Role of Menopause-Related Factors and Depression in Cardiovascular Disease Risk and Bone Loss in Postmenopausal Minority Women with HIV: A Secondary Analysis

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ABSTRACT

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An aging HIV population carries a magnified risk of degenerative conditions such as cardiovascular disease (CVD) and osteoporosis. Recent studies have demonstrated that the growing population of older HIV-infected women is at special risk for these complications. To what extent the metabolic consequences of menopause or other related disease predictors such as depression influence this gender disparity is not known. The overall goal of this dissertation is **to better characterize the etiology of low bone density and accelerated CVD in HIV-infected postmenopausal women.** Current gaps in knowledge surrounding women aging with HIV, particularly as it relates to CVD and bone loss, are discussed in Chapter One. Chapter Two reports findings from a systematic review of low bone density and fractures in HIV-infected postmenopausal women. To gain a better understanding of CVD risk in HIV-infected postmenopausal minority women, a cross-sectional study was conducted and is presented in Chapter Three. Quantitative findings from a longitudinal analysis of bone density in postmenopausal minority women with HIV are reported in Chapter Four. Finally, in Chapter Five, findings from the previous chapters are summarized and synthesized into a concluding chapter; implications and future recommendations are also discussed.

This dissertation was written following the format of the publication option, which includes versions of three full-length articles that have been submitted or will be submitted to peer reviewed journals for publication; two of the papers are data-based manuscripts and the third is a systematic review. The current status of these papers is as follows:

1. Chapter 2, (Paper #1)

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CHAPTER 1: INTRODUCTION

This chapter reviews the long-term complications of HIV infection and antiretroviral therapy, particularly cardiovascular disease and osteoporosis. The chapter evaluates the evidence for the potential additive effects of HIV and menopause. The impact of menopause and depression on cardiovascular disease and osteoporosis is also discussed. Additionally, a summary is provided of the dissertation's parent study. The chapter concludes by presenting the aims and hypotheses of this dissertation study, and its potential contributions to the current state of science on menopause-associated complications in women living with HIV.

HIV and Aging

With the development of highly effective antiretroviral therapy (ART), life expectancy has increased dramatically among people living with HIV. In addition, the incidence of HIV is increasing among older adults, with roughly 11% of annual HIV infections in the United States (U.S) occurring in people age ≥ 50 years (Prejean et al., 2011). Consequently, more than one-half of all persons living with HIV in the U.S (CDC, 2013a), and an estimated 4.2 million HIV-infected individuals worldwide (Mahy, Autenrieth, Stanecki, & Wynd, 2014), are aged 50 years and older. An aging HIV population is at greater risk of developing chronic conditions such as cardiovascular disease (CVD) and osteoporosis (Aberg, 2012). These age-related comorbidities may occur earlier in life in HIV-infected individuals compared with the general population (Guaraldi et al., 2011).

Cardiovascular Disease

CVD is a leading cause of death in HIV-infected individuals (D.A.D. Study Group et al., 2010). Persons living with HIV exhibit increased rates of multiple known risk factors for CVD including diabetes, body-fat abnormalities, dyslipidemia, hypertension and smoking (Grinspoon & Carr, 2005; Petrosillo & Cicalini, 2013; Worm et al., 2009). A number of studies have shown an increased risk of cardiovascular events in HIV-infected individuals compared to the general population (Bozzette, Ake, Tam, Change, & Louis, 2003; Armah et al., 2014). An observational study over a 13-year period found that the incidence of ischemic stroke was 5.27 per 1000 person years in HIV-infected patients compared with 3.75 in an uninfected comparison group (Chow et al., 2012). However, these previous studies were conducted mostly among HIV-infected men. Using a large data registry, a longitudinal analysis found that acute myocardial infarction rates were higher in HIV-infected persons, particularly women, after adjusting for traditional CVD

risk factors (Triant, Lee, Hadigan, & Grinspoon, 2007), but potential mechanisms for this sex-based difference were unclear. Studies of subclinical markers of CVD have found more advanced atherosclerosis in HIV-infected individuals, and at a younger age, than the general population (Currier et al., 2003). In addition, carotid-intima-media thickness, a measure of subclinical atherosclerosis, has been shown to be markedly greater in HIV-infected individuals even after controlling for age, race/ethnicity, and traditional CVD risk factors (Grunfeld et al., 2009; Hsue et al., 2004). While results from these studies suggest that HIV infection is an independent predictor of CVD risk, few studies have been conducted among older adult with HIV.

One widely studied hypothesis for the increased risk of CVD in HIV-infected individuals is the use of ART. While ART has improved survival among persons living with HIV, it is also associated with higher risk for age-related comorbidities and loss of potential years of life (Antiretroviral Therapy Cohort, 2008). Of specific concern is the use of non-nucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI), which have been associated with adverse lipid profiles known to increase risk of CVD (Friis-Moller, Weber, et al., 2003). In a cross-sectional study of age- and gender-matched HIV-infected individuals treated versus not treated with ART, patients on ART were twice as likely to be at high risk for coronary heart disease (Bergersen, Sandvik, Bruun, & Tonstad, 2004) according to a 10-year coronary heart disease risk-prediction measure. These findings highlight the importance of examining the effects of ART in any study of CVD in HIV-infected individuals.

