

**Bone Mineral Density in a Cohort of Young Adult Women Using
Depo-Provera and Tenofovir,
Kampala, Uganda**



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DECLARATION

I, Flavia Kiweewa Matovu declare that this thesis is my own work. This thesis is being submitted in fulfillment for the degree of Doctor of Philosophy in Public Health at the University of Witwatersrand, Johannesburg, South Africa. This work has not been submitted before for any degree or examination at this or any other university.

Signature:  _____

Date: 05 November 2021

DEDICATION

Your guidance and wise counsel still live on. Although our paths only crossed for the first 15 years of my life, your influence lives on. I would not be who I am today without the influence you had on my life. It is unfortunate that you were called home sooner than we ever expected; it was at a very tender age when your life was just beginning. We will never know why but we trust that God knows best. You believed in me and I am glad that I have not disappointed you. You would have been the proudest person today to know that all you wished for me has come true. Your efforts and tough love have yielded. You will always be valued and missed my sister and most impactful influence, **Robinah Nassaka Matovu**.

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PUBLICATION STATEMENT

All publications from this thesis have been conceptualized and written up as first author by the candidate. The status of the publications and contributions of other authors is detailed below. All published or submitted manuscripts are appended at the end of this thesis. Each manuscript is formatted in the style and guidelines of individual journals to which they were submitted.

Contributions of the authors in the different publications;

Flavia Kiweewa Matovu: Principal Investigator, Conceptualized and developed the protocol, lead the design, execution, analysis, and write up of all manuscripts as first author.

Malgorzata Beksinska: Supervisor, guided and supported the candidate in designing the protocol, data collection, analysis, interpretation and manuscript writing.

Prof. John M. Pettifor: Supervisor, guided and supported the candidate in designing the protocol, data collection including execution and interpretation of DXA scans, analysis and manuscript writing.

Todd T. Brown: Endocrinologist, Johns Hopkins Bloomberg School of Public Health, mentored and supported the student in grant writing for an NIH R01 award, provided expertise in the design and execution of the protocol as well as manuscript writing.

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Delia Scholes: Epidemiologist, supported the student in grant writing for an NIH R01 award, provided expertise in the design and execution of the protocol as well as manuscript writing.

Martin Nabwana: Was responsible for data management and analysis.

Prof. Noah Kiwanuka: Guided the analysis.

Stephen Bule and Andrew Kateregga: Performed the DXA scans.

Drs. Deo Wabwire, Betty Kamira and Samuel Kabwigu: Offered clinical consultation services to the study team.

Esther Isingel: Study coordinator, oversaw day to day running of the study, and assisted with data cleaning.

PUBLICATIONS AND CONFERENCE PRESENTATIONS

Two manuscripts have been published and one has been submitted for publication with The Lancet journal and favorable feedback received.

Publications

1. Matovu FK, Wattanachanya L, Beksinska M, Pettifor JM, Ruxrungtham K. Bone health and HIV in resource-limited settings: a scoping review. *Current Opinion in HIV and AIDS*. 2016 May;11(3):306-25.
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1. Bone Mineral Density in a Cohort of Young Adult Women Using Depo-Provera and Tenofovir, Kampala, Uganda, 19th International Workshop on Adverse Drug Reactions and Co-Morbidities in HIV in Milan, Italy, 23rd to 25th October 2017, *Oral Abstract no: ADRLH-79*.
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Media appearances and blogs related to this research

1. Video abstract published in the journal of the Current Opinion in HIV/AIDS as part of paper 1 of this PhD project entitled, "Bone health and HIV in resource-limited settings: a scoping review"
https://cdn-links.lww.com/permalink/coha/a/coha_2016_04_22_matovu_coh110306_sdc1.mp4
2. Makerere lecturer wins award on HIV research - The Observer (22 February 2016)
<https://observer.ug/news-headlines/42718-makerere-lecturer-wins-award-on-hiv-research>
3. CARTA fellow wins prestigious NIH grant (Makerere University News 27 January 2016)
<http://news2.mak.ac.ug/2016/01/carta-fellow-wins-prestigious-nih-grant/>
4. *Depo Provera* may worsen tenofovir-related bone loss in young women (aidsmap 9th July 2020)
<https://www.aidsmap.com/news/jul-2020/depo-provera-may-worsen-tenofovir-related-bone-loss-young-women>
5. Depo Provera may worsen tenofovir-related bone loss in young women – aidsmap (Osteoporosis International 9th July 2020) <https://osteoporosis-international.blogspot.com/2020/07/depo-provera-may-worsen-tenofovir.html>
6. Depo Provera associated with doubled bone loss in women starting ARVs (Medical brief 15th July 2020)
<https://www.medicalbrief.co.za/archives/depo-provera-associated-with-doubled-bone-loss- in-women-starting-arvs/>

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2. **“5 Pillars” On Spot Award** from the MU-JHU Executive Committee in recognition of outstanding achievements as an independent investigator. This includes all-round excellence contributing to each of the 5 research pillars: partnerships, implementing new research studies, prolific grant writing, disseminating findings and mentoring others (November 2017).
3. **David Cooper Travel Award**, a travel and conference participation grant at the 21st International Workshop on Adverse Drug Reactions and Co-Morbidities in HIV in Basel, Switzerland, 5th to 6th November 2019.
4. **Gilead Sciences Inc., ISR-UG-18-10327 Award**; A research grant to support a prospective study entitled, “Bone Mineral Density in a Cohort of African Women on Depo-provera and Tenofovir Disoproxil Fumarate Switched to Tenofovir Alafenamide Fumarate (TAF) based ART Anti-Retroviral Therapy”.
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6. **A further “5 Pillars extraordinary accomplishments award”** from the MU-JHU Executive Committee in recognition of extraordinary achievements and productivity as an independent investigator (December 2020).

LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral
BMD	Bone Mineral density
BMI	Body mass index
BONE: CARE	BONE: Contraception and Anti-retroviral Effects
CAPI	Computer-Assisted Personal Interviewing
CRF	Case Report Form
CTX	C-telopeptide
DAIDS	Division of AIDS
DMPA	Depo MedroxyProgesterone Acetate
DXA	Dual energy X-ray Absorptiometry
EC	Ethics Committee
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
FTC	Emtricitabine
IGF-1	Insulin-like growth factor 1
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
IDI	Infectious Diseases Institute
IRB	Institutional review board
KCCA	Kampala Capital City Authority
MJAP	Makerere University Joint AIDS Programme
MU-JHU	Makerere University – Johns Hopkins
NHANES	National Health and Nutrition Examination Survey
NIH	National Institutes of Health
NRTI	Nucleoside Reverse Transcriptase Inhibitor

ODK	Open Data Collection
P1NP	Procollagen type 1 N-terminal propeptide
PBM	Peak Bone Mass
PI	Principal Investigator
PLWH	Persons Living With HIV
PTH	Parathyroid hormone
PTID	Participant Identification
QC	Quality Control
RLS	Resource Limited Settings
RNA	Ribonucleic acid
RRL	Resource Rich Setting
SSA	Sub Saharan Africa
TAF	Tenofovir Alafenamide Fumarate
TASO	The AIDS Support Organization
TBS	Trabecular Bone Score
TDF	Tenofovir Disoproxil Fumarate
UNCST	Uganda National Council for Science and Technology
US	United States
UVRI	Uganda Virus Research Institute
WHO	World Health Organization
WLWH	Women Living With HIV
3TC	Lamivudine

PREFACE AND ACKNOWLEDGEMENTS

If I have seen further, it is by standing on the **SHOULDERS OF GIANTS**.

- Isaac Newton

What a fulfilling and transformative journey my PhD process has been! The journey began with getting admitted to Wits as Cohort 4 CARTA PhD Fellow – thanks to Professor Sharon Fonn. By then I was “sure” I knew what I wanted to do as an experienced researcher until I met my supervisors Professors Mags Beksinska and John M. Pettifor over a skype call. I was so convinced about my research idea that it took Mags and John to hit the point home. In his own words (John), “I know Flavia you have invested a lot of time into developing your PhD proposal but I am afraid this is not PhD material. A PhD is an opportunity to launch you into your professional career and you therefore need novel research using a prospective design.” This was not easy for me as expected but I later came to appreciate John’s fore sight. I cannot thank my supervisors enough as with their support the idea evolved to a level of an NIH R01 research grant. Through the grant application process, I in addition received overwhelming support from many other mentors including Scholes and Professor Mary Glenn Fowler who linked me to Professor Todd T. Brown who mentored me through the grant writing and award phase. Todd accepted to take time off his vacation in Greece to join the writing team. I would never have known how to write a specific aims page, respond to NIH summary critique or write a probing letter to the program officer. The team has been extremely supportive through the study implementation and data analysis phase through monthly calls, and prompt email responses. Unlike so many PhD students, I never ever had to send reminder emails to my supervisors and mentors. Mags, your support visit all the way from Durban to check on the progress of the BONE: CARE study was particularly humbling. I will forever be grateful and will always remain indebted to you all my mentors – Professors **John, Mags, Todd, Mary Glenn, Delia, and Dennis Israelski!**

Special thanks to my husband, Francis Kiweewa who selflessly forwarded me the CARTA PhD advert and encouraged to apply. Thank you for your love, patience, and support through this long journey. You willingly and ably assumed the roles of both father and mother to our lovely daughter (Flavia) and sons (Franklin, Favian and Fidel. The popular question,” Mummy when are you finishing your PhD?” has finally been answered.

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“The boundary lines have fallen for me in pleasant places; surely I have a delightful inheritance.”

Psalms 16:6



Flavia Kiweewa Matovu

April 2019, Johannesburg, South Africa

ABSTRACT

Antiretroviral therapy (ART) initiation with tenofovir disoproxil fumarate (TDF) leads to bone loss with greatest declines occurring within the first two years of use. Among women of reproductive age, the three-monthly contraceptive, intra-muscular depot medroxyprogesterone acetate (DMPA-IM, Depo Provera) also negatively impacts bone mineral density (BMD). To date, no studies have examined the combined impact of both in women living with HIV (WLWH). To address this gap, we evaluated the combined effect of DMPA-IM and TDF on BMD in a two-year follow-up study among young Ugandan women aged 18 to 35 years.

Women were recruited from health facilities around Kampala, Uganda between March 2015 and October 2017. They were classified into four groups based on their combination of HIV status, TDF use, and DMPA-IM use: i) WLWH using DMPA-IM and initiating TDF-containing ART (HIV+/IM-DMPA+/TDF+), ii) WLWH initiating TDF without DMPA-IM (HIV+/IM-DMPA-/TDF+), iii) WLWH using DMPA-IM not eligible for ART per local guidelines at the time of enrollment (HIV+/IM-DMPA+/TDF-), and iv) controls not using DMPA-IM (HIV-/IM-DMPA-/TDF-). BMD of lumbar spine (LS), total hip (TH) and femoral neck (FN) were measured using DXA at enrollment and at 6-monthly intervals thereafter. The primary outcome was percent (%) change from baseline in LS, TH, and FN BMD. BMD was measured at baseline, 6, 12, 18 and 24 months using dual energy x-ray absorptiometry. In addition, we conducted a review of published data synthesizing the current state of knowledge on bone health and HIV in resource limited settings (RLS) paying special attention to young women of reproductive age group. The review revealed important gaps in knowledge in bone health in RLS. There were very few studies of bone health in WLWH in RLS, the majority of which were cross-sectional in nature. No studies were found on the effect of TDF-containing ART initiation on BMD, nor on BMD among WLWH on DMPA-IM for contraception. In the prospective cohort, we enrolled 265 WLWH initiating ART (159 DMPA-IM users, 106 non-hormonal contraceptive users), 187 WLWH using DMPA-IM but not eligible for ART, and 69 controls without HIV. We assessed the association between HIV and/or baseline DMPA-IM and baseline BMD for this cohort. The mean age was 26.1 years (SD, 4.2) with a median duration of DMPA-IM use among current users of 24.0 months (12, 48). A higher proportion of previous (12.7%) or current DMPA-IM users (20.3%) and non-hormonal users living with HIV (15.0%) had

low BMD (Z-score ≤ -2 at any of the three sites) compared with age-matched control without HIV (2.9%). From the longitudinal analysis, there were significant declines in BMD from baseline in WLWH on TDF with or without DMPA-IM at the LS (-3.4% vs. -1.1%), TH (-3.9% vs. -1.7%), and FN (-4.4% vs -2.0%), $p < 0.001$, while significant gains (1.5% at LS) and non-significant declines (-0.1% at TH and FN) were observed among controls. Concurrent use of TDF and DMPA-IM resulted in significantly greater % BMD declines ($p < 0.001$) than TDF alone (LS (-2.7% (-3.7, -1.6)), TH (-2.5% (-3.6, -1.5)) and FN (-2.9% (-4.1, -1.7)) or controls (LS (-5.0% (-6.4, -3.6)), TH (-4.2% (-5.6, -2.7)), and FN% (-4.8 (-6.4, -3.1)). In summary, HIV-infection and DMPA-IM use were independently associated with significantly lower mean BMD Z-scores at all sites, with the greatest difference being among current DMPA-IM users living with HIV (5.6%-8.0%) versus non-hormonal controls without HIV. Concomitant DMPA-IM use was associated with a doubling of bone loss in WLWH initiating TDF-containing ART. Identification and use of safer contraceptive and bone-sparing ART options should be prioritized for the optimal care of WLWH.

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1.0 CHAPTER ONE - INTRODUCTION

1.1 Significance

1.1.1 Osteoporosis and Fractures in Persons Living with HIV

Although the burden of metabolic bone disease has not yet been quantified in sub-Saharan Africa (SSA) (1), osteoporosis would be expected in these populations in higher proportions than in developed countries due to higher Human Immunodeficiency Virus (HIV) infection rates and nutritional deficiencies(2). Data from developing countries estimate that up to two thirds of persons living with HIV (PLWH) exhibit osteopenia or osteoporosis at the time of HIV diagnosis(3). In SSA HIV incidence remains high(4), particularly among women, and many more adults are living longer with HIV due to expanded access to combination anti-retroviral therapy (ART)(5-8). These epidemiologic trends suggest that many more women living with HIV infection (WLWH) will experience menopause and its sequelae, including osteoporosis. Importantly, studies mainly in developed countries reporting increased fracture rates in the persons living with HIV (PLWH) are starting to emerge, with fracture rates 30%–70% higher than those among matched control without HIV(9, 10). Among the general population in the United States, fragility fractures are estimated to cost \$17 billion annually and are associated with significant loss of physical function, independence, and quality of life (11), as well as an increased risk of short term and long-term mortality(12-14). Because osteoporotic fractures are associated with significant prolonged or permanent morbidity and mortality(11-14), further risk of osteoporosis associated with HIV infection may have a dramatic impact on the health of women. Among women of reproductive age, the choice of contraception may also impact bone health(15). Greatest concern is among young WLWH concurrently using DMPA-IM, and tenofovir (TDF)-containing ART at the time of attaining their peak bone mass (PBM), a critical determinant of osteoporosis(16). Since DMPA-IM and TDF appear to be independently associated with reduced bone mineral density (BMD), there is concern that concurrent use of these two agents may potentiate each other's effect portending an increased burden of morbidity related to early osteoporosis and fragility fractures among WLWH in RLS with an already vulnerable skeletal profile.

1.1.2 Bone mass as a predictor of osteoporotic fractures

While osteoporosis is generally considered a condition of the aged, a lower PBM in the young is a major determinant of subsequent osteoporosis and fracture in older adults(17-20). Several studies support an independent, dose-response relationship between the BMD level at PMB and the risk of osteoporotic fractures(18, 21-31). For example, a 10% increase in PBM in young women is associated with an estimated 50% reduction in fracture risk after menopause(18). In general, prospective data consistently show, regardless of anatomic site and method of measurement, that a woman's risk of fracture is inversely related to bone mass(22), even among the young age group(16). Thus achieving maximal PBM is crucial in conferring immediate as well as future reduction of fracture risk, and the ongoing prevention of bone loss prior to the menopause is an important preventive strategy.

1.2 Literature review

1.2.1 HIV burden among young women

The HIV burden among young adult women in Uganda is high. SSA continues to bear the greatest burden of the HIV epidemic globally, with women accounting for approximately 57% of the estimated 34 million people living with HIV/AIDS(32). Compared to men, women are more likely to acquire HIV and at an earlier age. An estimated 6 out of every 10 new HIV infections in adults occur in women, and young women aged 15 to 24 are up to 8 times more likely than men in the same age group to be living with HIV(33). In eastern and southern Africa, the region most affected by HIV, young women accounted for 63% of all new infections in 2020(34). In Uganda, approximately 570 adolescent girls and young women (AGYW) get infected with HIV every week. This figure is only second to South Africa where 2,363 individuals get infected every week(35). Fortunately, the rollout of highly active antiretroviral therapy (HAART) in RLS has resulted in increased survival of HIV patients over the last two decades(5-8), and current treatment options like tenofovir (TDF) with simpler once a day dosing regimens promise even further increases in survival. The majority of African countries have made tremendous progress in increasing number of individuals tested for HIV, initiating ART, and achieving high levels of viral suppression(36). By the end of 2020, 96% of WLWH

aged 15 and over in Uganda were receiving ART(37), and about 79% globally(34). Admittedly, the expanding use of ART across the life span requires long-term monitoring to assess safety especially among women of reproductive age as newer regimens are rolled out. **Given that most women living with HIV are of reproductive age(32), provision of reproductive health services is a crucial part of their HIV care.** Prevention of unintended pregnancies in WLWH not only leads to a significant reduction in mother-to-child transmission of HIV but also improves the woman's overall health in much of the developing world where high maternal mortality continues to be an important health issue for women and their families. However, certain types of both hormonal contraception and antiretroviral drugs have been associated with long-term metabolic dysregulation, including negatively affecting bone mass. As access to both ART and long acting modern methods of contraception increases, WLWH may face a unique detrimental impact on their bone health when combining some of their contraceptive and ART options. Knowledge about how these agents act jointly has the potential to offer strategies to mitigate their effects.

1.2.2 DMPA-IM and bone loss

Emerging literature from observational studies on the association between DMPA-IM and fracture risk suggest an increased risk of fractures among DMPA-IM users(38, 39). For example a large population based control study by Meier et al showed an increased risk of incident fractures among 20–44 year old European DMPA-IM users compared to non-hormonal users, with an adjusted odds ratio of 1.54 (95%CI, 1.33–1.78) among those who had received 10 or more injections(38). A more recent case-control study found a similar increase in the risk of fractures in a national sample of British women with a first time fracture diagnosis(40).

Given as a 150mg intramuscular injection every 12 weeks, DMPA-IM is a safe and effective method of contraception(1, 41). In SSA countries with the highest unmet need for contraception, DMPA-IM is the most preferred contraceptive option across the different age groups(42), with approximately 16.5 million current users in the SSA region alone(42). By the end of 2016, Uganda's modern contraceptive prevalence rate among 15 to 49 year old women was estimated at 27.3%. According to the Uganda 2016 Health

Demographic and Health Survey Report, DMPA-IM is the most commonly used (>50%) modern contraceptive method in the country across all age categories(43). Specifically, the distribution of modern contraception use in Uganda by the end of 2016 was at 13.9% for injectables, 4.7% implants, 1.5% pills, and 1.1% intra-uterine device. More recently published regional data from the Multiple Indicator Cluster Surveys(44) conducted in 20 African countries showed that although the overall prevalence of modern contraceptive use was low (26%) and varied across the 20 countries, DMPA-IM was the most preferred method of modern contraception (32%) followed by oral pills(27%) and implants (16%)(45). Among 11, 177, 459 women aged 15–49 years old, the overall prevalence of modern contraceptive use ranged from 6% in Guinea to 62% in Zimbabwe. Among WLWH, in particular, DMPA-IM is considered safe(46) due to its lack of interactions with antiretroviral drugs(47-49). However, similar to the effects of TDF on bone, there is a strong body of evidence from longitudinal data that shows that DMPA negatively impacts BMD in current users(50-57). **Intramuscular DMPA is known to yield bone loss to such an extent as to warrant a “black box” warning from the U.S. Food and Drug Administration(58).** Due to the hypoestrogenemic effects of DMPA(59), women currently using DMPA have a lower average BMD than non-users with the greatest bone loss being experienced during the first few years of use(51, 53, 55, 56, 60-62) and which are more pronounced in younger women(52, 63-68).

A strong body of evidence from longitudinal studies shows that DMPA-IM negatively impacts BMD in current users with the greatest bone loss being experienced during the first few years of use(51, 53, 55, 56, 60). Longitudinal studies report bone losses at the hip and spine of 0.5–3.5% after 1 year of DMPA-IM use(56, 60) and of 5.7–7.5% after 2 years of use(53, 55). In one 3-year longitudinal study by Scholes et al, the mean change in BMD at each 6-month interval decreased as the number of cumulative months of DMPA-IM use increased(51). Those using DMPA-IM for 12 months or less lost BMD at a faster rate than those using DMPA-IM for 13 months or more(51). Another recent longitudinal study with a 4-year follow-up period demonstrated that almost 75% of the BMD lost at the hip and 90% lost at the spine occurred during the first 24 months of use(52). Women who continued DMPA-IM use beyond 24 months lost additional bone, but the magnitude of loss was smaller with each subsequent year of use(52).

A number of studies have however reported that the adverse effect of DMPA-IM on bone may be temporary and that bone mass may recover after discontinuation of the contraceptive, ultimately reaching levels close to those seen in women not on DMPA-IM(51, 69-73). It must also be noted that these studies were conducted in resource rich settings (RRS), where the decrease in BMD may be less than in RLS with their higher rates of background malnutrition and micronutrient deficiencies(74). Further, the better nutrition of subjects in RRS may hasten recovery of the BMD(75). Importantly, no studies assessed the effect of DMPA-IM among WLWH. Studies in adolescents up to age 21 years of age have found that among new DMPA-IM users, BMD decreased at a rate of about 1.5% per year while non-users gained BMD at an approximate rate of 2% per year, resulting in differences of up to 6.8% after two years of use(76-79). These findings suggest that the effect of DMPA-IM on BMD may be more pronounced in AGYW, compromising their ability to achieve normal PBM.

1.2.3 Effects of HIV and TDF-containing ART on BMD

HIV infection is associated with an increased risk of fractures(9, 10, 80, 81). A meta-analytic review of 11 studies by Brown et al involving 884 PLWH and 654 controls showed the prevalence of osteopenia and osteoporosis to be as high as 67% and 15% respectively. The magnitude of BMD reduction was 6.4 times greater and that of osteoporosis 3.7 times greater than in controls without HIV(3). Though underlying mechanisms leading to accelerated bone loss in PLWH are still unclear, they are believed to be multifactorial and include both traditional and HIV specific risk factors(3, 82-91). Due to physiologic, psychologic and lifestyle factors, PLWH are likely to have many of the traditional risk factors for low BMD such as physical inactivity, low body weight, nutritional deficiencies (including inadequate calcium and vitamin D intake), depression, smoking, heavy alcohol use, oligo/amenorrhea, and hypogonadism (92-97). Among the non-traditional causes, a direct effect of HIV and its treatment has been most often quoted(82, 84, 98-105). It is not yet known how much of the bone loss among PLWH is due to HIV infection per se and how much can be attributed to the consequences of ART. Chronic inflammation induced by HIV may impact bone metabolism(106), and in addition, ART also significantly contributes to bone loss among PLWH(107).

Though there have been a few controversies(108), the general conclusion from a number of studies is that TDF treatment decreases BMD with stronger associations being seen in children and adolescents than in adults(109-112); thus, bone mass may be more affected during the active period of bone growth and development. However, studies to date are limited by relatively small study sizes and the variability in BMD measurements in children and adolescents with dynamic rates of bone growth.

Of the different *antiretroviral drugs*, the potential effect of TDF is particularly concerning(112-115). With ART-initiation, there is a rapid acceleration of bone turnover; bone resorption outstrips bone formation, likely accounting for the decrease in BMD(116). Consistent with these findings, Brown and others have shown that ART initiation is associated with a 2-6% loss of BMD over the first 48-96 weeks of therapy which does not return to baseline after prolonged HIV RNA suppression and also re-occurs after re-initiation of ART after treatment failure(114, 115, 117). In another adult study comparing TDF-containing and non-containing regimens, Gallant et al observed increased bone resorption and loss in the TDF-containing arm compared to patients receiving an alternate stavudine, a nucleoside reverse transcriptase inhibitor (NRTI) at both the lumbar spine (LS) (-3.3% vs. -2.0%) and hip (-3.2% vs. -1.8%). Importantly, the majority of BMD loss was observed within the first 24 to 48 weeks of treatment, and thereafter, BMD loss slowed, but BMD did not recover over the 144 weeks of the study(112). Similarly, a study comparing TDF to abacavir, an NRTI, revealed a greater loss of BMD at total hip (-3.6% vs. -1.9%) and LS (-2.4% vs. -1.6%) in the TDF group. Again, BMD loss occurred closer to initiation of therapy and was maximal in the spine at 24 weeks and in the hip at 48 weeks(114). The mechanism through which TDF reduces bone mass is not clear. Though one potential mechanism known to be associated with NRTIs is that of mitochondrial dysfunction(118-124), there is more evidence suggesting that TDF induces renal dysfunction(125-137). TDF has been shown to induce proximal renal tubular dysfunction which results in excessive glomerular filtration, renal tubular acidosis phosphate loss and possible impairment in vitamin D hydroxylation in some patients(125, 126, 130, 136-144).

Again, all the above mentioned studies were conducted in RRS; and none evaluated dual TDF and DMPA-IM users. Given the paucity of data in SSA and globally on the combined effects of HIV DMPA-IM and TDF containing ART we conducted a longitudinal study to directly assess the combined effect of HIV, DMPA-IM and TDF containing ART on BMD among 18 to 30-year-old Ugandan women over a two-year follow-up period.

1.3 Conceptual framework

1.3.1 BMD loss due to ovarian estrogen suppression

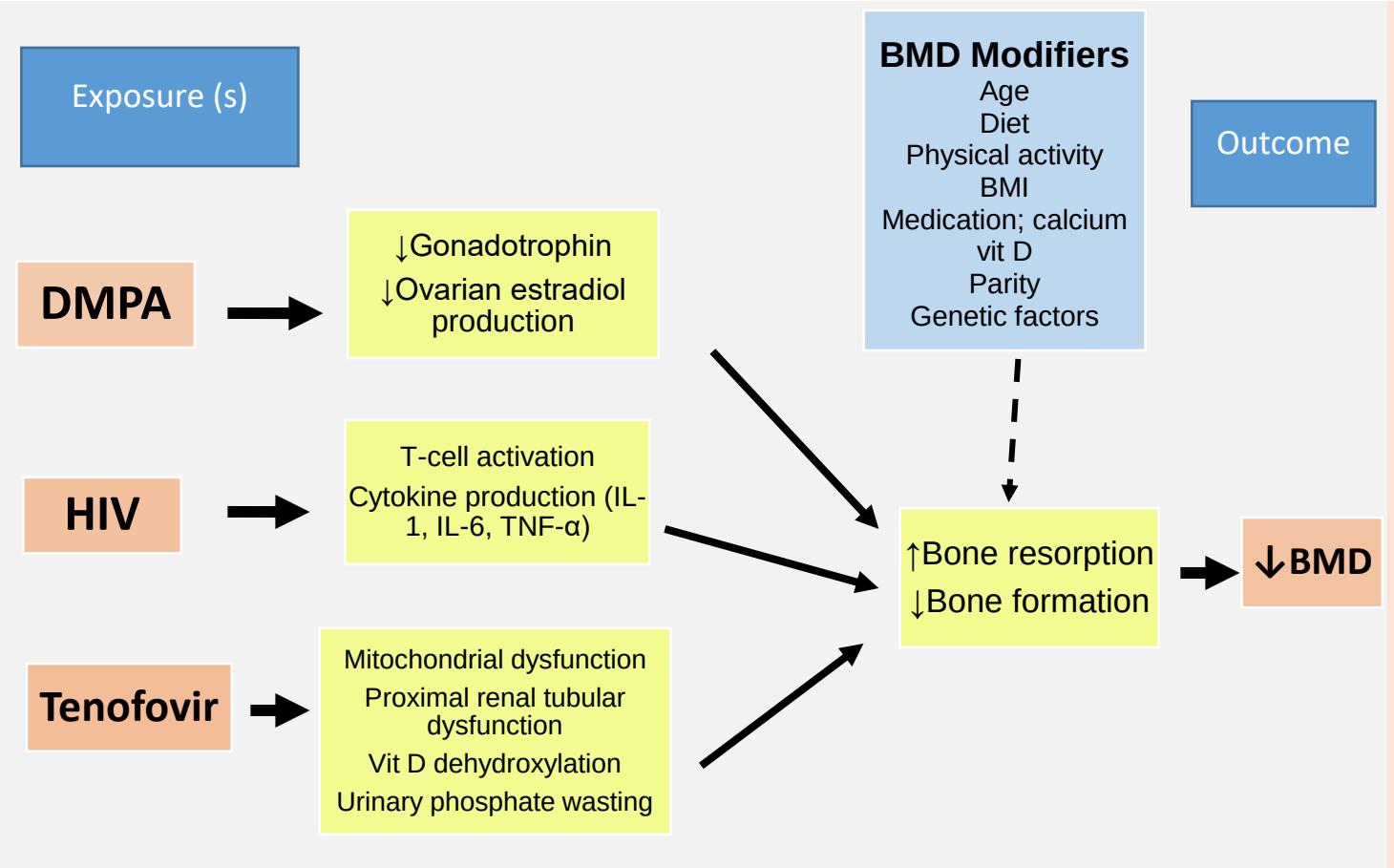
DMPA-IM, a progesterone only hormone confers its contraceptive effects by inhibiting gonadotropin secretion. This results in suppression of ovarian estrogen production, a hormone that plays an important role in the regulation of bone mass in premenopausal women. Bone turnover/ remodeling is a continuous process where old and/or damaged bone is removed (bone resorption) and new bone is formed (bone formation)(145). In adults, approximately 10% of the skeleton is replaced annually through bone remodeling(146). Following appropriate signaling by several hormones (estrogen, parathyroid hormone, vitamin D, and calcitonin), osteoclasts move to resorb the surface of the bone, followed by deposition of new bone by osteoblasts(147). Via its osteoclast activity, oestrogen down-regulates bone resorption, and in hypoestrogenemia (states of low estrogen levels such as during DMPA use), bone resorption exceeds bone formation. This imbalance between bone resorption and bone formation results in decreases in BMD over time[45].

1.3.2 Possible mechanisms of TDF associated bone loss

Among HIV treated patients, TDF has been shown to induce proximal renal tubular dysfunction which results in excessive glomerular filtration, renal tubular acidosis or possible impairment in vitamin D hydroxylation(125, 126, 140). Consequent to tubular epithelial damage, the kidneys fail to re-absorb glomerular filtration products leading to excessive renal phosphate, uric acid, and bicarbonate losses, as well as an impaired glomerular filtration rate as demonstrated by reduced creatinine clearance(137, 140,

148). Urinary phosphate wasting leads to low serum phosphate levels and impaired mineralization (osteomalacia).

Figure 1. 1: Conceptual Framework showing how DMPA-IM, HIV, and Tenofovir lead to a reduction in BMD



1.3.3 Possible mechanisms of TDF associated bone loss

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1.3.4 Other modifiers in the process of bone loss

There are a number of other possible contributory factors or modifiers in the process of bone remodeling. First, during the reproductive years, breastfeeding and repeated pregnancies (high parity) may adversely impact BM. Similarly, low levels of physical activity, low calcium diet, and insufficient vitamin D levels all lead to reduced BMD via a similar signaling pathway of increased osteoclastic activity and resultant bone resorption.

1.4 Problem statement and study justification

TDF and DMPA-IM are frequently used together in Uganda and in many other RLS, with TDF being included as a component of the most widely used first line ART regimens. Currently, the two preferred first line ART treatment regimens for adults and children over 15 years of age in Uganda and other RLS contain TDF; TDF, lamivudine (3TC) and efavirenz (EFV)/ nevirapine (NVP) or TDF, emicitritabine (FTC), and EFV/ NVP, which expose many PLWH to the negative impact of TDF on bone health(149, 150). Though reduced BMD has been an independent concern associated with both TDF and DMPA-IM use, no studies have examined their combined effects. Also, all the other studies examining TDF in PLWH that we are aware of have primarily included men (generally >85%). Relatively little is known about the effects of TDF on bone in women. Furthermore, there are very limited data in any geographic setting regarding longitudinal changes in BMD in PLWH not treated with ART, nor is there data from RLS where both TDF and DMPA-IM are very commonly used together (151). This study is highly relevant with important public health implications in wide geographic areas that remain understudied. BMD in young adulthood, which is the time of PBM attainment, accounts for approximately 50% of BMD variance at age 65 and is therefore a major, potentially modifiable, risk factor for fragility fractures in older populations(18, 41). It is indeed critical to identify risk factors that may impact PBM in order to decrease the risk of future fractures. DMPA-IM and TDF have independently been shown to negatively affect BMD in previous studies, but their combined effect is not known.

To address this gap, we conducted the BONE: Contraception and Anti-Retroviral Effects (CARE) study to evaluate the combined effect of DMPA-IM and TDF on BMD over a two-year period among WLWH aged 18

to 35 years enrolled at HIV treatment centers and general health care facilities in and around Kampala, Uganda. In order to develop the most effective strategies to mitigate bone loss among this key population, it is extremely important to determine the extent to which concurrent use of certain antiretroviral drugs and contraceptives that are associated with reduced BMD particularly TDF and DMPA-IM respectively, compromise BMD in young WLWH who are at a critical period of attaining their PBM. To our knowledge this is **the first study globally to define the combined effect of TDF and DMPA-IM on bone health of young WLWH**. The BONE: CARE study aimed to evaluate HIV treatment metabolic bone complications and addresses an important gap in HIV management highly relevant to RLS. Our findings will inform clinical practice guidelines about TDF-containing ART and DMPA-IM in RLS with a high burden of HIV among young women of reproductive age who frequently use DMPA-IM. A finding of a synergistic effect of DMPA-IM and TDF on BMD would lead to recommendations to use alternative contraception that preserve BMD in TDF treated women or conversely to start a non-TDF containing regimen (e.g., abacavir or tenofovir alafenamide (TAF)) in DMPA-IM-treated women.

1.5 Study aim and objectives

Aim: To determine the effect of HIV, DMPA-IM use and TDF-containing ART initiation on BMD over a two-year period.

Primary Objectives

Objective 1 To determine the association between HIV and DMPA-IM use and BMD among untreated WLWH. We hypothesized that BMD levels would be lower among WLWH using DMPA-IM (HIV+, DMPA+, ART-) compared to both WLWH (HIV+, DMPA-, ART-) and controls without HIV (HIV-, DMPA-, ART-) on non-hormonal contraception. This objective will establish the magnitude of the negative impact of DMPA-IM on bone health in WLWH prior to initiating TDF-containing ART and the effect on HIV infection on BMD prior to initiation of ART.

Objective 2 To determine the combined effect of HIV, DMPA-IM and TDF initiation on BMD. We hypothesized that WLWH initiating TDF-containing ART with DMPA-IM (HIV+, DMPA+, TDF+) would have greater BMD loss compared to controls without HIV (HIV-, DMPA-, ART-). This objective will establish the magnitude of the negative impact of DMPA-IM use and TDF-containing ART initiation on bone health in WLWH in comparison to the DMPA-IM unexposed control group without HIV.

Objective 3 To determine whether BMD loss with TDF-containing ART initiation over a two-year follow-up period is greater with DMPA-IM use. We hypothesized that WLWH initiating TDF-containing ART with DMPA-IM (HIV+, DMPA+, TDF+) would have greater BMD loss compared to WLWH initiating TDF-containing ART without DMPA-IM (HIV+, DMPA-, TDF+). This study will establish whether BMD loss is greater with DMPA-IM use among young WLWH initiating TDF containing ART.

Objective 4 To determine the effect of TDF based ART initiation on BMD among DMPA-IM users. We hypothesized that WLWH initiating TDF-containing ART with DMPA-IM (HIV+, DMPA+, TDF+) would have greater BMD loss compared to HIV infected DMPA-IM users not receiving TDF-containing ART (HIV+, DMPA+, TDF-). This study will establish in WLWH the magnitude of the negative impact on bone health of TDF-containing ART in comparison to HIV infected DMPA-IM users not on ART.

2.0 CHAPTER TWO - METHODS AND MATERIALS

2.1 Study Setting

This study was based at the Makerere University –Johns Hopkins University (MU-JHU) Research Collaboration located within Mulago National Referral Hospital complex, 3 km to the north of Uganda's capital city, Kampala. Potential participants were recruited from various established HIV care and treatment centers, as well as general health care facilities within a 20 km radius of Mulago Hospital between March 2015 and October 2017. These facilities included Makerere University Joint AIDS Programme (MJAP), The AIDS Support Organization (TASO), Reach out Mbuya, China-Uganda Friendship Hospital-Naguru and

Kampala Capital City Authority (KCCA) clinics (Kiswa, Kisenyi, Kawaala, Kitebi, Komamboga, Kisugu Health Centres, and City hall clinic).

Within these health units, the vast majority of clients are of low to very low educational and socio-economic status. These clinics provide sustainable and comprehensive HIV/AIDS prevention, care and treatment for PLWH residing in the Kampalan urban environment. Attendance at these clinics range between 100 to 350 HIV patients per day. Two of these recruitment sites, TASO and MJAP, are also located within Mulago Hospital complex, 1-5 minutes walking distance from MU-JHU where the study was implemented, while two other sites; KCCA clinics and

China- Uganda Friendship Hospital-Naguru serve as general government run health facilities *that care for both PLWH and uninfected patients (about 10%)*. Besides these two general facilities, all the other recruitment health facilities care only for approximately 5500 to 8000 PLWH, of whom 9% were not eligible for treatment at the time of planning this study, because of the government treatment guidelines. Prevalence of DMPA-IM use in these clinics at 50% of overall contraceptive use is similar to the national average (9). There were a number of advantages to recruiting from the above health facilities including: 1) a defined study population representative of the target population and comparable unexposed participants; 2) greater efficiency in follow-up as participants are retained within their care programs; 3) access to medical record and data on details of exposure status particularly HIV status and ART, and any other key medical history characteristics.

Figure 1. 2: Map of Uganda showing the location



2.2 Study Population

Women living with and without HIV aged 18 to 35 years attending the public and not-for-profit non-governmental health care facilities described in section 6.1 (study setting) above. Women were enrolled under the following 4 categories shown in **Figure 2.1** below;

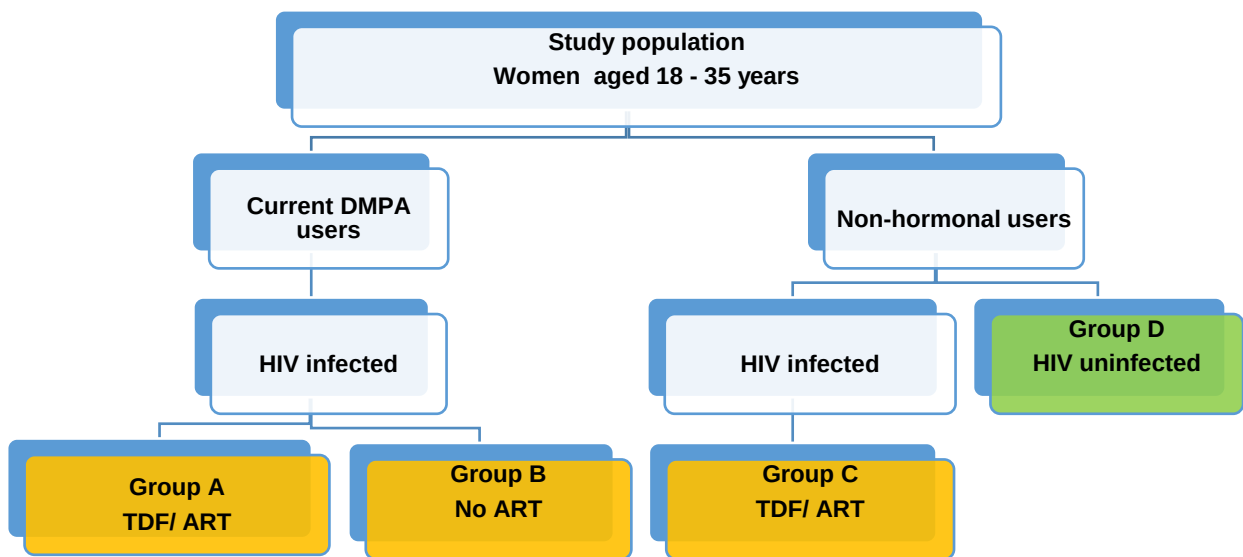
Group A: WLWH on DMPA-IM and initiating TDF-containing ART regimen (**HIV+/DMPA+/TDF+**)

Group B: WLWH on DMPA-IM but not yet eligible for ART (**HIV+/DMPA+/TDF-**)

Group C: WLWH not on DMPA-IM or any other hormonal contraception and initiating TDF-containing ART regimen (**HIV+/DMPA-/TDF+**)

Group D: Women without HIV and not on DMPA-IM or any other hormonal contraceptive method (**HIV-/DMPA-/TDF-**)

Figure 2. 1: Study Groups



2.3 Study Design

Over a five-year implementation period, we conducted an observational prospective cohort study to compare changes in BMD over a 24 months' follow-up period among women aged 18 to 35 years exposed and unexposed to HIV, DMPA-IM and/ or TDF. During this period, each participant was identified, enrolled, and followed up 6 monthly (except for the additional visits at week 4 and month 3) for a total of 24 months.

