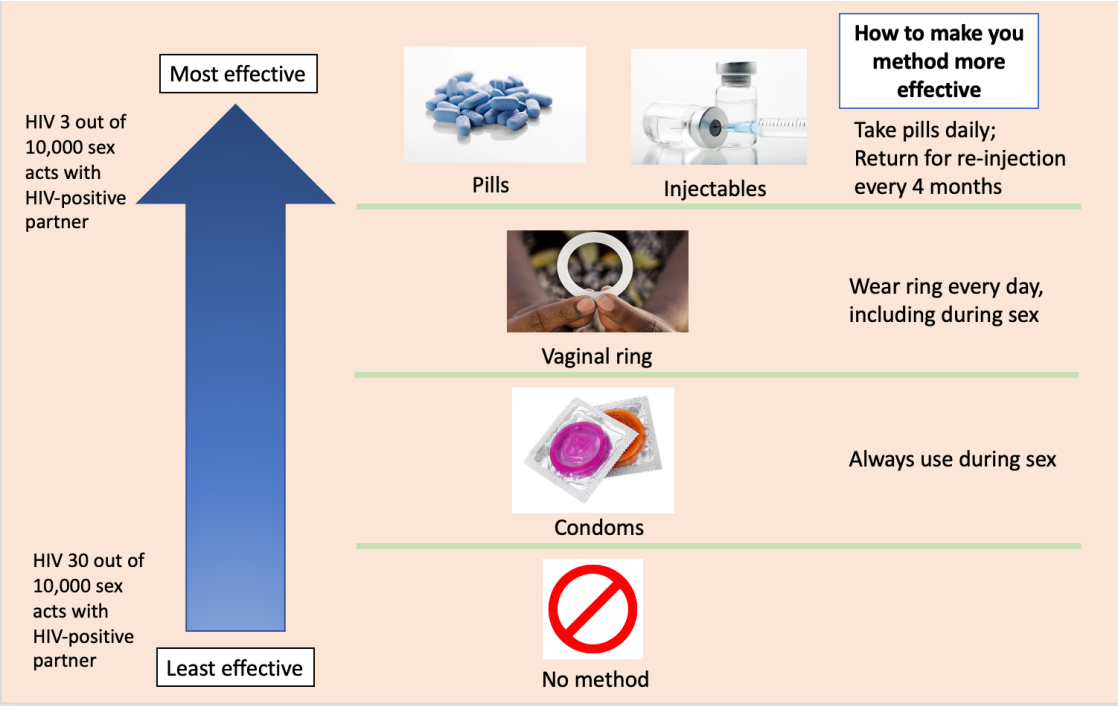
intracellular TFV-DP concentrations indicative of daily pill use [9,10]. Therefore, while the dapivirine ring provides substantial protection against HIV, it likely will not provide the same level of protection as daily oral PrEP or long-acting injectable PrEP with cabotegravir, which was recently shown to be highly effective in preventing HIV in the HPTN 084 study [125]. The implications of this discrepancy are worth considering on a patient-provider level. First, for many women, while use of the most efficacious option is ideal, this may not be the only factor influencing their decision. In qualitative interviews from the VOICE and FEM-PrEP studies, some women reported difficulties with managing relationship dynamics and concerns about what family and people in their community may think as reasons for not adhering to pills [129,141]. For these women, daily pill use may not be a realistic option and therefore an alternative method is needed. Some women also cited side effects as a reason they stopped taking their pills. These women may be reluctant to try an uninterruptable method like CAB-LA that would remain in their body for four months or more out of concern for experiencing additional side effects. In each of these cases, the dapivirine ring would have particular value for women and may lead to better product use, which could compensate for the gap in efficacy. This is reflected in the results from the recent TRIO Study, which asked women to use a placebo pill, ring and injection in a randomized order for one month and then gave them the choice to use one of the products for an additional two months to gauge preferences for multipurpose prevention technology product types [142]. In total, 15% of women chose to use the ring, which was not statistically different than the proportion of women that selected pills (21%). Product comfort as well as frequency of dosing and the peace of mind that came with the product were the most common reasons women gave for selecting it.

