PROTOCOL

Impact of Concurrent Initiation of DMPA Contraception and Tenofovir PrEP on Bone Loss in Young Women

The Kampala Women's Bone Study

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Contents

ACRONYMS

BMD	Bone mineral density
BMI	Body mass index
BTM	Bone turnover marker
CDC	Centers for Disease Control and Prevention
CKDE	Chronic kidney disease epidemiology
CTX	C-telopeptide
DMPA	Depo medroxyprogesterone acetate
DXA	Dual energy x-ray absorptiometry
EC	Ethics committee
EIA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FTC	Emtricitabine
eGFR	Estimated glomerular filtration rate
IDI	Infectious Disease Institute
IRB	Institutional review board
NTX	N-terminal telopeptide of type 1 collagen
P1NP	Procollagen type 1 N-terminal propeptide
PBM	Peak bone mass
PTH	Parathyroid hormone
PrEP	Pre-exposure prophylaxis
RNA	Ribonucleic acid
SHBG	Sex hormone-binding globulin
STI	Sexually transmitted infection
TBS	Trabecular bone score
TDF	Tenofovir disoproxil fumarate
VDBP	Vitamin D binding protein

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I. SUMMARY

For women with substantial HIV risk, tenofovir disoproxil fumarate (TDF)-based pre-exposure prophylaxis (PrEP) is a recommended HIV prevention strategy and recent approvals of TDF-based PrEP from sub-Saharan African drug authorities will accelerate roll-out. Unintended consequences of TDF-based PrEP use include decline in bone mineral density (BMD) and subclinical kidney injury, which might further enhance bone loss. In Africa, young women face substantial HIV risk from condomless heterosexual sex and many already use a highly effective injectable contraceptive, depot medroxyprogesterone acetate (DMPA), to prevent unintended pregnancy. DMPA is also known to cause BMD loss to a substantial degree, warranting a U.S. FDA "black box" warning. Thus, as PrEP becomes available in settings where DMPA is commonly used, such as Uganda, a large number of HIV-uninfected women will be concurrently exposed to two agents that may act additively or synergistically to adversely affect bone health. Furthermore, exposure to these agents during adolescence or early adulthood, critical periods for skeletal growth, may prevent the attainment of peak bone mass, a major determinant of increased fracture risk in later life. Bone fractures can result in the loss of independence, lost wages, and economic instability, severe consequences for young African women. The effect of concurrent use of DMPA and PrEP on bone health has not been explored in depth in PrEP research to date and is a key knowledge gap for women's health globally.

The primary objective of this study is to address critical safety questions with concurrent TDF-based PrEP and DMPA use. We hypothesize that young women using TDF-based PrEP and DMPA will have lower bone acquisition and altered bone metabolism. Bone mineral metabolism is in part regulated by the kidney, and we hypothesize that bone effects from concurrent PrEP and DMPA use will be driven by subclinical kidney injury, a known side effect of TDF, as well as DMPA-induced hypoestrogenism. To investigate our hypothesis, we will enroll a prospective cohort of approximately 500 HIV-uninfected women ages 16-25 years in Kampala, Uganda who have substantial HIV risk and are initiating DMPA or barrier method contraception. Over a 24-month period, we will offer TDF-based PrEP. We will use state-of-the-art radiologic, biochemical, and epidemiologic methods to test the hypothesis that concurrent TDF-based PrEP and DMPA use results in compounding adverse effects on bone health.

II. BACKGROUND

PrEP as a novel HIV prevention strategy for women

Women in sub-Saharan Africa are disproportionately affected by HIV and the greatest rates of HIV incidence are among women below age 25. In Uganda, 4% of young women were estimated to be living with HIV and 140,000 people became infected in 2013.[1] For women with substantial HIV risk, tenofovir disoproxil fumarate (TDF)-based pre-exposure prophylaxis (PrEP) is a daily oral medication highly protective against HIV acquisition.[2-7] Randomized trials of PrEP efficacy demonstrated high HIV protection at levels >90% in analyses of participants with high adherence.[4-7] Recent landmark approvals of TDF-based PrEP in sub-Saharan Africa and commitment from large-scale funding partners (including PEPFAR) will accelerate its roll-out.[8] WHO antiretroviral guidelines now include PrEP and clinical guidelines for Uganda are under development. A key advantage of PrEP for women is that it does not require the cooperation of a male partner, a challenging characteristic of condom use. Although two trials of PrEP conducted with African women showed null results, there is substantial objective evidence indicating that these studies were limited by low adherence to study medication.[3, 9, 10] Concerns about PrEP adherence by young women have been abated by data from PrEP delivery studies demonstrating very high adherence with open-label products with known efficacy and accompanied by risk-related counseling.[11]

Unintended consequences of PrEP

Like any biomedical intervention, the use of TDF-based PrEP could have unintended consequences, especially when added on top of medication already being used for other indications, such as the use of injectable depot medroxyprogesterone acetate (DMPA) to prevent pregnancy. TDF-based PrEP and DMPA have both been shown to have detrimental effects on bone health, including bone density and architecture. Reduced bone health (measured directly in bone density or through intermediate markers of bone turnover) results in increased risk for osteoporosis, osteopenia, and osteoporotic fracture. Bone fractures have severe consequences for the individual including loss of independence, reduced quality of life, increased frailty and depression, and results in less physical activity and social involvement, greater risk for subsequent fracture as well as high cost implications from a public health perspective.[12, 13] Fractures account for up to 2% of the global burden of non-communicable diseases and women account for more than half of this burden worldwide.[14] In resource-limited settings, the prevalence of low bone mineral density (BMD) in the general population is rarely described but HIV-infected individuals using common antiretroviral HIV treatment regimens appear to experience low BMD more frequently than individuals in high income regions.[15]

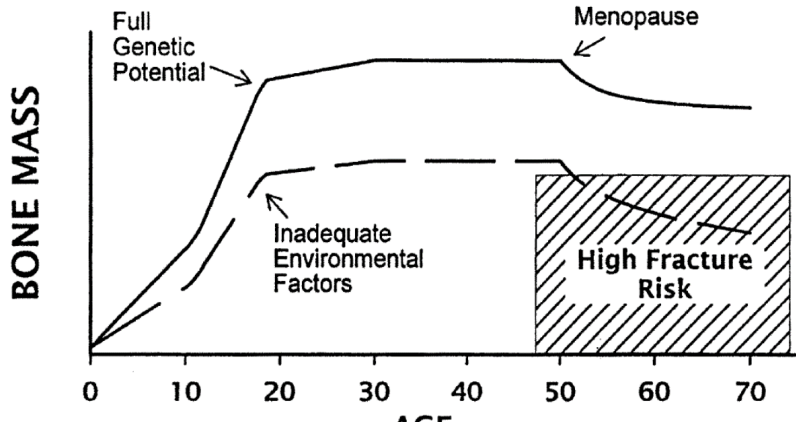
Implications of reduced peak bone mass

Peak bone mass is usually reached by the end of adolescence or early adulthood (Figure 1).[16] Reduced peak bone mass (PBM) is a frequent correlates of osteoporotic fracture later in life.[16-18] In epidemiological studies, a 10% gain in PBM in the female population was associated with 30-50% decreased risk of hip fracture and mathematical models indicate that relatively small increases (10%) in PBM acquisition in healthy females could delay onset of osteoporosis by as many as 13 years.[19, 20] Exposure to DMPA or TDF during adolescence or early adulthood, which unintended pregnancy and HIV risk is greatest, may result in low PMB in the hip, spine, or wrist with implications for immediate or long-term bone health.