2.4 Eligibility Criteria

2.4.1 Inclusion Criteria

Women were enrolled per inclusion criteria shown below, **Table 2.1**

Table 2. 1:Inclusion criteria for the four study groups

Groups A, B, C & D		
	1.	Able and willing to provide written informed consent.
	2.	Aged 18 to 35 years.
	3.	Having a documented HIV test result ever or within the past 3 months for WLWH or controls respectively.
HIV+/DMPA+/TDF+	4.	Documented HIV positive test.
	5.	Using DMPA-IM (new and prevalent users).
	6.	Naïve but eligible for ART at screening ($CD4 \leq 500/\mu L$ or $\geq$ WHO stage 3).
	7.	$CD4 \geq 100\text{cell}/\mu L$.
HIV+/DMPA+/TDF- **	8.	Documented HIV positive test
	9.	Using DMPA-IM (new and prevalent users)
	10.	Ineligible for ART per local guidelines ($CD4 \geq 500$ cells/ μL or $\geq$ WHO stage 3)
	11.	$CD4 \geq 650$ cells/ μL .*
HIV+/DMPA-/TDF+	12.	Documented HIV positive test.
	13.	Using non-hormonal contraception (e.g. Intra-uterine device (IUD), Female sterilization, or condoms †).
	14.	No history of use of hormonal contraception including DMPA-IM for more than 3 consecutive months in the last 2 years.
	15.	Naïve but eligible for ART at screening ($CD4 \leq 500$ cells/ μL or $\geq$ WHO stage 3)
	16.	$CD4 \geq 100$ cells/ μL .
HIV-/DMPA-/TDF-	17.	Documented HIV negative test.
	18.	Using non-hormonal contraception (e.g. IUD, Female sterilization, or condoms †).
	19.	No history of use of hormonal contraception including DMPA-IM for more than 3 consecutive months in the last 2 years
	20.	No history of use of post-exposure prophylaxis in the last 6 months.

† Women using condoms must have reported consistent use of condoms in the last eighteen months

** Women in the HIV+/DMPA+/TDF- group later initiated ART under the more recent Uganda's Ministry of Health test and treat policy was rolled out in Uganda effective February 2017. These women continued to be followed up in the study for an additional 2 years.

Additional eligibility criteria for the prospective groups (A, B, C) included;

1. Residence within a 20 km radius around MU-JHU clinic, Mulago, Kampala.
2. Residence in an area for 6 months and not planning to move out of the catchment area within the next 2 years (this criterion was augmented by questions regarding place of origin, date of arrival and residence of close relatives to reduce the possibility of selective attrition)
3. Agreeing to come to the study clinic for scheduled appointments and to be called by phone or

visited at home as needed to ensure follow up.

2.4.2 Exclusion Criteria

Regardless of the study group, women were excluded from the study if they were;

1. Currently pregnant/ breastfeeding or had been pregnant/ breastfeeding in the last 6 months prior to screening.
2. Having intentions of getting pregnant in next 2 years.
3. Having a pre-existing untreated or uncontrolled condition known to affect bone metabolism (e.g. thyrotoxicosis, tuberculosis, diabetes mellitus, liver (e.g., hepatitis B or C) or renal disease).
4. Taking medications known to interfere with bone metabolism (steroids, anti-convulsants, bisphosphonates, cancer drugs etc).
5. Hysterectomized and oophorectomized.
6. Having any other condition that in the investigator's opinion would preclude informed consent, make

study participation unsafe, complicate interpretation of study outcome data or otherwise interfere with achievement of the study objectives.

2.5 Sampling Procedure

Consecutive sampling technique was used to recruit all eligible study participants.

2.6 Participant Recruitment

Women were informed about the study by their counselors or nurses at the respective health care facility or treatment center. Women with no HIV were sequentially recruited from KCCA clinics and China-Uganda Friendship Hospital-Naguru. They were identified from the HIV Voluntary counseling and testing points, and family planning clinics. All potentially eligible women who were interested were referred to study specific recruiting study staff (designated as recruiters/ peer educators) who described the study in detail prior to giving them appointments to be screened at MU-JHU Research Clinic at Upper Mulago Hill Road. The recruiting study team wore a MU-JHU labelled apron that distinguished them from the HIV Clinic service providers. At the MU-JHU Research Clinic, participants were consented in their language of preference by trained nurse counselors. Assessment of participants' language preference for use during the informed consent process was

done by asking each participant in reference to the language the site consent forms are written in i.e. English and Luganda, and preference documented on informed consent cover sheet. Thereafter the prospective participant was screened to assess eligibility by a designated nurse clinician or medical officer. At enrolment, women had baseline evaluations related to assessment of BMD, **Table 2.2**. In order to capture locator information, women were accompanied back to their homes by a designated study health visitor.

2.7 Anthropometric and Clinical Procedures

Collection of socio-demographic information, medical history (concomitant medications, and other risk factors for osteoporosis), reproductive history (menarche, last normal menstrual period, parity, contraceptive etc.), lactation and physical activity (using the global physical activity questionnaire(GPAQ) (152, 153)), nutritional assessment (weight, height), clinical assessment for physical signs of malnutrition, and dietary intake assessment using a modified calcium rapid assessment method(154), that captures intake of common local foods and food supplements rich in calcium and phosphate, as well as vitamin D supplementation. The questionnaires were administered at baseline and at every semi-annual visit by trained clinicians (nurses and medical officer). They were brief enough not to add undue respondent burden. A detailed schedule of evaluations is provided under table 2.2 below.

2.8 Laboratory Assessments

1) Urine HCG test was performed at each visit prior to measuring BMD. 2) Serum and urine were also stored for markers of bone turnover and bone metabolism at baseline, week 4, 3, 6, 12, 18, and 24 months though this is not part of my PhD. The proposed future exploratory testing of these samples include but not limited to 25-hydroxy vitamin D, parathyroid hormone (PTH), follicle stimulating hormone (FSH), insulin-like growth factor-1 (IGF-1), estradiol, urinary phosphorus and creatinine. These measurements will provide information on the effects that DMPA-IM, HIV and TDF exposure may have on these important regulators of bone physiology. 3) RNA viral load testing was performed on baseline samples of WLWH and at months 12 and 24 using the COBAS AmpliPrep/COBAS TaqMan Assay, version 2.0 (Roche Molecular systems Inc., Pleasanton, CA, USA)

by the Infectious Diseases Institute (IDI) core laboratory, certified by College of American Pathologists. Data on pre-enrollment CD4 cell counts were abstracted from the participants' primary health facility records.

2.9 DXA Assessments

DXA is the most widely recognized technique for measuring BMD. It is quick, painless and non-invasive. This technique which uses low levels of x-rays, involves passing a scanner over the whole body. DXA can be used to determine BMD of the entire skeleton or at various fracture prone sites like the hip and spine. BMD by DXA of the hip and spine is generally considered the most reliable way to classify osteoporosis, assess changes in bone mass, and predict fracture risk(155).

In this study, BMD which is the primary outcome, was measured at L1-L4 vertebrae (LS), total hip (TH), femoral neck of hip (FN) and whole body using the same Hologic DXA machine for all participants at the MU-JHU research collaboration. Evaluation of bone mass at each of these sites allowed us to compare changes in both cortical bone (approximately 80% of total body calcium) and trabecular bone (approximately 20%). BMD was measured at baseline and at 6 months' intervals during study follow-up by two trained DXA technologists. Results were interpreted by study clinicians and the Principal Investigator. DXA technologists are trained biomedical radiologists trained at Makerere University. Together with the Principal Investigator, the DXA technologists receive continued medical education every two years through their attendance at the National Osteoporosis Foundation of South Africa Meeting. BMD was measured in g/cm² according to international densitometry (ISCD) guidelines(156). DXA scans were performed using the Hologic Discovery (Bedford, MA) interfaced with Apex System Software 2.3.2. The DXA equipment is standardized daily using a phantom and serviced every 6 months as prescribed by the manufacturer's instructions. Professor John M. Pettifor, a pediatric bone specialist based at the University of Witwatersrand, South Africa provided guidance on the planning and execution of the DXA investigations. Inter- and intra-tech variability were measured by the precision error of BMD measurements in 30 similar volunteers by the two DXA techs with repositioning between all replicate measurements. Coefficients of variation (% CV) were computed using a precision error calculator, and comparisons made between measurements by the same DXA technologist (intra-tech variability) as well as between average values between the two technologists (inter-tech variability). Both DXA

technologists met the minimum precision standards per ISCD(156). Throughout study implementation, Professor Pettifor performed a central read of 10% of DXAs as part of the quality measures. Any scans requiring correction were sent back electronically to replace the original scan results (less than 2%). Both he and the DXA technologists were blinded to the participant's exposure status.

2.10 Follow-up Visits

Participants had the following evaluations related to assessments of BMD 6 monthly for a total of at least 2 years; anthropometric and clinical, laboratory, and DXA procedures, **Table 2.2**. Participants were encouraged to receive their contraceptives (e.g. repeat injections, IUD insertions, condom refills) from MU-JHU Research Clinic, or Mulago Hospital. Otherwise, all potential recruitment sites had reproductive health services incorporated within their health package, and participants who received contraception from their HIV care centers were asked about receipt of contraception, and if there were any changes in their method(s) of contraception. All responses were verified against their HIV clinic records, and certified copies filed in the participants' study binders.

Participants who discontinued or switched contraceptive methods or ART continued to be followed up in the study. Participants with non-suppressed viral load above 1000 copies per ml were referred to the MU-JHU ART Clinic for intensive adherence counseling per WHO guidelines(149). DXA scans were held over for those who become pregnant, and were resumed after pregnancy termination if within the study timeframe.

Table 2. 2: Schedule of Evaluations¹

Data collection	Screening & Enrollment	Month 6	Month 12	Month 18	Month 24
Informed consent	X				
Eligibility	X				
Re-imbursement	X	X	X	X	X
Demographic data	X				
Medical History	X	X	X	X	X
Primary end point BMD DXA assessments	X	X	X	X	X
Urine HCG	X	X	X	X	X
Major co-variates Height, Weight Contraception Physical activity Dietary intake Lactation status HIV status ²	X	X	X	X	X
Viral load	X		X		X
Exposure HIV, DMPA-IM or TDF	X	X	X	X	X
Sample storage Plasma for viral load future testing Serum for biomarkers ³ Urine storage ³	X	X	X	X	X

¹ (All evaluations were conducted at the MU-JHU Research Clinic, Upper Mulago Hill Road

² Control group without HIV (HIV-/DMPA-IM-/TDF-) only.

³ Additional serum or urine was stored at week 4, and month 3 for bone biomarker testing.

In compliance with September 2015 WHO recommendations, Uganda's Ministry of Health launched the 'Consolidated guidelines for prevention and treatment of HIV in Uganda, November 2016' that promote a combination of interventions to manage a generalized HIV epidemic in the country on 29th November 2016(157). In February 2017, the new guidelines which eliminated all restrictions on ART initiation among PLWH were implemented in Uganda. All HIV infected populations and age groups became eligible for ART regardless of age, CD4 cell count, WHO staging or pregnancy status. By October 2017, all participants in the HIV+/ DMPA+/ TDF- group had been initiated on TDF-containing ART which modified their exposure

classification to HIV+/ DMPA+/ TDF+. The protocol was amended to follow-up these women for two years post ART initiation, and to have their semi-annual DXA scheduling modified to match the ART initiation dates.

2.11 Data management

Standard operating procedures were developed for all data management activities to ensure consistency and quality of data. I (the principal investigator) trained and certified data collectors/ research assistants in consent form and questionnaire administration. All questionnaires and other study implementation materials were pre-tested and the necessary revisions made prior to implementation. Interviewer administered questionnaires were translated to Luganda. These were administered to study participants in the language of their preference. All information containing personal identifiers, and recruitment and tracking information were maintained separately from the study data. Quantitative data were collected and entered by research staff on case report forms (CRFs) and/or Android electronic tablets (hand-held computers) using “ODK Collect”, a free and open source computer-assisted personal interviewing (CAPI) software(158, 159) developed by the University of Washington. The data were uploaded via internet onto a secure central server that is protected by multiple security layers. The data were stored securely on the central server and a back-up copy was automatically downloaded from the server into a secure central MySQL-server database at MU-JHU. The server is located in access-restricted rooms within MU-JHU and each computer and each database used to store the data were protected by separate passwords. Procedures to promote data quality included skip patterns, range and logical checks built into the ODK Collect entry program, and data validation and cleaning at the point of collection using two levels of quality controls (same-day check and feed-back by data collector and Principal Investigator/ designee).

Data that were initially captured onto CRFs, such as pre-screening data, were reviewed for completeness, accuracy and consistency by quality control reviewers prior to data entry. Quality control (QC) level I staff reviewed all records in real time before the participants left the clinic, and QC level II provided another layer of review within 5 days of the clinic visit prior to data entry. The data were key-double entered weekly and backed up on the MU-JHU server. CRFs were stored in a safe and restricted access area (the file room) within MU-

JHU while consent forms were stored in MU-JHU's limited access, secure regulatory office. Screening data were entered weekly into the visit schedule tracking system. Recruitment and follow-up appointment tracking reports were routinely generated by the data manager/ designee for weekly study team meeting discussions.

BMD data were stored in the Hologic database, backed up at weekly intervals and archived at six-month intervals. Scans were printed and filed in the participants' charts. Laboratory results were provided in two forms: 1) hard copies for filing in the subject's chart; 2) computerized spreadsheet files for merging with the analytic database.

2.12 Statistical considerations

2.12.1 Sample Size estimation and justification

In this two-year follow up study, there were 3 interrelated but separate sub-studies together aiming to determine the combined effect of DMPA-IM use and TDF-containing ART initiation on BMD and if BMD loss with TDF-containing ART initiation over a two-year follow-up period is greater with DMPA-IM use, **Figure 2.1**. The target number for each of the four groups was based on the sample size needed to answer the respective primary objective. Specifically, different sample sizes were obtained in the different groups due to estimated effect size between comparison groups, with larger differences leading to smaller sample sizes. All estimates below are based on type 1 error (α lpha) =0.05, power ($1-\beta$) = 0.8 and attrition rate (loss to follow-up and discontinuation of contraception), 20% over a two-year period. Computations were based on LS BMD estimates.

$$N = \frac{[1/q_1 + 1/q_2] S^2 (Z_{\alpha/2} + Z_{\beta})^2}{(\mu_1 - \mu_2)^2}$$

q1 - proportion of participants in group 1

q2 - proportion of participants in group 2

S - standard deviation

Z α - level of significance

Z β - power of the study

μ_1 - mean in group 1 and μ_2 is mean in group

Sub-study 1 compared the % change in BMD between **HIV+/DMPA+/ TDF+ WLWH**, versus the control group (**HIV-/DMPA-/TDF-**) (**Group A versus D**). There no available data for BMD changes among the

HIV+/DMPA+/TDF+ WLWH, but studies have reported BMD losses of 5.7% after 2 years of use among HIV-/DMPA+ women(53, 55), and 2-6.1% loss among treated PLWH in the absence of DMPA-IM(80, 114, 115, 117). Assuming an additive effect, we estimated a mean BMD loss among HIV+, TDF, DMPA+ of ~10% over 2 years. With a baseline mean LS BMD of 1.20 g/cm² in the HIV-, DMPA-IM- women, a 10% loss in BMD would lead to a BMD of 1.08 g/cm², yielding a sample size of 64 (32 per group) for this sub-study.

Sub-study 2 compared % change in BMD between **HIV+/DMPA+/TDF+ (Group A)** versus **HIV+/DMPA+/TDF- (Group B)**. With an average LS BMD of 1.08 g/cm² among HIV+/DMPA+/TDF+ and 1.147 g/cm² among HIV+/DMPA+/TDF- groups(53, 55, 160, 161), a sample size of 336 (168 per group) is needed for this sub-study.

Similarly, **sub-study 3** compared % change in BMD between **HIV+/DMPA+/TDF+ (Group A)** versus **HIV+/DMPA-/TDF+ (Group C)**. With an estimated average LS BMD of 1.08g/cm² among HIV+/DMPA+/ TDF+ and 1.164 g/cm² among HIV+/DMPA-/TDF+(160-162), a sample size of 170 (85 per group) is needed for sub-studies 3 and 4. This sample size is more than sufficient(116) to detect significant differences in changes in BMD between these two groups.

Since all sub-studies involve comparing the group with HIV+/TDF+/DMPA+ (Group A) participants to other groups, the overall sample size for this study is calculated as the total sum of **168 HIV+, TDF+, DMPA+ (Group A)** participants that covers all comparisons, with **32 HIV-, DMPA-, No ART- (group D)**, **168 HIV+, DMPA+ No TDF (group B)**, and **85 HIV +, DMPA-, TDF (group C)** making a **total of 453 participants**.

Since with a smaller sample size, variations from our estimated loss to follow up or switch in contraception for group C would make the study vulnerable to a loss of power, we made a conservative 25 - 40% adjustment in sample size due to anticipated attrition (loss to follow-up, pregnancies, contraceptive discontinuation or switches, and ART initiation (group B only)) bringing the final total sample size to **538 women** (**159 Group A, 204 Group B, 106 Group C, 69 Group D**). We enrolled 187 out of the planned 204 women prior to the

introduction and implementation of the test and treat policy which was rolled out in Uganda effective February 2017.

2.12.2 Analysis plan

Descriptive analyses were done using means (SD) or medians (IQR) for continuous variables, and percentages for categorical variables. Baseline characteristics were compared among the study groups using Pearson's chi-squared test or Fisher's exact test for categorical variables and one-way analysis of variance for continuous variables. Viral loads were log transformed prior to analysis.

Our co-primary outcomes were percent (%) change in mean BMD from baseline to two years at the LS, TH, and FN. Paired Student *t*-tests were used to compare baseline versus post-baseline mean BMD measurements at 6-monthly intervals. Multiple linear regression models were used to determine adjusted relative differences in BMD change between baseline and mean post-baseline measures, i.e., a difference of differences analysis. Estimates were made comparing WLWH using DMPA-IM (HIV+/DMPA+/TDF+) and WLWH using non-hormonal contraception (HIV+/DMPA-/TDF+) to controls (HIV-/DMPA-/TDF-), and WLWH using DMPA-IM (HIV+/DMPA+/TDF+) to WLWH using non-hormonal contraceptives (HIV+/DMPA-/TDF+). Women (HIV+/DMPA+/TDF-) who initiated ART under the test and treat guidelines introduced during the study were included in the HIV+/DMPA+/TDF+ group once initiated.

Models were adjusted for age, body mass index (BMI) (as a time varying covariate) and baseline BMD. A mixed-effects regression model was used to estimate the annualized rate of change in BMD within each of the three study groups adjusting for the same aforementioned variables. All the above analyses were conducted by intention to treat.

Multivariate random intercept and random slope models that account for correlations in repeated measures were used to estimate slopes and trajectories of BMD change overtime in each participant and to compare differences in slopes and trajectories of BMD change between various groups. We assessed if the change in BMD overtime follows a linear trend, a linear spline or a non-linear trend and modeled the data accordingly.

Change in BMD, the outcome variable, was determined by subtracting baseline BMD from the BMD at each post-baseline visit.

A post hoc sensitivity analysis was also performed to assess whether consistent users of DMPA-IM and TDF experienced more bone loss compared to inconsistent users. Consistent use of DMPA-IM was defined as receiving of ≥ 2 injections of DMPA-IM within one year or ≥ 4 injections in 2 years of follow-up, while consistent use of TDF-containing ART was defined as virologic suppression (<1000 copies/ml) at both the 12 and 24-month time points. Statistically significant differences between groups were tested at a p-value of ≤ 0.05 and all p-values are two-sided. Data for women who became pregnant were censored at first positive pregnancy test. All analyses were conducted using StataTM Release 16 (Stata Corporation, 4905 Lakeway Drive, College Station, Texas 77845 USA).

2.13 Ethical considerations

2.13.1 Ethical Review and Approval Process

The protocol was approved by the Uganda Virus Research Institute Ethics Committee (GC/127/16/09/524), Uganda National Council for Science and Technology (HS 1942), and the Human Research Ethics Committee at the University of the Witwatersrand, South Africa (M150858). Written informed consent for study participation and sample storage for future testing was obtained from each participant. Please see attached approval letters and informed consent forms both in English and Luganda language. Ethical committees overseeing the study are kept informed about the study through annual progress reports.

2.13.2 Informed Consent

Written informed consent forms were administered to all study participants describing the purpose of the study, the risks/ benefits of the study, confidentiality of information, voluntary participation and withdrawal from the study as well as contact information for further questions. Separate written consent was obtained for possible future testing of specimens collected during the study.

2.13.3 Confidentiality

Participants were assigned study IDs and were referred to by this number in all field notes and data collection for the study. The source data was stored securely under lock and key in a filing cabinet in a locked study room with limited access at MU-JHU to ensure confidentiality. Forms that contain names such as the informed consent form were stored separately in a locked filing cabinet. All local data bases were secured with password-protected access systems.

3.0 CHAPTER THREE – Bone Health and HIV in Resource Limited settings: A Scoping

Review

*Matovu FK, Wattanachanya L, Beksinska M, Pettifor JM, Ruxrungtham K. Bone health and HIV in resource-limited settings: a scoping review. **Current Opinion in HIV and AIDS.** 2016 May;11(3):306-25.*

The results presented in this chapter and subsequent two chapters highlight the key findings in the corresponding papers, **Appendices 9.5.**

This was an invited review of both published and presented data synthesizing the current state of knowledge on bone health and HIV in RLS. We aimed to perform the first review of published data on bone health among HIV-infected persons. Special attention was given to young women of reproductive age group whose choice of contraception particularly DMPA-IM may further compromise their bone health, as well as to perinatally infected adolescents with the greatest cumulative life-time exposure to HIV and its treatment.

We found very few studies on bone health in RLS, the majority of which were cross-sectional in design and lacked appropriate local reference data, which might have been due to a lack of appropriate diagnostic tools such as DXA machines. Compared to RRS, we found a higher prevalence of low BMD in RLS overall (**Table 3.1 and Figure 3.1**), and high but similar prevalence of low vitamin D among PLWH in both RLS and RRS (**Table 3.1 and Figure 3.2**).

The review highlights important gaps in our knowledge about HIV-associated bone health comorbidities in RLS, such as;

- no published data on the effect of TDF initiation on BMD despite widespread use of TDF among PLWH in RLS,
- no data on BMD among WLWH on DMPA-IM for contraception,
- no data on BMD among adolescents living with HIV,
- No data on HIV and fracture risk.

Given low clinical and research capacity for metabolic bone disease in RLS, their review emphasized the urgent special need for building capacity in bone health care and research in these communities. Expanding knowledge about bone health in RLS will not only provide significant insights into the burden of HIV related bone loss in RLS but also predictors and evolution of bone metabolic comorbidities during the time course of HIV infection and its lifelong treatment. An initial focus is needed to establish the epidemiology of metabolic bone diseases in both the general and HIV populations including women with gender specific safety issues.

Table 3. 1: Prevalence of low vitamin D in persons living with HIV in low-middle-income countries versus high-income countries

Country (region)	Reference	Type of study,	Patients	% Female	Age, mean or median (SD or IQR)	*Prevalence of			Key findings or Remarks (DXA machine)
						Low BMD	Osteopenia	Osteoporosis	
Low-middle-income countries (LMICs)									
Uganda (SSA)	Wandera et al. (2014), 21 st CROI (EARNEST trial) (163)	Cross-sectional analysis of prospective study, 2010-2014	181 PLWH failing their first line ART. (duration of first line ART 3.7 (2.7-5) years, 16.7% had used TDF, CD4 67 cells/ μ L (35-151)	69 (12.8% postmenopausal)	35 (31,41)	LS 50.9%, TH 24.8%	LS 42.9%, TH 23.7%	LS 8%, TH1.1%	Low BMD at LS was associated with both low BMI and use of TDF in first-line regimen. At TH, a low BMI was predictive of low BMD. (Discovery Hologic)
India (SA)	Dravid et al. (2014), Journal of the International AIDS Society (164)	Cross-sectional study, June to Dec 2013	536 patients: - 496 PLWH on ART - 40 ART naïve PLWH	34	42	LS or TH 80.4%	LS or TH 51%,	LS or TH 29.4%	Age, low BMI, current smoking, and menopause were associated with low BMD. Choice of ART use (TDF vs non-TDF, PI vs no PI) did not influence loss of BMD. (Lunar Prodigy)
Nigeria (SSA)	Alonge et al. (2013), J West Afr Coll Surg (165)	Cross-sectional study, Sept to Dec 2010	1005 HIV-positive patients (78.1% on ART; PI 12.6%), median CD4 371 cells/ μ L, median VL 200 copies/ml.	72	41.3 $\pm$ 10	n/a	Lt.distal radius 46.6%	Lt.distal radius 31.9%	Osteoporosis was higher in those aged >40 years, females, single, and underweight. There was no difference in BMD of those with or without PI containing medications and treatment naïve patients. (Specific DXA machine was not indicated.)
Indonesia (EA/P)	Masyeni et al. (2013), Acta Med Indones (166)	Cross-sectional study, Jan to June 2012	73 HIV-positive ART naïve patients (mean CD4 144.7 cells/ μ L and mean VL 272.3 copies/ml.	32.9	33.1 $\pm$ 8.3	LS or FN 43.8%	LS or FN 35.6%	LS or FN 8.2%	Low BMD was correlated with HIV stage (r=0.337; p<0.001) (Lunar DPX)
South Africa (SSA)	Hamill et al. (2013), Osteoporos Int (167)	Cross-sectional study, Feb 2010 to July 2010	75 ART naïve PLWH with low CD4 counts (200 cells/ μ L)	100	33.4 $\pm$ 6.5	No significant differences in BMD at LS, TH, or FN. Proportions with osteopenia or osteoporosis were not reported.			(Hologic QDR 4500A)
			74 HIV-positive ART naïve with relatively preserved CD4 counts (>350 cells/ μ L)	100	33.5 $\pm$ 6.1				
			98 healthy controls	100	30.0 $\pm$ 8.1				
Turkey (EU/CA)	Aydin et al. (2013), braz j infect dis(168)	Cross-sectional study, June 2010 to May 2011	126 PLWH (63.5% on ART; either AZT/3TC or TDF/FTC , LPV/r or EFV), mean CD4 313.8 cells/ μ L	16	40.1 $\pm$ 11.3	LS or TH 77.7%	LS or TH 53.9%	LS or TH 23.8%	Neither NNRTI nor PI containing regimens was associated with low BMD. High VL, using and duration of ART were associated with bone loss. (Norland)
China (EA/P)	Zhang et al. (2013), BMC Musculo skeletal Disorders (169)	Prospective study, April 2007 to March 2011	40 ART naïve PLWH 40 healthy controls	12.5 14.3	37.3 $\pm$ 9.9 37.2 $\pm$ 10.3	At baseline, LS BMD of PLWH was lower than controls (1.195 $\pm$ 0.139) vs (1.138 $\pm$ 0.112). Proportions with osteopenia or osteoporosis were not reported.			With ART initiation, LS, FN, and TH BMD reduced significantly in PLWH (annual percent decline 1.78-3.28%). (Lunar Prodigy Advance PA + 300388)
China (EA/P)	Wang et al. (2015), Future Virology (170)	Cross-sectional study, Jan 2010 to May 2014	21 ART naïve patients with acute HIV infection (mean CD4 420 $\pm$ 152 cells/ μ L, MSM 95.2%)	0	31.1 $\pm$ 6.9	LS or TH 33.3 %	LS or TH 33.3%	LS or TH 0%	TH and FN BMD in patients with chronic HIV infection were lower than the other 2 groups. HIV infection, older age, lower BMI and MSM were associated with low BMD. (MEDI LINK Osteocore)
				0	31.6 $\pm$ 5.9	LS or TH 63.4%	LS or TH 56.3%	LS or TH 7.1%	

			55 ART naïve patients with chronic HIV infection (mean CD4 286±168 cells/uL, MSM 81.7%) 71 healthy controls	0	33.7±5.7	LS or TH 45.1%	LS or TH 38%	LS or TH 7.1%	
Country (region)	Reference	Type of study,	Patients	% Female	Age, mean or median (SD or IQR)	*Prevalence of			Key findings or Remarks (DXA machine)
						Low BMD	Osteopenia	Osteoporosis	
Israel (ME/NA) (Ethiopian & Caucasian origin)	Shahar et al. (2013), Clinical nutrition(171)	Cross-sectional study, Summer 2009	43 Ethiopians living with HIV (mean CD4 233 cells/μL, 82% on ART, 20 subjects on PI)	100	35.9±8.2	LS 85%, TH 55%, FN 65%	n/a	n/a	Low BMD in PLW was associated with duration of HIV infection and ART use. (Lexxos, France)
			32 Caucasians living with HIV (mean CD4 264 cells/μL, 64% on ART, 21 subjects on PI)	100	34.8±8.7	LS 40%, TH 13.3%, FN 39.3%			
Thailand (EA/P)	Wattanachanya et al. (2014) ASBMR annual meeting (172)	Cross-sectional analysis of prospective study, 2010 to 2011	220 HIV-positive ART naïve patients (mean CD4 348 cells/μL)	46.8	Male 39±6 Female 39±4	n/a	LS, TH or FN 20.9% (male), 23% (female)	LS, TH or FN 0% (male), 2.3% (female)	No difference in BMD was found between HIV-positive patients and controls. (Hologic QDR4500)
			233 healthy controls	52.4	Male 40±6 Female 41±5	n/a	16.9%(male), 26.7% (female)	0.7% (male), 0.8% (female)	
South Africa, India, Thailand, Malaysia, Argentina	Martin et al. (2013), AIDS [12] and Haskelberget al. (2014) (Second Line trial) (173)	Prospective study, 2010 to 2011	210 PLWH failing their first line ART with current CD4 202 (104–307) cells/ μL (median duration of ART use 3.4 years, AZT 34%, d4T 48%, TDF 17% at baseline)	52	38.8 (32.9-44.2)	n/a	LS 31.3%, TH 19.7% (at baseline)	LS 5%, TH 1.5% (at baseline)	Reduced BMD was associated with longer duration of TDF and low BMI. An NRTI-sparing ARV regimen of LPV/r and raltegravir is associated with less bone loss than a LPV/r regimen containing NRTIs. BMD decrease was greatest at 48 weeks with stabilization to week 96, but no recovery. (Lunar-India, Malaysia, Argentina, Thailand or Hologic-Thailand, South Africa).
High-income countries (HICs)									
USA ¹ (NA)	Battalora et al. (2015), Antivir Ther (HOPS and SUN study) (174)	Cross-sectional analysis of prospective study, 2004 to 2012	1,006 PLWH with median CD4+ 461 cells/ μL (96.6% on ART, 67% non-Hispanic white)	17	43 (36, 49)	FN 40%	FN 36%	FN 4%	During 4,068 person-years of observation, 85 incident fractures occurred, predominantly rib/sternum, hand, foot, and wrist. Osteoporosis and current/prior tobacco use were associated with incident fracture. (Lunar or Hologic, reference standard: NHANES III database)
UK ^b (EU/CA)	Short et al. (2014), International Journal of STD & AIDS(175)	Cross-sectional study, May to Aug 2008	168 PLWH (63% on ART; PI 27%, NNRTI 45%, NRTI included TDF 47%)	0	45 (38, 51)	n/a	LS, TH, or FN 58%	LS, TH, or FN 12%	Number of fractures since HIV diagnosis was increased among those with osteoporosis. Duration of infection > 13 years was associated with osteoporosis. (Hologic QDR4500C)
Italy (EU/CA)	Mazzotta et al. (2015), AIDS Patient Care STDS (176)	Cross-sectional study, April 2009 to March 2011	163 PLWH (79.7% on ART; PIs 59.2%, TDF 70%)	29.4	44.2±10	LS or TH 63.2%	LS or TH 49.7%	LS or TH 13.5% LS or TH 19.6% (Z-score ≤ -2)	Low BMD was associated with lower BMI, AIDS diagnosis, HCV co-infection, ART, and non-traumatic fractures (NTBFs). Prevalence of NTBFs was 27.0%, predictors; male gender, HCV co-infection, lower FN Z-scores. (Hologic QDR 4500A)

Country (region)	Reference	Type of study,	Patients	% Female	Age, mean or median (SD or IQR)	*Prevalence of			Key findings or Remarks (DXA machine)
						Low BMD	Osteopenia	Osteoporosis	
Netherlands (EU/CA)	Kooij et al. (2015), JID(177)	Cross-sectional study, 2010 to 2012	581 HIV-positive patients (94.7% on ART; NRTI/TDF 96.4/77.1%, PI 43.6%, NNRTI/NVP 60.4/30.2%)	11.5	52.7 (48.3, 59.4)	n/a	LS 34%, FN 43%, TH 29%	LS 11%, FN 4%, TH 2%	Low BW was negatively associated with BMD in HIV-positive persons. Regardless of HIV status, younger MSM had lower BMD than older MSM, heterosexual men, and women. (Hologic QDR 4500 W, the reference standard: NHANES database)
			520 HIV-negative controls	15.2	52.0 (47.9, 58.0)	n/a	LS 35%, FN 34%, TH 16%	LS 6%, FN 1%, TH 0%	
Japan (EA/P)	Kinai et al. (2014), AIDS Res Hum Retro viruses (178)	Cross-sectional study, Feb 2012 to June 2013	184 men living with HIV (93% on ART; PIs 64%, TDF 62%), median CD4 493 cells/μL	0	43 (38, 51)	n/a	LS 46% FN 54%	LS 10% FN 12%	Low BMD was associated with long-term treatment with a PI and a low BMI. Patients who discontinued PI had a higher BMD than those who currently use PI at LS but not at FN. (Hologic QDR 4500 W)
USA ² ^a (NA)	Overton et al. (2015), Ann Intern Med(179)	Cross-sectional analysis of prospective study, September 2011 to February 2012	165 PLWH on EFV/FTC/TDF regimen (33% non-Hispanic black; median CD4 341 cells/μL) - Pre vitaminD/Calcium group (n=79) - Placebo group (n=86)	9.7	36 (28, 47) 31 (25, 44)	At baseline LS 9% TH 5% LS 10% TH 6%	n/a	n/a	Authors evaluated vitamin D3 (4000 IU daily) plus calcium (1000 mg calcium carbonate daily) supplementation on bone loss associated with ART initiation. BMD loss in the first year after ART initiation may be minimized by calcium and vitamin D supplementation. (Specific DXA machine was not indicated).
Mixed LMICs and HICs									
Australia, Belgium, Brazil, India, Ireland, Peru, South Africa, Spain, Thailand, UK, US	Carr et al. (2015), HIV Med. (START-Bone Mineral Density Sub-study) (180)	Cross-sectional analysis of prospective study, June 2011 to June 2013	424 ART-naïve participants with mean CD4 688±152 cells/μL	26	34±10.1	LS, TH or FN 35.1%, FN 18.8%	n/a	LS, TH or FN 1.9%, FN 0.5%	Lower BMD was associated with female sex, Latin/ Hispanic ethnicity, lower BMI and higher estimated GFR. Longer time since HIV diagnosis was associated with lower TH BMD, but not with CD4 cell count or viral load. (Lunar or Hologic, reference standard: NHANES III database (hip) and Hologic's reference data (spine))

Data shown in the table includes published articles and abstracts related to prevalence of low BMD in adolescents or adults living with HIV from RLS in 2014 and 2015 plus articles of special interest from 2013 ([†]). However for RRS, only the articles published in mid-2014 to 2015 and had more than 100 PLWH were included.

* BMD was assessed by DXA either central or peripheral sites.

In most studies, low BMD, osteopenia, and osteoporosis were defined as T-score < -1, T-score between -2.5 and -1, and T-score less than -2.5, respectively.

^a In this study, low BMD was defined as Z-score < -2 at LS or TH.

^b In this study, osteopenia and osteoporosis were defined as T- or Z-score < -1 and T- or Z-score < -2.5, respectively.

TH, total hip; FN, femoral neck. LS; lumbar spine; VL, viral load; n/a, not available.

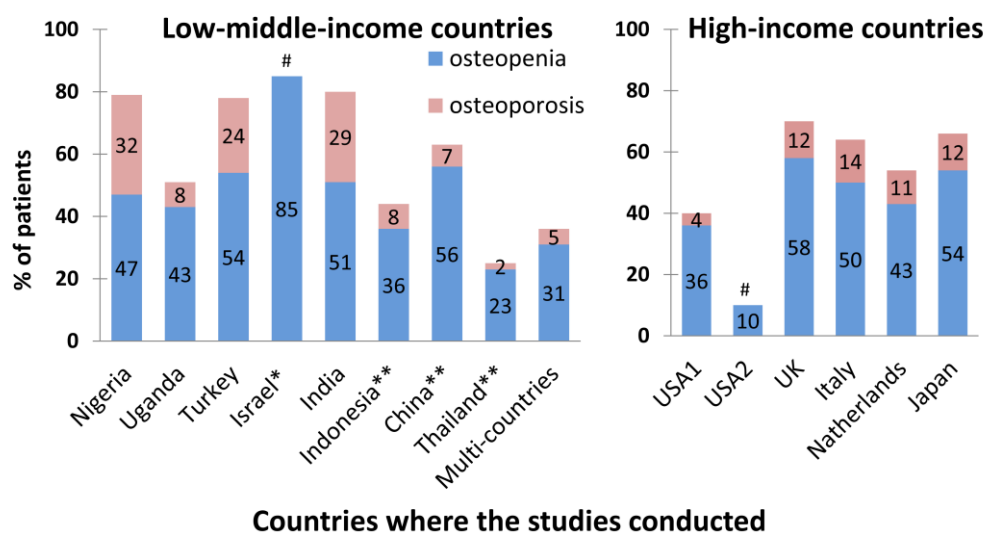
Table 3. 2: Prevalence of low vitamin D in persons living with HIV in low-middle-income countries versus high-income countries

Country (region)	Ref.	Type of study	Patients	% Female	Age (mean or median; SD, IQR)	Results	Remarks
Low-middle-income countries (LMICs)							
Turkey (EU/CA)	Aydin et al. (2013), braz j infect dis(168)	Cross-sectional study, June to Oct 2010	96 PLWH (80.2% on ART)	18	40.1 (range, 20-70)	Patients on ART: 14.3% had 25(OH)D <10 ng/mL 67.5% had 25(OH) D 10-20 ng/mL Patients without ART: 15.8% had 25(OH)D <10 ng/mL 73.7% had 25(OH) D 10-20 ng/mL	25(OH)D levels were low in women with veiled dressing style. No relation between low BMD and 25(OH) D levels.
Israel (ME/NA) (Ethiopian origin and Caucasian origin)	Shahar et al. (2013), Clinical nutrition(171)	Cross-sectional study, Summer 2009	43 Ethiopians living with HIV (mean CD4 233 cells/μL, 82% on ART, 20 subjects on PI)	100	35.9± 8.2	65% had 25(OH)D <10 ng/mL 25(OH)D 10-20 ng/mL	PIs used were LPV/r, invirase/r, IDV. All participants were living in Israel for at least 10 years. Significantly more Ethiopian than Caucasian women covered their face and hands.
			32 Caucasians living with HIV (mean CD4 264 cells/μL, 64% on ART, 21 subjects on PI)	100	34.8± 8.7	6.25% had 25(OH)D <10 ng/mL 25(OH) D 10-20 ng/mL	
Batswana (SSA)	Steenhoff et al. (2015), PLoS One(181)	Prospec -tive study, Dec 2011 to April 2012	60 PLWH (PI 25%, EFV 33%, NVP 42%, TDF/ NNRTI 13%)	50	19.5±12	At baseline: 5% had 25(OH)D < 20 ng/mL 26.5% had 25(OH)D 20-31 ng/mL. Mean 25(OH)D was 36.5 ng/mL in 4000IU group and 34.5 ng/mL in7000IU group. At 12 weeks: 1.5% had 25(OH)D < 20 ng/mL 16.8% had 25(OH)D 20-31 ng/mL Mean 25(OH)D was 54.8 ng/mL in daily 4000IU group and 56.5 ng/mL in daily 7000IU group.	Δ25D was 2-fold higher in subjects on EFV or NVP compared to those on PIs. At 6 weeks, both NNRTI regimens resulted in greater Δ25D than those on PIs. Subjects onTDF did not differ in Δ25D from subjects on other regimens.
Thailand1 (EA/P)	Chokephai bulkit et al. (2013), Pediatr Infect Dis J (182)	Cross-sectional study, Oct 2010 to Feb 2011	101 perinatally HIV-infected adolescents on ART (NNRTI-based: NVP 30%, EFV 20%, and PI-based: 50%), median CD4 646 cells/μL	50	14.3 (13, 15.7)	Median 25(OH)D was 24.8 ng/mL 24.7% had 25(OH)D < 20 ng/mL 46.5% had 25(OH)D 20–30 ng/mL	No associations between vitamin D deficiency and BMI, BMD, EFV use, HIV RNA, CD4, or self-reported sunlight exposure were observed.
Thailand2 (EA/P)	Avihingsanon et al. (2015), AIDS research and human retroviruses (183)	Cross-sectional analysis of cohort, July 2010 to June 2011	673 adults living with HIV (93% on ART; EFV 31%, TDF 79% and 57% of patients had previously used d4T), median CD4 571 cells/ μL	47	41.5 (37.2, 47)	40.6% had 25(OH)D < 20 ng/mL 29.9% had 25(OH)D 20-30 ng/mL	Female gender, age >37 years, and EFV use were independent predictors of hypovitaminosis D.
Country (region)	Ref.	Type of study	Patients	% Female	Age (mean or media; SD, IQR)	Results	Remarks
Thailand3 (EA/P)	Aurpibul et al. (2015), Journal of Pediatric Endocrinology and Metabolism (184)	Cross-sectional study, March to Sept 2011	80 perinatally HIV-infected children on ART (NVP-based 55%, EFV-based 31%, PI-based 14%), median CD4 784 cell/ μL	56	12.2 (9.1, 14.3)	Median 25(OH)D was 33.5 ng/mL, 10% had 25(OH)D < 20 ng/mL 33% had 25(OH)D 21–29 ng/mL	Only geographic location was significantly associated with low vitamin D level.
Brazil 1 (LA)	Sales et al. (2015), Sci Rep.(185)	Cross-sectional study, Aug 2011 to Dec 2013	32 WLWH (most were on ART but type of ART was not indicated) 66 men living with HIV	100	41.7	15.63% had 25(OH)D<10 ng/mL 65.63% had 25(OH)D 11-29 ng/mL 18.75% had 25(OH)D >30 ng/mL	Factors related to the virus itself and to the use of ART may have contributed for the low vitamin D levels.
				0	39	12.12% had 25(OH)D<10 ng/mL 71.43% had 25(OH)D 11-29 ng /mL, 15.31% had 25(OH)D >30 ng/mL	
Brazil 2 (LA)	Canuto et al. (2015), Arch Endocrinol Metab (186)	Cross-sectional study, Sept 2013	125 PLWH (83.2% on ART but type of ART was not indicated)	51.2	40.3±11	Mean 25(OH)D was 39.3 ng/mL 1.6% had 25(OH)D ≤ 20 ng/mL 22.4% had 25(OH)D 21-29 ng/mL	Higher 25(OH)D levels were associated with female gender, no use of sunscreen, and previous opportunistic infections. Lower values were associated with the use of ART, overweight and obesity.
High-income countries (HICs)							
USA1 (NA)	Schwartz, Moore et al. (2014), Journal of the Interna- tional Asso- ciation of Providers of AIDS Care(187)	Cross-sectional study, Oct 2009 to Jan 2010	507 HIV-negative subjects 358 HIV-positive ART naive patients	100 100	41.3 (33.6, 48.7) 42.9 (36.3, 49.6)	Median 25(OH)D was 14 ng/mL 72% had 25(OH)D < 20 ng/mL 18% had 25(OH)D 20-30 ng/mL Median 25(OH)D was 14 ng/mL 70% had 25(OH)D <20 ng/mL 20% had 25(OH)D 20-30	Vitamin D levels were lower if ART included efavirenz (15 vs 19 ng/mL, p<0.001).