From the perspective of the provider, discussion of differences in efficacy between methods is necessary but insufficient to make a clinical decision and should also be combined with exploration into a patient's unique circumstances and preferences on methods as part of a person-centered counseling approach [59]. However, when discussing efficacy, providers should use care and consult best practices that have been established for communicating risk to patients

[143]. For example, using plain language and presenting reductions in risk on both the relative and absolute scale would be helpful for patients, as relative risks can be easily misunderstood. In the case of oral PrEP versus the dapivirine ring, telling a patient that one method is 90% efficacious and the other is 50% efficacious could be taken to mean that 1 out of every 10 sex acts versus 4 out of every 10 sex acts with an HIV-positive partner will result in transmission, when in fact their baseline per-sex-act probability of acquiring HIV is orders of magnitude smaller. When discussing relative risk on the absolute scale, a consistent denominator should be used (e.g., 1 in 1000 sex acts will result in HIV transmission with PrEP versus 10 in 1000 without oral PrEP) [144]. Finally, establishing standardized vocabulary at the clinic and at the national level on words that should be used to communicate probabilities and efficacy is recommended to avoid mixed messaging and misunderstanding [144].

Research from the contraceptive field has shown that audio-visual aids were more effective than oral communication at communicating contraceptive method efficacy, which could be adapted for PrEP provision as more options become available [145,146]. When it came to the design of visual decision aids for contraception, presenting relative effectiveness of contraceptive methods on a continuous scale was found to be clearer to women than putting methods into discrete categories (e.g., “Less effective”, “Effective”, “More effective”) [146]. Research also recommends keeping visual aids simple; inclusion of absolute risks for every method could lead to confusion [147,148]. Based on these recommendations, and tested decision aids from the contraception field, an effective decision aid for HIV prevention methods may look like the one shown in Figure 1. This mock-up shows a simple display of the relative effectiveness of the future HIV prevention method mix on a continuum, while also providing some reference to the estimated absolute risks and giving clear suggestions on how to maximize effectiveness. Future implementation science research would be helpful to better understand the clinical effectiveness of using such decision aids in the context of HIV prevention.

Figure 1. Sample decision aid for counseling women on their selection of HIV prevention option



Safety of PrEP use for young women using hormonal contraception

As the number of PrEP options increases for young women in sub-Saharan Africa, so have the types of hormonal contraceptive methods. DMPA-SC, or Sayana Press, was first piloted in four countries in sub-Saharan Africa in 2014 and is now being scaled up in more than 15 low- and middle-income FP2020 countries, a collaborative which aims to expand women's access to effective contraception globally [149,150]. In Uganda specifically, over 3% of all women were using DMPA-SC in 2018, an increase of more than 100% from the previous year, and over a quarter of users were under the age of 25 [151]. In Chapter 5, we addressed concerns about the safety of combined DMPA and PrEP use for young women given past evidence showing reduced bone mineral density with use of these two medications, and specifically assess outcomes among DMPA-IM and DMPA-SC users. We found no substantial differences in changes in biomarkers of bone turnover, including CTX, P1NP and P1NP, among DMPA-IM or DMPA-SC users or PrEP

users within the first 6 months of product use. Additionally, no evidence of an interaction between DMPA dose and PrEP use was observed.

These results are reassuring, but more work is needed to better understand the effects of DMPA dose and PrEP use on bone turnover and overall bone health. Only one other previous analysis has compared bone health outcomes among DMPA-SC and DMPA-IM users. A significant difference in BMD of the lumbar spine was observed at Year 1 but not at other skeletal sites [26]. Therefore, it will be important to evaluate if differences in bone turnover biomarkers as well as bone mineral density are similarly observed at year 1 and later in follow-up in the Kampala Women's Bone Study cohort. To further develop the use of bone turnover biomarkers as a prediction tool, the correlation between biomarker concentrations and future BMD changes also needs to be evaluated among healthy African female populations. Several previous studies have identified significant correlations between changes in some bone turnover biomarkers, including P1NP and NTX, and BMD but results have been mixed and contradictory [152-155]. Additionally, these studies have mainly been conducted among postmenopausal women and male and female populations with underlying medical conditions, such as chronic kidney disease, in the US and Europe. Results from these studies may not be translatable to populations of women in Africa given that concentrations of bone biomarkers and can be influenced by age, underlying medical conditions and lifestyle factors such as diet, exercise, sun exposure and alcohol use [156].