Effects of TDF-based PrEP on bone metabolism and bone density

Prior studies, mostly in men, have established that TDF-based PrEP use leads to increased bone turnover, decline in BMD, and subclinical kidney injury, which might further enhance bone loss.[21-23] The mechanism of these toxicities is not well elucidated but is believed to be a complex interplay between renal, endocrine, and bone physiology, all of which affect calcium–phosphate homeostasis.[24-29] In HIV-infected adults, initiation of TDF for HIV treatment has been associated with a significant increase in bone turnover markers (BTM), including C-telopeptide (CTX) and procollagen type 1 N-terminal propeptide (P1NP).[30-32] These increases in CTX and P1NP were significantly correlated with BMD decline at the hip and spine, risk factors for later bone fracture.

Figure 1. Hypothetical representation of the bone mass life-line in individual who achieve their full genetic potential for skeletal mass and in those who do not. [Heaney et al. 2000]



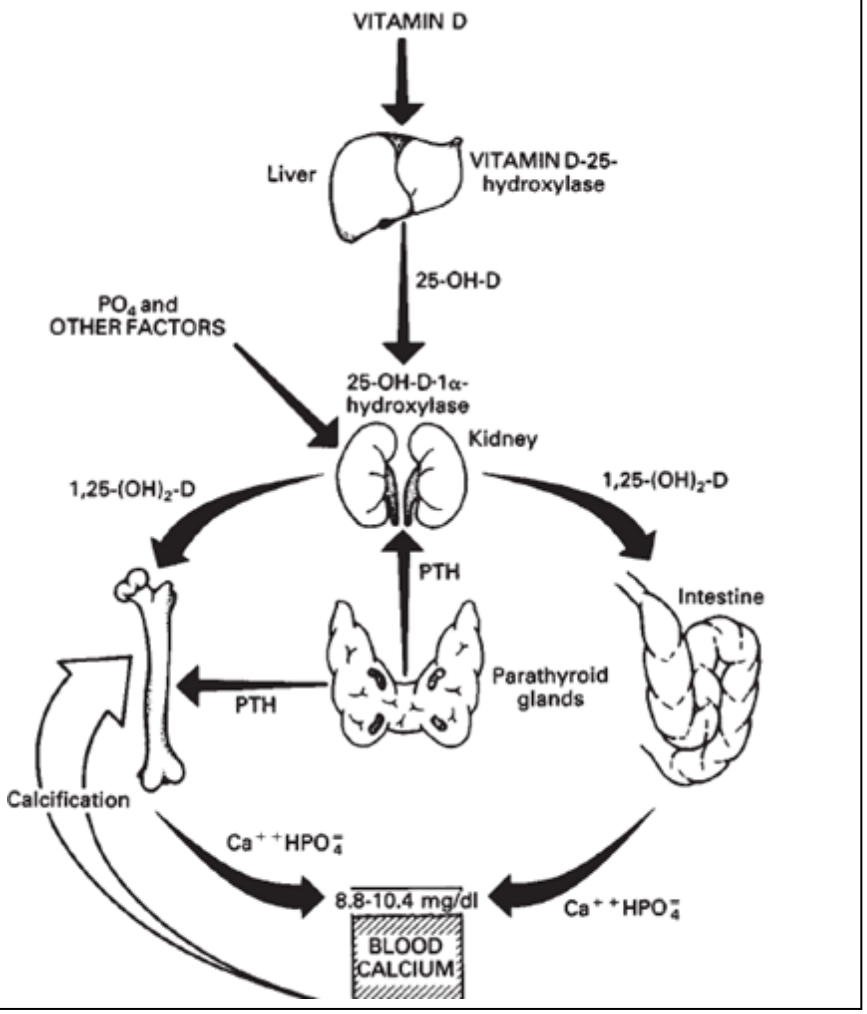
Three sub-studies within PrEP efficacy trials assessed BMD loss in comparisons of participants using active PrEP versus placebo (Table 1). The only one of these studies to include women (TDF2), found an overall net BMD decrease of up to 1.6% at the hip and spine with 30 months of TDF-based PrEP use. In this small study (N=114 women and 106 men), the proportion of participants with BMD losses >3.0% at any anatomic site was higher for PrEP versus placebo arms (50.0% vs 32.9%, p=0.04). However, this study was limited by low adherence to PrEP and high attrition rates. Complementary data from two studies among men, the iPrEx and CDC TDF Safety Study, provide consistent evidence, even in the face of low adherence.[21, 23]

Table 1: BMD loss in randomized clinical trials of TDF-based PrEP vs placebo in HIV-uninfected persons

Study, Authors [Year]	Anatomical site	Net %Δ in BMD (PrEP vs placebo)
TDF2 Study Kasonde [2014]	Hip	-1.55%; p=0.001
	Spine	-1.64; p<0.001
	Forearm	-0.86%; p <0.001
iPrEX Study Mulligan [2015]	Hip	-0.6%; p=0.01
	Spine	-0.91%; p=0.001
	Hip	-0.8%; p=0.001

Effects of TDF-based PrEP on subclinical kidney injury

Figure 2. Schematic representation of kidney Vitamin D processing and bone metabolism that are linked through vitamin D metabolism and calcium homeostasis pathways [Holick 1987]



Subclinical kidney injury is characterized by isolated abnormalities in some of markers of kidney function, but without generalized clinical disease manifestation. Kidney toxicity can result in phosphate wasting with hypophosphatemia and secondary hyperparathyroidism, leading to dysregulation of calcium-phosphate homeostasis and bone demineralization.[24-29, 33] Higher tenofovir plasma concentrations have been associated with higher vitamin D binding protein, lower 1,25-OH(2)D, lower estimated free 25-OHD, and increased parathyroid hormone (PTH) in HIV-infected persons. Vitamin D ensures a proper balance of calcium and phosphorus, key minerals for bone metabolism (Figure 2).[26, 27]

Our research has recently shown that TDF-based PrEP is associated with a small decline in estimated glomerular filtration rate (eGFR) in African men and women (Table 2).[34-36] It is not known whether this low level of kidney injury will be exacerbated with prolonged use of TDF-based PrEP or in women with small body mass. Studies are needed to address the effect of

concurrent use of DMPA and TDF-based PrEP on bone acquisition in young women who have generally small body structures that potentially elevate their risk for TDF-induced BMD decline and kidney injury.