			893 HIV-positive patients on ART (PI 61%, NRTI 98%, NNRTI 26%)	100	44.9 (39.3, 50.7)	ng/mL Median 25(OH)D was 17 ng/mL 25(OH)D <20 ng/mL ng/mL	57% had 24% had 25(OH)D 20-30	
USA2 (NA)	Hidron et al. (2015), PLoS One (188)	Cross-sectional study, 2007-2010	933 PLWH (82% on ART; TDF/EFV 31.6%, TDF without EFV 29.9 %, no TDF 20.6%) 5355 HIV-negative subjects	2.5 13.1	50 (range, 24-86) 63 (22–97)	Median 25(OH)D was 19 ng/mL ng/mL Median 25(OH)D was 24 ng/mL ng/mL	53.2% had 25(OH)D <20 38.5% had 25(OH)D <20	Risk factors for vitamin D deficiency in HIV-positive patients included black race, winter season and higher GFR, increasing age and TDF use.
USA3 (NA)	Lake et al. (2015), Open Forum Infect Dis(189)	Cross-sectional analysis of prospective study, June 2010 to April 2011	122 PLWH on ART (PI 34 %, NNRTI 58%, raltegravir 17%, TDF 80%, ABC 29%), mean CD4 520 cells/μL	5	49 (41, 55)	Median 25(OH)D was 20 ng/mL 25(OH)D <30 ng/mL	67.2% had	After 12 weeks of vitamin D supplementation (vitamin D3 50000 IU twice weekly for 5 weeks, then 2000 IU daily), 81% of insufficient persons achieved 25OHD ≥30 ng/mL. 25OHD repletion rates were comparable between HIV-positive patients and controls.
Country (region)	Ref.	Type of study	Patients	% Female	Age (mean or media; SD, IQR)	Results		Remarks
Australia (EA/P)	Klassen et al. (2015), Antivir Ther. (190)	Cross-sectional study, Jan 2008 to Dec 2012 May 2009-April 2010	997 PLWH (66% on ART; EFV 24%, NNRTI±PI 25%, no NNRTI/PI 17%) 3653 individuals without HIV	12 53	41 (32,48) 50 (39, 61)	Mean 25(OH)D was 24.8 ng/mL 25(OH)D < 20 ng/mL ng/mL	40% had 71% had 25(OH)D < 30	Males, Caucasian country of origin, summer/autumn, total cholesterol to HDL ratio >5 and HIV infection were associated with vitamin D deficiency.
United Kingdom (EU/CA)	Gedela et al. (2014),International Journal of STD & AIDS(191)	Cross-sectional study, Jan 2008 to Dec 2009	253 ART-naive PLWH (64.4% were white and 35.6% were black or other ethnicity) with median CD4 450 cells/μL	18	36 (range, 16-75)	12.6% had 25(OH)D ≤10 ng/mL ng/mL	58.5% had 25(OH)D ≤20	Vitamin D deficiency was common among ART naive patients, with those of nonwhite ethnicity at highest risk; no association was found with CD4 count, HIV viral load, and HIV clinical staging.
Belgium (EU/CA)	Theodorou et al. (2014), Clinical Nutrition Belgium(192)	Retrospec-tive study, Dec 2005 to March 2011	2044 PLWH (73.4% on ART; EFV 15.8%, 2NRTI/NNRTI 23.2%, 2NRTI/PI 35.9%, second line 14.2%)	41.5	43 (range, 20-85)	Median 25(OH)D was 13.8 ng/mL <10 ng/mL	32.4% had 25(OH)D 89.2% had 25(OH)D <30 ng/mL	25(OH)D <30 ng/mL is associated with general factors (female sex, winter season) and specific factors related to HIV (duration of treatment, second line treatments with multiple and complex combinations of ART). 25(OH)D <10 ng/mL is associated with a low CD4 count, a higher CDC stage and EFV therapy.
Spain (EU/CA)	Bañón et al. (2015), Endocrine (193)	Prospective study, 2012	365 PLWH (98% on ART;TDF/FTC 77%, the remaining on ABC/ 3TC, EFV 33%)	24	44 (range, 22-75)	At baseline: 15 % had 25(OH)D < 10 ng/mL 25(OH)D 10-19.9 ng/mL 29.9 ng/mL	48 % had 26 % had 25(OH)D 20-	After calcidiol supplementation (oral monthly dose of 16,000 IU), 25OHD levels increased in comparison with non-supplemented patients (+16.4 vs. +3.2 ng/ml; p < 0.01).

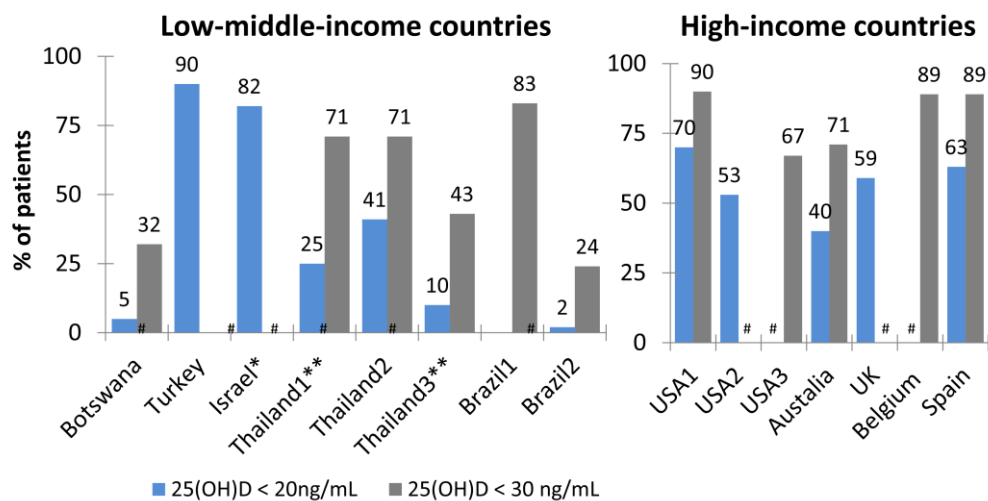
Data shown in the table includes published articles and abstracts related to prevalence of hypovitaminosis D in adolescents or adults living with HIV from RLS in 2014 and 2015 plus articles of special interest ⁽⁺⁾from 2013. However for RRS, only the articles published in mid-2014 to 2015 and had more than 100 PLWH were included.

Figure 3. 1: Proportion of persons living with HIV with low BMD, osteopenia, and osteoporosis



*Ethiopian only ; **patients were ARV-naïve; the others were ARV-treated.
Proportions with osteopenia or osteoporosis were not reported.

Figure 3. 2: Proportion of Antiretroviral-treated persons living with HIV with low vitamin D



*Ethiopian only ; **Children, adolescents; # value is not indicated

4.0 CHAPTER FOUR – Bone Mineral Density in Antiretroviral Therapy-Naïve HIV-1-

Infected Young Adults using Depot Medroxyprogesterone Acetate or Non-Hormonal Contraceptives in Uganda

*Matovu FK, Nabwana M, Kiwanuka N, Scholes D, Isingel E, Nolan ML, Fowler MG, Musoke P, Pettifor JM, Brown TT, Beksinska ME. Bone Mineral Density in Antiretroviral Therapy-Naïve HIV-1-Infected Young Adults using Depot Medroxyprogesterone Acetate or Non-Hormonal Contraceptives in Uganda. **JBMR Plus** 2020;5(2):e10446.*

This study was a cross-sectional analysis of baseline data for the BONE: CARE study, evaluating the association between HIV and/or DMPA-IM and BMD among untreated WLWH. We re-classified women based on their combinations of HIV status and DMPA-IM use at study entry: i) current DMPA-IM users living with HIV, ii) previous DMPA-IM users living with HIV, iii) non-hormonal contraceptive users living with HIV, and iv) non-hormonal contraceptive users with no HIV (controls). The primary outcome was mean baseline BMD-Z scores, calculated using data obtained from the local normal controls as the reference. We compared baseline BMD Z-scores among all treatment-naïve WLWH (non-hormonal, previous and current DMPA-IM users) to that of non-hormonal users living with HIV. We further compared BMD Z-scores among previous and current DMPA-IM users to that of non-hormonal users living with HIV as well as the non-hormonal comparison group with no HIV. Differences in mean BMD Z-scores between the aforementioned groups were assessed using Student *t*-tests. Multivariable linear regression was used to estimate adjusted differences between two groups. We further computed % differences in mean BMD of non-hormonal and previous and current DMPA-IM users living with HIV versus controls with no. Analyses were done using Stata™ Release 15.

Socio-demographic and Clinical Characteristics

Between March 2015 and October 2017, we screened 549 women. Of these, 15 were excluded due to pregnancy, and 13 due to other reasons (unstable location, unexplained amenorrhea for over 8 years, declined study participation, and use of combined oral contraceptive pills) resulting in a final analytical

sample of 521 women: 452 WLWH and 69 controls. The median time between ART initiation and enrollment was 0 (0, 2) days.

Baseline characteristics are shown in **Table 4.1**. The mean age was 26.1 years (SD: 4.2), 227 (43.6%) were married or co-habiting, 436 (83.7%) had ever been pregnant with a median parity of 2 (IQR: 1, 3) and 313 (60.1 %) had a history of DMPA use. Of the 313 women who had ever used DMPA, 176 (56.2%) were current users with a median duration of use of 24.0 months (IQR: 12, 48). The majority of participants (75.2%) reported a history of breastfeeding, with median cumulative breastfeeding duration of 26 months (IQR, 15 - 43). Compared with non-hormonal controls, WLWH were more likely to have less education, higher parity, and to have ever breastfed: $p < 0.001$ for all the variables. The median CD4 cell count among the groups of WLWH was 670 (411, 859) cells/ μ L. WLWH had significantly lower mean BMD compared with age matched comparison group with no HIV at all sites, with the current DMPA users having the lowest means, $p < 0.05$ for all, **Figure 4.1**. However, there were no differences in BMD between younger (18-24 years) and older women (25-35) in the different study groups (data not shown). Similarly, there was no difference in mean BMI across the different groups.

BMD Z-scores in Untreated WLWH Versus Women with no HIV

Low BMD (Z-score ≤ -2) at any of the three sites was demonstrated in 16.8% (76/452) of WLWH versus 2.9% (2/69) of the comparison group without HIV, **Table 4.2**. Mean BMD Z-score was lower in WLWH than women with no HIV at the LS: -0.442 (1.141) versus 0.000 (0.985) g/cm², $p = 0.002$, TH: -0.521 (1.125) versus 0.000 (0.985) g/cm², $p < 0.001$, and FN: -0.471 (1.094) versus 0.000 (-0.985) g/cm², $p = 0.001$ respectively, **Table 4.1**. Significant differences remained after controlling for age, and BMI, mean difference: -0.504 (95% confidence interval [CI] (-0.783, -0.226) at the LS, -0.613 (95% CI -0.877, -0.349) at the TH, and -0.541 (95% CI -0.802, -0.280) at the FN, $p < 0.001$ for all sites, **Table 4.3**.

BMD Z-scores in Untreated Non-Hormonal Users Living with HIV Versus Women no HIV

Non-hormonal users living with HIV had significantly lower mean BMD Z-scores compared with the age matched women with no HIV, adjusted mean Z-score difference at the LS was -0.361 (-0.658, -0.065),

p=0.017, at the TH -0.422 (-0.707, -0.137), p=0.004, and at the FN -0.363 (-0.640, -0.086), p=0.010, (Table 3B). A significantly higher proportion of non-hormonal users living with HIV had low BMD (Z-score <-2) compared with women with no HIV, p<0.001, **Table 4.2**.

BMD Z-scores in Untreated Previous and Current DMPA Users living with HIV Versus Non-hormonal controls with no HIV

A higher proportion of previous and current DMPA users living with HIV had low BMD compared with controls with no HIV, 12.7% (7/55) and 20.3% (36/177) versus 2.9% (2/69) respectively, **Table 4.2**. Compared with controls with no HIV, previous and current DMPA users living with HIV had significantly lower mean BMD Z-scores, mean difference at the LS: -0.419 (-0.830, -0.007), p=0.046, TH: -0.611 (-0.969, -0.253), p=0.001, and FN: -0.611 (-0.987, -0.234), p= 0.002. Greater differences were observed when we compared current DMPA users living with HIV to controls with no HIV, mean difference at the LS: -0.741 (-1.030, -0.452), TH: -0.873 (-1.144, -0.603), and FN: -0.788 (-1.064, -0.512), p<0.001 for all sites, (Table 3B). Similarly, current DMPA users living with HIV had the largest percentage difference in mean BMD between groups with and without HIV, **Table 4.4**.

BMD Z-scores in Untreated Previous and Current DMPA Users living with HIV Versus Non-Hormonal Users living with HIV

When we restricted the analysis to only WLWH, current DMPA users had a higher proportion of low BMD (Z-score <2) at any of the three sites compared with either previous DMPA users, or non-hormonal users living with HIV, **Table 4.2**.

After adjusting for age and BMI, there were no significant differences in mean BMD Z-scores at any of the sites between untreated DMPA previous users and non-hormonal users living with HIV, adjusted mean difference: -0.027 (-0.372, 0.318), p=0.878 at the LS, -0.167 (-0.490, 0.157), p=0.312 at the TH, and -0.170 (-0.486, 0.145), p=0.288 at the FN, **Table 4.3**. However significant differences were observed between current DMPA and non-hormonal users living with HIV at all sites, mean difference -0.395 (-0.610, -0.180) at the LS, -0.485 (-0.693, -0.277) at the TH, and -0.485 (-0.693, -0.277) at the FN, p<0.001)) after adjusting for age and BMI, **Table 4.3**.

Comparison with NHANES (2005 – 2008) non-Hispanic White and Black Reference Data

When we compared our data to the NHANES reference ranges rather than to the local Ugandan controls not living with HIV, a higher prevalence of low BMD was observed across all study groups regardless of DMPA or HIV status, **Table 4.2**. Using the NHANES black reference database, the mean BMD Z-scores in our control population were lower at the LS (-1.156 (0.758)), TH (-0.263 (0.783)), and FN (-0.409 (0.764)). Similar results were observed at the lumbar spine (-0.843 (0.904)) when the non-Hispanic White data was used, but slightly higher values at the total hip (0.226 (0.921)), and femoral neck (0.123 (0.963)) g/cm², **Table 4.5**. Compared to US white women, mean BMD for Ugandan women was 11.3% to 13.6% lower at the LS, as well as at the TH (1.3% to 2.0% lower) and FN (2.3% to 6.5% lower) except for the 30 to 35-year age category (1.0% and 1.4% higher at the TH and FN respectively). Greater differences were observed compared to US black women; mean BMD for black Ugandan women was lower at all sites for all age categories; 19.1% to 19.4% at the LS, 4.7% to 9.2% at the TH, and 5.5% to 14.6% at the FN.

Table 4. 1: Baseline Characteristics of Women in the BONE: CARE Study by HIV Status and Contraceptive Method

Characteristic ¹	Total (N=521)	Current DMPA-IM users living with HIV (n=177)	Previous DMPA-IM users living with HIV (N=55)	Non-hormonal users living with HIV(n=220)	Non-hormonal controls not living with HIV(n=69)	P-value ²
Age (years)	26.1 (4.2)	26.5 (3.8)	27.5 (4.0)	26.0 (4.4)	24.4 (4.0)	<0.001
18-24	202 (38.8)	62 (35.0)	13 (23.6)	93 (42.3)	34 (49.3)	
25-35	319 (61.2)	115 (65.0)	42 (76.4)	127 (57.7)	35 (50.7)	0.013
Education						
None	30 (5.8)	17 (9.6)	1 (1.8)	12 (5.5)	0 (0)	
Primary	198 (38)	81 (45.8)	31 (56.4)	74 (33.6)	12 (17.4)	
Secondary	250 (48)	75 (42.4)	19 (34.5)	117 (53.2)	39 (56.5)	
Tertiary	43 (8.3)	4 (2.2)	4 (7.3)	17 (7.7)	18 (26.1)	<0.001
Marital Status						
Single	112 (21.5)	26 (14.7)	10 (18.2)	59 (26.8)	17 (24.6)	
married/cohabiting	227 (43.6)	99 (55.9)	24 (42.6)	81 (36.8)	23 (33.3)	
Causal partner	126 (24.2)	36 (20.3)	10 (18.2)	53 (24.1)	27 (39.1)	
Divorce/separated/widow	56 (10.7)	16 (9.1)	11 (20.0)	27 (12.3)	2 (2.9)	<0.001
Earns income	362 (69.5)	137 (77.4)	37 (67.3)	154 (70.0)	34 (49.3)	<0.001
Median monthly income (USD)	40.7 (27.1, 70.6)	40.7 (27.1, 65.1)	27.1 (19.0, 48.9)	40.7 (27.1, 81.4)	54.3 (40.7, 81.4)	0.020
Ever used DMPA	315 (60.5)	177 (100)	55 (100)	69 (31.4)	14 (20.3)	<0.001
Median Duration of DMPA use (months)	N/A	24 (12, 48)	N/A	N/A	N/A	
Age at Menarche (years)	14.2 (1.6)	14.1 (1.6)	14.3 (1.7)	14.3 (1.6)	14.0 (1.4)	0.352
Ever Pregnant	436 (83.7)	171 (96.6)	52 (94.5)	180 (81.8)	33 (47.8)	<0.001
Parity	2.2 (1.6)	2.6 (1.5)	3.0 (1.8)	2.0 (1.5)	1.0 (1.4)	<0.001
Ever breastfed	390 (74.9)	163 (92.1)	49 (89.1)	153 (69.5)	25 (36.2)	<0.001
Median duration of breast feeding	26 (15, 43)	27 (15, 42)	30 (18, 48)	24 (15, 42)	28 (18,50)	0.760
Currently drinks alcohol	142 (27.3)	66 (37.3)	18 (32.7)	50 (22.7)	8 (11.8)	<0.001
Number of beers	4.8 (10.2)	3.7 (5.6)	5.2 (7.4)	4.4 (5.3)	16.1 (36.0)	0.975
Current smoker ³	12 (54.5)	6 (66.7)	0 (0.0)	6 (54.5)	0 (0.0)	0.447
Physical activity (minutes/week)⁴						
Vigorous (≥75)	55 (77.5)	17 (73.9)	11 (100)	18 (72)	9 (75)	0.245
Moderate (≥150)	169 (62.8)	63 (67.0)	20 (83.3)	58 (53.2)	28 (66.7)	0.027
Median CD4 cell count (cells/uL)	670 (411, 859)	745 (484, 927)	709 (480, 842)	560 (362, 795)	N/A	0.001
Body mass index (kg/m ²)	24.7 (4.4)	25.6 (4.6)	24.7 (4.1)	24.3 (4.2)	24.0 (4.1)	0.012
Median viral load (log10 copies/mL)	4.1 (3.2, 4.6)	4.1 (3.1, 4.5)	3.9 (3.6, 4.6)	4.1 (3.1, 4.6)	N/A	0.393
Mean BMD (g/cm²)						
Lumbar spine	0.938 (0.110)	0.914 (0.102)	0.949 (0.119)	0.947 (0.114)	0.966 (0.098)	0.002
Total Hip	0.960 (0.115)	0.931 (0.101)	0.965 (0.108)	0.970 (0.124)	0.995 (0.107)	<0.001
Femoral neck	0.863 (0.116)	0.837 (0.108)	0.861 (0.114)	0.873 (0.120)	0.896 (0.108)	0.001
Z-Score						
Lumbar spine	-0.383 (1.131)	-0.630 (1.070)	-0.353 (1.212)	-0.313 (1.162)	0.000 (0.985)	
Total hip	-0.452 (1.120)	-0.734 (1.012)	-0.524 (1.033)	-0.349 (1.205)	0.000 (0.985)	<0.001
Femoral neck	-0.409 (1.091)	-0.654 (1.034)	-0.519 (1.053)	-0.312 (1.131)	0.000 (0.985)	<0.001
Low BMD Z-score (≤-2)						
Lumbar spine	43 (8.2)	20 (11.3)	4 (7.3)	18 (8.2)	20 (11.3)	0.068
Total hip	45 (8.6)	22 (12.4)	2 (3.6)	21 (9.5)	0 (0.0)	0.002
Femoral neck	33 (6.3)	17 (9.6)	2 (3.6)	13 (5.9)	1 (1.4)	0.092

¹ mean or proportions unless stated otherwise, ² difference in baseline characteristics across groups ³ among those who have ever smoked, ⁴ vigorous physical activity over a shorter duration (at least 75 minutes) has the same health benefits as moderate physical activity performed over a longer period of time (at least 150 minutes). Some women contributed to both categories of physical activity.

Figure 4. 1: Mean bone mineral density at the different body sites by HIV infection and contraception status

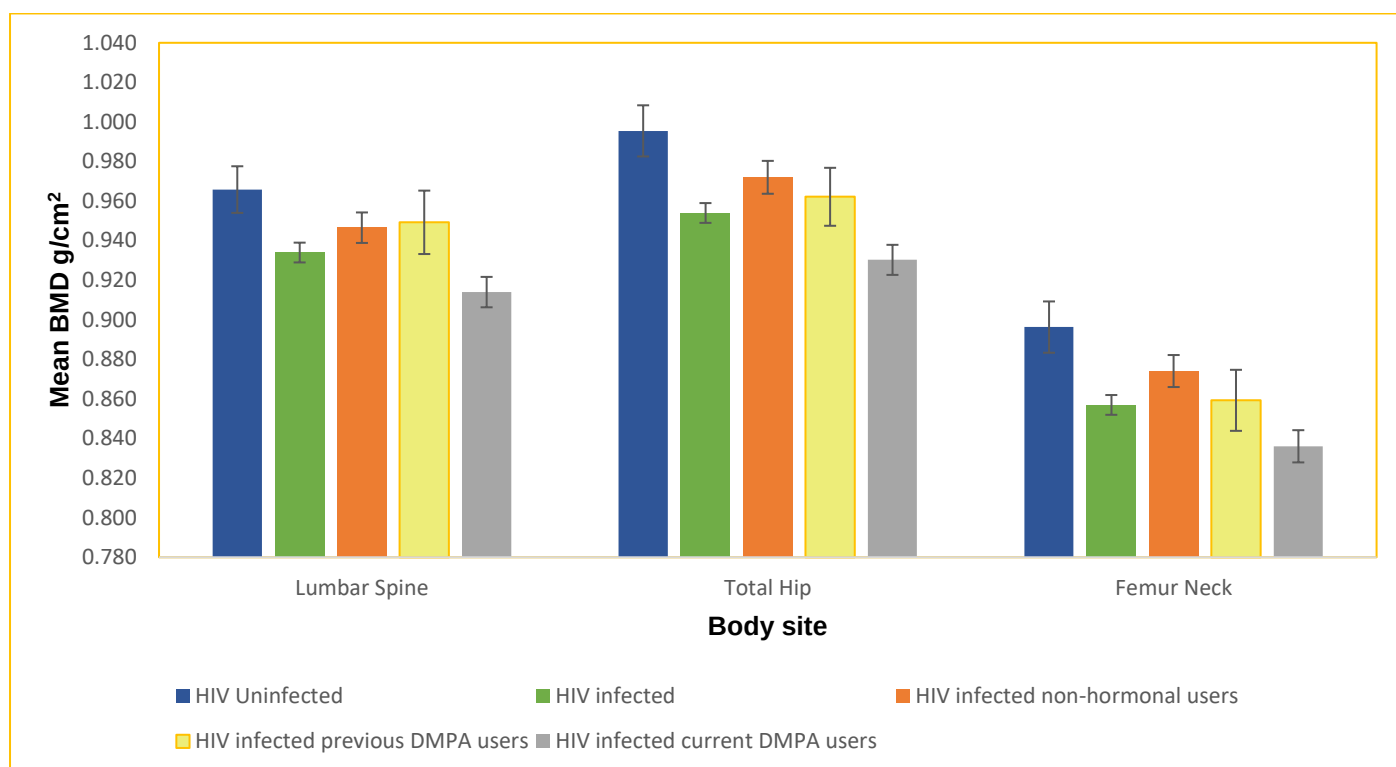


Table 4. 2: Proportion of women with low BMD based on local and NHANES (2005 – 2008) age matched

Study group by HIV Status and Contraception Status	Local Ugandan reference data	NHANES non-Hispanic white reference data	NHANES non-Hispanic black reference data
Non-hormonal controls with no HIV ¹	2.9 % (2/69)	8.7% (6/69)	14.5% (10/69)
Non-hormonal users living with HIV ²	15.0% (33/220)	19.1% (42/220)	26.4% (58/220)
Previous DMPA users living with HIV ³	12.7% (7/55)	16.4% (9/55)	21.8% (12/55)
Current DMPA users living with HIV ⁴	20.3% (36/177)	23.7% (42/177)	28.8% (51/177)

Low BMD was defined as Z-score ≤ -2 at any of the three sites: lumbar spine, total hip, and femoral neck

¹ Women not living with HIV using IUD or condoms at base line, and had not used DMPA or any other hormonal contraceptive method for more than three consecutive months in the last two years

² WLWH using IUD or condoms at base line, and had not used DMPA or any other hormonal contraceptive method for more than three consecutive months in the last two years

³ WLWH not using DMPA for at least 6 months prior to enrollment but had ever used DMPA in the past regardless of the duration

⁴ WLWH using DMPA at the time of enrollment for at least six consecutive months

Table 4. 3: Differences in adjusted mean BMD Z-scores at the lumbar spine, total hip and femoral neck by HIV status and contraceptive method

Comparison groups	Lumbar spine		Total Hip		Femoral Neck	
	BMD Z-score mean difference g/cm2 (95% CI) ⁵	P-value	BMD Z-score mean difference g/cm2 (95% CI) ⁵	P-value	BMD Z-score mean difference g/cm2 (95% CI) ⁵	P-value
WLWH ^{2, 3, 4} vs. uninfected non-hormonal users ¹ (N=521)	-0.504 (-0.783, -0.226)	<0.001	-0.613 (-0.877, -0.349)	<0.001	-0.541 (-0.802, -0.280)	<0.001
Non-hormonal users living with HIV ² vs. non-hormonal controls with no HIV ¹ (n=289)	-0.361 (-0.658, -0.065)	0.017	-0.422 (-0.707, -0.137)	0.004	-0.363 (-0.640, -0.086)	0.010
Previous DMPA users living with HIV ³ vs. non-hormonal controls with no HIV ¹ (n=124)	-0.419 (-0.830, -0.007)	0.046	-0.611 (-0.969, -0.253)	0.001	-0.611 (-0.987, -0.234)	0.002
Previous DMPA users ³ vs. non-hormonal users living with HIV ² (n=275)	-0.027 (-0.372, 0.318)	0.878	-0.167 (-0.490, 0.157)	0.312	-0.170 (-0.486, 0.145)	0.288
Current DMPA users living with HIV ⁴ vs. uninfected non- hormonal controls with no HIV ² (n=245)	-0.741 (-1.030, -0.452)	<0.001	-0.873 (-1.144, -0.603)	<0.001	-0.788 (-1.064, -0.512)	<0.001
Current DMPA users ⁴ vs. non-hormonal users living with HIV ¹ (n=398)	-0.395 (-0.610, -0.180)	<0.001	-0.485 (-0.693, -0.277)	<0.001	-0.485 (-0.693, -0.277)	<0.001

Codes below which correspond to the different study groups have been maintained from table 4.2 for consistency.

¹ Women not living with HIV using IUD or condoms at base line, and had not used DMPA or any other hormonal contraceptive method for more than three consecutive months in the last two years

² WLWH using IUD or condoms at base line, and had not used DMPA or any other hormonal contraceptive method for more than three consecutive months in the last two years

³ WLWH not using DMPA for at least 6 months prior to enrollment but had ever used DMPA in the past regardless of the duration

⁴WLW using DMPA at the time of enrollment for at least six consecutive months

⁵Adjusted for age and BMI

Table 4. 4: Percentage differences in mean BMD between WLWH and uninfected controls with no HIV

Body site	WLWH on non-hormonal contraception	Previous DMPA users living with HIV	Current DMPA users living with HIV
Lumbar spine	-2.7 %	-3.0 %	-5.6 %
Total hip	-3.3 %	-4.9 %	-7.5 %
Femoral neck	-3.3 %	-5.9 %	-8.0 %

Codes below which correspond to the different study groups have been maintained from table 4.2 for consistency.

² WLWH using IUD or condoms at base line, and had not used DMPA or any other hormonal contraceptive method for more than three consecutive months in the last two years

³ WLWH not using DMPA for at least 6 months prior to enrollment but had ever used DMPA in the past regardless of the duration

⁴WLWH using DMPA at the time of enrollment for at least six consecutive months

Table 4. 5: BMD Z-scores and Percent differences in mean BMD between the local Ugandan control group and NHANES (2005 – 2008) age matched reference data

Body site		NHANES non-Hispanic white reference data	NHANES non-Hispanic black reference data
Mean BMD Z-score (g/cm2)			
LS		-0.843	-1.156
TH		0.226	-0.263
FN		0.123	-0.409
Percent differences in mean BMD (g/cm2)			
LS	Below 20 years	-11.3	-19.4
	20-29 years	-13.6	-19.3
	30-35 years	-12.2	-19.1
TH	Below 20 years	-2.0	-9.2
	20-29 years	-1.3	-8.0
	30-35 years	1.0	-4.7
FN	Below 20 years	-6.5	-14.6
	20-29 years	-2.3	-11.3
	30-35 years	1.4	-5.5

5.0 CHAPTER FIVE – Intramuscular depot medroxyprogesterone acetate

accentuates bone loss associated with tenofovir disoproxil fumarate-containing ART initiation in young women living with HIV

Flavia Matovu Kiweewa, Noah Kiwanuka, Martin Nabwana, Delia Scholes, Philippa Musoke Mary Glenn Fowler, John M. Pettifor, Mags Beksinska, Todd Brown. **Submitted to The Lancet.**

This forms the primary analysis for my PhD work. The manuscript has been submitted to The Lancet (see Appendix 9.5.3). We evaluated the combined effects of DMPA-IM use and TDF initiation over a two-year follow-up period in 18 to 35-year-old women, compared to an age matched control group with no HIV. The primary outcome measure was % change in LS, TH, and FN BMD over the two-year interval following enrollment i.e., a difference of differences analysis. We used paired student *t*-tests were used to compare mean baseline BMD versus the mean BMD of all post-baseline BMD measures. Multiple linear regression models were used to determine adjusted differences in BMD change between baseline and mean post-baseline BMD measures. Models were adjusted for age, BMI (as a time varying covariate) and baseline BMD. To estimate the combined effect of DMPA-IM use and TDF initiation on BMD, estimates were made comparing WLWH initiating TDF-containing ART with DMPA-IM (HIV+/DMPA+/TDF+) to controls with no HIV (HIV-/DMPA-/TDF-). We further assessed whether BMD loss with TDF ART initiation over a two-year follow-up period is greater with DMPA-IM use among young women living with HIV, by comparing BMD changes in WLWH initiating TDF-containing ART with DMPA-IM (HIV+/DMPA+/TDF+) to WLWH initiating TDF-containing ART without DMPA-IM (HIV+/DMPA-/TDF+). A mixed-effects regression model was also used to estimate the annualized rate of change in BMD. Women who became pregnant were censored at first pregnancy. A post hoc analysis conducted excluding data for women who inconsistently used DMPA-IM (received less than 2 injections if terminated within one year after enrollment or received less than 4 injections if terminated within the second year) and TDF (virologically un suppressed with viral load level > 1000 copies/ml). We further explored whether BMD losses significantly differed among women who concurrently initiated DMPA-IM and TDF, defined as starting to use DMPA-IM or TDF within 6 months of the other. All analyses were done by intention to treat using StataTM Release 15 (College Station, TX, USA).

Socio-demographic and Clinical Characteristics

Of the 549 women screened, 15 were excluded due to current pregnancy, and 13 due to other reasons (unstable location, unexplained amenorrhea, refusal to participate, and use of combined oral contraceptive pills), **Figure 5.1**. A total of 521 women were enrolled: 452 were WLWH and 69 with no HIV. Of the WLWH, 265 initiated TDF-containing ART (159 DMPA-IM users, 106 non-hormonal users), and 187 were not eligible for ART at the time of their enrollment. Of these latter women, 166 later initiated ART under the revised Uganda test and treat policy and continued in the study, 21 of the original 187 women having been excluded from the analysis due to DMPA-IM discontinuation (10) and loss to follow-up (11) prior to ART initiation. Among the women that initiated ART (n=431), the majority (99%) were initiated on TDF/3TC/EFV, with only 1% on TDF/3TC/NVP. Two women switched to non-TDF regimens during the course of the study (ABC/3TC/ ATV/r and ABC/3TC/ EFV), but were included in the final analysis. The median time between ART initiation and enrollment was 0 (0, 2) days.

Baseline characteristics are shown in **Table 5.1**. The mean (SD) age at baseline was 26.1 (4.2) years, 43.6% were married or co-habiting and 83.7% had ever been pregnant with a median parity of 2 (IQR: 1, 3). Among DMPA-IM users, the majority (72.8%) were using DMPA-IM at the time of enrollment with a median duration of DMPA-IM use of 26.0 months (IQR: 12, 48). Compared with WLWH, controls were more likely to be younger, have more years of education, lower parity, and to not have ever breastfed. There was no difference in baseline viral load between the WLWH groups. WLWH had significantly lower baseline BMD at all sites than controls ($p<0.01$). Compared to the control group, a higher proportion of WLWH irrespective of the type of contraceptive use had low BMD Z-scores (defined as a Z-score ≤ -2.0 at any of the three sites) at enrollment; 2.9% vs $\approx 16.5\%$ respectively, ($p=0.018$).

Mean % change in BMD and BMD Z-scores among controls with no HIV and WLWH on TDF and DMPA-IM

No significant changes in BMD at FN and TH sites were observed among controls over the two-year follow-up period but a slight increase in BMD at the LS was noted at 6, 12, and 24 months ($p < 0.05$), **Figure 2**. On the other hand, WLWH experienced significant declines in BMD at the three sites at all time points post enrollment. The majority of BMD loss among WLWH initiating TDF-containing ART with or without DMPA-

IM was observed within the first 12 months of treatment at the LS, TH and FN. Thereafter, BMD tended to stabilize at the lower levels, **Figure 5.2**. Greater declines in BMD were observed among concurrent users of DMPA-IM and TDF compared to non-hormonal users on TDF-containing ART alone. Similar trends were observed in BMD Z-score changes within the different study groups, **Figure 5.3**. There were no differences in BMD changes among WLWH initiating TDF-containing ART with DMPA-IM prior to (n=159) or under the test and treat guidelines (n=166), p-value >0.16 (data not shown). Given that these two groups of DMPA-IM users ((HIV+/DMPA+/TDF+ (i) and (HIV+/DMPA+/TDF+ (ii), **Figures 5.2 and 5.3**)), were similar, we have combined their data for the rest of the results below.

Differences in mean % BMD change in WLWH initiating TDF-containing ART with (HIV+/DMPA+/TDF+) vs. without DMPA-IM or controls with no HIV (HIV-/DMPA-/TDF-)

Over the two-year study period, % BMD in the HIV+/DMPA+/TDF+ group declined significantly compared to controls (LS (-5.0% (-6.4, -3.6)), TH (-4.2% (-5.6, -2.7)), and FN% (-4.8 (-6.4, -3.1)), (p<0.001), **Table 5.2**. Similarly there were significant declines in BMD from baseline for the non-hormonal and DMPA-IM users on TDF at all the time points, **Table 5.2** and **Figure 5.2**. Compared to the use of TDF alone, concurrent use of both DMPA-IM and TDF resulted in greater BMD declines at all sites: -2.7% [95% CI -3.4, -1.6] at the LS, -2.5% [95% CI -3.6, -1.5] at the TH, and -3.0% [95% CI -4.1, -1.7] at the FN by 24 months, after adjusting for age, BMI and baseline BMD (p<0.001 for all), **Table 5.2**.

Annualized change in BMD among WLWH initiating TDF-containing ART with DMPA-IM, without DMPA-IM, and controls

The annualized rates of BMD loss were higher in WLWH using both DMPA-IM and TDF (HIV+/DMPA+/TDF+) than WLWH using non-hormonal contraceptives (HIV+/DMPA-/TDF+) or controls (HIV-/DMPA-/TDF-) at all sites. These changes were significantly different between the three groups after adjusting for baseline BMD, age, and BMI, p-value <0.001 at all sites, **Table 5.3**. The annualized rate of bone loss in the DMPA-IM users at between -1.5% and -2.3% at the different sites was approximately double that of the non-hormonal contraceptive users.

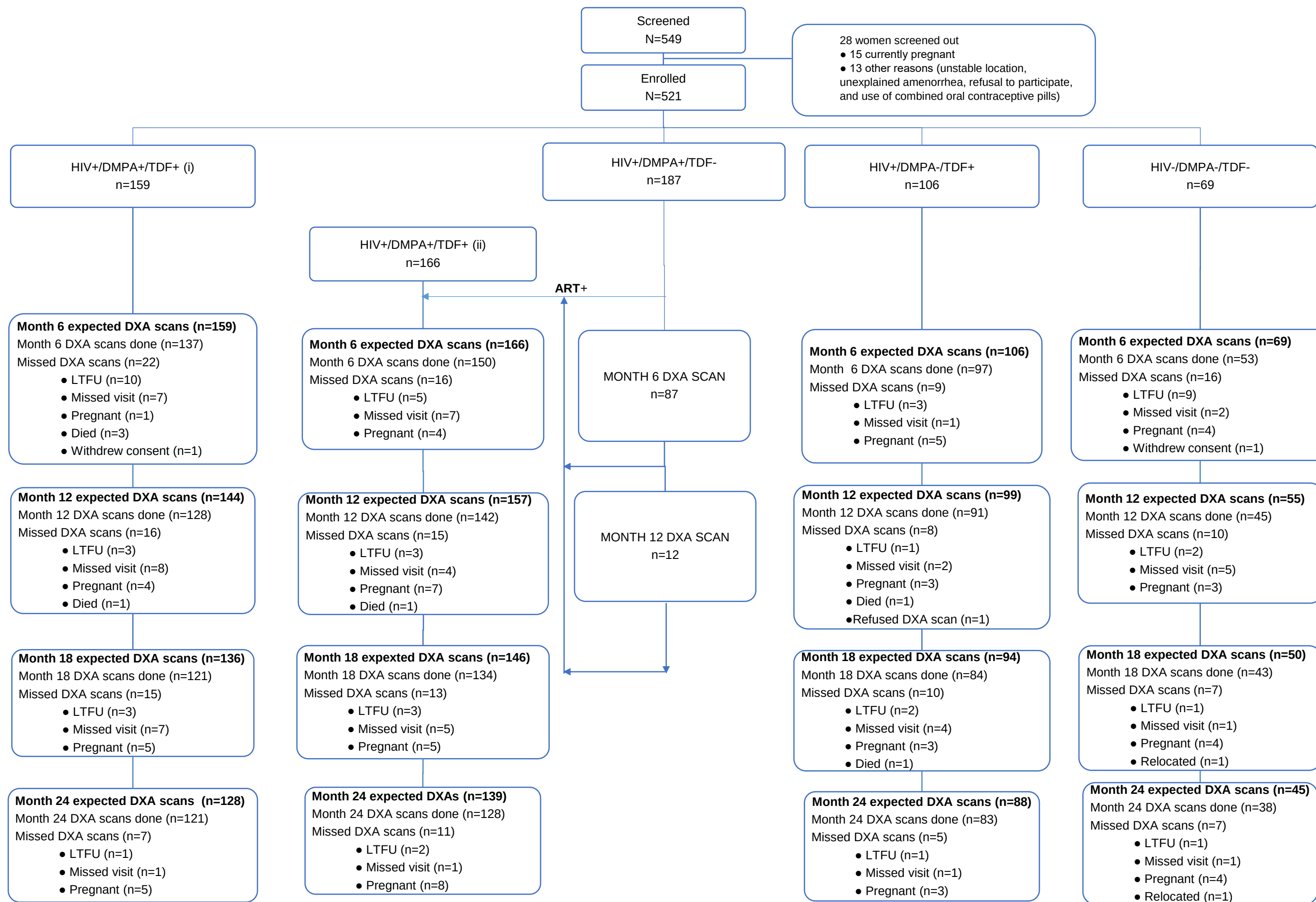
Post hoc analysis exploring BMD changes among consistent users of TDF-containing ART and DMPA-IM

High proportions of virologic suppression were achieved by both DMPA-IM (90%) and non-hormonal users (94%) at 12 and 24-month time points, **Figure 5.3**. Among DMPA-IM users, 252 WLWH (77.5%) met our definition for consistent use, while 73 (22.5%) did not. Consistent use of DMPA-IM and TDF resulted in greater declines in BMD at all sites compared to inconsistent use (p-value ≤ 0.02 at the 24-month time point), **Table 5.4**.