Additionally, while both PrEP and DMPA have been linked to reductions in BMD, the evidence is not conclusive about whether decreases in BMD translates to an increased risk for fracture. A Cochrane review on the topic concluded that the quality of evidence was not strong enough to link DMPA use and subsequent fracture [157]. This has led both the World Health Organization and the American College of Obstetrics and Gynecology to recommend that decisions on whether to prescribe DMPA to young women and the duration of use not be deterred by potential risks of bone loss [158,159]. However, the relationship between DMPA and PrEP use and fracture risk should continue to be explored, particularly with combined medication use

among populations in sub-Saharan Africa. This will be difficult to do using data from cohort studies like the Kampala Women's Bone Study, given the rarity of the outcome. Therefore, additional case-control studies using data from clinical chart reviews may be the best approach to addressing this question.

Demonstrating safety of combined DMPA and PrEP use will help to inform clinical recommendations for women who are seeking protection from HIV and pregnancy in sub-Saharan Africa. Additionally, it will hopefully encourage more women to consider using DMPA-SC as product rollout continues across the region. Data from three countries in sub-Saharan Africa, including Uganda, have shown that approximately half of DMPA-SC users were first-time users of modern contraception, indicating that the method could be instrumental in addressing need for contraception that is not currently met with current methods [151]. Similar to the critical niche that the dapivirine ring could occupy in the HIV prevention method mix, Sayana Press also has unique characteristics that would complement many women's needs and preferences. Most apparent is the added convenience due to the option of self-injection. This attribute eliminates the need to return to the clinic for re-injection and offers greater confidentiality than clinic-based injections, which may help young women cope with stigma around sex and contraception [61]. Studies have already demonstrated that self-injection is highly acceptable among young women and is associated with better method continuation in Uganda and elsewhere in sub-Saharan Africa [60,160-163].

Safety of PrEP use during pregnancy

On average, women in sub-Saharan Africa experience approximately 4.5 births over their lifetime, which equates to over three of their reproductive years if pregnancies are carried to term [164]. Making PrEP services equitable for women means ensuring they have access to products that are safe and effective during all stages of their life, including during pregnancy when women's risk of HIV is higher compared to non-pregnancy periods [38,39]. In Chapter 5, we reviewed the

key findings on the safety of TDF/FTC use during pregnancy to date. While data come mainly from secondary, observational analyses of women enrolled in PrEP efficacy trials and program evaluations, the data provide reassurance that TDF/FTC is safe for use peri-conception and during pregnancy. Most studies found no difference in pregnancy outcomes and early child growth indicators among women who were either exposed to PrEP up to detection of pregnancy or during pregnancy. Supporting data come from studies of TDF/FTC use for treatment of women living with HIV.

The review also highlights the wider barriers and disincentives to conducting research on biomedical interventions that involve pregnant women. Pregnant women were excluded from past PrEP efficacy trials and study product was withheld during incident pregnancies as a safety precaution. This is a common practice for studies of investigational products. Only 1% of pharmacokinetic trials of new drugs were found to involve pregnant women in a recent review [165]. Of all new medications approved by the FDA between 2000 and 2010, all but 3% had an 'undetermined risk' during pregnancy [166]. More effort is clearly needed to mitigate and eliminate the ethical, structural and political barriers designed to help women but ultimately do harm by perpetuating evidence gaps.

In biomedical research, pregnant women historically have been labeled a "vulnerable population" out of concerns for the health of the fetus [167]. While this categorization has been overturned in many jurisdictions, including by regulators in the United States in 2018, it is viewed as a primary contributor to lack of inclusion of pregnant women in past clinical studies and still has a lasting legacy in biomedical research [168]. The term vulnerable population is traditionally used for groups that are likely to have impaired decision-making capacity, such as children or adults with cognitive disabilities, which can diminish pregnant women's capacity to make informed decisions about their health and participation in the study. This label also leads researchers to be hesitant about including pregnant women in studies due to increased safety concerns and greater ethical review hurdles. There has been a move to reframe pregnant women as a 'complex'

population, to acknowledge that they experience unique physiological and ethical circumstances while not diminishing their ability to provide consent.

Another barrier to the inclusion of pregnant women in research is the unclear definition of "acceptable risk" [169]. The Common Rule, which is the set of baseline ethical guidelines for all federally-funded research in the US, states that if an intervention does not directly benefit the participant then risk must be minimal and any harm incurred should not exceed what is reasonably encountered in daily life or during routine medical examination [167]. However, this guideline is open to interpretation so many investigators may err on the side of caution and choose not to include pregnant women in a study to avoid ethical review delays or out of fear of legal ramifications.