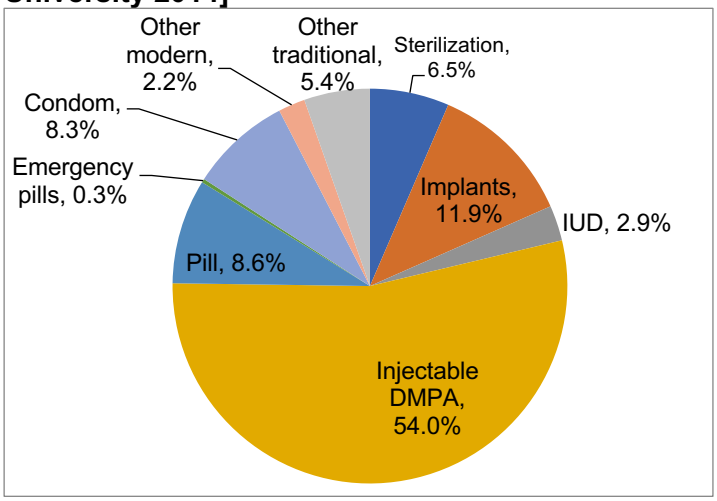
Table 2: Effect of TDF-based PrEP on estimated glomerular filtration rate in HIV-uninfected persons [Mugwanya et al. JAMA Int Med 2015]

	Net mean eGFR decline vs placebo (ml/min/1.73^2)	
	Randomized TDF-based PrEP agent	
	FTC-TDF	TDF
Women	-1.73; p=0.02	-1.47; p=0.05

Effects of DMPA on bone density

The most commonly used contraception in sub-Saharan Africa is DMPA, used by more than half of contracepting women in Uganda (Figure 3).[37-40] Intramuscular 150mg DMPA is often preferred because of it is easy to administer and can be used discretely. Intramuscular DMPA, as well as a lower dose subcutaneous formulation, is known to yield BMD loss to such an extent as to warrant a "black box" warning from the U.S. Food and Drug Administration.[38, 41-48] Since 2012, a global partnership of >50 governments, private sector, and multi-lateral organizations has made huge efforts to increase access to subcutaneous DMPA, implying that DMPA will remain the predominant contraceptive in this setting for the foreseeable future.[48] Women currently using DMPA have a lower average BMD than non-users and these effects are more pronounced in younger women.[41, 43, 44, 46, 49-51] Longitudinal studies have reported BMD losses up to 7.5% of BMD after ≥2 years of use (Table 3) and the greatest amount of loss during the first 1-2 years.[44]

Figure 3. Contraceptive method selection by contracepting Ugandan women [Makerere University 2014]



Effects of DMPA on bone metabolism

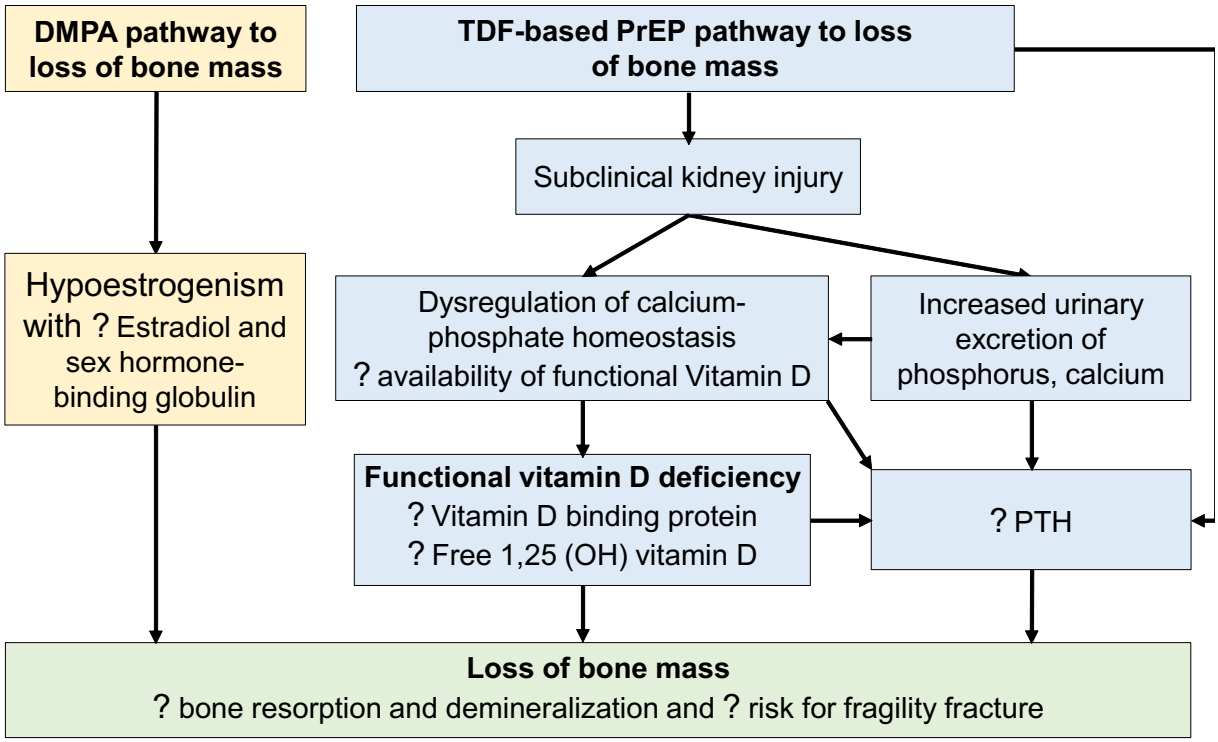
Studies of bone turnover in DMPA users have suggested that the effects of DMPA on the skeleton are at least partially mediated through increased bone turnover, with increases in markers of bone resorption, N-terminal telopeptide of type I collagen (NTX), and bone formation, P1NP. In addition, DMPA reduces estradiol and sex hormone-binding globulin (SHBG) levels, resulting in hypoestrogenism that is often accompanied by amenorrhea and alters the balance of bone resorption and formation yielding impairments in bone mass.[38, 52-56] Recent studies in animal models also suggest a role of estrogen to hasten recovery from kidney injury, leading to a hypothesis that subclinical kidney injury could be a consequence of hypoestrogenism.[57-59]

Table 3: Longitudinal studies of the association between DMPA and BMD loss

Study	Population	Anatomical site	Mean %Δ in BMD - DMPA vs Nonhormonal
Clark et al 2004	18-35 year old initiators	Hip	24 months:-5.7 vs <-0.4
		Lumbar spine	24 months:-5.7 vs <-0.4
Berenson et al 2004	18-33 year old initiators	Lumbar spine	24 months: -5.7 vs + 1.8
		Total hip	48 weeks: -1.6 vs 0.6; p<0.001
			240 weeks: -5.2 vs 0.2; p<0.001

Thus, as TDF-based PrEP is delivered in settings where DMPA is commonly used, a large number of HIV-uninfected women will be exposed to two agents that are each known to reduce BMD. Their concurrent use may act additively or synergistically through pathways of subclinical kidney injury and/or hypoestrogenism to adversely affect bone health and the risk of future fracture (Figure 4).