Post hoc analysis exploring BMD changes among untreated WLWH DMPA-IM users

WLWH who were ART-naïve but using DMPA had significantly lower BMD at the 6 months at the LS but not at the TH and FN, compared to the control group with no HIV. Their BMDs were greater than WLWH initiating TDF-containing ART without DMPA-IM at all sites, **Table 5.5**. Because of small numbers of WLWH not on TDF at 12 months, statistical comparisons were not made.

Figure 5. 1: Participant disposition in the BONE: CARE Study



One participant in the HIV+/DMPA+/TDF+ (ii) group has a missing baseline scan at the LS and another participant in the same group is missing a baseline scan at the TH & FN

Table 5. 1: Baseline Characteristics of Women enrolled in the BONE: CARE Study

Characteristic ¹	Total (N=521)	WLWH initiating TDF- containing ART with DMPA-IM at CD4 ≤ 500 cells/ µL (HIV+/DMPA+/TDF+) (n=159)	WLWH using DMPA-IM but NOT eligible for ART initiation (CD4 > 500 cells/µL) ² (HIV+/DMPA+/TDF-) (n=187)	WLWH initiating TDF- containing ART with DMPA-IM, including those following test and treat (HIV+/DMPA+/TDF+) (n= 325) ³	WLWH initiating TDF- containing ART without DMPA-IM (HIV+/DMPA-/TDF+) (n=106)	Women not living with HIV and not using DMPA-IM (non-hormonal controls) (n=69)	P-value ⁴
Age (years)	26.1 (4.2)	26.3 (3.9)	26.2 (4.0)	26.8 (4.0)	26.8 (4.6)	24.4 (4.0)	0.002
18-24	202 (38.8)	58 (36.5)	69 (36.9)	108 (33.2)	41 (38.7)	34 (49.3)	
25-35	319 (61.2)	101 (63.5)	118 (63.1)	217 (66.8)	65 (61.3)	35 (50.7)	0.280
Education level							
Below secondary	228 (43.8)	76 (47.8)	100 (53.5)	169 (52.0)	40 (37.7)	12 (17.4)	
≥Secondary	293 (56.2)	83 (52.2)	87 (46.5)	156 (48.0)	66 (62.3)	57 (82.6)	<0.001
Marital Status							
Not married	224 (43.0)	68 (42.8)	76 (40.6)	137 (42.2)	52 (49.1)	28 (40.6)	
Married	297 (57.0)	91 (57.2)	111 (59.4)	188 (57.9)	54 (50.9)	41 (59.4)	0.536
Earns income	362 (69.5)	111 (69.8)	141 (75.4)	227 (69.9)	76 (71.7)	34 (49.3)	0.001
Median monthly income (USD)	40.7 (27.1,70.6)	40.7 (27.1, 81.4)	40.7 (27.1, 67.8)	40.7 (27.1,70.6)	40.7 (27.1,71.9)	54.3 (40.7, 81.4)	0.098
Current use of DMPA-IM	177 (72.8)	82 (74.5)	95 (77.9)	168 (75.7)	0 (0.0)	0 (0.0)	-
Median duration of DMPA-IM use (months)	24 (12, 48)	24 (12, 48)	24 (9,48)	24 (12, 48)	N/A	N/A	0.936
Age at Menarche	14.2 (1.6)	14.2 (1.8)	14.2 (1.6)	14.2 (1.7)	14.3 (1.4)	14.0 (1.4)	0.860
Ever Pregnant	436 (83.7)	145 (91.2)	174 (93)	301 (92.6)	84 (79.2)	33 (47.8)	<0.001
Median parity	2 (1,3)	2 (1,3)	2 (1,3)	2 (1, 3)	2 (1,3)	0 (0,2)	<0.001
Ever breastfed	390 (74.9)	136 (85.5)	160 (85.6)	280 (86.2)	69 (65.1)	25 (36.2)	<0.001
Median duration of breast feeding	26 (15,43)	26 (16,43.5)	25.5 (15, 42.5)	26 (15.5,44)	26 (12,42)	28 (18,50)	0.991
Currently drinks alcohol	142 (27.3)	52 (32.7)	59 (31.6)	86 (26.5)	23 (21.7)	8 (11.8)	0.003
Ever smoked	22 (4.2)	7 (4.4)	9 (4.8)	10 (3.1)	5 (4.7)	1 (1.4)	0.691
Current smoker ⁵	12 (54.5)	4 (57.1)	6 (66.7)	5 (1.5)	2 (40.0)	0 (0.0)	0.727
Physical activity (minutes/week)⁶							
Vigorous (≥75)	55 (77.5)	15 (75.0)	22 (88.0)	32 (82.1)	9 (64.3)	9 (75.0)	0.346
Moderate (≥150)	169 (62.8)	46 (62.2)	63 (64.3)	102 (68.0)	32 (58.2)	28 (66.7)	0.831
Median CD4 cell count (cells/µL)	670 (411, 859)	452 (327, 605)	851 (754, 1024)	709 (454, 890)	442 (309, 641)	N/A	<0.001
Body mass index (kg/m ²)	24.7 (4.4)	24.8 (4.4)	25.2 (4.6)	25.2 (4.7)	24.3 (4.1)	24.0 (4.1)	0.160
Median viral load (log10 copies/mL)	4.1 (3.2, 4.6)	4.21 (3.56, 4.89)	3.90 (2.77, 4.40)	4.08 (3.35,4.64)	4.16 (3.29, 4.60)	N/A	0.115
Mean BMD (g/cm²)							
Lumbar Spine	0.938 (0.110)	0.927 (0.106)	0.926 (0.110)	0.923 (0.109)	0.960 (0.118)	0.966 (0.098)	0.006
Total Hip	0.960 (0.115)	0.942 (0.108)	0.954 (0.110)	0.948 (0.109)	0.975 (0.130)	0.995 (0.107)	0.005
Femoral Neck	0.863 (0.116)	0.847 (0.112)	0.855 (0.113)	0.849 (0.113)	0.877 (0.126)	0.896 (0.108)	0.011
Mean BMD Z-Score							
Lumbar Spine	-0.383 (1.131)	-0.502 (1.112)	-0.507 (1.146)	-0.544 (1.142)	-0.236 (1.162)	0.000 (0.985)	0.003
Total Hip	-0.452 (1.120)	-0.628 (1.070)	-0.502 (1.068)	-0.572 (1.070)	-0.394 (1.287)	0.000 (0.985)	0.001
Femoral Neck	-0.409 (1.091)	-0.553 (1.064)	-0.467 (1.042)	-0.533 (1.058)	-0.355 (1.221)	0.000 (0.985)	0.004
Low BMD Z-score (≤-2)							
Lumbar Spine	43 (8.3)	14 (8.8)	21 (11.2)	33 (10.2)	7 (6.6)	1 (1.4)	0.077
Total Hip	45 (8.6)	16 (10.1)	16 (8.6)	30 (9.2)	13 (12.3)	0 (0.0)	0.034
Femoral Neck	33 (6.3)	11 (6.9)	12 (6.4)	24 (7.4)	9 (8.5)	1 (1.4)	0.296
Low BMD Z-score (≤-2) at any of the 3 sites							
Normal (>-2)	443 (85.0)	135 (84.9)	152 (81.3)	268 (82.5)	89 (84.0)	67 (97.1)	-
Low BMD (≤-2)	78 (15.0)	24 (15.1)	35 (18.7)	57 (17.5)	17 (16.0)	2 (2.9)	0.018

¹ Mean or proportions unless stated otherwise.

²Participants not eligible for ART initiation at the time (CD4 cell count > 500 cells/u) later initiated ART under the test and treat guidelines.

³Combined total of WLWH initiated on TDF-containing ART with DMPA-IM is 325 (159 + 166) as 21 women of 187 in the group that later initiated ART due to change in the guidelines were excluded from analysis; 10 were lost to follow-up and 11 discontinued DMPA-IM prior to ART initiation.

⁴ Difference in baseline characteristics across groups.

⁵ Among those who have ever smoked.

⁶ Vigorous physical activity over a shorter duration (at least 75 minutes) has the same health benefits as moderate physical activity performed over a longer period of time (at least 150 minutes). Some women contributed to both categories of physical activity.

Figure 5. 2: Mean percent change in BMD among women in the BONE: CARE Study

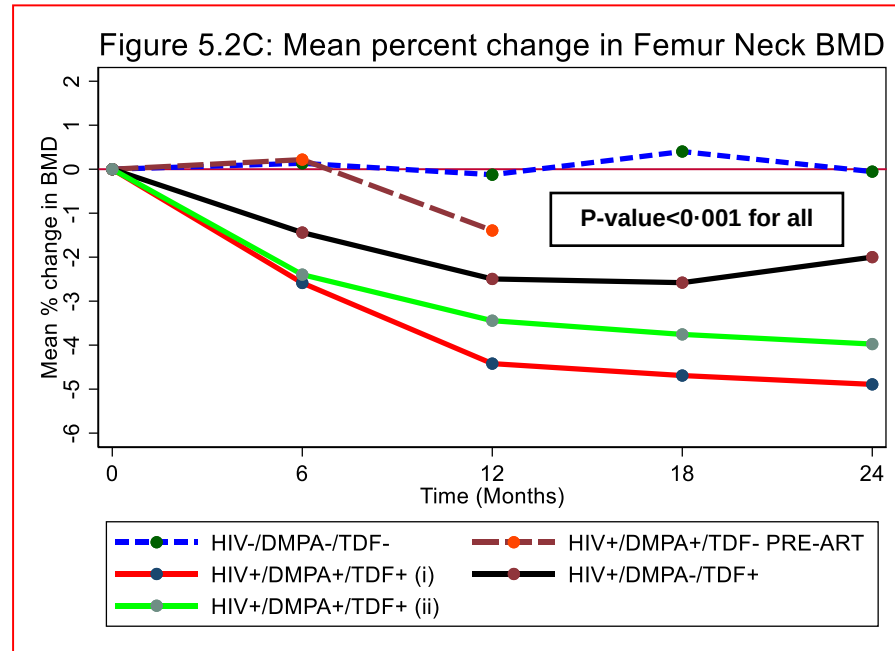
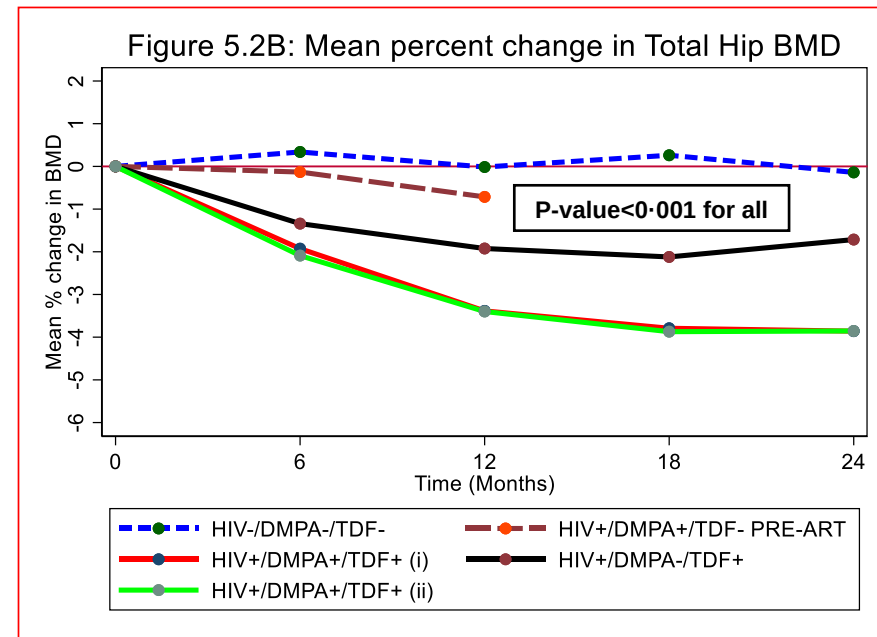
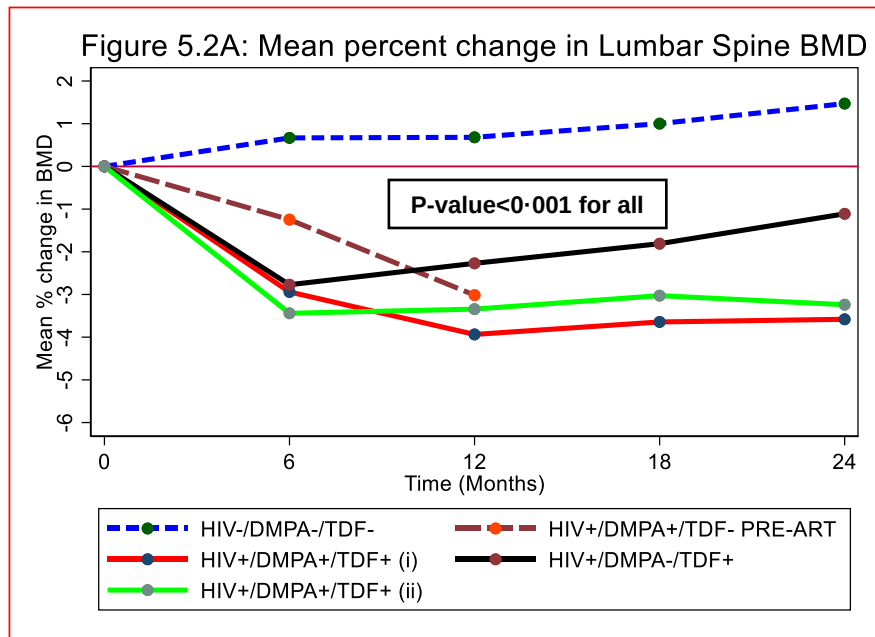


Figure 5. 3: Mean percent change in BMD Z-scores among women in the BONE: CARE Study

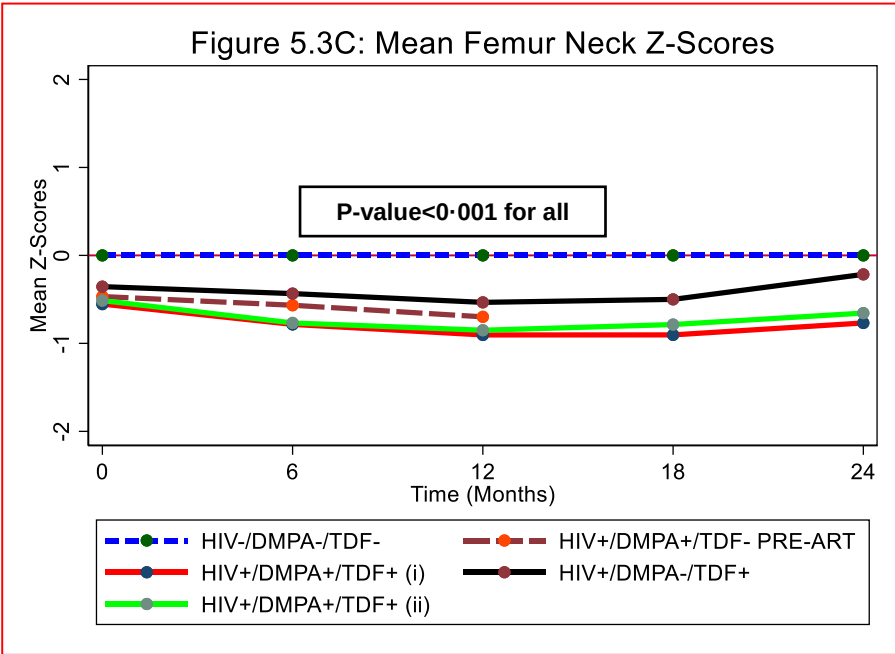
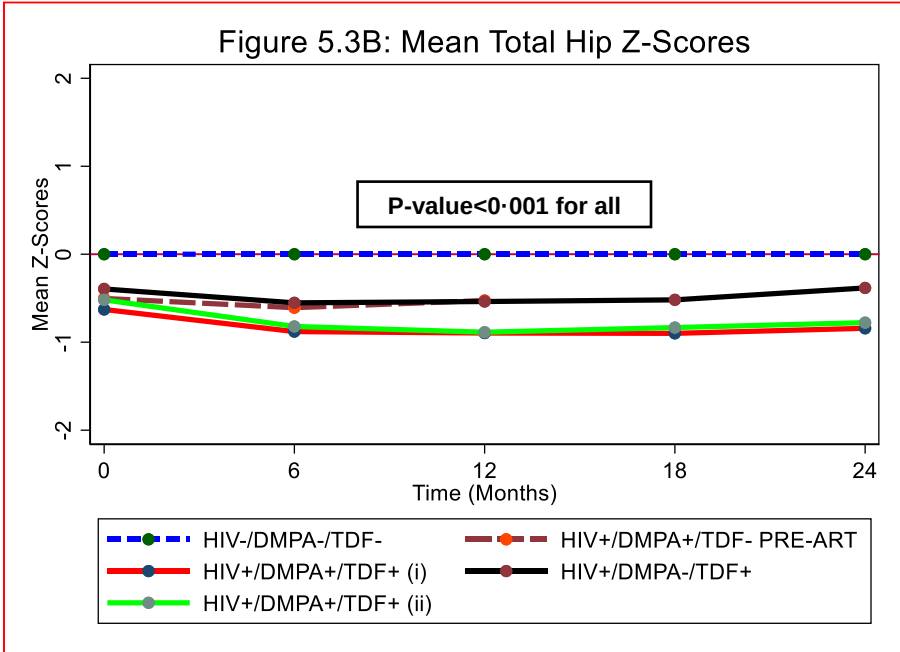
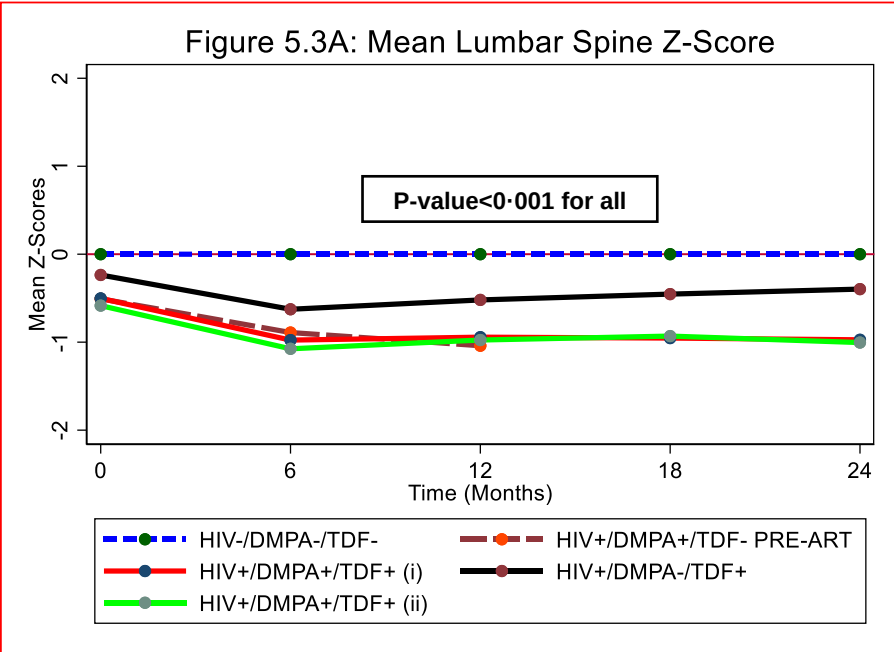


Table 5. 2: Adjusted differences in mean % BMD at the Lumbar Spine (L1 to L4), Total Hip, and Femoral Neck

Body site	N	% Changes in BMD from baseline (g/cm ²) (95% CI)	P-value ⁴	Adjusted % difference in BMD (g/cm ²) (95% CI) ⁵	P-value ⁴	Adjusted % difference in BMD (g/cm ²) (95% CI) ⁵	P-value ⁴
Lumbar spine							
Baseline to Month 6							
HIV-/DMPA-/TDF- ¹	53	0.667 (0.180, 1.154)	0.008	Ref			
HIV+/DMPA-/TDF+ ²	97	-2.772 (-3.258, -2.285)	<0.001	-3.302 (-4.228, -2.376)	<0.001	Ref	
HIV+/DMPA+/TDF+ ³	286	-3.201 (-3.551, -2.850)	<0.001	-3.972 (-4.793, -3.150)	<0.001	-0.673 (-1.339, -0.006)	0.048
Baseline to Month 12							
HIV-/DMPA-/TDF- ¹	45	0.682 (-0.040, 1.403)	0.063	Ref			
HIV+/DMPA-/TDF+ ²	91	-2.270 (-2.860, -1.681)	<0.001	-2.833 (-4.129, -1.538)	<0.001	Ref	
HIV+/DMPA+/TDF+ ³	269	-3.625 (-4.112, -3.138)	<0.001	-4.485 (-5.646, -3.323)	<0.001	-1.633 (-2.519, -0.747)	<0.001
Baseline to Month 18							
HIV-/DMPA-/TDF- ¹	43	1.001 (0.183, 1.820)	0.018	Ref			
HIV+/DMPA-/TDF+ ²	84	-1.811 (-2.540, -1.082)	<0.001	-2.521 (-3.992, -1.049)	0.001	Ref	
HIV+/DMPA+/TDF+ ³	254	-3.321 (-3.887, -2.755)	<0.001	-4.634 (-5.937, -3.331)	<0.001	-2.133 (-3.153, -1.113)	<0.001
Baseline to Month 24							
HIV-/DMPA-/TDF- ¹	38	1.471 (0.584, 2.359)	0.002	Ref			
HIV+/DMPA-/TDF+ ²	83	-1.111 (-1.929, -0.293)	0.008	-2.307 (-3.895, -0.719)	0.005	Ref	
HIV+/DMPA+/TDF+ ³	248	-3.406 (-3.969, -2.844)	<0.001	-4.970 (-6.391, -3.549)	<0.001	-2.677 (-3.743, -1.611)	<0.001

Body site	N	% Changes in BMD from baseline (g/cm ²) (95% CI)	P-value ⁴	Adjusted % difference in BMD (g/cm ²) (95% CI) ⁵	P-value ⁴	Adjusted % difference in BMD (g/cm ²) (95% CI) ⁵	P-value ⁴
Total Hip							
Baseline to Month 6							
HIV-/DMPA-/TDF- ¹	53	0.338 (-0.242, 0.919)	0.248	Ref			
HIV+/DMPA-/TDF+ ²	97	-1.341 (-1.790, -0.891)	<0.001	-1.568 (-2.488, -0.647)	0.001	Ref	
HIV+/DMPA+/TDF+ ³	286	-2.011 (-2.367, -1.655)	<0.001	-2.505 (-3.320, -1.690)	<0.001	-0.949 (-1.596, -0.303)	0.004
Baseline to Month 12							
HIV-/DMPA-/TDF- ¹	45	-0.015 (-0.756, 0.726)	0.968	Ref			
HIV+/DMPA-/TDF+ ²	91	-1.923 (-2.497, -1.349)	<0.001	-2.069 (-3.261, -0.877)	0.001	Ref	
HIV+/DMPA+/TDF+ ³	269	-3.392 (-3.832, -2.952)	<0.001	-3.769 (-4.836, -2.702)	<0.001	-1.697 (-2.507, -0.886)	<0.001
Baseline to Month 18							
HIV-/DMPA-/TDF- ¹	43	0.261 (-0.658, 1.179)	0.570	Ref			
HIV+/DMPA-/TDF+ ²	84	-2.121 (-2.817, -1.425)	<0.001	-2.491 (-3.850, -1.132)	<0.001	Ref	
HIV+/DMPA+/TDF+ ³	254	-3.833 (-4.337, -3.329)	<0.001	-4.553 (-5.755, -3.350)	<0.001	-2.060 (-2.979, -1.141)	<0.001
Baseline to Month 24							
HIV-/DMPA-/TDF- ¹	38	-0.140 (-1.121, 0.841)	0.774	Ref			
HIV+/DMPA-/TDF+ ²	83	-1.714 (-2.479, -0.949)	<0.001	-1.655 (-3.252, -0.058)	0.042	Ref	
HIV+/DMPA+/TDF+ ³	248	-3.856 (-4.449, -3.264)	<0.001	-4.151 (-5.579, -2.724)	<0.001	-2.518 (-3.575, -1.461)	<0.001

Body site	N	% Changes in BMD from baseline (g/cm ²) (95% CI)	P-value ⁴	Adjusted % difference in BMD (g/cm ²) (95% CI) ⁵	P-value ⁴	Adjusted % difference in BMD (g/cm ²) (95% CI) ⁵	P-value ⁴
Femoral Neck							
Baseline to Month 6							
HIV-/DMPA-/TDF- ¹	53	0.134 (-0.670, 0.938)	0.739	Ref			
HIV+/DMPA-/TDF+ ²	97	-1.440 (-2.073, -0.808)	<0.001	-1.599 (-2.787, -0.411)	0.008	Ref	
HIV+/DMPA+/TDF+ ³	286	-2.487 (-2.919, -2.056)	<0.001	-2.846 (-3.896, -1.796)	<0.001	-1.228 (-2.061, -0.396)	0.004
Baseline to Month 12							
HIV-/DMPA-/TDF- ¹	45	-0.125 (-1.112, 0.862)	0.799	Ref			
HIV+/DMPA-/TDF+ ²	91	-2.495 (-3.314, -1.676)	<0.001	-2.495 (-3.959, -1.031)	<0.001	Ref	
HIV+/DMPA+/TDF+ ³	269	-3.908 (-4.433, -3.383)	<0.001	-4.266 (-5.573, -2.958)	0.001	-1.748 (-2.738, -0.758)	0.001
Baseline to Month 18							
HIV-/DMPA-/TDF- ¹	43	0.402 (-0.649, 1.453)	0.444	Ref			
HIV+/DMPA-/TDF+ ²	84	-2.579 (-3.493, -1.665)	<0.001	-2.982 (-4.690, -1.274)	0.001	Ref	
HIV+/DMPA+/TDF+ ³	254	-4.201 (-4.831, -3.572)	<0.001	-5.068 (-6.576, -3.561)	<0.001	-2.077 (-3.247, -0.908)	0.001
Baseline to Month 24							
HIV-/DMPA-/TDF- ¹	38	-0.055 (-1.260, 1.151)	0.927	Ref			
HIV+/DMPA-/TDF+ ²	83	-1.999 (-3.022, -0.976)	<0.001	-1.872 (3.725, -0.018)	0.048	Ref	
HIV+/DMPA+/TDF+ ³	248	-4.422 (-5.078, -3.766)	<0.001	-4.773 (-6.424, -3.122)	<0.001	-2.907 (-4.132, -1.683)	<0.001
¹ Controls (N at baseline=69): Women with no HIV using non-hormonal contraception (IUD or condoms). ² WLWH initiating TDF-containing ART without DMPA-IM (N at baseline=106): WLWH using non-hormonal users (IUD or condoms) who are initiating TDF-containing ART. ³ WLWH initiating TDF-containing ART with DMPA-IM (N at baseline=325): Combined group of WLWH DMPA-IM users initiating TDF-containing ART at a CD4 cell count ≤ 500 and > 500 cells/μL. ⁴ Significant at ≤0.05. ⁵ Adjusted for age, BMI, and baseline BMD. Adjusting for baseline HIV RNA concentrations did not change the inferences.							

Table 5. 3: Mean annualized percent change in BMD over 24 months		
Body site	Percent annual rate of change (95% CI)⁵	P-value⁶
Lumbar spine		
HIV-/DMPA-/TDF- ¹	0.707 (0.278, 1.136)	0.001
HIV+/DMPA-/TDF+ ²	-0.338 (-0.756, 0.080)	0.113
HIV+/DMPA+/TDF+ ³	-1.532 (-1.820, -1.244)	<0.001
Total Hip		
HIV-/DMPA-/TDF- ¹	-0.053 (-0.487, 0.380)	0.810
HIV+/DMPA-/TDF+ ²	-0.887 (-1.291, -0.483)	<0.001
HIV+/DMPA+/TDF+ ³	-2.025 (-2.315, -1.735)	<0.001
Femoral Neck		
HIV-/DMPA-/TDF- ¹	0.017 (-0.510, 0.544)	0.949
HIV+/DMPA-/TDF+ ²	-1.111 (-1.621, -0.600)	<0.001
HIV+/DMPA+/TDF+ ³	-2.285 (-2.630, -1.939)	<0.001
¹ Controls (N at baseline=69): Women with n HIV using non-hormonal contraception (IUD or condoms). ² WLWH initiating TDF-containing ART without DMPA-IM (N at baseline=106): WLWH using non-hormonal users (IUD or condoms) who are initiating TDF-containing ART. ³ WLWH initiating TDF-containing ART with DMPA-IM (N at baseline=325): Combined group of DMPA-IM users LWH initiating TDF-containing ART at a CD4 cell count ≤ 500 and > 500 cells/μL. ⁴ Adjusted for age, BMI, and baseline BMD. ⁵ Significant at ≤0.05.		

Figure 5. 4: Proportion with VL Suppression over time

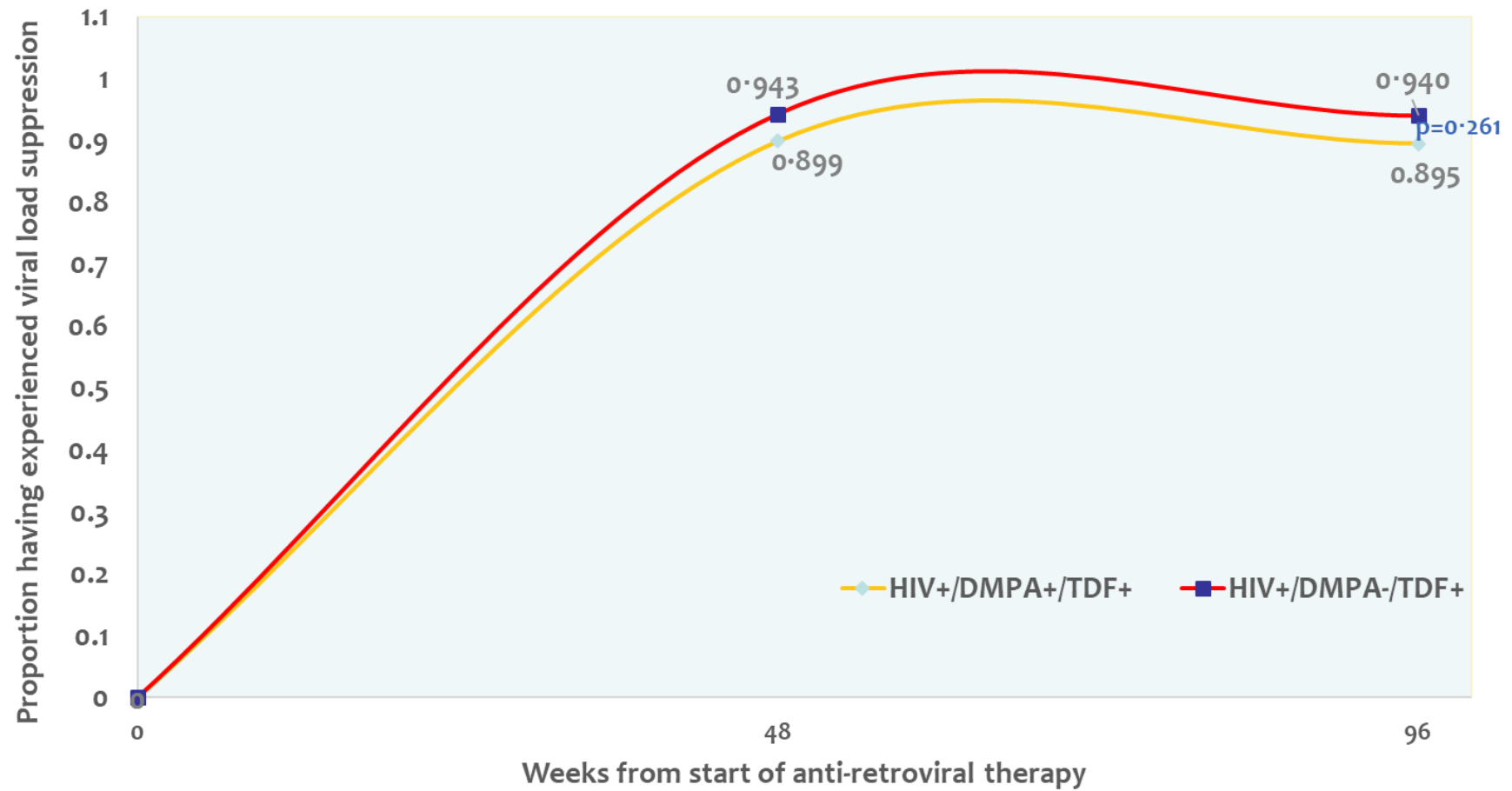


Table 5. 4: Mean % change in BMD at the Lumbar Spine (L1 to L4), Hip, and Femoral Neck among consistent and inconsistent users of DMPA-IM and TDF-containing ART

Body site	% Changes in BMD from baseline (g/cm ²)				Crude % Difference in BMD (g/cm ²) (95% CI)	P-value ³	Adjusted % difference in BMD (g/cm ²) 95% CI ⁴	P-value ³
	Consistent users of DMPA-IM and TDF-containing ART ¹ (N=252)		Inconsistent users of DMPA-IM and TDF-containing ART ² (N=73)					
	n	95% CI	n	95% CI				
Lumbar spine								
Baseline to Month 6	234	-3.505 (-3.852, -3.158)	52	-1.831 (-2.912, -0.750)	-1.674 (-2.562, -0.786)	<0.001	1.775 (-2.658, -0.892)	<0.001
Baseline to Month 12	225	-3.911 (-4.409, -3.412)	44	-2.163 (-3.675, -0.651)	-1.748 (-3.049, -0.446)	0.009	-1.788 (-3.056, -0.520)	0.006
Baseline to Month 18	214	-3.823 (-4.414, -3.232)	40	-0.633 (-2.137, 0.871)	-3.190 (-4.696, -1.685)	<0.001	-3.268 (-4.690, -1.845)	<0.001
Baseline to Month 24	210	-3.940 (-4.527, -3.353)	38	-0.459 (-1.906, 0.989)	-3.481 (-4.984, -1.978)	<0.001	-3.449 (-4.922, -1.977)	<0.001
Total Hip								
Baseline to Month 6	234	-2.138 (-2.534, -1.742)	52	-1.442 (-2.261, -0.624)	-0.695 (-1.616, 0.225)	0.138	-0.820 (-1.699, 0.059)	0.067
Baseline to Month 12	225	-3.512 (-3.985, -3.038)	44	-2.778 (-3.978, -1.579)	-0.733 (-1.922, 0.455)	0.225	-0.707 (-1.853, 0.439)	0.226
Baseline to Month 18	214	-4.138 (-4.681, -3.596)	40	-2.199 (-3.487, -0.911)	-1.939 (-3.305, -0.574)	0.006	-1.910 (-3.197, -0.623)	0.004
Baseline to Month 24	210	-4.263 (-4.902, -3.625)	38	-1.608 (-3.055, -0.162)	-2.655 (-4.270, -1.040)	0.001	-2.679 (-4.187, -1.171)	0.001
Femoral Neck								
Baseline to Month 6	234	-2.538 (-3.018, -2.058)	52	-2.260 (-3.274, -1.245)	-0.278 (-1.398, 0.842)	0.625	-0.429 (-1.535, 0.678)	0.446
Baseline to Month 12	225	-3.930 (-4.494, -3.366)	44	-3.795 (-5.256, -2.334)	-0.136 (-1.556, 1.285)	0.851	-0.102 (-1.469, 1.266)	0.884
Baseline to Month 18	214	-4.640 (-5.295, -3.985)	40	-1.853 (-3.674, -0.032)	-2.787 (-4.483, -1.091)	0.001	-2.768 (-4.389, -1.147)	0.001
Baseline to Month 24	210	-4.849 (-5.522, -4.177)	38	-2.060 (-4.100, -0.020)	-2.790 (-4.580, -1.000)	0.002	-2.744 (-4.453, -1.035)	0.002
¹ Consistent users of DMPA-IM and TDF containing ART (N at baseline=252): WLWH who achieved virologic suppression (<1000 copies/ml) at 12 and 24 months and received ≥ 2 injections of DMPA-IM if terminated within one year or ≥ 4 injections if terminated within the second year of follow-up.								
² Inconsistent users of DMPA-IM and TDF containing ART (N at baseline=73): WLWH who did not achieve virologic suppression (≥1000 copies/ml) at 12 and 24 months and received < 2 injections of DMPA-IM if terminated within one year or < 4 injections if terminated within the second year of follow-up.								
³ Signifcant at ≤0.05.								
⁴ Adjusted for age, BMI, and baseline BMD.								

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Table 5. 5: Adjusted differences in mean % BMD at the Lumbar Spine (L1 to L4), Total Hip, and Femoral Neck in WLWH using DMPA-IM and on or not on TDF-containing ART compared to controls

Body site	N	% Changes in BMD from baseline (g/cm ²) (95% CI)	P-value ⁵	Adjusted % difference in BMD (g/cm ²) (95% CI) ⁶	P-value ⁵	Adjusted % difference in BMD (g/cm ²) (95% CI) ⁶	P-value ⁵
Lumbar spine							
Baseline to Month 6							
HIV-/DMPA-/TDF- ¹	53	0.667 (0.180, 1.154)	0.008	Ref			
HIV+/DMPA-/TDF+ ²	97	-2.772 (-3.258, -2.285)	<0.001	-3.302 (-4.228, -2.376)	<0.001	Ref	
HIV+/DMPA+/TDF- ³	87	-1.247 (-1.800, -0.693)	<0.001	-2.258 (-3.118, -1.397)	<0.001	1.109 (0.346, 1.873)	0.005
Baseline to Month 12							
HIV-/DMPA-/TDF- ¹	45	0.682 (-0.040, 1.403)	0.063	Ref			
HIV+/DMPA-/TDF+ ²	91	-2.270 (-2.860, -1.681)	<0.001	-2.833 (-4.129, -1.538)	<0.001	Ref	
HIV+/DMPA+/TDF- ³	12	-3.015 (-4.140, -1.890)	<0.001	-4.228 (-5.776, -2.80)	<0.001	-1.594 (-3.239, 0.051)	0.057
Total Hip							
Baseline to Month 6							
HIV-/DMPA-/TDF- ¹	53	0.338 (-0.242, 0.919)	0.248	Ref			
HIV+/DMPA-/TDF+ ²	97	-1.341 (-1.790, -0.891)	<0.001	-1.568 (-2.488, -0.647)	0.001	Ref	
HIV+/DMPA+/TDF- ³	87	-0.132 (-0.689, 0.425)	0.638	-0.627 (-1.516, 0.262)	0.165	0.808 (0.080, 1.535)	0.030
Baseline to Month 12							
HIV-/DMPA-/TDF- ¹	45	-0.015 (-0.756, 0.726)	0.968	Ref			
HIV+/DMPA-/TDF+ ²	91	-1.923 (-2.497, -1.349)	<0.001	-2.069 (-3.261, -0.877)	0.001	Ref	
HIV+/DMPA+/TDF- ³	11	-0.714 (-3.431, 2.003)	0.571	-1.349 (-3.291, 0.592)	0.169	1.005 (-0.886, 2.897)	0.294

Body site	N	% Changes in BMD from baseline (g/cm ²) (95% CI)	P-value ⁵	Adjusted % difference in BMD (g/cm ²) (95% CI) ⁶	P-value ⁵	Adjusted % difference in BMD (g/cm ²) (95% CI) ⁶	P-value ⁵
Femoral Neck							
Baseline to Month 6							
HIV-/DMPA-/TDF- ¹	53	0.134 (-0.670, 0.938)	0.739	Ref			
HIV+/DMPA-/TDF+ ²	97	-1.440 (-2.073, -0.808)	<0.001	-1.599 (-2.787, -0.411)	0.008	Ref	
HIV+/DMPA+/TDF- ³	87	0.218 (-0.367, 0.803)	0.461	-0.098 (-1.111, 0.915)	0.848	1.413 (0.491, 2.335)	0.003
Baseline to Month 12							
HIV-/DMPA-/TDF- ¹	45	-0.125 (-1.112, 0.862)	0.799	Ref			
HIV+/DMPA-/TDF+ ²	91	-2.495 (-3.314, -1.676)	<0.001	-2.495 (-3.959, -1.031)	<0.001	Ref	
HIV+/DMPA+/TDF- ³	11	-1.393 (-3.909, 1.122)	0.245	-2.265 (-4.633, 0.102)	0.060	0.615 (-1.942, 3.173)	0.634
¹ Controls (N at baseline=69): Women with no HIV using non-hormonal contraception (IUD or condoms). ² WLWH initiating TDF-containing ART without DMPA-IM (N at baseline=106): WLWH using non-hormonal contraceptives (IUD or condoms) who initiated TDF-containing ART. ³ WLWH using DMPA-IM but not eligible for ART (N at baseline=87): WLWH using DMPA-IM but not TDF-containing ART because they were not eligible for HIV prior to the test and treat guidelines > 500 cells/μL. ⁴ significant at ≤0.05. ⁵ Adjusted for age, BMI, and baseline BMD.							