Many of these concerns have been expressed within the HIV/AIDS research community. Key barriers to the inclusion of pregnant women in HIV research were identified in a series of consultations with HIV experts carried out under the Pregnancy & HIV/AIDS: Seeking Equitable Study (PHASES) project, which aims to identify ethical solutions to the inequities surrounding involving women in HIV and reproductive health research [170]. As part of this investigation, more than 60 individuals across multiple disciplines of HIV/AIDS research and geographic locations were interviewed. Many acknowledged the current "Catch 22" predicament in which the limited safety data for women with regard to HIV biomedical intervention limits understanding about women's risks and therefore makes it difficult to justify inclusion of pregnant women in clinical studies.

Experts also discussed the financial and logistical barriers to inclusion of pregnant women in HIV research. For example, there may be a financial disincentive for pharmaceutical companies to develop drugs for pregnant women given they make up a much smaller portion of any potential market. Studies investigating medications can be more expensive to run as they may require longer follow up and provision of extra antenatal services. Some even voiced their belief that public funding agencies were biased against studies involving pregnant women due to the added

complexities and costs. Logistically, recruitment of pregnant women can be difficult and loss-to-follow-up could be more frequent.

As part of the Choices in Pregnancy (ChIP) project, a diverse group of professionals in the HIV prevention field with expertise in research, policy, and bioethics were interviewed to get their perspective on the disparities in recruitment of pregnant women in HIV clinical research [171]. Many of the interviewees said that pregnant women should be prioritized in HIV prevention research, especially when evaluating a severe disease that affects women during pregnancy, stating that it would be unethical to not include them. Given the lack of data from pregnant women, many experts admit that they would have to carefully weigh the benefits and risks posed to the mother and child before deciding to implement an HIV intervention. Factors that come into play include the severity of the illness as well as social and economic factors. Regardless, experts believe that women should be brought into the decision-making process and that they should be empowered to make their own choices in cases where the data are uncertain or unclear.

While there have been recent changes at the policy level to increase representation of pregnant women in biomedical research, including at the NIH and FDA [169,172-174], studies have shown that women and minorities are still significantly underrepresented in many areas of biomedical research [175]. Therefore, better strategies are needed to optimize the data that are available on pregnant women while also developing innovative ways to overcome systemic barriers and increase inclusion of pregnant women in new trials, including upcoming pharmacokinetic, safety and efficacy trials of new HIV prevention products. These strategies may include the use of inclusive trial designs, which involve actively recruiting women, providing women with the informed option to stay on study product should they experience an incident pregnancy, and/or tracking unintended exposures during pregnancy [128]. When following incident pregnancies, it is important that data should be collected not only on fetal outcomes but also maternal outcomes as well. In larger research networks, harmonizing data collection and management will help to maximize data and improve power to detect safety signals. Finally,

pushing drug developers to move up preclinical animal studies to assess potential fetal harm, a prerequisite for inclusion of pregnant women in clinical studies, will also help to improve inclusion.

Conclusion

The findings from this dissertation help to advance understanding and development of strategies to improve women's uptake of and adherence to PrEP in the coming years. Real-time drug-level testing is now possible with the development and validation of a novel urine-based immunoassay. It will be important to continue to evaluate the acceptability and clinical effectiveness of the assay within routine clinical care settings. Messaging to users based on assay results should also be carefully crafted, evaluated and refined to ensure it is having the intended effect on pill use. Expansion of the HIV prevention method mix to include the dapivirine ring may also improve PrEP use by introducing an option better suited to some women's needs and preferences. In the first dose-response model to evaluate the association between ring exposure and HIV risk reduction, we estimated a 55% per-sex-act relative risk reduction for HIV acquisition with continuous ring use. Despite lower estimated efficacy relative to oral PrEP and injectable cabotegravir, the ring still fills an important niche in HIV prevention services. Developing effective ways to communicate the difference in efficacy to potential users while also prioritizing women's own preferences will be an important next step as the ring reaches the market. Reassuring safety data may also help to expedite product rollout and integration of PrEP into family planning and antenatal care services. We found no evidence of detrimental short-term changes in bone turnover with combined PrEP and DMPA use, supporting the option for women to use both medications to prevent HIV and pregnancy. Evaluating bone health with longer durations of combined medication use is needed. Finally, the data on oral PrEP use during pregnancy are encouraging. However, data gaps exist due to pregnant women being under-represented in pharmacokinetics and efficacy studies of new medications, which delays women's access to these products. As new

HIV prevention products emerge from the development pipeline, investigators and funders should develop strategies to involve pregnant women in clinical studies to prevent these gaps.

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