Figure 4. Schematic of pathways through which concurrent TDF-based PrEP and DMPA use could impact bone density



Summary of the potential for joint effects of PrEP and DMPA on bone health

For young women who equally need TDF-based PrEP to prevent HIV infection and effective contraception to prevent unintended pregnancy, concerns about TDF-induced reductions in bone mass and associated kidney injury must be heightened. Both are known to have minor effects on bone density and bone metabolic markers but through separate pathways. However, it is unknown if there is a joint effect of concurrent use of PrEP and DMPA on bone density and markers of bone metabolism and whether there are any cross-pathway effects that would result in clinically relevant changes to young women's bone health. This risk is particularly salient for women below age 25 who have not yet acquired peak bone mass and thus studying the effects of concurrent PrEP and DMPA use on bone health in this population is especially paramount.

Public health impact

This study will provide the first opportunity to evaluate reductions in bone mass and the preceding mechanisms triggered by the simultaneous initiation of PrEP and DMPA during a critical time of bone maturation in a cohort of young women. As PrEP delivery is brought to scale in multiple African settings where young women are a predominant risk population and DMPA use is widespread and will remain common, safety findings from our study will address a key knowledge gap and be pertinent to recommendations about concurrent use and how to best incorporate PrEP into the lives of young women.

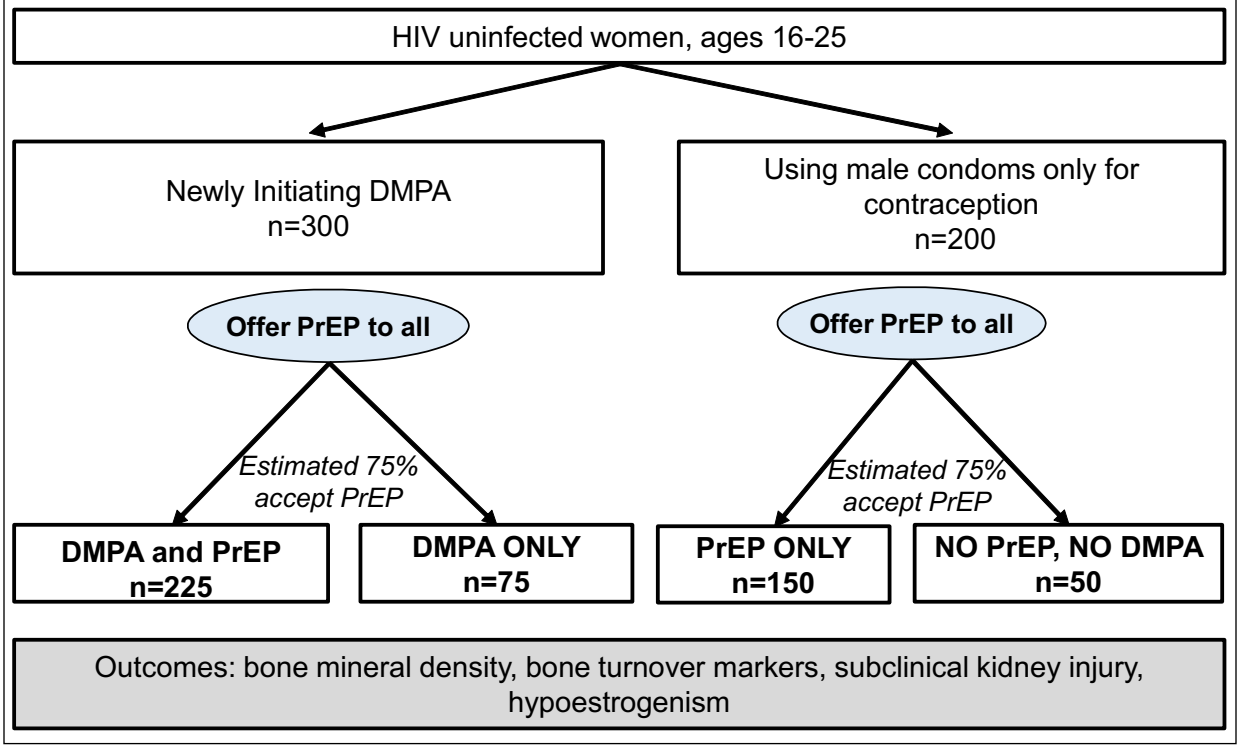
III. STUDY METHODS

Overall Design

The proposed research will investigate the hypothesis that TDF-based PrEP and DMPA initiated concurrently by HIV-uninfected women will enhance each other's effects on bone metabolism. We will conduct an open-label prospective study of approximately 500 HIV-uninfected women at substantial risk for HIV-infection who are newly initiating DMPA or using condoms as their only contraceptive in Kampala, Uganda (Figure 5). We will offer PrEP to all of these women and determine the joint effect of PrEP and DMPA on changes in bone mass (Aim 1), bone turnover, subclinical kidney injury, and hypoestrogenism (Aim 2), and conduct mediation analysis to assess the contribution of each pathway to overall losses in bone mass (Aim 3).

Aims

Figure 5. Study design for 2-year prospective follow study among healthy, HIV-uninfected women in Kampala, Uganda



1. **We will assess whether young women using TDF-based PrEP and DMPA concurrently attain lower peak bone mass over a 24-month period and have evidence of disrupted microarchitecture, relative to women using either agent singly or neither agent**

   We will use dual energy x-ray absorptiometry (DXA) scans to measure BMD annually at 3 anatomical sites (lumbar spine, total hip, and wrist). Additionally, we will derive the trabecular bone score (TBS), an index of lumbar spine trabecular microarchitecture.

   Hypothesis: Relative to women using DMPA only (without tenofovir exposure) and women using PrEP only (without DMPA exposure), women concurrently using TDF-based PrEP and DMPA will have lower bone mass and more disruptions in bone microarchitecture.

2. **We will investigate whether young women concurrently using TDF-based PrEP and DMPA experience: 1) higher rates of bone turnover, a direct precursor to bone loss and 2) subclinical kidney injury and hypoestrogenism, markers of two mechanistic pathways between TDF and DMPA use and bone mass**

   At baseline, 3, 6, 12, and 24 months, we will measure:

   1) Markers of bone formation and resorption (e.g. NTX, P1NP, serum intact parathyroid hormone, total and bioavailable 25-OH-vitamin D)
   2) Markers of kidney function (phosphate, glucose, creatinine, total protein, albumin) and
   3) Markers of estrogen (serum estradiol, sex hormone binding protein, and the occurrence of amenorrhea)

   Hypothesis: Relative to women using DMPA only and PrEP only, women concurrently using TDF-based PrEP and DMPA will have increased bone turnover markers and PTH. In addition, women concurrently using TDF-based PrEP and DMPA will have more frequent subclinical kidney injury (relative to women without tenofovir exposure) and reduced serum estrogen (relative to women without DMPA exposure).