6.0 CHAPTER SIX - DISCUSSION

Our review underscored critical gaps in knowledge about bone health in RLS, and consequently the lack of policies and clinical management guidelines on minimizing bone loss for WLWH. My PhD work addresses critical gaps in bone health knowledge among WLWH in RLS. We observed a significantly higher prevalence of low BMD and mean differences in BMD Z-scores among ART naïve Ugandan WLWH compared with uninfected women independent of age and BMI(194, 195). At baseline, current DMPA-IM users had lower BMD at all three sites than previous DMPA-IM users or non-hormonal contraceptive users suggesting BMD recovery post DMPA-IM discontinuation.

The above findings of low BMD among ART naïve WLWH are consistent with the majority of published studies that have reported HIV to be an independent risk factor for low BMD(196, 197) and can be explained by both the direct effect of the HIV virus as well as chronic immune activation. Our longitudinal analysis showed that TDF-containing ART initiation was significantly associated with bone loss among non-hormonal users. Consistent with these findings, Brown and others have shown that ART initiation is associated with a 2-6% loss of BMD over the first 48-96 weeks of therapy which does not return to baseline after prolonged HIV RNA suppression(198, 199). In a recent two-year longitudinal study comparing BMD among black South African WLWH initiating TDF-containing ART versus uninfected controls, Hamill et al observed that exposure to TDF-containing ART was associated with a loss in BMD and an increase in bone turnover that largely stabilizes by 24 months, similar to what we observed in our current study. However, although our data suggest that bone loss stabilizes after 12 months in these young adult women, this trajectory was exaggerated in WLWH concurrently using TDF and DMPA-IM, with on-going BMD loss observed at the 24 months particularly at the FN the site of greatest importance from the standpoint of future fracture morbidity in women. We provide the first evidence on the combined effect of DMPA-IM and HIV on bone mass. Our finding of a strong negative effect of current DMPA-IM use on BMD is consistent with previous studies conducted among new users the general population, in whom BMD losses of up to 5.7 % of BMD after 2 or more years of use were recorded with the greatest loss occurring during the first 1-2 years(65, 200). Though our study included both new and current users which may have masked the bone loss, concurrent DMPA-IM and TDF users experienced greater BMD loss (double) compared to non-hormonal contraceptive

TDF users. Due to the hypo-estrogenic effects of DMPA-IM, current DMPA-IM users have been shown to have lower mean BMD than non-users and these effects are more pronounced in younger women(200, 201). Similar to TDF associated BMD loss, studies of bone turnover in DMPA-IM users have suggested that the effects of DMPA-IM on the skeleton are at least partially mediated through increased bone turnover, with increases in markers of bone resorption, s-CTX, and reduction in markers of bone formation, P1NP.

The above findings are of high public health significance. During the early menopausal transition, the loss of BMD accelerates with annual rates of bone loss between 1.0% and 1.6% per year in the general population(202). The magnitude bone loss that we observed with ART initiation alone (0.9% to 1.8%) approximates that which would be expected by aging alone, and exceeds that seen in late menopause with concurrent use of TDF and DMPA-IM (2.1% to 2.5%). The 4.2% to 5.4% cumulative loss in BMD by 24 months among TDF+/DMPA+ users relative to the uninfected group is potentially clinically significant, and further studies are therefore needed to determine if this degree of bone loss is associated with an increased immediate or later risk of fracture.

This study incorporated a number of methodological features in order to provide the most rigorous observational data on the research questions being addressed. One of the main strengths of our study is the use of a suitable comparison group of women who are uninfected, and not exposed to hormonal contraception in the previous 2 years which enables evaluation of combined effect of the three exposures (HIV+, DMPA+, TDF+). This group of women provides the initial local reference data for the healthy HIV-uninfected young Ugandan women which is currently unavailable. In addition, the prospective nature of the design allows for assessment of changes in BMD over time. Two potential sources of error, temporal sequence and recall bias, are also minimized by the prospective design and the relatively short time (quarterly) periods participants are usually asked to recall their details about ART adherence and contraceptive use. Lastly, our large sample size of 521 women including 431 WLWH initiating TDF-containing ART, and high retention overall provides high power to detect significant differences for our current analyses. BMD was evaluated at several anatomic sites, including LS, TH and FN. Other indices of bone metabolism will also be examined. Though changes

caused by estrogen, HIV or TDF are usually most rapid in trabecular bone (seen early at the spine)(22, 203), it is important to document any changes in both kinds of bone, since eventual fractures may occur with loss of either type, and the hip is the site of greatest importance from the standpoint of future fracture morbidity.

The study has some limitations including lack of randomization for the groups of WLWH to the different contraceptive methods. Bias related to differences between women who choose DMPA-IM versus non-hormonal contraception was minimized by collecting data on exposures known to affect BMD in young women. During analysis, careful assessment of potential confounders and construction of multivariable models was central to our evaluation of the DMPA-IM, TDF- BMD association. Though the data are not included here, our study design has further advantages of addressing possible selection bias by examining the effects of discontinuation on BMD in the same women who have been members of the exposed cohort. We included prevalent users (current as well as newer users) an epidemiological approach which enables better observation of the effects of on-going use and of discontinuation on BMD or bone metabolism. These analyses are planned in addition to trabeculae bone score assessments as well as laboratory evaluations on parathyroid hormone, 25 hydroxyvitamin D, and bone turnover biomarkers which are important mediators in bone metabolism that will further support more detailed conclusions about the underlying mechanisms of the observed associations. In order to account for contraceptive switches, and poor ART adherence in a small proportion of women, further per protocol analyses were conducted that complement the intention-to-treat analyses, and we demonstrate that consistent use of both TDF and DMPA resulted in greater declines in BMD compared to inconsistent use. Lastly, our study was comprised of only Ugandan women which may limit the generalizability of our findings. However, the results are more likely to be generalizable to other populations in SSA than studies done on other continents. Of note, although injectable NET-EN a progestin only contraception administered every 2 months (204, 205) may be a feasible alternative long acting injectable contraceptive for WLW on ART, this contraceptive is not available in most SSA except South Africa. Though both DMPA and NET-EN are highly effective contraceptive methods, their pharmacokinetic profiles differ with amenorrhoea and delayed return to ovulation being more common in DMPA users compared to NET-EN users(206, 207). One cohort study reported significant

differences in the time it took for ovulation to return among DMPA and NET-EN users following cessation; 5.5 versus 2.6 months, $p < 0.001$ (206), while another study conducted in South Africa reported higher preference for NET-EN compared to DMPA particularly among young women who wanted children in future (208). Based on differences in their pharmacodynamic properties, it has been suggested that NET-EN might be bone protective compared to DMPA (209). Unfortunately, most studies looking at the effect of contraception on bone health to date have focused on DMPA, and the only two studies among HIV-uninfected women in South Africa that have assessed the effect of NET-EN on BMD have provided conflicting results due to methodological limitations such as sites measured, equipment used, and the study design. One cross-sectional study among pre-menopausal women in Cape Town found lower calcaneal BMD among current NET-EN users compared to non-hormonal users (210), while cross-sectional analysis from another study among peri-menopausal women in Durban found no differences in forearm BMD between non-users and long term current users of NET-EN (211). Robust data on BMD changes and micro-architecture among new users initiating NET-EN or DMPA users who switch to NET-EN is currently unavailable.

7.0 CHAPTER SEVEN - CONCLUSION

In summary, adult Ugandan ART-naïve WLWH demonstrated low BMD compared to the controls. Significantly lower BMD was seen among WLWH currently using DMPA-IM. As HIV is a chronic disease, our findings provide insight into the prevalence of low BMD in untreated WLWH on DMPA-IM and non-hormonal contraception in a RLS before long-term exposure to ART. The longitudinal analysis provides further evidence of additional BMD loss among WLWH with a doubling of loss among women on TDF-containing ART who are concurrently using DMPA-IM for contraception compared to those on non-hormonal contraception. Although improved life expectancy, viral suppression and the prevention of viral transmission remain key goals of HIV care for PLWH, treatment choices and management practices that balance optimal efficacy with improved long-term safety are needed for WLWH using hormonal contraception. Therefore, as the HIV treatment landscape continues to evolve, there are potential gender specific safety issues that should be considered when initiating women on ART including their contraceptive choices. Identification and use of safer contraceptive and ART options for WLWH is a high priority. These may include options such as an alternative long-acting injectable like

norethisterone enanthate (NET-EN, Noristerat®) as well as non-hormonal methods relevant to WLWH in RLS. In addition, newer bone sparing treatment interventions like tenofovir alafenamide fumarate (TAF) may have a promising role in delivering the vision of sustained undetectable viral load, while minimizing comorbid risks. An extension investigator sponsored clinical trial that's currently assessing the effect of switching from TDF to TAF-containing ART on bone mass and turnover among DMPA-IM users over a two-year period provides a timely opportunity to obtain comprehensive safety data on TAF- containing ART in the context of hormonal (DMPA-IM) and non-hormonal contraceptive use prior to TAF roll out in RLS. Another on-going NIH R01 grant assessing the impact of concurrent initiation of DMPA Contraception and Tenofovir PrEP on Bone Loss in Young Women in Uganda will provide data concomitant use of DMPA and TDF in women without HIV.

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9.0 APPENDICES

9.1 Plagiarism Check

9.2 Regulatory Ethics Approvals/ Clearance

9.2.1 Wits HREC Approval

9.2.2 Uganda Virus and Research Ethics Research Ethics Committee Approval

9.2.3 Uganda National Council for Science and Technology Clearance

9.3 Informed consent forms

9.3.1 English Screening and Enrollment ICF

9.3.2 Luganda Screening and Enrollment ICF

9.3.3 English Storage ICF

9.3.4 Luganda Storage ICF

9.4 Questionnaires/ Case Report Forms

9.5 Publications

9.5.1 Paper 1

9.5.2 Paper 2

9.5.3 Paper 3

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R14/49 Dr Flavia Kiweewa Matovu et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150858

NAME: Dr Flavia Kiweewa Matovu et al
(Principal Investigator)

DEPARTMENT: School of Public Health
Kampala Uganda

PROJECT TITLE: Bone Mineral Density in a Cohort of Young Adult
Women using Depo-provera and Tenofovir,
Kampala, Uganda

DATE CONSIDERED: 28/08/2015

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Dr Malgorzata Beksinska and Prof John Pettifor

APPROVED BY: 
Professor P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 04/09/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**


Principal Investigator Signature

10 SEP 15
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES



R14/49 Dr Flavia Kiweewa Matovu et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150858

NAME: Dr Flavia Kiweewa Matovu et al
(Principal Investigator)

DEPARTMENT: School of Public Health
Kampala Uganda

PROJECT TITLE: Bone Mineral Density in a Cohort of Young Adult
Women using Depo-provera and Tenofovir,
Kampala, Uganda

DATE CONSIDERED: 28/08/2015

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Dr Malgorzata Beksinska and Prof John Pettifor

APPROVED BY: 
Professor P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 04/09/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

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Principal Investigator Signature

10 SEP 15
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES



Uganda Virus Research Institute

Plot 51-59, Nakiwogo Road, Entebbe
P.O. Box 49, Entebbe-Uganda
Tel: +256 414 320 385 / 6
Fax: +256 414 320 483
Email: directoruvri@uvri.go.ug



Our Ref : GC/127/15/10/518

Your Ref:

26th October 2015

Dr. Flavia Matovu,

RE: UVRI REC review of protocol titled “Bone Mineral Density in a Cohort of Young Adult Women Using Depo-Provera and Tenofovir, Kampala, Uganda, Version 1.0

Thank you for your responses to the queries addressed to you by the UVRI REC.

This is to inform you that your responses dated 20th October 2015 were reviewed and met the requirements of the UVRI REC.

UVRI REC annual approval has been given for you to conduct your research up to 26th October 2016. Annual progress report and request for extension should be submitted to UVRI REC prior to the expiry date, to allow timely review.

The reviewed and approved documents included;

1. UVRI REC Application form
2. Study protocol v. 1.0
3. Informed Consent documents.
4. Applicant's CVs

You can now continue with your study after registration with the Uganda National Council for Science and Technology (UNCST).

Note: UVRI REC requires you to submit a copy of the UNCST approval letter for the above study before commencement.

Yours sincerely,

Dr. Alice Namale
Vice Chair, UVRI REC
C.C Secretary, UVR



Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: HS 1942

4th January 2016

Flavia Matovu Kiweewa
MU-JHU Care Limited
Kampala

Re: Research Approval:

Bone Mineral Density in A Cohort of Young Adult Women Using Depo-Provera and Tenofovir, Kampala, Uganda

I am pleased to inform you that on 16/11/2015, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period 16/11/2015 to 16/11/2020.

Your research registration number with the UNCST is HS 1942. Please, cite this number in all your future correspondences with UNCST in respect of the above research project.

As Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the research protocol or the consent form (where applicable) must be submitted to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval prior to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local REC for review with copies to the National Drug Authority.
4. Unexpected events involving risks to research subjects/participants must be reported promptly to the UNCST. New information that becomes available which alters the risk/benefit ratio must be submitted promptly for UNCST review.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. A progress report must be submitted electronically to UNCST within four weeks after every 12 months. Failure to do so may result in termination of the research project.

Below is a list of documents approved with this application:

	Document Title	Language	Version	Version Date
1.	Research proposal	English	1.0	June 2015
2.	Information and Consent Form for Storage and Future Use of Blood and Urine	English and Luganda	1.0	June 2015
3.	Participant Screening and Enrollment Informed Consent Document	English and Luganda	1.0	June 2015
4.	Study Informed Consent Comprehension Checklist	English and Luganda	1.0	June 2015

Yours sincerely,


Hellen N. Opolot
for: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

cc. Chair, Uganda Virus Research Institute, Research Ethics Committee

Received at MUJHU
Date: 07 Jan 16
Initials: Mg

LOCATION/CORRESPONDENCE

Plot 6 Kimera Road, Ntinda
P. O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

TEL: (256) 414 705500
FAX: (256) 414-234579
EMAIL: info@uncst.go.ug
WEBSITE: <http://www.uncst.go.ug>



MU-JHU CARE LTD
MU-JHU Research Collaboration

Makerere University, Kampala
The Johns Hopkins University, Baltimore
P. O Box 23491, Kampala, Uganda

Telephone: +256-414-541044 Facsimile: +256-414-543002



PARTICIPANT SCREENING AND ENROLLMENT INFORMED CONSENT DOCUMENT

Title of the study: Bone Mineral Density in a Cohort of Young Adult Women Using Depo-provera and Tenofovir, Kampala, Uganda. Version 1.0 dated 26 June 2015

Sponsors: U.S. National Institutes of Health

Principal Investigator: Dr. Flavia Matovu Kiweewa

Phone Number: 0414-541044

Date: 24 April 2018

1) What should you know about this study?

- Hello, my name is _____. I am inviting you to take part in a research study. The study is being conducted by researchers from Makerere University-Johns Hopkins University (MU-JHU) Research Collaboration– Kampala, Uganda, in collaboration with Makerere University School of Public Health – Kampala, Uganda, Johns Hopkins Bloomberg School of Public Health- Baltimore, USA, University of Washington- Seattle, USA and University of Witwatersrand – Johannesburg, South Africa.
- You are requested to take part in this study because are using contraception and/or may be starting on HIV treatment any time. We are recruiting non-pregnant young women aged 18 to 35 years with no intention of getting pregnant in the next 2-3 years. We plan to recruit about 516 women, that is 476 HIV-infected and 40 HIV uninfected women. Two groups of HIV infected women will be using depoprovera for contraception, among which one group of 168 women will be starting HIV treatment, while the second group of 204 women will not need to start HIV treatment soon. The last two groups of women will be using a non-hormonal contraceptive method like intra-uterine contraceptive device (IUD). However unlike the first two groups of women, one group of 104 women will be HIV infected and the other group of 40 women will be HIV uninfected. The HIV infected group will be starting HIV treatment.
- Before you decide if you want to join, we would like to explain the purpose, procedures, risks and benefits of participating, and what will be expected of you and us in this study.

- Please read this form with care. You may also have this form read to you.
- Ask questions about the things that are not clear to you now or when you think about them later.
- After the study has been fully explained to you and all your questions answered, you may decide freely if you want to join this study or not.
- If you agree to take part in the study, you will be requested to sign this consent form to show that you have understood what you have been informed and you agree to take part. If you cannot sign, you will be requested to place your thumbprint on this consent form in the presence of a witness.
- You will be given a copy of this form to keep.

2) Why is this study being done?

The main purpose of this study is to find out changes in the thickness of bones for women who start on HIV medications containing tenofovir, and at the same time are using depo-provera injection for contraception. Some studies have reported bone loss among HIV uninfected women using depo-provera for contraception. While other studies have independently reported bone loss in HIV-infected people within the first two years of starting HIV treatment. To date, no studies have been conducted among women who are using both depo-provera and HIV treatment at the same time. This study will help us better understand changes in thickness of bones for women using both depo-provera and HIV treatment containing tenofovir.

The Uganda Virus Research and Ethics Committee, the Human Research and Ethics Committee at the University of the Witwatersrand, South Africa, and the Uganda National Council for Science and Technology have approved the study. The IRB is a group of people who review the activities of research that involves human volunteers. It is the role of this IRB to protect the rights of volunteers participating in research studies.

3) What will happen if you agree to take part in the study?

Study procedures:

If you decide to join this study, after placing your signature or thumbprint on this form, this is what will be done;

- Urine exam to check whether you are pregnant or not
- Measurement of your height and weight
- DXA scan; this is an X-ray that examines the strength of your bones. This exam is not painful. You will lie on the table and have a special X-ray of your bones.
- Physical examination
- HIV test (if in the HIV uninfected group)
- Collection of blood for baseline viral load testing (if in the HIV infected groups, and do not have a test result from your HIV care center).

- Collection of blood (15mls or 3 teaspoons) and urine that will be stored and will be later tested to help researchers better understand the DXA results. The study doctors will decide which blood and urine samples will be tested during or after the study. If any tests are done, the study doctors do not plan to give you the results of the tests unless they feel the results are important to your health.
- Every 6 monthly visit (that is once every 6 months), a trained study staff member will cut a total of about 100 Strands of hair from the back of your head. Humans normally lose about 100 hairs from their head every day (mostly without realizing it). Removing this amount of hair should not disrupt your hairstyle and will not affect the normal new growth of your hair.

You will also answer questions about the following;

- The food you eat or if you have ever had eating problems in the past. If you can benefit from changing your diet, study doctors will talk with you about ways of improving your diet for it to be more useful
- Breastfeeding
- Contraception
- Physical activity
- Any illnesses
- And medications you may be taking

Some information about your health will be obtained from your health care records.

The total time that it will take you to complete the consent form, the questionnaires, the physical examination and the DXA scan will be approximately 3 hours.

From today, you will be in this study for up to 2 years. You may be requested to stay in the study for up to 3 years if you start your ARVs while already participating in the study. You will have the procedures listed above today and every 6 months while you are in this study from MU-JHU clinic. Because we need to be sure that you are getting your contraception and HIV medications, study staff will conduct a short visit from care facility at week 4, months 3, 9, 15 and 21 to collect this information. We will also be able to know if there are any changes to your contact address. These procedures will be done on the same day as your regular care visits, when possible and if agreeable to you.

At each of the following visits; enrollment, week 4, month 3 and every 6 months you will give 15 mls or 3 teaspoonful of blood that will be used for future measurement of markers of bone turnover and viral load. A total of about 75 mls or 15 teaspoons of blood will be drawn in the first year and 60 mls or 12 teaspoons in the second year and/or third year.

It may be necessary for you to make additional visit(s) during your participation in this study to have any of the study procedures listed above repeated in the event of unforeseen or unanticipated abnormal results; difficulties in sample processing, or testing; and/or if you experience any changes in your physical condition. If you start HIV medications during study participation, you may be requested to have a bone scan at that time.

If you choose to leave this study early, you will be asked to come to the clinic for a final set of tests.

Before each DXA scan and at any time in the study, if you or the study staff think you might be pregnant, you will give urine for a pregnancy test. The DXA test should not be done if you are pregnant.

If at any time in the study your DXA test results show that you might benefit from taking calcium and vitamin D medications, these will be given to you.

Sample storage and testing

Storage of your blood, hair and urine for testing is required as part of your study participation. Your blood and urine may be stored here in Uganda or in the United States until all testing has been done. Some tests will be done in the United States.

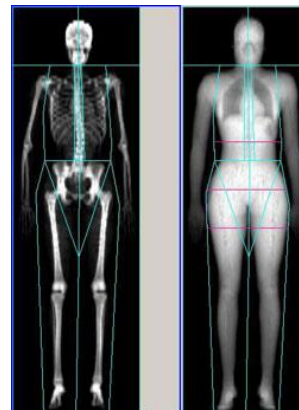
4) What is a bone scan (DXA)?

The DXA is a special X-ray that measures how much mineral your skeleton contains in the whole body or at particular parts of the body like the hip and spine. It also measures how much of your body is fat, muscle and bone.

While the DXA scan is being done, you will lie still on a bed for up to 15 minutes while a machine passes over your body and takes a picture of your bones. At each DXA scan visit, you will have scans of the whole body, spine, hip and extremities. You will not feel anything while this DXA scan is being done.



Whole-body DXA position



Sample Whole body DXA scan

5) What additional study procedures can you take part in?

You may also be asked to participate in a few optional study activities. If you choose to participate, you will be asked questions about your use of family planning, your preferences and opinions, your experiences with using family planning, and any problems you may have had using family planning and HIV medications. Approximately 35-50 women will be asked questions either in a group or individually.

You do not have to agree to participate in these activities to join this study. The interviews will be audio-recorded to make sure we record your words exactly how you said them. The audio recording, notes, and analyses from these materials will be kept confidential and will only use study numbers or fake names.

In-depth Interview(s) and Group Discussions

You may be asked to participate in an interview with a trained staff member or you may be asked to participate in a group discussion with other study participants about opinions that you or other participants have. If you choose to participate, you will be asked to discuss your use of family planning and HIV medications, and other questions that can help researchers to better understand women's experiences using family planning while on HIV treatment. These discussions will last about half an hour to an hour.

Please mark one of the following boxes to show whether you agree or not agree to participate in in –depth interview or group discussions.

☐

I DO agree to participate in in-depth interviews or group discussions

☐

I DO NOT agree to participate in in-depth interviews or group discussions

6) Are you willing to have a study staff come visit you at home?

If you decide to participate in this study, a study staff called a health visitor will accompany you to your home to know where you live. This is important so the health visitor can help remind you of clinic visits if you are not able to come to the clinic on your planned day or if a test result is not normal and you are required to be reviewed by the study clinician at the study clinic. If you do not come for a scheduled visit or if a test result comes back abnormal, the study health visitor will contact you.

7) What are the risks of participating in the study?

Taking part in this study may involve some risks or discomforts. There is a risk of minor bruising from the collection of the blood samples but this is minimized as only well-trained and experienced staff will take off the samples. There are no risks in collection of urine, although sometimes it may be uncomfortable for some people.

A. Risk of the DXA Scans

No complications are expected with the DXA procedures which measure the mineral content of your bones. Care is taken during DXA procedure to use the lowest radiation dose possible while producing the

best pictures for evaluation. National and international radiology protection councils continually go back and look and update the technique standards used in DXA procedure. DXA systems have tightly controlled X-ray beams with significant filtration and dose control methods to reduce stray or scatter radiation. This ensures those parts of a participant's body not being imaged receive very little radiation exposure. The radiation dose from DXA is very low so the staff operating the scanners do not have to wear special protective clothing and the room does not have to be shielded.

There is a risk from being near X-rays, also known as radiation. Every person is exposed to a small level of natural radiation from the sun, air, food and soil (called background radiation). Exposure to X-rays, particularly at high doses, can lead to cancer. However, the level of X-rays used in a DXA scan is very low and is about the same dose the average person receives from natural radiation in one day. Study staff have been trained to do the DXA scan using the smallest amount of X-ray possible.

B. Pregnancy

We do not know if the DXA test has any effects on the fetuses of women who have a DXA test when pregnant. Because of this, pregnant women cannot join this study.

All women who join this study should use family planning methods. For this study we are enrolling women on **depo-provera, intrauterine contraceptive device (IUD), condoms and sterilization.**

If you become pregnant during the study, you will stop having DXA scans but continue with the rest of the procedures as originally planned. You will be able to have the DXA scans after your pregnancy.

C. Risks of information about you becoming known by others

Study staff will make every effort to protect your privacy while you are in this study. Your visits here will take place in private. There is a risk to your privacy if a study staff member or someone else in the study knows you. Study staff will make every possible effort to be sure that others do not learn of your HIV status. However, coming here for study visits could make others wonder if you have HIV. Because of this, others may learn of your study participation and treat you unfairly, or talk bad things about you. If you have any problems, study staff will talk with you to try to help resolve them.

D. Risks of collecting hair

There is a very small risk that the study staff may accidentally cut your skin or disturb your hair style when collecting the hair sample. Staff will take extra care when cutting your hair.

8) Are there benefits of participating in this study?

You may or may not benefit directly from being in this study. You will get to know whether your bones are healthy. You may benefit when you learn of how to change to a good diet. You will be given calcium and vitamin D medications, if you need them. Above all, your participation in this study will benefit the wider society, because it will enable us to gain valuable information on bone health of HIV infected young women on depo-provera and tenofovir based HIV treatment. This has the potential to improve care for HIV-infected women in Uganda and other countries all over the world.

9) Do you have to take part in this study?

Taking part in the study is completely voluntary. You may decide not to take part now or take time to think through before you make a decision. If you don't want to take part or later change your mind, you will not be penalized or lose benefits of your regular care. If you decide to withdraw from the study in future, with your consent, samples and data obtained may be kept and used as part of the study results or for future studies. However, should you request your samples and data to be destroyed along with any other information relating to you, we will ensure that this takes place.

Study investigators may also decide take you out of the study if you are unable to follow study procedures, or if the study sponsors decide to stop paying for the study.

10) Will you pay anything to participate in this study?

The study procedures, DXA exams, and all tests will be done without you paying anything.

11) Will you be paid if you take part in this study?

We shall not pay you for taking part in this study. However, we will give you 20,000 Shillings (80 South African rand) as compensation for travel costs for each scheduled study visit.

12) What information is kept private and what information may be given out?

The study doctors and study staff will keep your personal information and information about your participation in this study as confidential as they can. On your study records, a hidden number will be used instead of your name. It is only the study staff who will know this hidden number. All written publications from this study will not use your name or identify you. Some of the government health institutions, study sponsors, study review committees, and other approved institutions may see details about your health in the study records.

Study staff will not give out any record that identifies you without your consent in writing. In some situations, including some unexpected occurrences, the law or work ethics, may force us to share your personal information.

13) What happens if you are injured in this study?

It is extremely unlikely that there will be any harm caused to you as a result of participating in this study. If study staff determines that you have been harmed as a direct result of participating in this study, you will be given immediate treatment for those injuries at no cost to you. There are no other special compensation arrangements. By signing this consent form you will not be giving up any of your legal rights in such situations.

14) Who can you contact if you have questions or any concerns about this study?

If you have any problems or questions during the study, the study counselors and other staff will be happy to help you contact the right person to respond to your questions.

If you ever have questions about this study or if you have a study related medical problem or injury, you should contact Dr. Flavia Matovu Kiweewa or Dr. Clemensia Nakabiito at Makerere University-Johns Hopkins University (MUJHU) Research Collaboration, Upper Mulago Hill Road.or call them to the on Tel. 0414-541044 or 0772 544759 or 0702 544759.

If you have questions about your rights as a research participant, you may contact the Uganda Virus and Research Ethics Research Committee chair, Mr. Tom Lutalo on 0404320385 or 0404320386

MU-JHU also has an advisory committee (CAB) that helps study doctors, study volunteers, or communities where the study is done, to jointly work together on important issues and risks in the study. CAB includes people from the community, religious leaders, past study participants, and other influential people. They can also help you with the problems or issues you may have. If you want to talk to any of the CAB, you are requested to inform the study counselor, they will tell you how you will contact one of the CAB members.

15) What does your signature imply?

Placing your signature or thumb print below means that you have understood the information contained in this consent form, and that you have voluntarily agreed to take part in the study. If you sign or thumbprint the form, it means that you agree to join the study. By signing this consent form, you have not waived any of the legal rights that you would have as a participant in a research study.

WE SHALL GIVE YOU A COPY OF THIS CONSENT FORM

Do not sign later than the following date: _____

IT IS NOT VALID IF IT DOES NOT BEAR THE IRB APPROVAL STAMP

Bone Mineral Density in a Cohort of Young Adult Women Using Depo-provera and Tenofovir, Kampala, Uganda. Version 1.0 dated 26 June 2015

STATEMENT OF CONSENT: I have read (or someone has read and explained to me) this consent form. I understand why this study is being done, study procedures, as well as the risks and benefits as they have been explained in this written summary. I agree voluntarily to take part in this study.

Participant's name
(Print)

Participant's Signature
or thumb print

Date

For Illiterate volunteers:

Participant's names and date written by

Name of person obtaining
consent (Print)

Empid of person
obtaining consent

Date

For all participants: I have explained the purpose of the study to the participant and have answered all her questions. To the best of my knowledge, she understands the purpose, procedures, risks and benefits of this study.

Name of person obtaining
consent (Print)

Signature of person
obtaining consent

Date

For illiterate participants: I attest that the information contained in this written consent form has been read and explained to the participant. To the best of my knowledge, the information provided is complete and accurate. The participant appears to understand the purpose, methods, risks and benefits of taking part in the study, and she willingly agrees to take part.

For those Placing Thumb Print only. I attest that the study participant who states that her name is _____ has placed her thumb print on this consent form of

her own free will on this day _____

Witness to consent process
Name (Print)

Witness' Signature

Date

Appendix 9.3.2: Luganda Screening and Enrollment ICF

BONE: Contraception & Anti-Retroviral Effects (BONE: CARE) Study-
Kampala, Uganda. Protocol version 1.0 dated 26 June 2015

Patient ID Sticker



MU-JHU CARE LTD
MU-JHU Research Collaboration

Makerere University, Kampala
The Johns Hopkins University, Baltimore
P. O Box 23491, Kampala, Uganda

Telephone: +256-414-541044 Facsimile: +256-414-543002



EKIWANDIIKO KYABANAKYEWA EKY'OKUKKIRIZA OKUSUNSULWA N'OKWETABA MU KUNOONYEREZA

Omutwe gw'okunoonyereza:	Obugumu bwa'magumba mu kibinja kya' bakyala abato abakozesa Depo-provera ne Tenofovir, Kampala, Uganda. Version 1.0, dated 26 June 2015.
Asasulira okunoonyereza:	Ekitongole ky'America ekye by'obulamu (NIH)
Akulira okunoonyereza:	Dr. Flavia Matovu Kiweewa
Ennamba y'essimu:	0414-541044
Ennaku z'omwezi:	24 April 2018

1) Kiki ky'olina okumanya ku kunoonyereza kuno?

- Nkulamusizza nyabo. Erinya lyange nze _____. Nkwaniza okwetaba mu kunoonyereza. Okunoonyereza kukolebwa abanoonyereza okuva mu Makerere Univasite –Johns Hopkins Univasite (MU-JHU) Research Collaboration – Kampala, Uganda, nga bakolaganira wamu ne Univasite ye Makerere School of Public Health – Kampala, Uganda, John's Hopkins Bloomberg School of Public Health, mu America, Univasite ye Washington –Seattle, mu America ne Univasite ye Witwatersrand – Johannesburg, mu Sausi Afirica.
- Osabibwa okwetaba mu kunoonyereza kuno kubanga obadde okozesa enkola ey'okuziyiza okufuna olubuto era/ oba oyinza okutandika okumira eddagala eriwezeza ku akawuka ka mukenenya ekiseera kyona. Tusingiza abakyala abato wakati w'emyaka 18 ne 35 nga tebali lubuto

era nga tebalina ntekateeka ya kufuna lubuto mu myaka ebiri kw'esatu egigya. Tuteekateeka okuyingiza abakyala nga 516, bebakyala 476 abalina akawuka ka mukenenya n'abakyala 40 abatalina kawuka kamukenenya. Ebibinja 2 eby'abakyala abalina akawuka kamukenenya bigya kubanga bikoze empiso (depo-Provera) olw'o kuziyiza okufuna olubuto naye ate nga mu bano ekibinja ekimu ekyabakyala 168 bagya kuba batandiika eddagala eriwezeza ku kawuka kamukenenya (ART) ate

nga ekibinja eky'o kubiri eky'abakyala 204 bagya kuba tebaneetaaga kumira ddagala liwezeza kawuka ka mukenenya mu bwangu. Ebibinja by'abakyala ebibiri ebisembayo bigya kuba nga bakozesa enkola eyokwegema okuzaala etalimu ddagala (non hormonal) okufaanana ko nga akaweta akatekebwa munabaana (IUD). Wabula, okwawukanako n'ebibinja bya bakyala ebibiri ebisooka, ekibinja ekimu ekyabakyala 104 bagya kuba na kawuka kamukenenya ate ekibinja ekirala eky'a bakyala 40 nga tebatalina kawuka kamukenenya. Ekibinja kya balina akawuka kamukenenya bagya kuba nga batandika okumira eddagala eriziyiza akawuka kamukenenya.

- Nga tonasalawo oba nga oyagala okwetaba mu kunoonyera, twandyagade okukunyonnyola ekigendererwa, emitendera, obuzibu n'emigaso gy'okwetabamu nakiki ekinkusubirwamu era naffe ekitusubirwamu mu kunoonyereza kuno.
- Tukasaba okusoma ekiwandiiko kino n'obwegendereza. Oyinza era okusomerwa ekiwandiiko kiino.
- Buuza ebibuuzo kubintu byona by'otategedde kati oba nga obirowoozezaako oluvanyuma.
- Oluvanyuma nga okunoonyereza kumaze okukunyonnyolwa mu bujjuvu era nga n'ebibuuzo byo
- byona bididdwamu, osobola okusalawo kyeyagalire obanga oyagala okwetaba mu kunoonyereza kuno oba nedda.
- Ssinga osalawo okwetaba mu kunoonyereza, ogya kusabibwa okuteeka omukono kukiwandiiko ky'okukkiriza kino okulaga nti otegedde ebikunyonnyondwa era okkiriza okukwetabamu. Bwoba tosobola kuwandiika, ogya kusabibwa okuteekako ekinkumu kyo ku kiwandiiko ky'okukkiriza nga waliwo omujjulizi.
- Ogya kuweebwa kopi yekiwandiiko kino ogitereke.

2) Lwaki okunoonyereza kuno kukolebwa?

Ekigendererwa ky'okunoonyereza kuno ekikulu kwekuzuula enkyukakyuka mu bugumu bwa magumba g'abakyala abatandika okumira eddagala eriwezeza akawuka ka mukenenya nga mulimu tenofovir era nga mukiseera kyekimu bakozesa empiso ya depo-provera olw'o kwegema okuzaala. Okunoonyereza okumu kulaze nti abakyala abatalina kawuka kamukenenya nga bakozese empiso ya depo-provera bakendera obugumu bwa amagumba. Ate nga era okunoonyereza okulala okwetengeredde kulaga nti

abantu abalina akawuka kamukenenya bakendera obugumu bwa amagumba mubanga ery'emwaka 2 nga batandise okukozesa eddagala eriweze akawuka kamukenenya. Mukiseera kino, tewali kunoonnyereza kwalikukolekwa mu bakya abakozesa depo-provera era neddagala eriweze akawuka kamukenenya mu kiseera kyekimu. Okunoonnyereza kuno kugya kutuyamba okwongera okutegeera enkyukakyuka mu bugumu bwamagumba g'abakya abakozesa depo-provera neddagala eriweze akawuka kamukenenya eririmu tenofovir.

Akakiiko ka Uganda akakwasisa amateeka kuby'okunoonnyereza ku bawuka (Uganda Virus Research and Ethics Committee), akakiiko akakwasisa amateeka kuby'okunoonnyereza ku Universite ye Wit Waters rand e Sausi Afirika, era n'ekitongole kya Uganda ekyo sayansi namagezi agekikugu (Uganda National Council for Science and Technology) bakkiriza okunoonnyereza kuno kubereho. IRB kekakiiko akalabirira enzirikanya y'o kunoonnyereza okulimu abantu banakyewa. Mulimu gwa kakiiko kano okukuma eddembe lya banakyewa abetaba mu kunoonnyereza.

3) Kiki ekinabaawo ssinga okkiriza okwetaba mu kunoonnyereza?

Emitendera gy'okunoonnyereza:

Ssinga osalawo okwetaba mu kunoonnyereza kuno, oluvanyuma lw'okusa omukono oba ekinkumu ku kiwandiiko kino, bino bye bigya okukolebwa:

- Okukebera omusulo okulaba oba olina olubuto oba nedda
- Okupima obuwanyu bwo n'obuzito
- Okukebererwa kwa DXA; eno yengeri y'okukubwa ekifananyi eky'ekenya obugumu bwa magumba go. Okukebererwa kuno tekuluma. Ogya kugalamira ku meeza era okubwe ekifananyi ekyenjwulo ekyo magumba go
- Okwekebegyerwa omubiri
- Okukebererwa akawuka kamukanya (ssinga obeera mu kibinja ekyabatalina kawuka kamukanya)
- Okugibwako omusaayi ogw'okukebererwa okupima obungi bwa kawuka kamukanya akali mu musaayi kuntandiikwa (ssinga obeera mu bibinja ebya balina akawuka kamukanya ate nga tokebedwa bungi bwakawuka kamukanya okuva mu dwalirolyo)
- Okugyako omusaayi (miruzi 15 oba obujjiko bwa caayi 3) era n'omusulo ogugya okuterekerwa nga gugya kukebererwa gyebugya, okuyamba abasawo b'okunoonnyereza okwongera okutegeera ebivudde mu DXA. Abasawo b'okunoonnyereza bagya kusalawo musaayi ki oba musulo ki ebinakebererwa mu kunoonnyereza oba oluvanyuma. Singa okukebera kwonna kukolebwa abasawo b'okunoonnyereza tebategese kukuwa binava mu bikebererwa okugyako nga balowooza nti ebivuddemu bya mugaso eri obulamu bwo.
- Buli luvanyuma lw'emyezi mukaaga (omulundi gumu buli myezi mukaaga), omusawo mukunoonnyereza atandekeddwa agya kusala omugatte gwa nviiri nga kikumi okuva enkoto. Mu mbeera eyabulijjo abantu bavaako enviiri nga kikumi buli lunaku ate nga kino tebakimanya.

Okujjako enviiri zino tekyandikyusiza musono gwa nviirizo era tekigya kukosa kumerakwa nviiri zo.

Ogya era kudamu ebibuuzo ebikwata kubino;

- Emmere gy'olya oba wali ofunye obuzibu bw'okulya mu kiseera ekiyise. Bw'oba oyinza oganyulwa mu kukyusa mu mere gy'olya, abasawo mu kunoonyereza bagya kwogera nawe mungeri y'okulongosaamu ebyendyayo okwongera okubeera eby'omugaso.
- Okuyonsa
- Okwegema okuzaala
- Okukozesa omubiri
- Obulwadde bwonna
- Era neddagala lyona ly'oyinza okuba ng'omira

Ebimu kubikwata ku bulamu bwo bigya kugyibwa mubiwandiiko byo eby'obujjanjabi.

Obudde bw'onomala mu kusoma ekiwandiiko ky'okukkiriza, okuddamu ebibuuzo, okwekebegyebwa omubiri n'okukubwa ekifananyi ky'amagumba (DXA) bwona awamu bugya kuba ssaawa nga 3.

Okuva olwaleero, ogya kubeera mu kunoonyereza kuno okumala wakati w'emyaka 2. Oyinza okusabibwa okumala mukunoonyereza ebanga eriwera emyaka 3 ssinga otandika eddagala eriweweza akawuka kamukenenya nga omaze okweyunga mukunoonyera. Emitendera egy'ogeddawako waggulu gigya kukukolebwako olwaleero era nabuli myezi 6 ng'oli mu kunoonyereza kuno ku kirinika ya MU-JHU. Olw'okuba twagala okukakasa nti ofuna bulungi enkola eziyiza okuzaala n'eddagala eriweweza akawuka kamukenenya, abakozi mu kunoonyereza kuno bagya kukolayo olukyala olumpimpi mu ddwaliro w'ofunira obujjanjabi ku wiiki 4, ku myezi 3, 9, 15 ne 21 okusobola okukunganya ebikukwata bino. Era tugya kusobola okumanya oba waliwo enkyukakyuka mu ndagiriroyo. Bino bigya kukolebwa ku lunaku lwerumu nga enkyala zo ezabulijjo ez'okufuna obujjanjabi, bwekiba kisobose era ng'okiriziganya nakyo.