3. **Using mediation analysis, we will identify the degree to which the pathways through subclinical kidney injury and hypoestrogenism account for changes in bone density among women concurrently using TDF-based PrEP and DMPA**

   We will conduct mediation analysis to determine the degree to which changes in the pathways through subclinical kidney injury, hypoestrogenism and the combination of these pathways account for changes in bone density.

   Hypothesis: The pathway through hypoestrogenism will be a stronger link between concurrent TDF-based PrEP and DMPA use and bone density changes.

Population

The proposed study will enroll approximately 500 HIV negative Ugandan women ages 16-25. This age group has a high risk for sexual HIV transmission and they also need effective contraceptive options and thus they will be an important part of the study population. Secondly, bone mass is acquired until approximately age 25 and there may be differences in the attainment of peak bone mass if PrEP and DMPA use is started at age 16 with up to 9 years of use prior to reaching peak bone mass versus age 18. Thus, since this research question is of particular importance to younger females and they will benefit most from the information obtained through this study, it is ideal to include them in the study population.

Location

Study visits will take place at the Infectious Disease Institute (IDI)-Kasangati research clinic, located in the Kasangati area of Kampala in close proximity of several public health care facilities. DXA scans will be conducted at the Makerere University-John Hopkins University Research Collaboration clinical site (MUJHU), ~5 km from the IDI-Kasangati research clinic Women will be transported to MUJHU for scans by study vehicles which has comprehensive insurance that covers bodily injury of all vehicle occupants and third parties and drivers.

Eligibility

Inclusion criteria:

- Age 16-25
    - If age 16-17:
        - qualification as an emancipated minor (due to past pregnancy, being married, having a child, or catering for their own livelihood) or
        - a mature minor (due to having a sexually transmitted infection) or
        - able to have a parent/guardian provide informed consent
- HIV-uninfected
- Initiated DMPA within the past 90 days or using condoms only for contraception
- Willing and able to provide written informed consent
- Not planning to get pregnant in the next 24 months
- Sexually active
- Planning to remain in the study area for the next 2 years

Exclusion criteria:

- Currently enrolled in a biomedical HIV-1 prevention study
- Current or prior use of PrEP consecutively in the last 3 months
- Abnormal renal function (creatinine clearance <60 min/ml)
- Hepatitis B infection
- Currently pregnant or breastfeeding
- If history of DMPA use, 1) DMPA use of greater than 90 days with last injection within 2 years of enrollment or 2) continuous DMPA use of greater than 2 years duration at any time
- Current use of implant, IUD, or oral contraceptives
- Past hysterectomy, oophorectomy, or tubal ligation
- Current or recent history of primary or secondary amenorrhea
- Taking medications known to interfere with bone metabolism (steroids, anti-convulsants, bisphosphonates, cancer drugs).
- Has any other condition that would preclude the ability to provide informed consent, make study participation unsafe, complicate the interpretation of study findings or otherwise interfere with achievement of the study objectives, in the investigator's discretion.

Sample size and study power

Power calculations are based on the number of participants needed to achieve aim 1. Our target sample size is 500 women, of which 300 will be using DMPA and 200 will be using condoms for contraception. We calculate that with 300 women newly initiating DMPA and 75% of women choosing to initiate PrEP, thus with 225 women concurrently using PrEP and DMPA and 75 women using DMPA without PrEP, we will have >90% power to detect changes as low as 2.2% in BMD (with 1.0 g/cm^2 mean BMD loss) at any anatomical site between women using PrEP and DMPA concurrently and women using DMPA only during the 24 month follow up period (Table 4). Should more than 90% of women choose to initiate PrEP (270 women concurrently using PrEP and DMPA and 30 women using DMPA without PrEP), we will have 80% power to detect changes of >2.7% or more and should initiation rates be closer to 50% (150 women concurrently using DMPA and PrEP and 150 women using DMPA without PrEP), we will have >90% power for all comparisons.

Table 4. Power calculations. N=500: 300 on DMPA and 200 on condoms, shown with a range of PrEP acceptance for the primary comparison of DMPA + PrEP vs DMPA (α=0.05)

	Outcome: BMD (Aim 1)			Outcome: NTX (Aim 2)		
Expected PrEP acceptance	Mean %Δ (std dev range)	Mean Δ	**Power**	Mean %Δ	Mean Δ	**Power**
75% (225 DMPA+PrEP, 75 DMPA)	2.2 (4–5)	0.5 (0.5–1.0)	91%	2.2 (4–5)	0.10 (0.2 – 0.5)	>99%
75% (225 DMPA+PrEP, 75 DMPA)	2.5 (4–5)	0.5 (0.5–1.0)	>96%	2.5 (4–5)	0.20 (0.2 – 0.5)	>96%
75% (225 DMPA+PrEP, 75 DMPA)	3 (4–5)	1.0 (0.5–1.0)	>99%	3 (4–5)	0.50 (0.2 – 0.5)	>99%
90% (270 DMPA+PrEP, 30 PrEP)	2.5 (4–5)	0.5 (0.5–1.0)	74-90%	2.5 (4–5)	0.10 (0.2 – 0.5)	>99%

Recruitment

Recruitment strategies will include collaboration with local family planning clinics, gynecologists, women's health clinics, HIV counseling and testing centers, and public and private health clinics. Additionally, we will conduct outreach activities during community markets to recruit women newly initiating contraception. Recruitment materials will educate women about the benefits and potential adverse effects of contraceptives and PrEP, based on available data.

General study procedures

Over a 24-month period, we will follow women quarterly with HIV rapid testing, PrEP adherence counseling and refills, DMPA injections, and provision of other contraceptives as desired. At enrollment and quarterly visits, we will assess physical activity, sexual behavior, and menstrual cycle characteristics. Annually, we will conduct physical exams and anthropometric readings. Sexually transmitted infections will be routinely assessed syndromically, according to national guidelines. Women will be encouraged to consider their HIV risk and fertility desires at every visit and counseled about contraception and PrEP accordingly. Dual energy x-ray absorptiometry (DXA) scans will be performed at baseline and annually thereafter. Blood and urine samples will be obtained quarterly, aliquoted, and archived at minus 80^{o} Celsius.

DXA and TBS assessments

DXA scans will be conducted at study enrollment (within 1 week of contraceptive initiation if possible), 12, and 24 months after enrollment. DXA is the clinical standard for measuring BMD. It is painless and non-invasive. Women will be exposed to a relatively low level of radiation which is <1/10 the dose of a standard chest x-ray, and less than a day's exposure to natural radiation. BMD by DXA of the hip and spine is generally considered the most reliable way to classify osteoporosis, assess changes in BMD, and predict fracture risk. We will measure areal BMD of the lumbar spine (L1-4), hip, whole body and body composition (total and % fat and lean mass) using a DXA machine. For the TBS analysis, no additional image acquisition is required. TBS will be performed as a re-analysis of the lumbar spine taken as part of the standard DXA procedure with specialized software.

Contraceptive provision

Women will initiate contraception at the study clinic or a family planning clinic, as they are seeking services. Throughout study follow up, the study will assume responsibility for contraceptive provision, including DMPA injections, and care of all other methods desired by women. The staff at the IDI-Kasangati research clinic have extensive experience with the provision of contraception and contraceptive counseling during previous HIV prevention studies.

PrEP provision and adherence monitoring

Co-formulated emtricitabine (FTC)-TDF (Truvada®) will be provided as PrEP by the study through donation from Gilead Sciences, LLC. PrEP counseling and provision will follow WHO guidelines, or Ugandan clinical guidelines when they are available.[60] We will measure adherence using three different methods:

1. Pill counts will be conducted by the pharmacist during study visits to determine the percentage of expected pills that were used
2. Real time monitoring will be conducted using an electronic monitoring system that captures a date-time stamp each time the pill container is opened
3. The primary circulating form of TDF, tenofovir (TFV), will be quantified in blood archives from 1) a random sample of up to 15% of women who initiate PrEP to characterize the adherence in the cohort overall and 2) a targeted group of women who have no BMD change and women who experience a fracture

Laboratory processing to measure markers of bone turnover, subclinical kidney injury, and hypoestrogenism

Using state-of-the-art assay techniques on archival samples, we will measure 1) established markers of bone turnover and bone health, 2) markers of subclinical kidney injury, and 3) markers of hypoestrogenism (Table 5). Archived samples will be batch-analyzed.

Table 5. Measurements for markers of bone turnover, subclinical kidney injury, and hypoestrogenism

Outcome	Biomarkers	Primary Outcome definition	Secondary Outcome definition
Bone turnover and bone health	NTX, P1NP	Percent change from baseline in NTX and P1NP	Percent change from baseline in serum levels of: • intact PTH • vitamin D binding protein, • 25-(OH) vitamin D
Subclinical kidney injury	• Urine: phosphate, glucose, creatinine, total protein, albumin • Serum: phosphate, glucose, creatinine	eGFR decline calculated using Chronic Kidney Disease Epidemiology equation (≥25% decline from baseline or creatinine clearance <60 mL/min)	• Tubular proteinuria: elevated urine protein with urine albumin: protein ratio <0.4, • Euglycemic glycosuria, • Phosphaturia defined as increased fractional excretion of phosphorus >15% or maximum rate of tubular phosphate reabsorption to the glomerular filtration rate
Hypoestrogenism	• Serum estradiol • Sex hormone-binding globulin	Percent change from baseline of serum estradiol with control for SHBG	Frequency of amenorrhea, defined as the absence of menses for at least 3 menstrual cycles

Retention and study exit

Upon completion of study follow up, women will be provided with referrals to clinics of their choice to continue contraceptive and PrEP provision. Since the interim guidelines for HIV prevention now include PrEP, by the time this study is completed, we anticipate that PrEP will be available through public facilities. We will provide participants with information about which facilities are actively providing PrEP and link them to services at that clinic via a referral.

Special circumstances

a. HIV seroconversion

HIV rapid testing will be conducted quarterly during follow up. We expect the rate of HIV seroconversion to be very low, due to the use of PrEP and repeated counseling about HIV prevention and PrEP. However, in the event of HIV positive rapid test results, PrEP will be stopped immediately and additional blood samples will be drawn for confirmatory EIA testing, plasma HIV RNA quantification, drug resistance, and/or drug levels. When with confirmed HIV seroconversion will be extensively counseled and referred to local clinics providing antiretroviral medications and HIV care. At the visit when HIV seroconversion is confirmed, women will be exited from the study once the set of study procedures for an exit visit are completed. HIV seroconverters who are pregnant will be referred for services to prevent mother to child HIV transmission and study staff will go to great lengths to ensure that women experience no delays in accessing these services.

b. Pregnancy

We expect that some women who stop using contraception will become pregnant. Women who become pregnant while using PrEP will be counseled about the known and unknown risks and benefits of PrEP use during pregnancy, in accordance with U.S. CDC and WHO guidelines.[60, 61] Women who are pregnant will not undergo DXA scans, and their DXA scans will be postponed until their pregnancy is complete.

Visit-specific procedural tables

	SCR	ENR	M1	M3 & M15	M6 & M18	M9 & M21	M12 & M24 & EXIT
ADMINISTRATIVE & REGULATORY PROCEDURES							
Obtain informed consent	X	X					
Collect/update locator information	X	X	X	X	X	X	X
Collect/update demographic information	X	X					X
CLINICAL & COUNSELING PROCEDURES							
HIV pre and post-test counseling	X	X	X	X	X	X	X
Risk reduction counseling & condom provision	X	X	X	X	X	X	X
Contraceptive counseling		X	X	X	X	X	X
Contraceptive provision			[X]	X	X	X	X
PrEP counseling, including adherence counseling	X	X	X	X	X	X	X
PrEP provision		X	X	X	X	X	X
PrEP pill count			X	X	X	X	X
Physical exam		X	X	[X]	[X]	[X]	X
Anthropometric readings		X					X
DXA scan (unless pregnant)		X					X
Syndromic management of STIs	X	X	X	X	X	X	X
LABORATORY PROCEDURES							
Creatinine testing when using PrEP	X				X (M6 only)		X
HBV surface antigen	X						
HIV rapid test (EIA testing and HIV RNA quantification, if rapid test positive)	X	X	X	X	X	X	X
Urine pregnancy test	X	X	X	X	X	X	X
Collect urine specimen for archive		X		X	X	X	X
Collect blood specimen for archive		X		X	X	X	X
CLINICAL & BEHAVIORAL DATA COLLECTION							
Medical history questionnaire, including occurrence of fractures	X	X	X	X	X	X	X
Sexual behavior & contraceptive use questionnaire		X	X	X	X	X	X
Physical activity & dietary questionnaire		X		X	X	X	X

Menstrual cycle questionnaire	X	X		X	X	X	X
X required procedure; [X] if clinically indicated							

Safety monitoring

Kidney function will be monitored 6 months after initiation and annually thereafter, in accordance with Ugandan guidelines, using creatinine clearance calculated by Cockroft–Gault equation. DXA scans will be reviewed in real time and women with bone density below -2.0 standard deviation of the z-score will be referred locally for vitamin D assessment and provided with any necessary therapy. The investigators will be responsible for continuous close monitoring of all adverse events that occur among study participants. For the purposes of this study, only serious adverse events (SAEs) and adverse events felt related to PrEP will be documented on case report forms. Adverse events and the severity of clinical symptoms will be scored using the DAIDS Table for Grading the Severity of Adult and Pediatric AEs.[62] The study safety monitor will review clinical and laboratory SAEs in real time as they are reported by the site clinicians on CRFs and through email within 48 hours of occurrence. As needed, the safety monitor will work provide rapid consultation with clinicians regarding the management of toxicities. A Safety Review Team comprised of the study investigators and lead clinicians will review clinical and laboratory safety reports summarizing SAEs via conference call on an approximately quarterly basis.