Kubuli lukyala olumu ku zinno; olw'o kwetaba mu kunoonyereza, wiiki 4, emyezi 3 ne buli myezi 6 ogya kuwaayo omusaayi miruzi 15 oba obujjiko 3 obutabula caayi ogugya okukozesebwa gyebugya okupima ebiramba enkyukakyuka mu magumba n'obungi bwakawuka kamukenya akali mumusaayi.

Omusaayi ogwenkanga miruzi 75 oba obujjiko 15 obutabula caayi gugya kugyibwako mumwaka ogusooka ne miruzi 60 oba obujjiko 12 obutabula caayi mu mwaka ogw'okubiri era/oba ogw'okusatu.

Kiyinzika okukwetaagisa okugya emirundi egisingawo mu kiseera ky'okunoonyereza kuno tusobole okuddamu egimu ku mitendera egimennyedwa waggulu ssinga wabaawo ekitalengereerwawo oba ekitasuubirwa mu bivuddde mu kukebera, obuzibu mu kutegekka ebyakugyibwako, oba okukebera; ne/oba ssinga ofuna enkyukakyuka mu mubiri gwo. Ssinga otandika okumira eddagala eriweweza akawuka ka silimu ng'oli mukunoonyereza, oyinza okusabibwa n'okukubwa ekifananyi ky'amagumba (DXA) mukiseera ekyo.

Ssinga osalawo okuva mu kunoonyerereza kuno amangu, ogya kusabibwa okugya ku kirinika okufuna okukeberegwa okusembayo.

Nga okukeberegwa kwa DXA tekunakolebwa, nekiseera kyona mu kunoonyerereza, ssinga ggwe oba abakozi mu kunoonyerereza balowooza nti oyinza okubanga oli lubuto, ogya kuwaayo omusulo okukeberegwa oba oli lubuto. Okukebera kwa DXA tekulina kukolebwa nga oli lubuto.

Ssinga ekiseera kyona mu kunoonyerereza ebiva mu kukebera kwo okwa DXA biraga nti oyinza okuganyulwa nga omira amakerenda ga calcium n'evitamin D, bino bigya kuweebwa.

Okutereka ebiggyibwako n'okukeberegwa

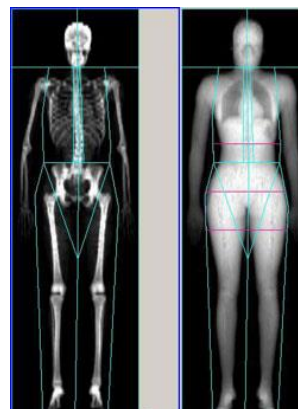
Okutereka omusayi gwo, enviiri n'omusulo olwokukebera gyebugya kyetaagisa nga ekimu kubitundu by'okwetaba mu kunoonyerereza kuno. Omusayi gwo n'omusulo biyinza okuterekebwa wano mu Uganda oba mu America okutuusa nga okukeberegwa kwona kuwedde. Okeberegwa okumu kugya kukolebwa mu America.

4) Ekifananyi ky'amagumba (DXA) kyeki?

DXA kwe kukeberegwa mukifananyi okwenjawulo okupima obungi bwe birungo amagumba byegalina mu mubiiri gwo gwonna oba mu bitundu by'omubiri ebimu nga ekisambi ne nkizi. Era kipima n'obungi bwamasavu enyama n'amagumba ebiri mu mubiri gwo.



Engeri omubiri gwonna gyegukeberegwa mu kyuma kya DXA



Ekifananyi kya DXA eky'omubiri gwonna

Nga okukebera kwa DXA kukolebwa, ogya kwebaka ku kitanda okumala eddakika 15 nga ekyuma bwekiyita waggulu w'omubiri gwo era kikube ekifananyi ky'amagumba go. Buli lukyala olwo kukeberegwa kwa DXA ogya kukubibwa ekifananyi ky'omubiri gwonna, enkizi, ekisambi, emikono n'amagulu. Togya kuwulira kintu kyona nga ekifananyi ky'okukebera amagumba kikolebwa.

5) Mitenderaki emirala gy'oyinza okwetabamu?

Oyinza okusabibwa okwetaba mu mitendera emitono egyikolebwa egy'okunoonyereza nga sikyateeka okugyetabamu. Ssinga osalawo okwetabamu, ogya kubuuzibwa ebibuuzo ku nkozesaayo eya famile, ebikusingira n'ebirowoozi byo, by'oyiseemu ng'okozesa famile n'ebizibu ebirala byona by'oyinza okuba nga wafuna ng'okozesa famile ne ddaga eriweweza akawuka k'amukenenya. Abakyala nga 35-50 be bagya okubuuzibwa ebibuuzo nga bali mu kibinja oba nga ssekinoomu.

Tolina kukkiriza kwetaba mu mitendera ginno okweyunga mu kunoonyereza kunno. Ebibuuzo bigya kukwatibwa mu maloboozi ku katambi okukakasa nti by'oyogedde bikwatibwa nga bwo by'ogedde. Okukwata amaloboozi, okuwandiika ate n'okwekeneenya okunakolebwa ku bintu binno bigya ku kumibwa nga byakyama era tugya kukoseza namba z'okunoonyereza oba amanya agatali matuufu.

Ebibuuzo eby'omunda n'okukubaganya ebirowoozi okwawamu

Oyinza okusabibwa okwetaba mu bibuuzo eby'omunda n'omusawo omutendeke, oba oyinza okusabibwa okwetaba mu kukubaganya ebirowoozi okwawamu nebanakyewa abalala ku bikwata ku ndowooza gwe oba banakyewa abalala zemuyina. Ssinga osalawo okwetabamu, ogya kusabibwa okukubaganya ebirowoozi ku nkozesaayo eya famile ne ddaga eriweweza akawuka k'amukenenya, n'ebibuuzo ebirala ebiyinza okuyamba abanoonyereza okutegeera obulungi abakyala byebayitamu nga bakozesa famile nate nga bali ku ddagala eriweweza akawuka k'amukenenya. Okukubaganya ebirowoozi kunno kugya kutwaala wakati we kitundu ky'esaawa n'essaawa ng'emu.

Mwattu, lamba akabokisi kamu kw'obwo okulaga nti okkiriza oba tokkiriza kwetaba mu kubuzibwa ebibuuzo eby'omunda oba okukubaganya ebirowoozi okwawamu.

☐ NZIKKIRIZA okwetaba mu kubuzibwa ebibuuzo eby'omunda oba okukubaganya ebirowoozi okwawamu.

☐ SIKKIRIZA kwetaba mu kubuzibwa ebibuuzo eby'omunda oba okukubaganya ebirowoozi okwawamu.

6) Okkiriza omukozi mu kunoonyereza okugya okukukyalira awaka?

Ssinga osalawo okwetaba mu kunoonyereza kuno, omukozi mu kunoonyereza ayitibwa omusawo w'ebiyobulamu akyala awaka (health visitor) agya kugenda naawe ewakaawo okumanya gy'obeera. Kino

kyamugaso okusobozesa omusawo akyala awakaawo okukuyamba okukujjukiza enaku z'okugya ku kirinika ssinga oba tosobodde kugya ku kirinika kulunaku lwo olutegeke oba ssinga ebiva mukeberegwa biba nga biri mu mbeera etali yabulijjo era nga wetaagisa okwekenenzebwa omusawo w'okunoonyereza ku kirinika. Ssinga togya kulukyala lwo olutegeke oba ssinga ebiva mu kukeberegwa bikomawo nga biri mumbeera etali yabulijjo, omusawo w'okunoonyereza akyala ewaka agya kukubira essimu.

7) Buzibu ki obuli mu kwetaba mu kunoonyereza kuno?

Okwetaba mu kunoonyereza kuno kuyinza okubaamu obuzibu oba obutewulira bulungi. Waliwo obuzibu obutonoto obw'okuzimba webagyeko omusaayi naye kino kikendezebwa kubanga abakozi abatendeke obulungi era abalina obumanyirivu bokka bebagya okugyako omusaayi. Tewali buzibu mu kuggyako omusulo, newankubadde ebiseera ebimu kiyinza okusumbuwa abantu abamu.

A. Obuzibu bw'ekifananyi kya DXA

Tewali buzibu busuubirwa mu mitendera gya DXA egyipima ebirungo bya magumba go. Bafaayo nnyo nga bakola emitendera gya DXA nga bakozesa ekipimo ky'amaanyi g'enjuba agasinga okuba amatono agasoboka nga ate bafulumya ebifaananyi ebisinga okuba ebirungi eby'okukozesa mu kwekkennnya. Obukiiko bw'eggwanga n'ensi yonna obukebera amaanyi g'enjuba agabeera mu byuma bya DXA buli kiseera buddayo nebwongerwa ku bukodyo obw'omutindo obukozesebwa mu mitendera gya DXA. Enkola ya DXA erina amaanyi g'enjuba agaziyizibwa n'amaanyi n'okusengegya okw'omugaso n'enkola z'okuziyiza ekipimo okukendeeza amaanyi g'enjuba okugenda ewala oba okusaasaana. Kino kikakasa nti ebitundu by'omubiri gw'eyetaba mu kunoonyereza ebitakubiddwa kifaananyi bifuna amaanyi g'enjuba matono nnyo. Ekipimo ky'amayengo g'amaanyi g'enjuba agava mu kyuma kya DXA matono nnyo era nga n'omukozi akozesa ekyuma ky'ekifaananyi talina kwesabika mu ngoye ez'enjawulo era n'ekisenge tekyetaagisa kukisabika.

Waliwo obuzibu obuva mu kubeera okumpi ne X-ray ekimanyiddwa nga amanyi ge njuba (radiation). Buli muntu afuna ekigero ky'amaanyi agobutonde amatono okuva mu musana, mumpewo, mummere ne mutaka (ekimanyiddwa nga background radiation). Okuyitibwamu amaanyi ga X-ray mu bipimo ebinene kisobola okuleeta kookolo (cancer). Wabula, ekigero ky'amaanyi ga X-ray ekikozesebwa mu kyuma kya DXA kitono nnyo era kyenkana nga ekyo omuntu kyafuna okuva mu mayengo g'omusana mu lunaku lumu. Abakozi mu kunoonyereza batendekeddwa okukuba ekifaananyi kya DXA nga bakozesa omuwendo gw'amaanyi g'enjuba amatono ennyo nga bwekisoboka.

B. Okubeera Olubuto

Tetumanyi oba okukebera kwa DXA kulina obulabe bwonna ku baana abali mu mbuto za bakyala abakerebwa DXA nga bali mbuto. N’olwekyo kino, abakyala abali embuto tebasobola kwetaba mu kunoonyereza kuno.

Abakyala bonna abetaba mu kunoonyereza kuno bateekwa okukozesa enkola ezigema okuzaala ezesigibwa. Mu kunoonyereza kuno tuyingiza abakyala abo bokka abakozesa **empiso y’okwegema okuzaala, akaweta akatekebwa munabaana, obupiira bu kalimpitawa oba okusala obuseke.**

Ssinga ofuna olubuto nga oli mu kunoonyereza, ogya kulekeraawo okukebererebwa kwa DXA naye ogya kugenda mumaaso n’emitendera emirala nga bwegyategekebwa mukusooka. Ogya kusobola okudamu okukebererebwa kwa DXA oluvanyuma, nga tokyali lubuto.

C. Obuzibu bw’ebikukwatako okumanyibwa abalala

Abakozi mu kunoonyereza bagya kukola kyona ekisoboka okukuuma ebyama byo ng’oli mu kunoonyereza kuno. Enkyala zo zigya kukolebwa nga za kyama. Waliwo obuzibu ku byama byo ssinga omukozi mu kunoonyereza oba omuntu omulala yena mu kunoonyereza abeera akumanyi. Abakozi mu kunoonyereza bagya ku kola buli kyebasobola okukakasa nti abalala tebamanya bw’oyimiridde ku bikwata ku kawuka kamukenenya. Wabula, okugya wano kunkyala z’okunoonyereza kiyinza okuletera abalala okwewunya oba olina akawuka ka mukenenya. Olwokubeera kino, abantu abalala bayinza okumanya ku kwetabakwo mu kunoonyereza ne bakuyisa mungeri etali yabwenkanya oba nebakwogerako ebintu ebiibi. Bwoba olina obuzibu bwona. Omukozi mu kunoonyereza agya kwogera nawe okugezaako okuyamba okubigonjola.

D. Obuzibu bw’okukujjako enviiri

Waliwo obuzibu butono nnyo obuyinza okubaawo nti omukozi mukunoonyereza ayinza okusala olususu lwo mu butanwa oba okutataganya omusono gw’enviirizo ng’akugyako enviiri zino.

8) Waliwo emigaso mu kwetaba mu kunoonyereza kuno?

Oyinza okuganyulwa oba obutaganyulwa buterevu olw’okubeera mu kunoonyereza kuno. Ogya kumanya oba amagumba go malamumu. Oyinza okuganyulwa bw’onyoyiga engeri y’o kukyusa endya yo okuba enungi. Ogya kuwebwa amakerenda ga calcium oba vitamin D ssinga onooba ogetaaga.

Ekisinga byona, okwetaba kwo mu kunoonyereza kuno kugya kuyamba abantu abasingawo, kubanga kugya kutuyamba okufuna amawulire ag’omugaso ku bulamu bwa magumba ga bakyala abato abalina akawuka kamukenenya abakozesa depo-provera n’eddagala erijjanjaba akawuka kamukenenya eririmu tenofovir. Kino kirina obusobozi bw’okutumbula omutindo gw’obujjanjabi bw’abakyala abalina akawuka kamukenenya mu Uganda ne mu mawanga amalala okwetolola ensi yonna.

9) Oteekwa okwetaba mu kunoonyereza kuno?

Okwetaba mu kunoonyereza kuno kwa kyeyagalire ddala. Oyinza okusalawo obutetabamu kati oba okutwala obudde okukirowoozako nga tonasalawo. Ssinga toyagala kwetabamu oba oluvanyuma n'okyusa endowoozayo, togya kubonerezebwa oba okufiirwa emigaso gy'obujjanjabi bwo obwabulijjo. Ssinga osalawo okuva mu kunoonyereza gyebujja, bwokkiriza, ebibwako n'ebikukwatako ebifuniddwa biyinza okukumibwa n'ebikozesebwa wamu nebiva mu kunoonyereza oba mu kunoonyereza okulala gy'ebujja. Wabula, ssinga osaba ebyakugyibwako n'ebiwandiiko okusaanyizibwawo wamu n'ebikukwatako ebirala byona, tugya kukakasa nti kino kikolebwa.

Abakulira okunoonyereza bayinza era okusalawo okukujja mu kunoonyereza ssinga tosobola kugoberera mitendera gy'okunoonyereza, oba ssinga abasulira okunoonyereza balekeraawo okukusasulira.

10) Olina ky'onasasula kyona okusobola okwetaba mu kunoonyereza kuno?

Emitendera gy'okunoonyereza, okukebera kwa DXA, n'okukeberegwa okulala kwona bigya kukolebwa nga tolina kyosasudde.

11) On'osagulwa ssinga wetaba mu kunoonyereza kuno?

Tetugya kukusasula olw'okwetaba mu kunoonyereza kuno. Wabula, tugya kukuwa sillingisi emitwalo ebiri, 20,000 (rand ze Sausi Afirika 80) olw'okusasula ebisale byentambula kulwenkyala entegeke ez'okujja ku Kirinika.

12) Biki ebikumibwa nga byakama era biki ebiyinza okufulumizibwa?

Abasawo n'abakozi mu kunoonyereza bagya kukuuma ebikukwatako n'okwetabakwo mu kunoonyereza nga byakyama ddala nga bwebasobola. Kubiwandiko byo eby'okunoonyereza, ennamba enekusifu yegya okukozesebwa mukifo kyamanya go. Abakozi mu kunoonyereza bokka bebagya okumanya ennamba eno enekusifu. Ebiwandiko byona ebinafulumizibwa okuva mu kunoonyereza kuno tebigya kukozeza linya lya oba okukwatukkiriza gwe ng'omuntu. Ebimu kubitongole bya gavumenti eby'obulamu, abasulira okunoonyereza, obukiiko obwekekenenya okunoonyereza n'ebitongole ebirala ebyakakasibwa biyinza okulaba kalonda akwata kubulamu bwo mubiwandiko by'okunoonyereza.

Abakozi mu kunoonyereza tebagya kuwayo mawulire ga kwatukkiriza nga towadde lukusa mu buwandiko. Mubiseera ebimu nga mw'otwalidde ebigwa bitalaze, oba amateeka agafuga emirimu, bayinza okutuwaliriza okugabana ebikukwatako.

13) Kiki ekibaawo ssinga ofuna obuvune oba obukosefu mu kunoonyereza kuno?

Tekisubirwa nakamu nti wagya kubaawo obuvune bwonna kugwe olw'okwetaba mu kunoonyereza kuno. Ssinga abakozi mu kunoonyereza bazuula nti olumiziddwa buterevu olw'okwetaba mu kunoonyereza kuno, ogya kuwebwa obujjanjabi obwamangu olw'obuvune obwo awatali kubusasulira. Tewali ntekateeka ndala ezenjawulo ez'okukuliyirira. Bw'oteeka omukono gwo kukiwandii kino ogya kuba tewegyeko dembelyo mumateeka mu mbeera ng'e zo.

14) Ani gw'oyinza okutuukirira ssinga oba n'ebibuuzo oba ky'oyagala okumanya kyona kubikwata ku kunoonyereza kuno?

Ssinga obeera n'ebizibu oba ebibuuzo byona ng'oli mu kunoonyereza, omukozi mu kunoonyereza abudabuda n'abakozi abalala bagya kuba basanyufu okukuyamba okutuukirira omuntu omutuufu okuddamu ebibuuzo byo.

Bw'oberanga n'ebibuuzo ebikwata ku kunoonyereza kuno oba ssinga ofuna ekizibu ekikwata kubulamu oba obuvune oyina kutuukirira dokita Flavia Matovu Kiweewa oba dokita Clemensia Nakabiito ku MU-JHU Research collaboration, kukasozi e Mulago oba bakubiire ku ssimu ku 0414-541044 oba 0772544759 oba 0702544759.

Ssinga oba n'ebibuuzo ebikwata ku ddembe lyo nga nnakyewa mu kunoonyereza, oyinza okutuukirira ssentebe w'akakiiko ka Uganda akakwasisa amateeka kuby'okunoonyereza ku buwuka (Uganda Virus Research and Ethics Committee), mwami Tom Lutalo ku ssimu 0404320385 oba 0404320386.

MU-JHU nate erina akakiiko akawi kamagezi (CAB) akayamba abasawo b'okunoonyereza, abantu mu kunoonyereza n'emubitundu okunoonyereza mwekukolebwa, okukolera awamu okutunuulira ensonga enkulu ez'okunoonyereza era n'ebizibu mu kunoonyereza. CAB erimu abantu okuva mu batuuze b'omukitundu, bannaddini, abantu abaali babaddeko mu kunoonyereza n'abantu abalala abamanyifu. Basobola nabo okukuyamba kubizibu oba ensonga z'oyinza okuba nazo. Bwoba oyagala okwogera n'omu kubali ku kakiiko ka CAB, osabibwa obulire omusawo abudabuda mu kunoonyereza, agya kubuulira engeri gy'onotukiriramu omu kubatuula ku kakiiko ka CAB.

15) Okussaako omukono gwo kitegeeza ki?

Okussaako omukono gwo oba ekinkumu ku kiwandiiko kinno kitegeeza nti otegeera ebiri mu kiwandiiko ky'okukkiriza kino. Era okkiriza kyeyangalire okwetaba mu kunoonyereza. Ssinga oteeka omukono gwo oba ekinkumu ku kiwandiiko, kitegeeza nti okkiriza okwetaba mu kunoonyereza. Mu kuteeka omukono gwo ku kiwandiiko ky'okukkiriza kino, tewegyako ddembe lyo lyona mu mateeka lyewandibadde nalyo nga nnakyewa mu kunoonyereza.

TUGYA KUKUUWA KOPI Y'EKIWANDIIKO KY'OKUKKIRIZA

Tokeekako mukononga ennaku z'omwezi zino ziyiseeko: _____

SIKITUUFU NGA TEKULI SITAMPU YA IRB ERAGA OBUKKAKAFU

Bone Mineral Density in a Cohort of Young Adult Women Using Depo-provera and Tenofovir, Kampala, Uganda. Version 1.0 dated 26 June 2015

OKUKKIRIZA: Nsomye (oba omuntu ansomedde era nanyinnyonyola) ebiri mu kiwandiiko ky'okukkiriza kino. Ntegeera lwaki okunoonyereza kuno kukolebwa emitendera gy'okunoonyereza era, n'obuzibu n'emigaso nga bwebinyonyoddwa mu buwandiike buno obufunze. Nzikkiriza nga neesaliddewo okwetaba mu kunoonyereza kuno.

Erinnya lya nnakyewa (kyapa)

Omukono oba ekinkumu
kya nnakyewa

Ennaku z'omwezi

Kulwabannakyewa abatamanyi kusoma n'okuwandiika:

Amannya ga nakyewa ne nnaku z'omwezi biwandiikidwa

Erinnya ly'omuntu afuna okukkiriza
(Kyapa)

EMPID

Ennaku z'omwezi

Ku lwa banakyewa bonna: Nyinyonnyodde ekigendererwa ky'okunoonyereza kuno eri nnakyewa era muzzemu ebibuuzo bye byona. Okusinziira ku kumanya kwange, ategeera ekigendererwa, emitendera, obuzibu n'emigaso egy'o kunoonyereza kuno.

Erinnya ly'omuntu afuna okukkiriza
(Kyapa)

Omukono gw'afuna okukkiriza

Ennaku z'omwezi

Kulwabannakyewa abatamanyi kusoma n'okuwandiika: Njulira nti ebiri mu kiwandiiko ky'okukkiriza kino bisomeddwa era binnyonyoddwa nnakyewa. Okusinzira ku kumanya kwange, ekiwandiiko kyona kisomeddwa mu bujjuvu era mubutuufu bwakyo. Nnakyewa alabika ategeera ekigendererwa, emitendera, obuzibu n'emigaso gy'o kwetaba mu kunoonyereza, era akkiriza nga yeyagalidde okwetaba mu.

Ku lwabo abassako ekinkumu bokka. Njulira nti nnakyewa agamba nti erinnya lye ye _____ attadde ekinkumu kye ku kiwandiiko ky'okukkiriza kino nga yesaliddewo yekka ku lunaku luno nga _____

Erinnya ly'omujjulizi
mu mitendera gy'okukkiriza
(kyapa)

Omukono gw'omujjulizi

Ennaku z'omwezi

Appendix 9.3.3: English Storage ICF

BONE: Contraception & Anti-Retroviral Effects (BONE: CARE) Study-
Kampala, Uganda. Protocol version 1.0 dated 26 June 2015

Patient ID Sticker



MU-JHU CARE LTD
MU-JHU Research Collaboration

Makerere University, Kampala

The Johns Hopkins University, Baltimore

P. O Box 23491, Kampala, Uganda

Telephone: +256-414-541044 Facsimile: +256-414-543002



INFORMATION AND CONSENT FORM FOR STORAGE AND FUTURE USE OF BLOOD, HAIR AND URINE

Title of the study: Bone Mineral Density in a Cohort of Young Adult Women Using Depo-provera and Tenofovir, Kampala, Uganda. Version 1.0 dated 26 June 2015

Sponsors: U.S. National Institute of Health

Principal Investigator: Dr. Flavia Matovu Kiweewa

Phone Number: 0414-541044

Date: 24 April 2018

1) What you should know about this research study:

- Hello, my name is _____. You have decided to join the BONE: CARE study that is being done by doctors from the Makerere University –Johns Hopkins University Research Collaboration (MUJHU) - Kampala, Uganda, in collaboration with Makerere University School of Public Health - Kampala, Uganda Johns Hopkins University - Baltimore, USA, University of Washington- Seattle, USA, and University of Witwatersrand – Johannesburg, South Africa.
- While you are in this study, there may be some leftover blood, hair and urine samples taken from you that are not needed for this study, but that may be helpful for future research.
- Researchers can learn a lot from a study but as time goes by more information can be learned by using left overs from specimens.
- You are being asked to agree to let us store these samples for future use.
- This consent form explains how the samples will be collected, stored, and used.
- Please read it with care and take as much time as you need. You may also have this consent form read to you.
- Ask questions about things that are not clear to you now or when you think of them later.
- After the specimen storage has been fully explained to you and all of your questions have been answered, you can decide freely if you allow storage of your specimens for future testing.

- Allowing storage of these samples is entirely your free choice. You can decide if you want to allow your specimens to be stored or not. You may be a part of the BONE:CARE study even if you do not agree to storage of these specimens.
- If you agree, you will be asked to sign this consent form. If you cannot sign, you will be asked to place your thumb print on the consent form in the presence of a witness. You will be offered a copy of this form to keep.

2) How will we get the samples?

There will be NO ADDITIONAL samples taken from you for storage. By joining the BONE: CARE study, you agreed to have blood, hair and urine taken and tested as explained in the study consent form. After all the tests are done for the BONE: CARE study, there may be some samples left that have not been used. If you agree, these samples will be kept and used for future research.

3) How will we use these samples?

Your samples will only be used to look for evidence of the mechanisms through which HIV and its treatment as well as depo-provera injection affect bones, or your body's response to these agents. Tests to be performed will include but are not limited to; 25-OH Vitamin D, calcium, phosphorus, parathyroid hormone (PTH), follicle stimulating hormone (FSH), insulin-like growth factor-1, estradiol levels, urinary phosphorus and creatinine.

If a researcher wants to do other tests on your stored specimens, they will write up the idea and it will have to be approved by the Principal Investigator, other study team leaders and institutional review boards to make sure that the research is worthwhile. They would never know your name.

The researchers do not plan to contact you or your regular doctor with the results from tests done on the stored samples. This is because research tests are often done with experimental procedures, so the results from one research study are generally not useful for making decisions on managing your health. Should a rare situation come up in which the researchers decide that a specific test result would provide important information for your health, the researchers will try to contact you. If you wish to be contacted with this type of test result, you must give the study doctor or nurse any change to your address and/or phone number. If you want your regular doctor to be told about this type of test result, you must provide the study doctor or nurse with your regular doctor's name and contact information.

Your samples will not be sold or used directly to produce commercial products. Research studies using your samples will be reviewed by the sponsor of this study (the US National Institutes of Health), the Uganda Virus and Research Ethics Research Committee, the Human Research and Ethics Committee at the University of the Witwatersrand, South Africa, and other special committees at the researchers' institutions (Institutional Review Boards) prior to being carried out. The role of the Institutional Review Boards is to protect you and other research volunteers from harm.

4) How long will we keep the samples?

There is no time limit on how long your samples will be stored.

5) How will the samples be stored?

The samples will be stored in special facilities that are designed to store samples safely and securely called repositories. Some of these facilities are outside of Uganda. The facilities are designed so that only

approved researchers will have access to the samples. Some employees of the laboratories will need to have some access to your samples in order to store them and to keep track of where they are, but these people will not have information that directly identifies you.

6) Will there be any benefit to you from storing the samples?

You will not directly benefit from storing these samples. The results of the studies using stored specimen may help other people in the future.

7) What are the risks?

These specimens are being collected as part of the BONE: CARE study in which you are taking part. We are not asking you to give any additional specimens for storage, so there is no additional risk associated with collection. The specimens are stored only by code number (not your name), so there is no risk of loss of privacy.

8) What information is kept private and what information may be given out?

To keep your information private, your samples will be labeled with a code that can only be traced back to this research clinic. Your personal information (name, address, phone number) will be protected by the research clinic. When researchers are given your stored samples to test they will not be given your personal information.

The results of future tests will not be included in your health records. Also, some U.S and Uganda government health agencies, the study sponsor and their authorized agents may see details in research records.

Any publication of this study will not use your name or identify you personally.

9) What are your rights?

Letting your samples to be stored is your free choice. You may choose not to have any samples stored other than what is needed to complete the BONE: CARE study. You can still be in the BONE: CARE study or any future study if you decide not to have your sample stored. No matter what you decide it will not affect your usual medical care.

If you decide now that your samples can be stored for future research, you may change your mind at any time. You must contact your study doctor or nurse and let them know that you do not want your samples used for future research. Your samples will then not be used and will be destroyed.

10) What should you do if you have questions?

If you ever have questions about the storage of samples or need any more information, you should contact the study doctor, Dr. Flavia Matovu Kiweewa or Dr. Clemensia Nakabiito at Makerere University-Johns Hopkins University (MU-JHU) Research Collaboration, Upper Mulago Hill Road or call them to the on Tel. 0414-541044 or 0772 544759 or 0702 544759.

If you have questions about your rights as a research participant, you may contact the Uganda Virus and Research Ethics Research Committee chair, Mr. Tom Lutalo on 0404320385 or 0404320386

MU-JHU also has an advisory committee (CAB) that helps study doctors, study volunteers, and communities where the study is done; to jointly work together on important issues and risks in the study. CAB includes people from the community, religious leaders, past study participants, and other influential people. They can also help you with the problems or issues you may have. If you want to talk to any of the CAB, you are requested to inform the study counselor, they will tell you how you will contact one of the CAB members.

11) What does your signature mean?

Your signature below means that you understand the information given to you in this consent form about taking, storing, and using samples later and you have chosen freely to allow storage of specimens for future testing. You will not give up your legal rights by signing this consent.

WE SHALL GIVE YOU A COPY OF THIS CONSENT FORM

Do not sign after the expiry date of: _____

NOT VALID WITHOUT THE IRB STAMP CERTIFICATION

Bone Mineral Density in a Cohort of Young Adult Women Using Depo-provera and Tenofovir, Kampala, Uganda. Version 1.0 dated 26 June 2015

Please carefully read the statements below or have them read to you and think about your choices. No matter what you decide, it will not affect your participation in the BONE CARE study or your medical care. Please initial or mark your choices and sign or place your thumbprint below.

I agree to all the following leftover samples to be stored for future research testing:

	YES	NO
Blood	☐	☐
Hair	☐	☐
Urine	☐	☐

I have read (or someone has read and explained to me) this consent form. I understand the purpose of the storage of specimens collected in this study for future use and the risks and benefits as described in this written summary.

Participant's Name (print)

Participant's Signature or

Date

Thumbprint

Name of person obtaining consent (Print)	Empid of person obtaining consent	Date
--	-----------------------------------	------

For all volunteers: I have explained the purpose of storing specimens from the study to the volunteer and have answered all of her questions. To the best of my knowledge, she understands the purpose, methods, risks and benefits.

Name of person obtaining consent (Print)	Signature of person obtaining consent	Date
--	---------------------------------------	------

For illiterate volunteers: I attest that the information contained in this written consent form has been read and explained to the participant. To the best of my knowledge, the information provided was complete and accurate. The participant appears to understand the purpose, methods, risks and benefits and has voluntarily made her choice indicated above.

For those placing thumbprint only: I attest that the participant who states that her name is _____ has placed her thumbprint on this consent form of her own free will

on this day _____.

Witness to consent process Name (print)	Witness' Signature	Date
---	--------------------	------



MU-JHU CARE LTD
MU-JHU Research Collaboration

Makerere University, Kampala
The Johns Hopkins University, Baltimore
P. O Box 23491, Kampala, Uganda

Telephone: +256-414-541044 Facsimile: +256-414-543002



AMAWULIRE N'EKIWANDIIKO KY'OKUKKIRIZA OKUTEREKA N'OKUKOZESA OMUSAAYI, ENVIIRI N'OMUSULO GYEBUGYA

Omutwe gw'okunoonyereza:	Obugumu bw'amagumba mu kibinja ky'abakyala abato abakozesa Depo-provera ne Tenofovir, Kampala, Uganda Version 1.0, dated 26 June 2015.
Asasulira okunoonyereza:	Ekitongole ky'America ekye by'obulamu (NIH)
Akulira okunoonyereza:	Dr. Flavia Matovu Kiweewa
Ennamba y'essimu:	0414-541044
Ennaku z'omwezi:	24 April 2018

1) Kiki ky'olina okumanya ku kunoonyereza kuno?

- Nkulamusizza nyabo. Erinya lyange nze _____. Osazeewo okwetaba mu kunoonyereza okwa BONE: CARE okukolebwa abasawo okuva mu Makerere Univasite –Johns Hopkins Univasite (MU-JHU) – Kampala, Uganda, nga bakolaganira wamu ne Univasite ye Makerere School of Public Health- Kampala, Uganda, Univasite ye John's Hopkins Bloomberg School of Public Health, mu America, Univasite ye Washington –Seattle, mu America ne Univasite ye Witwatersrand – Johannesburg, mu Sausi Afirica.
- Ng'oli mu kunoonyereza kuno, wayinza okubaawo ebifisse ku musaayi, enviiri n'omusulo ebikugyibwako nga tebyetaagibwa mu kunoonyereza kuno, naye nga biyinda okuba eby'omugaso mu kunoonyereza gyebugya.
- Abanoonyereza bayiga bingi okuva mu kunoonyereza naye ekiseera bwekiyitawo basobola okuyiga ebisingawo nga bakozesa ebisigalira ku byakugyibwako ebyaterekebwa.
- Osabibwa ottukirize tutereke ebikugyibwako bino olw'okukozesebwa gyebugya.

- Ekiwandiiko ky'okukiriza kino kinyonnyola engeri ebigyibwako gyebinakunganyizibwamu okuterekebwa era n'okukozesebwa.
- Osabibwa okisome n'obwegendereza era otwale ekiseera kyonna kyewetaaga. Era oyinza n'okusomerwa ekiwandiiko kyo'okukiriza kino.
- Buuza ebibuuzo ku bintu ebitegerekeka gy'oli kati oba oluvanyuma nga obiroowoozezaako.
- Oluvanyuma lw'okunonyolwa entereka yebikugyibwako era nga n'ebibuuzo byo byonna nga bididdwamu, osobola okusalawo kyeyagalire oba okkiriza tutereke ebikugyibwako olw'okukeberekwa gyebugya.
- Okukkiriza okutereka ebikugyibwako bino kya kyeyagarire ddala. Bwoba oyagala osobola okusalawo oba nga okukkiriza ebikugyibwako okuterekebwa oba nedda. Oyinza okwetaba mu kunoonyereza kwa BONE: CARE yadde nga tokkiriza kutereka ebikugyibwako bino.
- Bwokkiriza ogya kusabibwa okussa omukono ku kiwandiiko ky'okukkiriza kino. Bw'oba tosobola kusaako mukono, ogya kusabibwa okussako ekinkumukyo ku kiwandiiko ky'okukiriza nga waliwo omujjulizi. Ogya kuwebwa kopi y'ekiwandiiko kino ogitereke.

2) Tunafuna tutya ebikugyibwako?

Tewajja kubaawo BIKUGYIBWAKO BIRALA byonna olw'okuterekebwa. Bwewetaba mu kunoonyereza kwa BONE: CARE, wakkiriza okugyibwako omusaayi, enviiri n'omusulo era bikeberekwe nga bwe kinyonyoddwa mu kiwandiiko eky'okukkiriza okwetaba mu kunoonyereza. Ng'okukebera kwonna okw'okunoonyereza kwa BONE: CARE kuwedde, wayinza okubaawo ebyakugyibwako ebifisse nga tebikozeseddwa. Ssinga okkiriza, ebikugyibwako bino bigya kuterekebwa era bikozebebe olw'okunoonyereza gyebugya.

3) Tunakozesa tutya ebikugyibwako bino?

Ebikugyibwako bigya kukozebebe mu kunoonyereza ku ngeri y'akawuka ka mukenenya n'enzijanjabayaako era wamu ne mpiso ya depo-provera bwebikosa amagumba, oba engeri omubiri gwo gyegweyisaamu ku bintu bino. Okukebera okunaakolebwa kugya kubaamu bino naye sibyokka; 25-OH Vitamin D, Calcium, phosphorus, parathyroid hormone (PTH), follicle stimulating hormone (FSH), insulin-like growth factor-1, estradiol levels, ebirungo bya phosphorus awamu ne creatinine ebiri mu musulo.

Ssinga abanoonyereza baagala okukola okukebera okulala kuby'akugyibwako ebyaterekebwa, bagya kuwandiika ekirowoozo era kirina okukakasibwa akulira okunoonyereza era n'obukiiko obwekebejja okunoonyereza okukakasa nti okunoonyereza kusanidde. Ttebalimanya linya lyo.

Abanoonyereza tebatega ku kutuukirira oba omusawo wo owa buligyo kubinava mu kukebera kwebyakugyibwako ebyaterekebwa. Kino kiri bwekityo kubanga okukebera kw'okunoonyereza kutera kukolebwa mitendera gya byakugezesa n'olwekyo ebiva mu kunoonyereza okumu okutwalira awamu si byamugaso mu kusalawo ku byendabirira y'obulamu bwo. Ssinga wabawo embeera etali yabuligyo abanoonyereza nebasalawo nti ebivudde mu kukebera okumu biyinza okuba eby'omugaso eri obulamu bwo, abanoonyereza bagya kugezaako okukutuukirira. Bwoba oyagala okukutuukirira nebyava mu kukeberekwa eby'ekika kino oteekwa okuwa omusawo mu kunoonyereza oba nansi endagiro gy'obeera

ne/oba ennamba y'essimu. Ssinga oba oyagala omusawo wo owabulijjo okubuulirwa kubinaaba bivudde mu kukeberegwa okwekika kino, oteekwa okuwa omusawo mu kunoonyereza oba nansi erinya n'endagiriro y'omusawo wo owabulijjo

Ebikugyibwako tebijja kutundibwa oba okukozesebwa buterevu mu kufulumya ebyamaguzi. Okunoonyereza okunakozesa ebikyidwako kugya kwekenenyezebwa اساسلرا okunoonyereza kuno ekitongole kya America eky'ebyobulamu (the US National Institutes of Health), akakiiko ka Uganda akakwasisa amateeka kuby'okunoonyereza ku buwuka (Uganda Virus Research and Ethics Committee), akakiiko akakwasisa amateeka kuby'okunoonyereza ku Universite ye Wit Waters rand e Sausi Afirika, ekitongole kya Uganda ekya sayansi n'amagezi ag'ekikugu (Uganda National Council for Science and Technology), n'obuukiko obulala obwenjawulo awakolebwa okunoonyereza (Institutional review boards) nga okunoonyereza tekunakolebwa. Omulimu gw'obukiiko buno kwe kukukuumu nebanakyewa abalala obutatusibwako bulabe.

4) Ebikyibwako tunabitereka kumala bbanga ki?

Tewaliwo bbanga ggere ebikugyibwako ly'ebinaterekebwa.

5) Ebikyibwako binaterekebwa bitya?

Ebikyibwako bigya kuterekebwa mu bifo ebyenjawulo ebyategekebwa okutereka ebikyibwako obulungi era mu bukusifu ebityibwa eteerekero (repositories). Ebimu ku bifo bino biri bweru wa Uganda. Ebifo byategekebwa nga abo bokka abanoonyereza abakirizidwa be bokka abasobola okutuuka eri ebikyibwako. Abamu ku bakazi aba mu laabu bagya kwetaaga okutukirira ebikyibwako basobole okubitereka n'okumanya wa gyebiri, naye abantu bano tebajja kuba na bikwatako bikwatukiriza buterevu.

6) Wanabaawo omugaaso gwonna eri gwe okuva mu kutereka ebikyibwako?

Togya kuganyurwa buterevu olw'okutereka ebikyibwako bino. Ebinava mu kunoonyereza okukozesa ebikyibwako ebiterekebwa biyinza okuyamba abantu abalala gyebujja.

7) Buzibu ki?

Ebikyibwako bino bikunganyizibwa nga ekitundu mu kunoonyereza kwa BONE: CARE kwe wetabamu. Tetukusaba kuwayo bigyibwako birala olw'okuterekebwa n'olwekyo tewali buzibu bweyongera bukwatagana n'okugyako ebiterekebwa. Ebikyibwako biterekebwa na namba enekusifu yokka (so si linya lyo) kale tewali buzibu bwakufiirwa ebyamabyo.

8) Biki ebikumibwa nga byakyama era biki ebinyinza okufulumizibwa?

Okukuma ebikukwatako nga byakyama, ebikugyibwako bigya kulambibwa ne namba esobola okukwataganyizibwa wanno wokka mu kirinika y'okunoonyereza. Ebikukwatako (erinya, gy'obeera, ennamba ye ssimu) bigya ku kumibwa kirinika y'okunoonyereza. Abanoonyereza bwebanawebwa ebyakugyibwako ebyaterekebwa okubikebera tebajja kuweebwa bikukwatako.

Ebinaava mu kukebera gyebugya tebajja kutekebwa mu biwandiiko byo eby'obulamu. Era ebitongole ebimu ebyagavumenti y'America ne Uganda eby'obulamu, asasulira okunoonyereza era nabo abaweredwa olukusa bayinza okulaba kalonda mu biwandiiko by'okunoonyereza.

Ebinafulumizibwa okuva mu kunoonyereza kuno tebija kukozeza linyalyo oba okukwatukiriza ng'omuntu.

9) Ddembe ki ly'olina?

Okukkiriza okutereka ebikugyibwako kwa kyeyagalire. Oyinza okusalawo obutatereka bikugyibwako byonna okugyako ebyo ebyetaagibwa okumaliriza okunoonyereza kwa *BONE: CARE*. Osobola era okusigala mu kunoonyereza kwa *BONE: CARE* oba okunoonyereza okulala kwonna gyebugya bwosalawo nti ebikugyibwako tebiterekebwa. Ssi nsonga ki kyosalawo tekijja kukosa ndabirira y'obulamu bwo eyabulijjo.

Bw'osalawo kati nti ebikugyibwako biyinda okuterekebwa olw'okunoonyereza gyebugya, osobola okukyusa endowoozayo obudde bwonna. Oteekwa okutukirira omusawo mu kunoonyereza oba nansi era obategeze nti toyagala bikugyibwako kuterekebwa olw'okunoonyereza gyebugya. Olwo ebikugyibwako tebigya kukozezebwa era bigya kusanyizibwawo.

10) Kiki ky'olina okukola bwoba olina ebibuuzo?

Bwoba nga n'ebibuuzo ebikwata ku kutereka ebikugyibwako oba nga wetaaga amawulire amalala gonna, tuukirira dokita Flavia Matovu Kiweewa oba dokita Clemensia Nakabiito ku MU-JHU Research collaboration, kukasozi e Mulago oba bakubiire ku ssimu ku 0414-541044 oba 0772544759 oba 0702544759.

Ssinga oba n'ebibuuzo ebikwata ku ddembe lya nga nnakyewa mu kunoonyereza, oyinza okutuukirira ssentebe w'akakiiko ka Uganda akakwasisa amateeka kuby'okunoonyereza ku buwuka (Uganda Virus Research and Ethics Committee), mwami Tom Lutalo ku ssimu 0404320385 oba 0404320386.

MU-JHU nate erina akakiiko akawo kamagezi (CAB) akayamba abasawo b'okunoonyereza, bannakyewa mu kunoonyereza, n'emubitundu okunoonyereza mwekukolebwa; okukolera awamu okutunuulira ensonga enkulu ez'okunoonyereza era n'ebizibu mu kunoonyereza. CAB erimu abantu okuva mu batuuzo b'omukitundu, bannadini, abantu abaali babaddeko mu kunoonyereza n'abantu abalala abamanyifu. Basobola nabo okukuyamba ku bizibu oba ensonga zoyinda okuba nazo. Bwoba oyagala okwogera n'omu

kubali ku kakiiko ka CAB, osabibwa obulire omusawo abudabuda mu kunoonyereza, ajja okukubulira engeri gy'onotukiriramu omu kubatuula ku kakiiko ka CAB.

11) Okussaako omukono gwo kitegeeza ki?

Okusaako omukono gwo wamanga kitegeeza nti otegeera ebikunyonyoddwa ebiri mu kiwandiiko ky'okukkiriza kino ebikwata ku kujjako, okutereka era n'okukozesa ebigyibwako oluvanyuma, era osazeewo kyeyangalire okukkiriza okutereka ebigyibwako olw'okukeberegwa gyebugya. Togya kwegyako dembe lyo mu mateeka bwoteeka omukono ku kiwandiiko ky'okukkiriza kino.

TUGYA KUKUWA KOPI Y'EKIWANDIIKO KY'OKUKKIRIZA KINO.

Tosaako mukono nga ennaku zino ziyiseeko eza nga: _____

SIKITUUFU NGATEKULI SITAMPU YA IRB EKAKASA

Bone Mineral Density in a Cohort of Young Adult Women Using Depo-provera and Tenofovir, Kampala, Uganda. Version 1.0 dated 26 June 2015

Osabibwa n'obwegendereza okusoma bino wammanga oba babikusomere era olowooze ku by'osalawo. Ssi nsonga ki ky'osalawo, tekigya kukosa kwetabaakwo mu kunoonyereza kwa BONE: CARE oba eby'obujjanjabi bwo. Osabibwa okulamba ku ky'osazeewo era oteekeko omukono oba ekinkumu wamanga.

Nzikkiriza ebinasigalawo ku binagyibwako byonna okuterekebwa olw'okukeberegwa kw'okunoonyereza gyebugya.

	Yee	Nedda
Omusaayi	☐	☐
Enviiri	☐	☐
Omusulo	☐	☐

Nsomye (oba omuntu ansomedde era nanyinyonnyola) ebiri mu kiwandiiko ky'okukkiriza kino. Ntegeera ekigendererwa ky'okutereka ebigyibwako mu kunoonyereza kuno olw'okukozesebwa gyebugya era nebizibu, n'emigaso nga bwebinnyoonnyoddwa mu buwandiike buno obufunze.

Erinnya lya nnakyewa (kyapa)
kya nnakyewa

Omukono oba ekinkumu

Ennaku z'omwezi

Kulwabannakyewa abatamanyi kusoma n'okuwandiika:

Amanya ga nakyewa ne nnaku z'omwezi biwandiikidwa

Erinnya ly'omuntu afuna okukkiriza
(Kyapa)

EMPID

Ennaku z'omwezi

Ku lwa banakyewa bonna: Nyinyonnyodde ekigendererwa ky'okutereka ebigyibwako mu kunoonyereza eri nnakyewa era muzzemu ebibuuzo bye byonna. Okusinziira ku kumanya kwange kwonna, ategeera ekigendererwa, emitendera, obuzibu n'emigaso egy'o kunoonyereza kuno.

Erinnya ly'omuntu afuna okukkiriza
afuna okukkiriza

Omukono oba ekinkumu

Ennaku z'omwezi

Kulwa bannakyewa abatamanyi kusoma n'okuwandik:a Njulira nti ebiri mu kiwandiiko ky'okukkiriza kino bisomeddwa era binnyonyoddwa nnakyewa. Okusinziira ku kumanya kwange, ekiwandiiko kyona kisomeddwa mu bujjuvu era mubutuufu bwakyo. Nnakyewa alabika ategeera ekigendererwa, emitendera, obuzibu n'emigaso, era akkiriza kyeyagalire era n'alondawo ngabwekiragiddwa waggulu.

Ku lwabo abassako ekinkumu bokka: Njulira nti nnakyewa agamba nti erinnya lye ye _____ attadde ekinkumu kye ku kiwandiiko ky'okukkiriza kino nga yesaliddewo yekka ku lunaku luno nga _____

Erinnya ly'omujjulizi
mu mitendera gy'okukkiriza

Omukono gw'omujjulizi

Ennaku z'omwezi

(Kyapa)

Appendix 9.4: Questionnaires/ Case Report Forms

BONE: CARE Study

Facility Number: _____

Screening ID: BMD - ____ - ____ - ____

PRE-SCREENING ELIGIBILITY CHECKLIST

Date - -

day month year

Hello, my name isand I am going to take you through a few questions to find out whether you can be screened for the study (**BONE: CARE Study**) that you have been told about to be conducted at MU-JHU Research site.

1.	How old are you?	Less than 18 ☐ 18-35 ☐ 36 and above ☐ (<i>ineligible</i>)	
2.	What is the name of the village where you stay?		
3.	For how long have you lived in this village?	months	
3.	Are you currently pregnant?	YES ☐	No ☐
4.	Are you currently using any of the following family planning methods; a) Depo-provera for 2 years or less b) Intrauterine contraceptive device (IUD) c) Female sterilization <i>Women on IUD and female sterilization should not have used hormonal contraception like pills, Depo-Provera and implants for more than 3 months in the last 2 years.</i>	YES ☐ YES ☐ YES ☐	No ☐ No ☐ No ☐
5.	Have you breastfed in the last 6 months?	YES ☐	No ☐
6.	Have you delivered a baby in the past 6 months?	YES ☐	No ☐
7.	Have you ever tested for HIV?	YES ☐	No ☐
8.	If yes, what is your HIV status?	HIV Infected ☐	HIV uninfected ☐
9.	Would you be interested in hearing more about the BMD-TDF study?	YES ☐	No ☐
10.	Eligibility status	Eligible ☐	Ineligible ☐
11.	If ineligible, state screen out reason (s)		
12.	If eligible, state outcome study group		
13.	Comments (if any):		
14.	Next appointment date (if applicable)/ dd-mm-yy		
15.	Staff initials & date:		
16.	QC I reviewer's initials & date:	QC II reviewer's initials & date:	

Demographics Questionnaire

Participant ID: BMD - ____ - __

Visit Date

--
 day month year

1. What is your date of birth..... -- If un known, record age:
 day month year years

2. What is your current relationship/ marital status?

- ☐ Married (traditional, religious or civil), monogamous
☐ Married (traditional, religious or civil), polygamous
☐ Cohabiting
☐ Regular partner, not cohabiting
☐ Casual partner
☐ Single, no partner
☐ Divorced
☐ Separated
☐ Widowed

3. What is your highest level of education?

- ☐ no schooling
☐ primary school, not complete
☐ primary school, complete
☐ secondary school, not complete
☐ secondary school, complete
☐ completed college or university
☐ don't know or not sure

Yes No

4. Do you earn an income? ☐ ☐ (If no skip to item 6)5. What is your average monthly income? Shs.

Demographics Questionnaire

Participant ID: BMD - ____ - ____

Visit Date

--
 day month year

6. What kind of work do you do, or what kind of activities keep you busy during an average day, whether you get money for them or not? (*Code up to two responses*)

- ☐ Agriculture for home use/barter
- ☐ Agriculture for selling
- ☐ Housework in own home (*If no other work, go to item 7*)
- ☐ Housekeeper (for relative or employer)
- ☐ Shopkeeper
- ☐ Trading/vending
- ☐ Government/clerical/teaching/ medical worker
- ☐ Student
- ☐ Military/police
- ☐ Bar worker or owner
- ☐ Unemployed (*skip to item 7*)
- ☐ Waitress/Waiter/restaurant owner
- ☐ Hair dresser/Salon owner
- ☐ Commercial sex worker
- ☐ Other (specify)_____

Yes No

7. Do you own a mobile phone? ☐ ☐ (*If no, go to item 8*)

Yes – phone present No – phone not present

a. Do you have your phone on you? ☐ ☐

8. What is your spouse's/partner's average monthly income? Shs *If no partner, skip to item 9.*

- ☐ No income
- ☐ Do not know/ not sure

Yes No

9. Do you, or does someone in your family, own the house you are currently living in? ☐ ☐

Demographics Questionnaire

Participant ID: BMD - ____ - __

Visit Date

--
 day month year

10. How many rooms are in the house you are currently living excluding the kitchen and bathroom?
 # of rooms

11. What is the tenure status for your house?

- ☐ Owned
☐ Rented (Normal)
☐ Rented (subsidized)
☐ Supplied free by employer
☐ Supplied free or rent paid by other person
☐ Other (specify)

12. How many people including adults and children live in your house?

13. Comments if any.....

.....

.....

.....

.....

 Staff initials and date

QC I reviewer's initials & date		QC II reviewer's initials & date	
---------------------------------	--	----------------------------------	--

Enrollment Eligibility Form

VISIT CODE .

Participant ID BMD - ____ - ____

Visit Date: --
day month year

Instructions: Use the table below to document a participant eligibility status at the enrollment visit.

INCLUSION CRITERIA		Yes	No
	Items 1 to 6 apply to all participants		
1.	Age 18 to 35 years (inclusive). <i>Source copy of identification card or other documents as specified in SOP.</i>		
2.	Able and willing to provide written informed consent. <i>Source: signed/marked screening and enrollment consent form.</i>		
3.	Have a documented HIV test result from the parent health care center within the last 3 months. <i>Source: Parent health care provider medical records.</i>		
4.	Resident within a 20 km radius around MU-JHU clinic, Mulago, Kampala. <i>Source: Participant locator form.</i>		
5.	Resident in an area for 6 months and not planning to move out of the catchment area within the next 2 years. <i>Source: Participant self-report - this document is source</i>		
6.	Agrees to come to the study clinic for scheduled appointments and to be called by phone or visited at home as needed to ensure follow up. <i>Source: signed/marked screening and enrollment consent form AND Participant locator form.</i>		
	Items 7 to 10 apply to participants in GROUP A ONLY		
7.	Has documented HIV positive test result <i>Source: Parent health care provider medical records.</i>		
8.	Has been using depo-provera for $\leq$ 2yrs (new and current users) <i>Source: Parent family planning provider medical records.</i>		
9.	Is naive but eligible for anti-retroviral therapy at screening <i>Source: Parent health care provider medical records.</i>		
10.	Her current CD4 performed within the last 3 months is 200cell/ul or greater <i>Source: Parent health care provider medical records.</i>		
	Items 10 to 14 apply to participants in GROUP B ONLY		
11.	Has documented HIV positive test <i>Source: Parent health care provider medical records.</i>		
12.	Has been using depo-provera for $\leq$ 2yrs <i>Source: Parent family planning provider medical records.</i>		

Enrollment Eligibility Form

VISIT CODE .

Participant ID BMD - ____ - ____

Visit Date: --
day month year

13.	Is ineligible for antiretroviral therapy per local guidelines (currently CD4 $\geq$ 500 cells/ μ l performed in previous 3 months) <i>Source: Parent health care provider medical records.</i>		
14.	CD4 performed in the last 3 months > 700 cells/ μ l <i>Source: Parent health care provider medical records.</i>		
	Items 15 to 19 apply to participants in GROUP C ONLY		
15.	Has documented HIV positive test performed in last three months <i>Source: Parent health care provider medical records.</i>		
16.	Is using non-hormonal contraception (Intra uterine device, or female sterilization) <i>Source: Parent family planning records, or medical examination.</i>		
17.	Is naïve but eligible for antiretroviral therapy at screening (CD4 < 500 cells/ μ l performed in last 3 months) <i>Source: Parent family planning records, or medical examination.</i>		
18.	Her most recent CD4 performed in last 3 months is greater than 200 cells/ μ l <i>Source: Parent health care provider medical records.</i>		
	Items 20 to 21 apply to participants in GROUP D ONLY		
19.	Has a documented HIV negative test performed in the last 3 months		
20.	Is using non-hormonal contraception (Intra uterine device, or female sterilization) <i>Source: Parent family planning records, or medical examination.</i>		
21.	Has not used post exposure prophylaxis in the last 6 months		

In order to be eligible, all responses to items 1 to 21 above must be Yes

EXCLUSION CRITERIA		Yes	No
	<i>Items 22 to 28 apply to all participants</i>		
22.	Is currently pregnant/ breastfeeding or was pregnant/breastfeeding in the last 6 months prior to screening. <i>Source: Baseline medical history questionnaire</i>		
23.	Intends to get pregnant in next 2 years. <i>Source: Self report/ counseling notes</i>		
24.	Has a pre-existing untreated or uncontrolled condition known to affect bone metabolism		

Enrollment Eligibility Form

VISIT CODE .

Participant ID BMD - ____ - ____

Visit Date: --
day month year

	(e.g. thyrotoxicosis, tuberculosis, diabetes mellitus, liver (e.g., hepatitis B or C) or renal disease).		
	<i>Source: Baseline medical history questionnaire</i>		
25.	Is taking the following medications known to interfere with bone metabolism (steroids, anti-convulsants, bisphosphonates, cancer drugs).		
	<i>Source: Baseline medical history questionnaire</i>		
26.	Was hysterectomized and oophorectomized.		
	<i>Source: Baseline family planning and menstrual history questionnaire</i>		
27.	Has any other condition that in the investigator's opinion, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data or otherwise interfere with achievement of the study objectives.		
	<i>Source: Combination of the various study records</i>		
	Item 28 applies to GROUP C and D ONLY (HIV+, DMPA-, TDF ART and HIV -, no DMPA-, no ART)		
28.	Has used hormonal contraception for more than 3 consecutive months in the past 2 years		

In order to be eligible, all responses to items 22 to 28 above must be No.

29.	AFFIRM PARTICIPANT'S STUDY GROUP:		GROUP A
			GROUP B
			GROUP C
			GROUP D

Clinician's initials & date _____/_____

QC I's initial's & date _____/_____ QC II's initials & date _____/_____

Baseline Family Planning and Menstrual History Questionnaire

Participant ID BMD - _____ - _____

Visit Date --

1. Over the next 2 years do you have intentions to get pregnant? Yes ☐ (*Ineligible, end form*) No ☐
2. Now I am going to ask you some questions about the different methods of family planning you may have ever used, the method (s) you have used in the last two years, and the methods (s) you are currently using. (*Mark all that apply*).
- 1a) What family planning methods have you ever used?
- 1b) What family planning method(s) have you used in the last 2 years?
- 1c) What family planning method(s) are you currently using?

Method	1a) Ever used	2a) Used in the last 2 years	Currently using
3-month injectable (Depo-provera)	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Combined oral contraceptive pills (COCs)	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Progestin-only contraceptive pills (POPs)	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
IUD	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Hormonal implant	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Female Sterilization	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Sterilization for the partner	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Female condoms	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Male condoms	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Withdrawal	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Emergency contraception	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Lactation amenorrhea (breastfeeding)	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Rhythm method/safe days	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Abstinence	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Abortion	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
None	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
Other, SPECIFY _____	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No

**Baseline Family Planning and Menstrual History
Questionnaire**

Participant ID BMD - _____ - _____

Visit Date --

3. Have you ever changed your family planning method? Yes ☐ No ☐

4. If you have ever changed your family planning method what are the reasons that caused you to change? *(Mark all that apply)*

- a. Heavy menses
- b. Prolonged bleeding
- c. Frequent menses (describe) _____
- d. Infrequent menses (describe) _____
- e. Absence of menses
- f. Other menstrual changes (specify) _____
- g. Reduced libido
- h. Vaginal dryness
- i. Back ache and other bone pains
- j. Pelvic pain
- k. Infection
- l. Headaches
- m. Weight gain
- n. Weight loss
- o. Increased appetite
- p. Partner objection
- q. Desire to conceive
- r. Contraceptive failure associated with poor adherence (specify) _____
- s. Contraceptive failure despite perfect adherence

**Baseline Family Planning and Menstrual History
Questionnaire**

Participant ID BMD - -

Visit Date --

- t. Method stock outs (from supplier)
- u. Advice from health worker (describe) _____
- v. Advice from a relative or friend (describe) _____
- w. Other specify _____

5. If you plan to start a new family planning method, which of the following family planning method(s) would you like to use over the next 2 years?
Mark all that apply.

- ☐ 1a. None
- ☐ 1b. Natural methods such as withdrawal or rhythm method
- ☐ 1c. Implants
- ☐ 1d. Combined oral contraceptive pills
- ☐ 1e. Progesterone only pills
- ☐ 1f. Intrauterine device (IUD) → **Record on contraceptives log**
- ☐ 1g. Depo-provera → **Record on contraceptives log**
- ☐ 1h. Female sterilization → **Record on contraceptives log**

→ **In eligible, end form.**

**Baseline Family Planning and Menstrual History
Questionnaire**

Participant ID BMD - ____ - ____ - ____

Visit Date --

☐ 1i. Male condoms

☐ 1j. Female condoms

Must be combined with another method (1f.

→ to 1g.) for participant to be eligible

We would now like to ask you some questions about your menstrual cycle,

6. When did you have your first menstrual period? (*mmm-yy*): - → *If unknown record age* years

7. Do you menstruate regularly? By regular cycle we mean a periodic bleeding cycle occurring every 21-35 days. Yes ☐ No ☐

8. When did you start your last menstrual period? (*dd-mmm-yy*), *record any date regardless of interval*: --

9. When did you complete your last menstrual period? (*dd-mmm-yy*): -- OR On-going

10. (For interviewer) If no menses in the last 35 days, is it unexpected or unexplained? Yes ☐ No ☐

a. If unexplained, state most likely explanation: _____

11. How many times have you been pregnant including live births, miscarriages, still births, or tubal pregnancies? → *If 00, end of form.*

**Baseline Family Planning and Menstrual History
Questionnaire**

Participant ID BMD - _____- _____

Visit Date --a. How many times have you given birth? It does not matter if the baby will alive or still born. 12. Did you breast feed any of your children? Yes ☐ No ☐ (*if no, end form*)

13. For how long did you breastfeed each of your children?

Children	Month and year of birth (mmm-yy)	Total breastfeeding (months)
1 st child		
2 nd child		
3 rd child		
4 th child		
5 th child		
6 th child		

14. (For interviewer) Participant's cumulative breastfeeding period in months: months

Comments: _____

Staff initials & date _____/_____/_____

QC I's initial's & date _____/_____/_____

QC II's initials & date _____/_____/_____

BONE: CARE Study

VISIT CODE .

Baseline Medical History Questionnaire

Participant ID BMD_TDF - _____

Visit Date --

1. Do you have or have you been told that you have any of the following conditions? (mark all that apply)

Medical problem	Yes or No		If yes, onset date (dd/mm/yy)	Description (include outcome date, if applicable)
1a. Liver disease including chronic hepatitis B or C	☐	☐		
1b. Diabetes mellitus	☐	☐		
1c. Chronic kidney disease	☐	☐		
1d. Thyroid disease	☐	☐		
1e. Parathyroid disease	☐	☐		
1f. Cancer	☐	☐		
1g. Pituitary disease	☐	☐		
1h. Bone disease (including rickets)	☐	☐		
1i. Bone fracture in the last year	☐	☐		
1j. Weight loss (5kg or more in the last 6 months)	☐	☐		
1k. Chronic diarrhea (in the last 6 months)	☐	☐		
1l. Tuberculosis	☐	☐		
1m. Condition leading to bed rest for 6	☐	☐		

VISIT CODE

Participant ID BMD_TDF - _____

Visit Date --

weeks or longer in the last 12 months				
---------------------------------------	--	--	--	--

- medications log)*

Medication	Currently taking medication		Taken medication for more than 3 months in the past	
a) Anticonvulsants	Yes ☐	No ☐	Yes ☐	No ☐
b) Systematic corticosteroids	Yes ☐	No ☐	Yes ☐	No ☐
c) Drugs for hypo- or hyper-thyroidism	Yes ☐	No ☐	Yes ☐	No ☐
d) Drugs for hypo- or hyper-parathyroidism	Yes ☐	No ☐	Yes ☐	No ☐
e) Vitamin D	Yes ☐	No ☐	Yes ☐	No ☐
f) Multivitamins	Yes ☐	No ☐	Yes ☐	No ☐
g) Calcium supplements	Yes ☐	No ☐	Yes ☐	No ☐
h) Thiazide diuretics	Yes ☐	No ☐	Yes ☐	No ☐
i) Antacids	Yes ☐	No ☐	Yes ☐	No ☐
j) Others (specify)	Yes ☐	No ☐	Yes ☐	No ☐

3. Have you ever smoked tobacco / cigarettes? Yes ☐ No ☐ (*skip to question 9*)

5. When did you start smoking? (*Current episode only*) *mmm/yy* /

Baseline Medical History Questionnaire

Participant ID BMD_TDF - _____

Visit Date --

6. What is the average number of cigarettes you have smoked per day in the last month? cigarettes
7. What is the average number of tobacco pipes you have smoked per day in the last month? tobacco pipes
8. If no longer smoking now when did you stop? *mmm/yy* /
9. Sometimes people use drugs of recreation for different reasons. Have you used any of these drugs that make people feel high? Are you currently using them?

	Yes	No	Currently using
Marijuana	☐	☐	☐
Amphetamines	☐	☐	☐
Aero fuels ("glue")	☐	☐	☐
Mayirungi	☐	☐	☐
Heroin	☐	☐	☐
Other (specify)_____	☐	☐	☐

10. Do you drink alcohol? Yes ☐ No ☐

11. How many bottles of the following do you drink in a typical week: (code 00 if none)

Beer	bottles / cans	
Local brew	500 ml cups	
Wine	glasses	
Spirits	25 ml shots	

BONE: CARE Study

VISIT CODE .

Baseline Medical History Questionnaire

Participant ID BMD_TDF -

Visit Date --

Other (specify) bottles/cans

12. Does the participant have any history of obstetric/gynecologic/reproductive problems/ procedures not recorded elsewhere?

Yes ☐ No ☐

a. If yes, specify:

13. Comments:

BONE: CARE Study

VISIT CODE .

Baseline Medical History Questionnaire

Participant ID BMD_TDF - _____

Visit Date --

Clinician's initials & date _____/_____

QC I's initial's & date _____/_____

QC II's initials & date _____/_____

BONE: CARE Study

VISIT CODE .

Physical Exam Case Report Form

Participant ID: BMD - ____ - ____

Visit Date --

VITAL SIGNS				
1. Weight kg	2. Height . cm		3. Blood Pressure / mmHg	
SYMPTOM-DIRECTED FINDINGS				
SYMPTOM	Not done	Normal	Abnormal	Comments
4. General appearance				
5. Abdomen/ Gastrointestinal				Tenderness: Hepatomegaly: Splenomegaly:
6. Lymph nodes (size and location)				Neck: Axilla:
7. Heart/ cardiovascular				
8. Lungs/Respiratory				
9. Extremities				

BONE: CARE Study

VISIT CODE .

Physical Exam Case Report Form

Participant ID: BMD - ____ - ____

Visit Date --

SYMPTOM	Not done	Normal	Abnormal	Comments
10. Neurological				
11. Skin				
12. Eyes				
13. Musculoskeletal				Tenderness: Swelling:
14. Ears, Nose, Throat				
15. Head				
16. Other (specify)				

Staff initials & date

BONE: CARE Study

VISIT CODE .

Physical Exam Case Report Form

Participant ID: BMD - -

Visit Date --

QC I reviewer's initials & date		QC II reviewer's initials & date	
---------------------------------	--	----------------------------------	--

Clinical Chart Review Form

Participant ID BMD_TDF - _____

Visit Date --

1. Current (most recent) CD4 count and date	CD4 count: Sample collection date: - -
2. Current (most recent) HIV Viral load	Viral load: OR ☐ Not detected Sample collection date: - -
3. ART Regimen	☐ TDF + 3TC +EFV ☐ TDF+ FTC +EFV (Atriplar) ☐ TDF + 3 TC + NVP
4. Bactrim (PCP) prophylaxis (Cotrimoxazole or Dapsone)	YES ☐ NO ☐
5. Has the participant changed her ARV combination since last visit? 5.1 If yes, record new combination, and update ART medications log: (If changed regimen inform PI)	YES ☐ NO ☐ (skip to item6) N/A ☐ New medication: _____

Clinical Chart Review Form

Participant ID BMD_TDF - Visit Date --

5.3. Reason for change in ART regimen	[1] Substitution due to side effects [2] Switch to second line (failed 1 st line) [3] Drug stock outs [4] Other (specify).....
6. WHO clinical staging (refer to WHO staging chart)	
7. Drug Adherence at current visit (Pill count) (%) 6.1 ART 6.2 Cotrimoxazole/ Dapsone	# #

 Staff initials and date

QC I reviewer's initials & date		QC II reviewer's initials & date	
---------------------------------	--	----------------------------------	--

VISIT CODE .

Contraceptives Log

Participant ID BMD - ____ - _

Visit Date --

1.

Medication (<i>trade name</i>)		Staff Initials/ Date
Indication		
Date Started - -	Date Stopped OR ☐ continuing at end of study - -	
Frequency ☐ qd ☐ tid ☐ qhs ☐ Cont. ☐ once ☐ bid ☐ qid ☐ other specify: _____		
Dose/Units _____ OR ☐ N/A	Route ☐ PO ☐ IM ☐ INTRA ☐ TOP ☐ SC ☐ Other, specify: _____	

2.

Medication (<i>trade name</i>)		Staff Initials/ Date
Indication		
Date Started - -	Date Stopped OR ☐ continuing at end of study - -	
Frequency ☐ qd ☐ tid ☐ qhs ☐ Cont. ☐ once ☐ bid ☐ qid ☐ other specify: _____		
Dose/Units _____ OR ☐ N/A	Route ☐ PO ☐ IM ☐ INTRA ☐ TOP ☐ SC ☐ Other, specify: _____	

Concomitant Medications Log

Participant ID BMD - _____ - _____

Visit Date --

1.	Medication (trade name)		Staff Initials/ Date
	Indication		
	Date Started - -	Date Stopped OR ☐ continuing at end of study - -	
	Frequency ☐ prn ☐ qd ☐ tid ☐ qhs ☐ once ☐ bid ☐ qid ☐ other specify: _____		
	Dose/Units	Route ☐ PO ☐ IM ☐ IV ☐ TOP ☐ VAG ☐ Other, specify: _____	
2.	Medication (trade name)		Staff Initials/ Date
	Indication		
	Date Started - -	Date Stopped OR ☐ continuing at end of study - -	
	Frequency ☐ prn ☐ qd ☐ tid ☐ qhs ☐ once ☐ bid ☐ qid ☐ other specify: _____		
	Dose/Units	Route ☐ PO ☐ IM ☐ IV ☐ TOP ☐ VAG ☐ Other, specify: _____	

Anti-Retroviral Therapy Log

Participant ID BMD - _____ - _____

Visit Date --

1.

Medication (trade name)		Staff Initials/ Date
Date Started - -	Date Stopped OR ☐ continuing at end of study - -	
Frequency ☐ prn ☐ qd ☐ tid ☐ qhs ☐ once ☐ bid ☐ qid ☐ other specify: _____		
Dose/Units	Route ☐ PO	

2.

Medication (trade name)		Staff Initials/ Date
Date Started - -	Date Stopped OR ☐ continuing at end of study - -	
Frequency ☐ prn ☐ qd ☐ tid ☐ qhs ☐ once ☐ bid ☐ qid ☐ other specify: _____		
Dose/Units	Route ☐ PO	

VISIT CODE .**Physical Activity Questionnaire**

Participant ID BMD - _____ - _____

Visit Date --

Physical Activity	
Next I am going to ask you about the time you spend doing different types of physical activity in a typical week. Please answer these questions even if you do not consider yourself to be a physically active person. Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, farming, harvesting food/crops, to get from place to place and in your spare time. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.	
Questions	Response
Activity at work	
1. Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like heavy lifting, digging, carrying heavy loads, cutting and fetching firewood, collecting water for household use, farming activities, or fast bicycling for at least 10 minutes continuously? (USE SHOWCARD)	Yes ☐ No ☐ If No, go to item 4
2. In a typical week, on how many days do you do vigorous-intensity activities as part of your work?	Number of days
3. How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes : hrs mins
4. Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking, carrying light loads, bicycling at a regular pace, and household chores such as sweeping, mopping, and polishing floors for at least 10 minutes continuously? (USE SHOWCARD)	Yes ☐ No ☐ If No, go to instructions above item 7
5. In a typical week, on how many days do you do moderate-intensity activities as part of your work?	Number of days
6. How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours : minutes :
Travel to and from places	
The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship, [insert other examples if needed]	
7. Do you walk or use a bicycle (pedal cycle) for at least 10 minutes continuously to get to and from places?	Yes ☐ No ☐ If No, go to item instructions above 10
8. In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days
9. How much time do you spend walking or bicycling for travel on a typical day?	Hours : minutes :

VISIT CODE .**Physical Activity Questionnaire**

Participant ID BMD - _____ - _____

Visit Date --**Recreational activities**

The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (leisure).

10.	Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate like running or netball for at least 10 minutes continuously? (USE SHOWCARD)	Yes ☐ No ☐ If No, go to P 13
11.	In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (leisure) activities?	Number of days
12.	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours : minutes : hrs mins
13.	Do you do any moderate-intensity sports, fitness or recreational (leisure) activities that causes a small increase in breathing or heart rate such as brisk walking, cycling, swimming, volleyball for at least 10 minutes continuously? (USE SHOWCARD)	Yes ☐ No ☐ If No, go to instructions above item 16
14.	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (leisure) activities?	Number of days
15.	How much time do you spend doing moderate-intensity sports, fitness or recreational (leisure) activities on a typical day?	Hours : minutes : hrs mins

Sedentary behavior

The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent sitting at a desk, sitting with friends, weaving baskets and mats, sorting grain, sewing or knitting, travelling in car, bus, train, reading, playing cards, listening to the radio or watching television, but do not include time spent sleeping. (USE

16.	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes : hrs min s
-----	---	--

Staff initials & date _____/_____/_____

QC I's initial's & date _____/_____/_____ QC II's initials & date _____/_____/_____

Bone CARE study

VISIT CODE .

Food Frequency Questionnaire

Participant ID BMD - _____ - ____

Visit Date --

I would like to ask you about the foods and drinks you ate or drank in the last week whether at home or outside of your home. Your participation in this part of the study will help us to understand how the study tablets might affect your bones. In addition to the bone test that you will have, it is important to know how much calcium you eat. Calcium is a nutrient found in some foods and drinks and it helps your bones and teeth stay strong. I'm only going to ask you about a few things you may eat or drink because these are foods that contain high amounts of calcium.

1. Have you eaten any of the following dairy foods in the last week including Saturday and Sunday? If yes, how many times a day did you drink or eat this food? How much would you eat or drink at a time? In the last 7 days, on how many days did you drink or eat this food? ***[SHOW CUPS]. For amount in cups, interviewer to fill in how much participant drunk or ate on a typical day in the previous 7 days.***

DAIRY FOODS	None	1 day	2 days	3 days	4 days	5 days	6 days	Every day	Amount in cups/ day
1a. Cheddar cheese (hard cheese)									
1b. Cottage cheese									
1c. Milk									
1d. Ice Cream									
1e. Yoghurt									
1f. Macaroni and cheese									
1g. Pizza									

Bone CARE study

VISIT CODE .

Food Frequency Questionnaire

Participant ID BMD - _____ - _____

Visit Date --

2. Have you eaten any of the following fruits and vegetables in the last week including Saturday and Sunday? If yes, how many times a day did you eat this food? How much would you eat at a time? In the last 7 days, on how many days did you eat this food? **[SHOW CUPS]. For amount in cups, interviewer to fill in how much participant drank or ate on a typical day in the previous 7 days.**

FRUITS AND VEGETABLES	None	1 day	2 days	3 days	4 days	5 days	6 days	Every day	Amount in cups/day
2a. Dark greens									
Spinach									
Sukuma wiki									
Nakati									
Entula									
Jjobyo									
Avocado									
Eggplant (biringanya)									
Broccoli									
pumpkin leaves									
lettuce									
2b. Peas (such as pigeon peas, field peas)									
2c. Beans (such soybeans, lentils, cowpeas, and butter beans)									
2d. Ground nuts									
2e. Millet									
2f. Sorghum									
2g. Fruits (like oranges, apples, pears, guava, watermelon, jackfruit)									
2h. Sugar cane									
2i. Sweet potatoes									

Bone CARE study

VISIT CODE .

Food Frequency Questionnaire

Participant ID BMD - _____ - _____

Visit Date --

3. Have you eaten any of the following foods in the last week including Saturday and Sunday? If yes, how many times a day did you eat this food? How much would you eat at a time? In the last 7 days, on how many days did you eat this food? **[SHOW CUPS]. For amount in cups, interviewer to fill in how much participant drank or ate on a typical day in the previous 7 days.**

MEAT-FISH -POULTRY	None	1 day	2 days	3 days	4 days	5 days	6 days	Every day	Amount in cups/day
3a. Small whole fish (such as enkejje, and mukene)									
3b. Fish without bones									
3c. Liver									
3d. Eggs									

4. Have you eaten any of the following foods in the last week including Saturday and Sunday? If yes. How many times a day did you eat this food? How much did you eat at a time? In the last 7 days, on how many days did you drink or eat this food? **[SHOW CUPS]. For amount in cups, interviewer to fill in how much participant drank or ate on a typical day in the previous 7 days.**

BREADS-CEREALS-RICE	None	1 day	2 days	3 days	4 days	5 days	6 days	Every day	Amount in cups/day
4a. Bread									
4b. Cereal									
4c. Biscuits									
4d. Rice									

Bone CARE study

VISIT CODE .

Food Frequency Questionnaire

Participant ID BMD - ____ - ____ - ____

Visit Date --

5. Now I would like to ask you about the foods and drinks you ate or drank yesterday during the day and at night (24 hours), whether at home or outside of home. Please recall all foods and beverages you consumed beginning with yesterday morning when you woke up.

[For interviewer: Write down all food and drinks mentioned by the respondent. When the respondent has finished, probe for snacks, fruits, vegetables, and alcoholic and non-alcoholic beverages, if not yet mentioned]

Breakfast	Snack	Lunch	Snack	Supper	Snack

Bone CARE study

VISIT CODE .

Food Frequency Questionnaire

Participant ID BMD - _____ - _____

Visit Date --

6. Now, I’m going to ask you more details about the foods and beverages you just listed. I want you tell me “how much you ate”, “which ingredients were added in the food during preparation, where possible”, and “how the food was prepared”. **[SHOW CUPS]**. When you remember anything else you ate or drank as we go along, please tell me.

Food Item (As listed in the table above)	How much of this food did you actually eat or drink?	Ingredients and Additions	Cooking Method (Boiled, fried, roasted, raw etc)	CODER USE ONLY	
				Food code	Weight

Bone CARE study

VISIT CODE .

Food Frequency Questionnaire

Participant ID BMD - _____ - _____

Visit Date --

7. Was the amount of food that you ate yesterday about usual, less than usual, or more than usual?

[1] Usual (skip to Qn 10) [2] Less than usual [3] More than usual (skip to Qn 9)

8. What is the main reason the amount you ate yesterday was less than usual?

[1] Nausea

[2] Vomiting

[3] Diarrhoea

[4] Lack of appetite/ Not hungry

[5] Other Sickness/illness

[6] At a social function, special meal/ special day

[7] Fasting

[8] Short of money/no food at home

[9] Other reason: _____

9. What is the main reason the amount you ate yesterday was more than usual?

[1] Away from home

[2] At a social function, special meal, or on a special day

[3] Very hungry/increased appetite

[4] Bored or stressed

[5] Other reason: _____

10. How could you describe your current dietary habit?

(1) No special diet, I eat almost everything

(2) Vegetarian

(3) Special diet: _____

Bone CARE study

VISIT CODE .

Food Frequency Questionnaire

Participant ID BMD - _____ - _____

Visit Date --

11. In the past 7 days, have you taken a Vitamin D supplement or multivitamin containing Vitamin D? Yes ☐ No ☐ (If yes, record on concomitant medications log)

12. In the past 7 days, have you taken a calcium supplement or multivitamin containing calcium? Yes ☐ No ☐ (If yes, record on concomitant medications log)

Staff I initials and date:

QC I reviewer's initials & date		QC II reviewer's initials & date	
---------------------------------	--	----------------------------------	--

DXA Scan CRF

VISIT CODE .

Participant ID BMD - _____ - _____

Visit Date: - -
 day month year

1. Was a DXA scan performed? ☐ Yes ☐ No (If no end form?

	Scan results	Z-score for age
2. Lumbar Spine 2a. BMD (g/cm ²) 2a.1 L1 2a.2 L2..... 2a.3 L3..... 2a.4 L4..... 2a.5 Average:.....		+ or -
2b. BMC (g) 2b.1 L1 2b.2 L2..... 2b.3 L3..... 2b.4 L4..... 2b.5 Average.....		+ or -
2c. Bone area (cm ²) 2c.1 L1 2c.2 L2..... 2c.3 L3..... 2c.4 L4..... 2c.5 Average:.....		+ or -

DXA Scan CRF

VISIT CODE .

3. Total Hip 3a. BMD (g/cm ²)..... 3b. BMC (g)..... 3c. Bone area (cm ²).....	Scan # 1 . . .	Z-score for age + or - . + or - . + or - .
4. Femoral Neck of Hip 4a. BMD (g/cm ²)..... 4b. BMC (g)..... 4c. Bone area (cm ²).....	Scan # 1 . . .	Z-score for age + or - . + or - . + or - .
5. Whole body 5a. BMD (g/cm ²) 5b. BMC (g) 5c. Bone area (cm ²)	Scan # 1 . . .	Z-score for age + or - . + or - . + or - .
6. Total Body Fat Mass (g) ...	.	
7. Trunk Fat Mass	.	
8. Lower Extremity Fat Mass (Lt) (g)...	.	
9. Lower Extremity Fat Mass (Rt) (g)...	.	
10. Upper Extremity Fat Mass (Lt) (g)...	.	
11. Upper Extremity Fat Mass (Rt) (g)...	.	
12. Total Body Lean Mass (g)...	.	
13. Upper Extremity Lean Mass (Lt) (g)...	.	
14. Upper Extremity Lean Mass (Rt) (g)...	.	
15. Lower Extremity Lean Mass (Lt) (g)...	.	
16. Lower Extremity Lean Mass (Rt) (g)...	.	

DXA Scan CRFVISIT CODE .

Comments including quality of scan, and positioning (were L1-L4 correctly identified, was the femur appropriately rotated?)

Clinician's initials & date _____/_____/_____

QC I's initial's & date _____/_____/_____ QC II's initials & date _____/_____/_____

**Follow-up Family Planning and Menstrual
History Questionnaire**

Participant ID BMD - _____ - _____

Visit Date --

1. Since the last visit, have you changed/stopped or started a new family planning method? ☐ Yes (*update contraceptives log*) ☐ No (*If yes, go to item 3*)

2. What method(s) of contraception/family planning have you been using since the last visit? Mark "none" or all that apply.