IV. DATA COLLECTION

Clinical data including anthropometric, dietary, physical activity and other lifestyle and sexual behaviors, will be recorded electronically on case report forms (CRFs) and data will be uploaded to a secure server. Automated legal range checks will be programmed to reduce data entry errors and internal quality control reports will be run on a monthly basis. Results from laboratory testing will be returned to the data center at the University of Washington and stored alongside other key study data. DXA scan images will be downloaded to project-specific databases and sent to the University of Washington via secure file transfer system. TBS measurements will be performed using the lumbar spine DXA images. PrEP adherence data from the electronic monitoring device will be stored on an encrypted website and downloaded to the University of Washington server on a regular basis.

V. DATA ANALYSIS

Aim 1. We will assess whether young women using TDF-based PrEP and DMPA concurrently attain lower peak bone mass over a 24-month period and have evidence of disrupted microarchitecture, relative to women using either agent singly or neither agent

The primary analysis will be separate comparisons of the annualized rates of change in BMD and TBS of the spine and hip between women using PrEP and DMPA concurrently versus women using DMPA only (comparison 1), women using PrEP only (comparison 2), and women using neither (comparison 3). Analyses will account for baseline BMD.

Aim 2, part 1. We will investigate whether young women concurrently using TDF-based PrEP and DMPA experience higher rates of bone turnover relative to women using either agent singly or neither agent

This outcome will be defined by the percent change from baseline of serum levels of NTX and P1NP over 24 months. Secondary outcomes will include: 1) percent change from baseline in serum levels of intact PTH, total 25-(OH)D, bioavailable (i.e. unbound or free) 25-(OH)D calculated from vitamin D binding protein (VDBP) using the standard equation.

$$\text{Free } 25(\text{OH})\text{D} = \frac{\text{total } 25(\text{OH})\text{D}}{1 + (6 \times 10^3 \times \text{albumin}) + (7 \times 10^8 \times \text{DBP})}$$

To test the hypothesis that combined PrEP and DMPA use is associated with greater bone turnover than DMPA use alone, we will use time-dependent linear mixed-effects regression models to compare mean percent changes in NTX and P1NP between concurrent users of PrEP and DMPA versus users of DMPA only. Secondary powered comparisons will be between: a) concurrent users of PrEP and DMPA versus users of PrEP only; b) users of PrEP only versus users of condoms only; c) users of DMPA only vs users of condoms only. Analysis of the percent change from baseline in serum levels of intact PTH, total 25-OH-D and bioavailable 25-OH-D over 24 months will follow a similar approach. All analyses will be adjusted for confounders and we will consider a broad range of demographic, medical, lifestyle, and sexual behavior characteristics as potential confounders.

Aim 2, part 2. We will investigate whether young women concurrently using TDF-based PrEP and DMPA experience elevated markers of subclinical kidney injury relative to women using TDF-based PrEP alone

This outcome will be defined as having either:

1) eGFR decline (≥25% decline from baseline or creatinine clearance <60 mL/min). GFR will be calculated using the Chronic Kidney Disease Epidemiology (CKDE) collaboration equation
2) proximal tubular injury (tubular proteinuria defined as elevated urine protein with urine albumin- protein ratio <0.4, euglycemic glycosuria, phosphaturia defined as increased urinary excretion of phosphorus >15% or maximum rate of tubular phosphate reabsorption to the glomerular filtration rate).

These markers were selected because they are routinely available in clinical practice and have been used in prior studies. The cutoff of ≥25% for eGFR decline was adapted from established criteria for the diagnosis of acute kidney injury;[41] eGFR decline of this magnitude has been associated with increased morbidity and mortality.[22-24]

To determine whether young women using PrEP alone or concurrently with DMPA experience higher rates of subclinical kidney injury, we will perform a qualitative comparison of the incidence of individual markers of kidney injury among women using PrEP (alone or concurrently with DMPA) versus women who have no exposure to TDF.

Aim 2, part 3. We will investigate whether young women concurrently using TDF-based PrEP and DMPA experience elevated markers of hypoestrogenism relative to women using DMPA alone

This outcome will be defined as the percent change from baseline serum estradiol over 24 months and we will adjust for sex hormone-binding protein.[56] The secondary outcome will be based on self-reported missed menses, with >3 missed menses in a row categorized as amenorrhea.

To determine whether young women concurrently using DMPA alone or concurrently with PrEP experience greater frequency of hypoestrogenism, we will conduct time-dependent logistic

regression among women using DMPA and women not exposed to DMPA, comparing the proportion of visits with hypoestrogenism.

Aim 3. Using mediation analysis, we will identify the degree to which the pathways through subclinical kidney injury and hypoestrogenism account for changes in bone density among women concurrently using TDF-based PrEP and DMPA

Our goal for this analysis is to assess the relative magnitude of each pathway on BMD. To define each mediator pathway, we will use the set of markers that is most predictive of the mean percent change in BMD. For the subclinical kidney injury pathway, the set of markers may include phosphate, glucose, creatinine, total protein, and albumin. Because the relationship between TDF-induced subclinical kidney injury and dysregulation of PTH and vitamin D metabolism remains unclear, we will also consider bioavailable 25-OH vitamin D, and/or intact PTH measurements as potential markers of this pathway. In secondary analyses, we will consider markers of bone turnover as an alternative outcome to BMD changes. For the hypoestrogenism pathway, the set of markers may include estradiol, sex hormone-binding globulin, and/or amenorrhea.

We will estimate the crude and adjusted direct and indirect effects of concurrent TDF-based PrEP and DMPA use on mean percentage change in BMD through each pathway by constructing separate regression models of BMD conditional on TDF and DMPA exposure, the mediator's occurrence, and baseline covariates. We will compute the proportion mediated by each mediator individually as well as the both mediators concurrently closed form estimators. Bootstrapping will be used to compute standard errors. Our interpretation of these results will carefully specify model assumptions including no unmeasured confounding between the exposure and outcome, exposure and mediators, and mediators and outcome.[63]

VI. HUMAN SUBJECTS CONSIDERATIONS

The protocol, informed consent forms (for cohort participation and for interviews of providers), and patient education and recruitment materials will be reviewed and approved by the IRBs/ECs responsible for oversight. Subsequent to initial review and approval, the responsible IRBs/ECs will review the study at least annually.

Study oversight

An independent Data Monitoring Committee will be established to protect patient safety by monitoring study outcomes, implementation, and data quality. The committee will consist of expert clinicians, statisticians, and scientists, including Ugandans, in the field of PrEP and family planning delivery in sub-Saharan African settings. The committee will meet via teleconference every 6 months and be charged with the decision to recommend stopping the study early if interim analyses yield any safety concerns. In addition, the committee will provide guidance about results from international studies of PrEP and contraception that may spur Ugandan policy change. Reports from all reviews will be submitted to overseeing institutional review boards and ethics committees.

Informed consent

We will perform paper-based or electronic-based consenting of participants. After we have ensured that participants have read and understood the consent forms, we will ask them to append their signature on a tablet or on paper. Each participant will receive a participant information sheet that they can use for reference. However, any participant who wants their consent form to take home will have it printed and given to them. Using electronic-based consenting will greatly reduce the burden of storing paper-based consent forms.

Risks

Risks for this cohort include side effects from PrEP provided to the young women through the study clinic, DMPA and other contraceptives provided through Ugandan public health clinics according to the national guidelines for contraceptive provision in Uganda, and discomfort associated with blood draws, physical exams, interviews, and HIV counseling and testing. Intensive and ongoing counseling and monitoring of side effects and adverse events limit the risk for participants from the direct study procedures. Women will have three DXA scans during the course of the study and be exposed to radiation during each scan. The effective radiation dose from the DXA procedure, approximately 0.01 millisievert (mSv), is similar to that received during a 5-hour airplane flight. The total dose over these 3 scans will be several orders of magnitude less than the radiation exposure women will have from their natural environments (from sources such as sun, air, food, and soil). Women who become pregnant will not undergo DXA scans.

For clinical data collection, standardized surveys will be used that will include questions on sensitive topics, such as sexual behavior. All participants will be extensively counseled about the risk for HIV transmission with sex unprotected by a condom and/or PrEP and the importance of using condoms consistently and correctly. We expect that the risk of HIV transmission will actually be lower in the proposed novel cohort than the general population of young women due to the careful attention that will be given to counseling women about HIV risk and the provision of PrEP. Study staff will be aware of the potential for confidentiality issues with regards to test results from HIV testing that are part of a participant's study documents. For minors who enroll with parental consent, results of HIV testing will be confidential and not available to the parent or guardian. But the study will stipulate that minors will be encouraged to disclose positive results to a responsible adult, which need not be their legal guardian. Support will be provided for such disclosures.

There is the potential for confidentiality issues with regards to study data. Particularly, since parental consent will be required for some participants under the age of 18, these participants would have to share their choice to participate in a study that will follow their contraceptive and PrEP use choices and collect detailed sexual behavior data, whereas otherwise they may not have chosen to disclose this information. Should there be any psychosocial stress associated with participation in the study, counseling by study staff will be provided, or referral to appropriate mental health professionals will be done. The age of consensual sex is 16 years, and therefore underage sex is not an issue in this protocol.

Benefits

Participants will receive individualized HIV risk-reduction counseling and counseling about pregnancy planning and prevention of unintended pregnancy, access to PrEP medication, contraceptives, and free condoms. In addition, participants may benefit in the future from information learned from this study.

Care for persons identified as HIV positive

Women who become infected with HIV will be referred immediately to HIV care centers and encouraged to initiate antiretrovirals in line with Ugandan national antiretroviral guidelines.

Seroconverters who become pregnant will be referred for prevention of mother to child HIV transmission services.

Treatment for injury

Participants will be asked to inform the clinic staff if they feel they have been injured because of taking part in the study. Injuries may also be identified during laboratory testing, medical histories, and physical examinations. Treatment for adverse events related to study participation will be provided by the treatment clinic. If treatment is required that is beyond the capacity of the clinic, the clinic staff will refer the participant to appropriate services or organizations that can provide care for the injury. The study based at IDI will cover the costs of the referral and treatment costs up to the time the participant is stable or injury is resolved.

Study records

Complete, accurate, and current study records will be maintained and stored in a secure manner throughout the study. Study records include administrative documentation and regulatory documentation as well as documentation related to each participant enrolled in the cohort, including informed consent forms, data forms, notations of all contacts with the participant, and all other source documents.

Confidentiality

Every effort will be made to protect participant privacy and confidentiality to the extent possible. Personal identifying information will be retained at the local study site.

VII. CLINICAL RESEARCH SITE

Site location

The Infectious Disease Institute (IDI)-Kasangati research clinic is part of the Infectious Diseases Institute, Makerere University College of Health Sciences. The facility is located next to Kasangati Health Center IV and has adequate space and equipment for ongoing and planned research studies. The Infectious Diseases Institute training team offers specialized courses in HIV management for health providers in Africa. For this study the focus of the training will be combination HIV prevention services including PrEP.

Clinical facilities

The site has a large training room that can accommodate up to 50 people, 3 clinical rooms, 6 counseling rooms, a pharmacy, a phlebotomy room, a side lab, secure data room with data archive, administrative office space, wireless internet, telephone intercom system, community education offices, and a large waiting area which provide sufficient space for the execution of this study.

Staff

The Principal Investigator is a seasoned HIV/AIDS researcher and trainer, of local and international repute. He will lead the study team to maximize the scientific, ethical integrity of the study and ensure the training provided is of high quality. The study employs in total 3 doctors, 2 nurse counselors, 1 pharmacy technician, 2 laboratory staff and 2 community educators/counselors, 2 data personnel, 4 qualitative research associates as well as support and administrative staff. The respective staff are overseen by a site coordinator, training coordinator and administrator. The study site previously implemented the Partners PrEP Study and the

Partners Demonstration Project. The staff have substantial experience in the use of PrEP and its accompanying supportive services. The staff have been involved in the conduct of protocol specific training, development of outreach materials, and have skills in training of adult learners.

Administrative procedures

All administrative procedures regarding protocol compliance, study coordination, study activation, study monitoring, study records, and use of information and publications will be done according to good clinical practice.

Laboratory considerations

We will as much as possible use the public health laboratory facilities for routine laboratory monitoring. In situations where this is not possible, specimens will be collected and transported to the study collaborating laboratories. All specimen collection, transport, processing, testing, archiving, and results reporting will be conducted in accordance with good clinical and laboratory practice standards. The collaborating laboratories for the research clinic are the MUJHU-IDI Core Laboratory, the Makerere University Walter Reed Laboratory, and the Molecular Biology Laboratory in the Department of Medical Microbiology at Makerere College of Health Sciences. These are well-established labs with extensive experience in supporting clinical trials. External Quality Assurance (EQA) procedures will be followed throughout the study and are overseen by the University of Washington International Clinical Research Center.

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