☐ 2a. None →

counsel appropriately

☐ 2b. Natural methods such as withdrawal or rhythm method

☐ 2c. Implants

☐ 2d. Combined oral contraceptive pills

☐ 2e. Progesterone only pills

☐ 2f. Intrauterine device (IUD)

☐ 2g. Depo-provera

☐ 2h. Female sterilization → Update contraceptives log if applicable

☐ 2i. Male condoms

☐ 2j. Female condoms

→ *Update Contraceptives Log*

If not used in combination with another method,

→ *counsel appropriately*

**Follow-up Family Planning and Menstrual
History Questionnaire**

Participant ID BMD - -

Visit Date --

3. Why did you change your family planning method? *(Mark all that apply)*

- a. Heavy menses
- b. Prolonged bleeding
- c. Frequent menses (describe) _____
- d. Infrequent menses (describe) _____
- e. Absence of menses
- f. Other menstrual changes (specify) _____
- g. Reduced libido
- h. Vaginal dryness
- i. Back ache and other bone pains
- j. Pelvic pain
- k. Infection
- l. Headaches
- m. Weight gain
- n. Weight loss
- o. Increased appetite
- p. Partner objection
- q. Desire to conceive
- r. Contraceptive failure associated with poor adherence (specify) _____
- s. Contraceptive failure despite perfect adherence
- t. Method stock outs (from supplier)

**Follow-up Family Planning and Menstrual
History Questionnaire**

Participant ID BMD - -

Visit Date --

- u. Advice from health worker (describe) _____
- v. Advice from relative or friend (describe) _____
- w. Other specify _____

4. Do you have intentions to get pregnant in the next ____ months (remaining period of the study)? Yes ☐ (*Counsel appropriately*) No ☐

We would now like to ask you some questions about your menstrual cycle,

5. When did you start your last menstrual period? (*dd-mmm-yy*): -- OR Amenorrheic for past 3 months

6. When did you complete your last menstrual period (*dd-mmm-yy*) -- OR On-going

7. (For interviewer) If no menses in the last 35 days, is it unexpected or unexplained? Yes ☐ No ☐

a. If explained, state most likely explanation: _____

8. Since your last visit, have you breastfed? Yes ☐ No ☐ → *If no, end form.*

1a. How many **days** have you spent breast feeding since your last visit? days

Comments: _____

Staff initials & date _____/_____

QC I's initial's & date _____/_____

QC II's initials & date _____/_____

Follow up Medical History Questionnaire

Participant ID BMD - _____ - _____

Visit Date --1. Since the last visit, have you had or have you been told that you have any of the following conditions? (*mark all that apply*)

Medical problem	Yes or No		If yes, onset date (dd/mm/yy)	Description (include outcome date, if applicable)
1a. Liver disease including hepatitis B or C	☐	☐		
1b. Diabetes mellitus	☐	☐		
1c. Kidney disease	☐	☐		
1d. Thyroid disease	☐	☐		
1e. Parathyroid disease	☐	☐		
1f. Cancer	☐	☐		
1g. Pituitary disease	☐	☐		
1h. Bone disease (specify):	☐	☐		
1i. Bone fracture	☐	☐		
1j. Weight loss (>10%)	☐	☐		
1k. Chronic diarrhoea (> 3 months)	☐	☐		
1l. Tuberculosis	☐	☐		
1m. Condition leading to bed rest for 6 weeks or longer	☐	☐		

Follow up Medical History Questionnaire

Participant ID BMD - _____ - _____

Visit Date --2. Are you taking, any of the following medications currently or for more than 3 months in the past? *(If yes, record on con med log)*

Medication	Currently taking medication		Taken medication for more than 3 months in the past	
a) Anticonvulsants	Yes ☐	No ☐	Yes ☐	No ☐
b) Systematic corticosteroids	Yes ☐	No ☐	Yes ☐	No ☐
c) Drugs for hypo- or hyper-thyroidism	Yes ☐	No ☐	Yes ☐	No ☐
d) Drugs for hypo- or hyper-parathyroidism	Yes ☐	No ☐	Yes ☐	No ☐
e) Vitamin D	Yes ☐	No ☐	Yes ☐	No ☐
f) Multivitamins	Yes ☐	No ☐	Yes ☐	No ☐
g) Calcium supplements	Yes ☐	No ☐	Yes ☐	No ☐
h) Thiazide diuretics	Yes ☐	No ☐	Yes ☐	No ☐
i) Antacids	Yes ☐	No ☐	Yes ☐	No ☐

We would like to ask you some questions about smoking, alcohol, and recreational drugs.3. Since the last visit, have you smoked tobacco / cigarettes? Yes ☐ No ☐4. What is the average number of cigarettes you have smoked per day in the last month? cigarettes5. What is the average number of tobacco pipes you have smoked per day in the last month? cigarettes

6. Have you used any of these drugs of recreation since the last visit?

	Yes	No
Marijuana	☐	☐
Amphetamines	☐	☐
Aero fuels ("glue")	☐	☐
Mayirungi	☐	☐

Follow up Medical History Questionnaire

Participant ID BMD - _____ - _____

Visit Date --

Heroin ☐ ☐
Other (specify): _____ ☐ ☐

7. Have you drunk alcohol since the last visit? Yes ☐ No ☐

8. How many bottles of the following did you drink in the last 7 days: (code 00 if none)

Beer	bottles / cans	
Local brew	500 ml cups	
Wine	glasses	
Spirits	25 ml shots	
Other (specify) _____	bottles/cans	

9. Does the participant have any changes to her obstetric/gynecologic/reproductive history since the last visit? Yes ☐ No ☐

a. If yes, specify: _____

10. Comments: _____

Clinician's initials & date _____/_____

QC I's initial's & date _____/_____

QC II's initials & date _____/_____

Antiretroviral Medication Adherence Form

Participant ID BMD - _____

Visit Date --

Most people with HIV have many pills to take at different times during the day.

Many people find it hard to always remember their pills:

- Some people get busy and forget to carry their pills with them.
- Some people find it hard to take their pills according to all the instructions, such as “with meals” or “on an empty stomach,” or “every 8 hours,”.
- Some people decide to skip pills to avoid side effects or to just not be taking pills that day.

We need to understand how people with HIV are really doing with their pills. Please tell us what you are **actually** doing. Don't worry about telling us that you don't take all your pills.

This questionnaire asks about the medications that you are currently taking.

	i)	ii)	lii)	iv
1. Name of drug				
2. How many TIMES did you actually take this medication?				
2i) Yesterday	① ② ③ ④ ⑤	① ② ③ ④ ⑤	① ② ③ ④ ⑤	① ② ③ ④ ⑤
2ii) 2 days ago	① ② ③ ④ ⑤	① ② ③ ④ ⑤	① ② ③ ④ ⑤	① ② ③ ④ ⑤
2iii) 3 days ago	① ② ③ ④ ⑤	① ② ③ ④ ⑤	① ② ③ ④ ⑤	① ② ③ ④ ⑤
2iv) 4 days ago	① ② ③ ④ ⑤	① ② ③ ④ ⑤	① ② ③ ④ ⑤	① ② ③ ④ ⑤
3. Is this pattern typical of your recent use of [DRUG]	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes
4. Was there any time in the last 4 days when you took fewer PILLS per dose (time) than were prescribed?	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes
5. When was the last time you skipped any of your medications?	☐ Never skip medications → Skip to question 7 ☐ Within the past week ☐ 1-2 weeks ago ☐ 3-4 weeks ago ☐ 1-3 months ago ☐ More than 3 months ago			

Antiretroviral Medication Adherence Form

Participant ID BMD - _____

Visit Date --

6. People miss taking their medications for various reasons. How often have you missed your current medications because you:	Never	Rarely	Sometimes	Other
a) Were away from home?	☐	☐	☐	☐
b) Were busy with other things?	☐	☐	☐	☐
c) Simply forgot?	☐	☐	☐	☐
d) Had too many pills to take?	☐	☐	☐	☐
e) Wanted to avoid side effects?	☐	☐	☐	☐
f) Did not want others to notice you taking medications?	☐	☐	☐	☐
g) Had a change in daily routine? (e.g., vacation, holiday, non-work day)?	☐	☐	☐	☐
h) Felt like the drug was toxic or harmful?	☐	☐	☐	☐
i) Fell asleep/slept through dose time?	☐	☐	☐	☐
j) Felt sick or ill?	☐	☐	☐	☐
k) Felt depressed or overwhelmed?	☐	☐	☐	☐
l) Had problems taking the pills?	☐	☐	☐	☐
m) Ran out of pills?	☐	☐	☐	☐
n) Don't want to take pills?	☐	☐	☐	☐
o) Have special instructions that conflict?	☐	☐	☐	☐
p) Other specify:	☐	☐	☐	☐

7. Most medications need to be taken on a schedule, such as "2 times a day" or "3 times a day" or "every 8 hours." How closely did you follow your specific schedule over the last four days?

☐ Never
☐ Some of the time
☐ About half of the time
☐ Most of the time
☐ All the time

8. Do any of your medications have special instructions, such as "take with food" or "on an empty stomach" or "with plenty of fluids"?

☐ Yes
☐ No, skip to item 10

Antiretroviral Medication Adherence Form

Participant ID BMD - _____

Visit Date --

9. If Yes, how often did you follow those special instructions over the last **four** days?

- ☐ Never
- ☐ Some of the time
- ☐ About half of the time
- ☐ Most of the time
- ☐ All the time

10. How do you remember to take your medications?

- ☐ Calendar/diary
- ☐ Pill box
- ☐ Alarm
- ☐ Friend/family member
- ☐ Memory only
- ☐ Other (specify):

MISSED VISIT FORM

VISIT CODE .

Participant ID BMD - _____ - _____

Visit Date: --
dd mmm yy

--

1. Target visit date (dd-mmm-yy):

2. Reason visit was missed. *Mark only one*

- ☐ 2a. unable to contact participant
- ☐ 2b. unable to schedule appointment(s) within allowable window
- ☐ 2c. participant refused visit
- ☐ 2d. participant incarcerated
- ☐ 2e. participant admitted to a health care facility
- ☐ 2f. participant withdrew from the study → **complete termination form**
- ☐ 2g. participant deceased → **complete termination form**
- ☐ 2h. other, specify: _____
- ☐ 2i. participant relocated

3. Who provided this information?

- ☐ 3a. Participant
- ☐ 3b. Partner
- ☐ 3c. Close family member (specify): _____
- ☐ 3d. Neighbor
- ☐ 3e. Friend
- ☐ 3d. Other (specify): _____

Comments: _____

Clinician's initials & date _____ / _____

QC I's initial's & date _____ / _____ QC II's initials & date _____ / _____

TERMINATION FORM

VISIT CODE .

Participant ID BMD_TDF - ____

Visit Date: --
dd mmm yy

--

1. Termination date: (*dd-mmm-yy*):

2. Reason for termination. **Mark only one.**

- ☐ 2a. scheduled exit visit/end of study → **End form**
- ☐ 2b. unable to schedule appointment(s) within allowable window
- ☐ 2c. participant refused further participation, specify: _____
- ☐ 2d. participant relocated, no further follow-up planned
- ☐ 2e. unable to contact participant
- ☐ 2f. inappropriate enrollment
- ☐ 2g. death, *indicate date and cause if known* → **complete termination form**

2g1. date of death -- **OR** cause unknown

2g2. Cause of death _____ **OR** date unknown

- ☐ 2h. investigator decision, specify: _____
- ☐ 2i. other, specify: _____

Comments: _____

Clinician's initials & date _____/_____

QC I's initial's & date _____/_____ QC II's initials & date _____/_____

Thailand (EA/P)	Wattanachanya <i>et al.</i> [54]	Cross-sectional analysis of prospective study, 2010–2011	220 HIV-positive ART-naïve patients (mean CD4 348 cells/μl)	46.8	Male 39 ± 6; female 39 ± 4	n/a	LS, TH or FN 20.9% (men), 23% (women)	LS, TH or FN 0% (men), 2.3% (women)	No difference in BMD was found between HIV-positive patients and controls (Hologic GDR4500).
South Africa, India, Thailand, Malaysia, Argentina	Martin <i>et al.</i> [12]; Haselberger <i>et al.</i> (second-line trial) [55]	Prospective study, 2010–2011	233 healthy controls 210 HIV-infected patients failing their first line ART with current CD4 202 (104–307) cells/μl; median duration of ART use 3.4 years; AZT 34%, d4T 48%, TDF 17% at baseline	52.4	Male 40 ± 6; female 41 ± 5	n/a	LS 16.9% (men), 26.7% (women) TH 19.7% (at baseline) FN 1.5% (at baseline)	LS 0.7% (men), 0.8% (women) TH 5% FN 1.5% (at baseline)	Reduced BMD was associated with longer duration of TDF and low BMI. An NRTI-sparing ARV regimen of LPV/r and raltegravir is associated with less bone loss than a LPV/r regimen containing NRTIs. BMD decrease was greatest at 48 weeks with stabilization to week 96, but no recovery. (Lunar-India, Malaysia, Argentina, Thailand or Hologic-Thailand, South Africa.)
High-income countries (HICs)									
USA1 (NA)	Battalora <i>et al.</i> (HOPS and SUN study) [56]	Cross-sectional analysis of prospective study, 2004–2012	1006 HIV-infected patients with median CD4 ⁺ 461 cells/μl (96.6% on ART, 67% non-Hispanic White)	17	43 (36, 49)	FN 40%	FN 36%	FN 4%	During 4,068 person-years of observation, 85 incident fractures occurred, predominantly rib/sternum, hand, foot, and wrist. Osteoporosis and current/prior tobacco use were associated with incident fracture. (Lunar or Hologic, reference standard: NHANES III database.)
UK ^a (EU/CA)	Short <i>et al.</i> [57]	Cross-sectional study, May to August 2008	168 HIV-infected patients (63% on ART; PI 27%, NNRTI 45%, NRTI included TDF 47%)	0	45 (38, 51)	n/a	LS, TH, or FN 58%	LS, TH, or FN 12%	Number of fractures since HIV diagnosis was increased among those with osteoporosis. Duration of infection > 13 years was associated with osteoporosis. (Hologic GDR4500C).

(Continued)

Table 1 (Continued)

Country (region)	Reference	Type of study	Patients	% Women	Age, mean or median (SD or IQR)	Prevalence* of			Key findings or Remarks (DXA machine)
						Low BMD	Osteopenia	Osteoporosis	
Italy (EU/CA)	Mazzotta <i>et al.</i> [58]	Cross-sectional study, April 2009 to March 2011	163 HIV-infected patients (79.7% on ART; Pls 59.2%, TDF 70%)	29.4	44.2 ± 10	LS or TH; 63.2%	LS or TH; 49.7%	LS or TH; 13.5%; LS or TH 19.6% (Z-score ≤ -2)	Low BMD was associated with lower BMI, AIDS diagnosis, HCV co-infection, ART, and nontraumatic fractures (NTBFs). Prevalence of NTBFs was 27.0%, predictors: male sex, HCV co-infection, lower FN Z-scores. (Hologic QDR 4500A.)
The Netherlands (EU/CA)	Kooij <i>et al.</i> [59]	Cross-sectional study, 2010–2012	581 HIV-positive patients (94.7% on ART; NRTI/TDF 96.4/77.1%, PI 43.6%, NNRTI/NVP 60.4/30.2%)	11.5	52.7 (48.3, 59.4)	n/a	LS 34%, FN 43%, TH 29%	LS 11% FN 4%, TH 2%	Low BW was negatively associated with BMD in HIV-positive persons. Regardless of HIV status, younger MSM had lower BMD than older MSM, heterosexual men, and women. (Hologic QDR 4500 W, the reference standard; NHANES database.)
			520 HIV-negative controls	15.2	52.0 (47.9, 58.0)	n/a	LS 35%, FN 34%, TH 16%	LS 6% FN 1%, TH 0%	
Japan (EA/P)	Kinai <i>et al.</i> [60]	Cross-sectional study, February 2012 to June 2013	184 HIV-infected men (93% on ART; Pls 64%, TDF 62%), median CD4 493 cells/ μ l	0	43 (38, 51)	n/a	LS 46% FN 54%	LS 10% FN 12%	Low BMD was associated with long-term treatment with a PI and a low BMI. Patients who discontinued PI had a higher BMD than those who currently use PI at LS but not at FN. (Hologic QDR 4500 W.)
USA2 ^b (NA)	Overton <i>et al.</i> [61]	Cross-sectional analysis of prospective study, September 2011 to February 2012	165 HIV-infected patients on EFV/FTC/TDF regimen (33% non-Hispanic Black; median CD4 341 cells/ μ l)	9.7		At baseline			Authors evaluated vitamin D3 (4000 IU daily) plus calcium (1000 mg calcium carbonate daily) supplementation on bone loss associated with ART initiation. BMD loss in the first year after ART initiation may be minimized by calcium and vitamin D supplementation. (Specific DXA machine was not indicated.)
			Pre vitamin D/Calcium group (n = 79) Placebo group (n = 86)		36 (28, 47) 31 (25, 44)	LS 9%; TH 5% LS 10%; TH 6%	n/a	n/a	

USA3 ^c (NA)	Collier <i>et al.</i> [62]	Cross-sectional analysis of prospective study February 2011 to July 2012	210 HIV-positive patients (40% African)	41	39 (33, 46)	At baseline: LS 24.3%, TH 13.8%, FN 23.8% LS 12.5%, TH 5.7%, FN 11.7%	n/a n/a	n/a	HIV was independently associated with lower BMD at femoral neck, total hip and lumbar spine. Lunar Prodigy DXA (GE Medical Systems, Madison, Wisconsin, USA.)
Mixed LMICs and HICs									
Australia, Belgium, Brazil, India, Ireland, Peru, South Africa, Spain, Thailand, UK, US	Carr <i>et al.</i> (STARTBone Mineral Density Substudy) [63]	Cross-sectional analysis of prospective study, June 2011 to June 2013	424 ART-naïve participants with mean CD4 688 ± 152 cells/μl	26	34 ± 10.1	LS, TH or FN 35.1%, FN 18.8%	n/a	LS, TH or FN 1.9%, FN 0.5%	Lower BMD was associated with female sex, Latin/Hispanic ethnicity, lower BMI and higher estimated GFR. Longer time since HIV diagnosis was associated with lower TH BMD, but not with CD4 cell count or viral load. [Lunar or Hologic, reference standard: NHANES III database (hip) and Hologic's reference data (spine).]

Data shown in the table include published articles and abstracts related to prevalence of low BMD in HIV-infected adolescents or adults from RLS in 2014 and 2015 plus articles of special interest from 2013. However, for RRS, only the articles published in mid-2014 to 2015 and had more than 100 HIV-infected participants were included. BMD, bone mineral density; FN, femoral neck; HICs, high-income countries; LMICs, low-middle-income countries; LS, lumbar spine; n/a, not available; TH, total hip; VL, viral load.

^aBMD was assessed by DXA either central or peripheral sites. In most studies, low BMD, osteopenia, and osteoporosis were defined as T-score < -1, T-score between -2.5 and -1, and T-score less than -2.5, respectively.

^bIn this study, low BMD was defined as Z-score < -2 at LS or TH.

^cIn this study, osteopenia and osteoporosis were defined as T- or Z-score < -1 and T- or Z-score < -2.5, respectively.

^dIn this study, low BMD was defined as T-score < 1 in those older than 40 years or Z < 2.0 in those younger than 40 years, respectively.

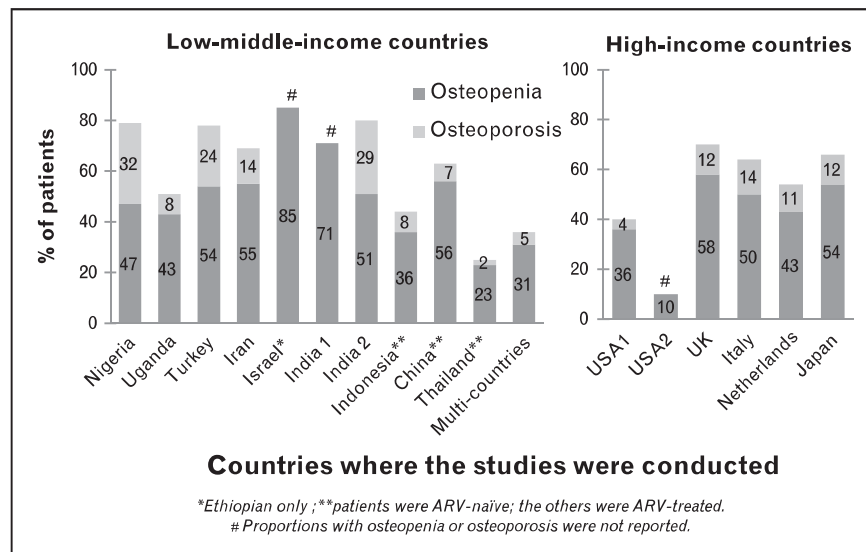


FIGURE 1. Proportion of HIV-infected patients with low bone mineral density, osteopenia, and osteoporosis. Overlapping prevalence of osteopenia and osteoporosis in low-/low-middle-income countries and high-income countries was found, with a generally higher prevalence of low bone mineral density in low- to middle-income countries overall compared to high-income countries.

dysfunction that results in excessive glomerular filtration, renal tubular acidosis phosphate loss [83] and possible impairment in vitamin D hydroxylation [75,76,80,86–94].

In RLS, the two WHO recommended first line ART treatment regimens for adults and children above 15 years contain TDF; TDF, lamivudine (3TC) and EFV or TDF, emtricitabine (FTC), and EFV, which exposes many HIV-infected individuals to the negative impact of TDF on bone health [3,95]. Conversely, there are scarce data on the effect of TDF-based ART on BMD in these settings. Martin *et al.* [55] reported that HIV-infected patients who initiated a second-line regimen had a greater bone loss if they were on TDF for longer duration during the 48 weeks of the study. For every 1 year of TDF use, the femur BMD reduced by 1.58% and spine BMD by 1.65% ($P < 0.001$).

Vitamin D and bone health in HIV

Worldwide, it's estimated that more than one billion people are characterized as having vitamin D deficiency (<20 ng/ml), or insufficiency (<30 ng/ml) regardless of the economic setting. According to a recent review by Mansueto *et al.* [96] the prevalence of vitamin D deficiency among HIV-infected individuals in both RLS and RRS varies widely across studies ranging from 25 to 93%, with an overall prevalence of 70.3 to 83.7%. Similarly, our review yielded high but similar prevalence of low vitamin D among HIV individuals regardless of ART use in both RLS [50,53,97–102] and RRS [61,103–109] with insufficient levels of up to 90% in Turkey [50] and

the USA [103], Belgium [108], Spain [109] (Table 2 and Fig. 2). The authors ascribed the high prevalence of vitamin D deficiency seen among Turkish [50], and Israeli [53] to skin coverage with resultant reduced sunlight exposure. Among individuals on ART, several cross-sectional studies from both RRS and RLS have shown an association between EFV use and low 25-hydroxyvitamin D (25(OH)D) [30,97,104,110–113]. NNRTIs, especially EFV which are widely used to treat HIV infection in RLS are hypothesized to enhance 25(OH)D catabolism through the induction of cytochrome P450 enzymes (CYP24A) [112] which reduce 25(OH)D concentrations. Among HIV-infected individuals, vitamin D insufficiency has been associated with a higher risk of HIV disease progression, death and virologic failure after ART [96,114]. In addition, vitamin D deficiency has been reported to independently increase the risk of low BMD [115]. In view of this, supplementation with vitamin D has been reported to mitigate bone loss [61,105]. In a recent randomized trial Overton *et al.* [61] found that BMD loss in the first year after ART initiation may be minimized by calcium and vitamin D supplementation D. By way of contrast, none of the studies we reviewed supported an association between vitamin D insufficiency and low BMD [61]. Though a cross-sectional study by Shahar *et al.* [53] among HIV-infected Israeli women of Ethiopian and Caucasian origin reported lower levels of BMD among vitamin D deficient individuals, there findings were limited by the small sample size in addition to lack of an HIV-uninfected control group. Larger studies with a suitable comparison of HIV-uninfected controls are

Table 2. Prevalence of low vitamin D in HIV-infected individuals in low- to middle-income countries versus high-income countries

Country (region)	Reference	Type of study	Patients	% Women	Age (mean or median; SD, IQR)	Results	Remarks
Low-middle-income countries (LMICs)							
Turkey (EU/CA)	Aydin <i>et al.</i> [50]	Cross-sectional study, June to October 2010	96 HIV-infected patients (80.2% on ART)	18	40.1 (range, 20–70)	Patients on ART: 14.3% had 25(OH)D <10 ng/ml, 67.5% had 25(OH)D 10–20 ng/ml; patients without ART: 15.8% had 25(OH)D <10 ng/ml, 73.7% had 25(OH)D 10–20 ng/ml	25(OH)D levels were low in women with veiled dressing style. No relation between low BMD and 25(OH)D levels.
Israel (ME/NA) (Ethiopian origin and Caucasian origin)	Shahar <i>et al.</i> [53]	Cross-sectional study, Summer 2009	43 HIV-infected Ethiopians (mean CD4 233 cells/ μ l, 82% on ART, 20 study participants on PI) 32 HIV-infected Caucasians (mean CD4 264 cells/ μ l, 64% on ART, 21 study participants on PI)	100	35.9 $\pm$ 8.2	65% had 25(OH)D <10 ng/ml 16.6% had 25(OH)D 10–20 ng/ml 6.25% had 25(OH)D <10 ng/ml 15.6% had 25(OH)D 10–20 ng/ml	PIs used were LPV/r, zidovudine/r, IDV. All participants were living in Israel for at least 10 years. Significantly more Ethiopian than Caucasian women covered their face and hands.
Botswana (SSA)	Steenhoff <i>et al.</i> [97]	Prospective study, December 2011 to April 2012	60 HIV-infected study participants (PI 25%, EFV 33%, NVP 42%, TDF/ NNRTI 13%)	50	19.5 $\pm$ 12	At baseline: 5% had 25(OH)D <20 ng/ml 26.5% had 25(OH)D 20–31 ng/ml. Mean 25(OH)D was 36.5 ng/ml in 4000 IU group and 34.5 ng/ml in 7000 IU group. At 12 weeks: 1.5% had 25(OH)D <20 ng/ml 16.8% had 25(OH)D 20–31 ng/ml. Mean 25(OH)D was 54.8 ng/ml in daily 4000 IU group and 56.5 ng/ml in daily 7000 IU group.	Δ 25D was two-fold higher in study participants on EFV or NVP compared to those on PIs. At 6 weeks, both NNRTI regimens resulted in greater Δ 25D than those on PIs. Study participants on TDF did not differ in Δ 25D from study participants on other regimens.

(Continued)

Table 2 (Continued)

Country (region)	Reference	Type of study	Patients	% Women	Age (mean or median; SD, IQR)	Results	Remarks
Thailand1 (EA/P)	Chokephaibulkit et al. [98]	Cross-sectional study, October 2010 to February 2011	101 perinatally HIV-infected adolescents on ART (NNRTI-based: NVP 30%, EFV 20%, and PI-based: 50%), median CD4 646 cells/ μ l	50	14.3 (13, 15.7)	Median 25(OH)D was 24.8 ng/ml, 24.7% had 25(OH)D <20 ng/ml, 46.5% had 25(OH)D 20–30 ng/ml	No associations between vitamin D deficiency and BMD, BMD, EFV use, HIV RNA, CD4, or self-reported sunlight exposure were observed.
Thailand2 (EA/P)	Avihingsanon et al. [99]	Cross-sectional analysis of cohort, July 2010 to June 2011	673 HIV-infected adults (93% on ART; EFV 31%, TDF 79% and 57% of patients had previously used d4T), median CD4 571 cells/ μ l	47	41.5 (37.2, 47)	40.6% had 25(OH)D <20 ng/ml, 29.9% had 25(OH)D 20–30 ng/ml	Female sex, age >37 years, and EFV use were independent predictors of hypovitaminosis D.
Thailand3 (EA/P)	Aurpibul et al. [100]	Cross-sectional study, March to September 2011	80 perinatally HIV-infected children on ART (NVP-based 55%, EFV-based 31%, PI-based 14%), median CD4 784 cell/ μ l	56	12.2 (9.1, 14.3)	Median 25(OH)D was 33.5 ng/ml, 10% had 25(OH)D <20 ng/ml, 33% had 25(OH)D 21–29 ng/ml	Only geographic location was significantly associated with low vitamin D level.
Brazil 1 (LA)	Sales et al. [101]	Cross-sectional study, August 2011 to December 2013	32 HIV-infected women (most were on ART but type of ART was not indicated) 66 HIV-infected men	100 0	41.7 39	15.63% had 25(OH)D <10 ng/ml; 65.63% had 25(OH)D 11–29 ng/ml 18.75% had 25(OH)D >30 ng/ml; 12.12% had 25(OH)D <10 ng/ml, 71.43% had 25(OH)D 11–29 ng/ml, 15.31% had 25(OH)D >30 ng/ml	Factors related to the virus itself and to the use of ART may have contributed for the low vitamin D levels.

Brazil 2 (LA)	Canuto <i>et al.</i> [102]	Cross-sectional study, September 2013	125 HIV-infected patients (83.2% on ART but type of ART was not indicated)	51.2	40.3 ± 11	Mean 25(OH)D was 39.3 ng/ml; 1.6% had 25(OH)D ≤ 20 ng/ml; 22.4% had 25(OH)D 21–29 ng/ml	Higher 25(OH)D levels were associated with female sex, no use of sunscreen, and previous opportunistic infections. Lower values were associated with the use of ART, overweight and obesity.
High-income countries							
USA 1 (NA)	Schwartz, Moore <i>et al.</i> [103]	Cross-sectional study, October 2009 to January 2010	507 HIV-negative study participants 358 HIV-positive ART naive patients 893 HIV-positive patients on ART (PI 61%, NRTI 98%, NNRTI 26%)	100 100 100	41.3 (33.6, 48.7) 42.9 (36.3, 49.6) 44.9 (39.3, 50.7)	Median 25(OH)D was 14 ng/ml; 72% had 25(OH)D < 20 ng/ml; 18% had 25(OH)D 20–30 ng/ml; Median 25(OH)D was 14 ng/ml; 70% had 25(OH)D < 20 ng/ml; 20% had 25(OH)D 20–30 ng/ml; Median 25(OH)D was 17 ng/ml; 57% had 25(OH)D < 20 ng/ml; 24% had 25(OH)D 20–30 ng/ml	Vitamin D levels were lower if ART included efavirenz (15 vs. 19 ng/ml, $P < 0.001$).
USA 2 (NA)	Hidron <i>et al.</i> [104]	Cross-sectional study, 2007–2010	933 HIV-infected patients (82% on ART; TDF/EFV 31.6%, TDF without EFV 29.9%, no TDF 20.6%) 5355 HIV-negative study participants	2.5 13.1	50 (range, 24–86) 63 (22–97)	Median 25(OH)D was 19 ng/ml; 53.2% had 25(OH)D < 20 ng/ml Median 25(OH)D was 24 ng/ml; 38.5% had 25(OH)D < 20 ng/ml	Risk factors for vitamin D deficiency in HIV-positive patients included black race, winter season and higher GFR, increasing age and TDF use.

(Continued)

Table 2 (Continued)

Country (region)	Reference	Type of study	Patients	% Women	Age (mean or median; SD, IQR)	Results	Remarks
USA 3 (NA)	Lake <i>et al.</i> [105]	Cross-sectional analysis of prospective study; June 2010 to April 2011	122 HIV-infected patients on ART (PI 34%, NNRTI 58%, raltegravir 17%, TDF 80%, ABC 29%), mean CD4 520 cells/ μ l	5	49 (41, 55)	Median 25(OH)D was 20 ng/ml; 67.2% had 25(OH)D <30 ng/ml	After 12 weeks of vitamin D supplementation (vitamin D3 50000 IU twice weekly for 5 weeks, then 2000 IU daily), 81% of insufficient persons achieved 25OHD $\geq$ 30 ng/ml. 25OHD repletion rates were comparable between HIV-positive patients and controls.
Australia (EA/P)	Klassen <i>et al.</i> [106]	Cross-sectional study, January 2008 to December 2012 May 2009 to April 2010	997 HIV-infected patients (66% on ART; EFV 24%, NNRTI $\pm$ PI 25%, no NNRTI/PI 17%) 3653 HIV-uninfected individuals	12 53	41 (32, 48) 50 (39, 61)	Mean 25(OH)D was 24.8 ng/ml; 40% had 25(OH)D <20 ng/ml; 71% had 25(OH)D <30 ng/ml Mean 25(OH)D was 27.6 ng/ml; 22% had 25(OH)D <20 ng/ml; 63% had 25(OH)D <30 ng/ml	Men, Caucasian country of origin, summer/autumn, total cholesterol to HDL ratio >5 and HIV infection were associated with vitamin D deficiency.
United Kingdom (EU/CA)	Gedela <i>et al.</i> [107]	Cross-sectional study, January 2008 to December 2009	253 HIV-infected ART-naïve study participants (64.4% were white and 35.6% were black or other ethnicity) with median CD4 450 cells/ μ l	18	36 (range, 16–75)	12.6% had 25(OH)D $\leq$ 10 ng/ml; 58.5% had 25(OH)D $\leq$ 20 ng/ml	Vitamin D deficiency was common among ART naïve patients, with those of nonwhite ethnicity at highest risk; no association was found with CD4 cell count, HIV viral load, and HIV clinical staging.

Belgium (EU/CA)	Theodorou <i>et al.</i> [108]	Retrospective study, December 2005 to March 2011	2044 HIV-infected study participants (73.4% on ART; EFV 15.8%, 2NRTI/NNRTI 23.2%, 2NRTI/PI 35.9%, second line 14.2%)	41.5	43 (range, 20–85)	Median 25(OH)D was 13.8 ng/ml, 32.4% had 25(OH)D <10 ng/ml, 89.2% had 25(OH)D <30 ng/ml	25(OH)D <30 ng/ml is associated with general factors (female sex, winter season) and specific factors related to HIV (duration of treatment, second line treatments with multiple and complex combinations of ART). 25(OH)D <10 ng/ml is associated with a low CD4 cell count, a higher CDC stage and EFV therapy.
Spain (EU/CA)	Bañón <i>et al.</i> [109]	Prospective study, 2012	365 HIV-infected patients (98% on ART; TDF/FTC 77%, the remaining on ABC/3TC, EFV 33%)	24	44 (range, 22–75)	At baseline: 15% had 25(OH)D <10 ng/ml; 48% had 25(OH)D 10–19.9 ng/ml; 26% had 25(OH)D 20–29.9 ng/ml	After calcitriol supplementation (oral monthly dose of 16 000 IU), 25(OH)D levels increased in comparison with nonsupplemented patients (+16.4 vs. +3.2 ng/ml; $P < 0.01$).

Data shown in the table includes published articles and abstracts related to prevalence of hypovitaminosis D in HIV-infected adolescents or adults from RLS in 2014 and 2015 plus articles of special interest ⁽⁺⁾ from 2013. However, for RRS, only the articles published in mid-2014 to 2015 and had more than 100 HIV-infected participants were included. HICs, high-income countries; LMICs, low- to middle-income countries; SSA, sub-Saharan Africa.

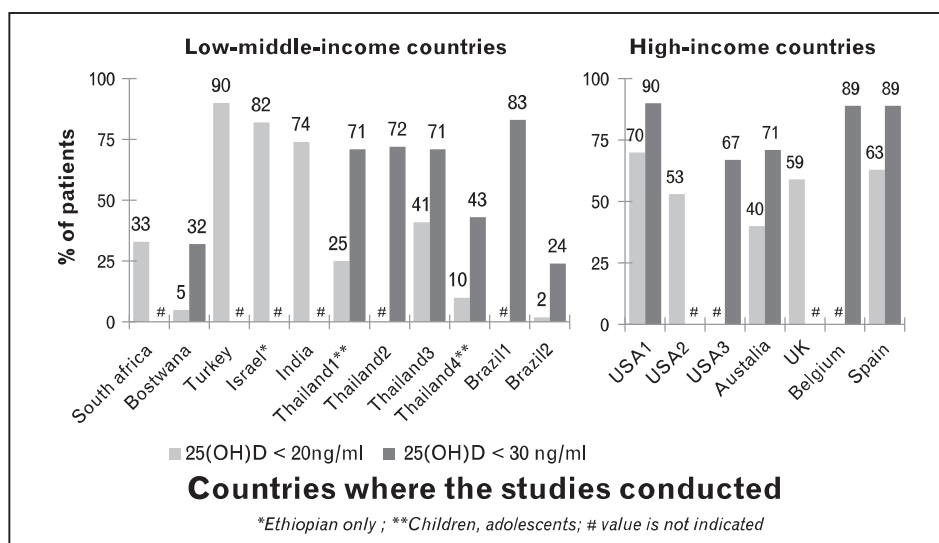


FIGURE 2. Proportion of antiretroviral-treated HIV infected patients with low vitamin D. The prevalence of low vitamin D among HIV infected individuals in both low-/low- to middle-income countries and high-income countries varies widely across studies, regardless of ART use, with insufficient levels of up to 90% in Turkey, the USA, Belgium, and Spain.

needed to quantify the association between vitamin D status and BMD or fracture risk in HIV populations in RLS, and whether vitamin D supplementation mitigates bone loss.

Bone health among HIV-infected young adult women

In RLS, the HIV burden among young adult women is high [2]. Women account for approximately 57% of the 34 million people living with HIV/AIDS. Most women living with HIV are of reproductive age [2], and the provision of reproductive health services is a crucial part of their HIV care. However, certain types of hormonal contraception have been associated with long-term metabolic dysregulation, particularly low BMD. In RLS with the highest unmet need for contraception, depot medroxyprogesterone acetate (DMPA) is the preferred contraceptive option across the different age groups [116] with approximately 15 million current users in the sub-Saharan African region alone [117]. Among HIV-infected women in particular, DMPA remains effective [118] because of its lack of interactions with antiretroviral drugs [119–121]. However, owing to its hypoestrogenemic effects [122], DMPA has also been associated with reduced BMD [123–130]. The few published observational studies on the association between DMPA and fracture risk in RRS suggest increased risk of fractures among DMPA users [131,132]. For example, a large population-based control study by Meier *et al.* [131] showed a 50% increased risk of incident fractures among 20 to

44-year-old European DMPA users receiving 10 or more injections compared to nonhormonal users, among those who had received 10 or more injections. It must also be noted that all the above studies were conducted among HIV-negative individuals. Our review did not yield any published data on the effect of DMPA on BMD or fracture risk among HIV-infected women either in RRS or RLS. This presents a critical gap in policy and clinical management guidelines for HIV infected women.

Bone health among HIV-infected adolescents

With the scale up of ART, more HIV-infected children are surviving into adolescence. In 2012, an estimated 2.1 million adolescents (10–19 years) were living with HIV in RLS [2,133], constituting over 95% of all HIV infections in this age group [2]. Although global data on ART coverage for adolescents are not available, the WHO ‘Early Release Guideline’ recommending initiation of ART in all individuals living with HIV, regardless of CD4 cell count raises further the number of adolescents in need of treatment. Perinatally infected individuals have the greatest cumulative life-time exposure to HIV and its treatment which results in increased risk of associated comorbidities, including possible reduced bone mass at a critical time of peak bone mass (PBM) accrual. Data show that a lower PBM in the young is a major determinant of subsequent osteoporosis and fracture in older adults [134–137]. Several studies from RRS support an independent, dose–response relationship between BMD and

risk of osteoporotic fractures [135,138–148]. For example, a 10% increase in PBM in young women is associated with an estimated 50% reduction in fracture risk after menopause [135]. Although there have been a few controversies among HIV individuals on ART [133,149], the general conclusion from a number of studies in RRS is that TDF treatment decreases BMD with stronger associations being seen in children and adolescents than in adults [64,150–152]. Thus, BMD may be more affected during the active period of bone growth and development. Among HIV-infected adolescents living in RLS, additional highly prevalent factors, including protein and energy malnutrition, micronutrient deficiencies, and childhood infections that are known to adversely affect bone mass accrual may pose additional threats to bone acquisition. To date, there are currently no published data in RLS where over 90% of infected adolescents live. This has inadvertently lead to lack of prevention and clinical management guidelines for this unique age group who may be at considerable risk of bone complications during a critical period of PBM attainment and subsequent lifelong ART exposure.

Constraints to diagnosis and management of bone loss in resource-limited settings

In 2015, 11 out of the 16 million people receiving ART globally were in the WHO Africa region alone [153]. However, unlike RRS where medications such as EFV are no longer preferred, and alternatives to TDF with less bone toxicity are likely to be more frequently used, there are currently no strategies in RLS for minimizing bone loss among HIV-infected individuals. The already limited funding, poor healthcare infrastructures, and sparse personnel pose tremendous challenges toward prevention and management of metabolic bone complications in RLS. As the standard assessment tool for BMD, DXA has only limited value as a single assessment. Serial assessments during HIV patient monitoring while on ART provide more information on the pattern of BMD changes [154]. In RLS, use of DXA scans in assessing BMD is limited by availability, cost, and training. In addition, once the diagnosis is obtained, the current cost of treatment medications for osteoporosis, for example, bisphosphonates is prohibitive. Furthermore, most healthcare personnel in most RLS lack the expertise to make appropriate diagnoses and provide relevant care.

Research needs

With more people starting ART [153] and living longer with HIV than ever before, more individuals

will continue to experience osteoporosis and its sequelae, including fragility fractures [155]. Given low clinical and research capacity for metabolic bone disease in RLS, there is urgent special need for building capacity in bone healthcare and research. Expanding knowledge about bone health in RLS will not only provide significant insights into the burden of HIV-related bone loss in RLS but also predictors, and evolution of bone metabolic comorbidities in the time course of HIV infection and its lifelong treatment. An initial focus is needed to establish the epidemiology of metabolic bone diseases in both the general and HIV populations. We recommend prioritization of the following research agenda in RLS:

- (1) Cost-effective and feasible strategies to prevent osteoporosis for both HIV-infected and non-infected populations.
- (2) Identification of simple-low cost tools to detect early osteopenia.
- (3) Strategies to minimize or avoid ARV-associated bone loss such as ART choice, dose optimization, and ARV switching.
- (4) Research among HIV-infected populations focusing on women of reproductive age and special populations such as perinatally infected children and adolescents.

To successfully conduct research addressing the above mentioned gaps in bone health comorbidities in RLS, there is need to work through several existing research networks either regionally or globally. This will ensure effective design and quality implementation approaches are employed. Importantly, involving key policy makers both domestically and regionally upfront will make the future policy implementation more successful.

CONCLUSION

The review reveals overlapping prevalence of low BMD in RLS and RRS, with a generally higher prevalence of low BMD in RLS overall compared to RRS. We highlight important gaps in our knowledge about HIV-associated bone health comorbidities in RLS. In particular, there are scarce data on bone health mainly from cross-sectional studies that call for urgent need for research that can inform management guidelines in metabolic bone complications in RLS.

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Conflicts of interest

There are no conflicts of interest.

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