HIV infection itself may contribute to atherosclerotic disease. HIV induces a state of chronic inflammation, which can be compounded by the aging process in older HIV-infected individuals. HIV infection is associated with elevated levels of inflammatory cytokines such as

C-reactive protein (CRP), interleukin-6 (IL-6), and tumor-necrosis-factor-alpha (TNFα), regardless of ART use and low HIV RNA levels (Neuhaus et al., 2010; Triant, Meigs, & Grinspoon, 2009). In a sample of HIV-infected and uninfected women, CRP and IL-6 levels were elevated in women with HIV (Dolan et al., 2005). Inflammatory markers, such as CRP have also been observed in advanced age (Sarkar & Fisher, 2006; Walston et al., 2002) and CVD (Hansson & Libby, 2006). Despite the potential association between inflammation and CVD, particularly in older adults, the mechanism by which HIV infection and aging increase risk of CVD remains unclear.

Osteoporosis

Osteoporosis is a skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in susceptibility to fractures (Christiansen, 1993; Christiansen & Riis, 1991). Osteoporosis is associated with functional impairment and lower health-related quality of life (Wilson, Sharp, & Davie, 2012). In the US, there are approximately 10 million people over the age of 50 years with osteoporosis and an additional 34 million with osteopenia (US Department of Health and Human Services, 2004). By 2025 the annual health care cost due to osteoporosis-related fractures is estimated to be $25 billion per year (Burge et al., 2007; Dempster, 2011).

Osteoporosis is a common complication of HIV and ART (Brown and Qaqish, 2006). Researchers have estimated an osteoporosis prevalence rate of 15% among HIV-infected individuals (Bonjoch et al., 2010; Dolan, Kanter, & Grinspoon, 2006; Fessel, Chau, & Leong, 2011), and a 58% higher fracture rate in persons with HIV compared to the general population (Shiau, Broun, Arpadi, & Yin, 2013). HIV infection is independently associated with reduced bone mineral density (BMD) in aging men (Arnsten et al., 2007) and women (Arnsten et al.,

2006). In younger HIV-infected individuals initiating ART, there is an initial period of short-term accelerated BMD loss followed by a longer period of BMD stability (Bolland, Wang, Grey, Gamble, & Reid, 2011). In older individuals, however, reduced BMD at the lumbar spine has been associated with duration of ART (Arnsten et al., 2007; Arnsten et al., 2006; Sharma, Cohen, Freeman, Santoro, & Schoenbaum, 2011). With HIV-infected individuals living longer on ART, there is a need to clarify the potential long-term morbidity related to ART.

As in CVD, persistent inflammation due to HIV infection and advanced age may lead to reduced BMD. Cytokine activation has been noted to increase risk of fractures (Ding, Parameswaran, Udayan, Burgess, & Jones, 2008). HIV infection is correlated with high levels of receptor of activated NF-Kb ligand (RANKL) and TNFα, which are associated with osteoclast differentiation and bone turnover (Wei, Kitaura, Zhou, Ross, & Teitelbaum, 2005). Elevated levels of RANKL have been observed in HIV-infected individuals (Brown, Ross, Storer, Labbato, & McComsey, 2011), suggesting the role of HIV infection in reduced BMD. Studies of bone in HIV have found that older age is associated with lower BMD regardless of ART use (Li Vecchi et al., 2012; Pinto Neto et al., 2011; Tsai et al., 2012).

Other factors associated with decreased BMD in HIV-infected individuals include lower body mass index (BMI), drug abuse, smoking, and menopause (Sharma et al., 2011; Yin, Kendall, et al., 2012). Studies have shown that HIV-infected postmenopausal women are at increased risk for osteoporosis compared with uninfected or premenopausal women (Anastos et al., 2007; Cazanave et al., 2008; Gomes et al., 2014). However, a common limitation across these studies was these assessment of postmenopausal status by self-report. Further information on the increased risk of low BMD and fractures in HIV-infected postmenopausal women is presented in Chapter 2.

HIV and Menopause

In 2010, over 55,000 HIV-infected women in the U.S were age ≥50 years, with over 75% of these women identifying as African-American or Hispanic (CDC., 2013a). An aging HIV population means that a greater number of HIV-infected women are now transitioning through menopause. Although HIV disproportionately affects minority women, they are especially underrepresented in HIV research.

Menopause, a time of waning ovarian function, rising follicular stimulating hormone (FSH) and diminished estrogen (Mack et al., 2004; Neer, 2010; Sutton-Tyrrell et al., 1998; Vaananen & Harkonen, 1996), leads to some of the same metabolic manifestations as HIV, including central adiposity, adverse lipid profiles, and insulin resistance (Looby, 2012). Existing research suggests that HIV-infected women transition through menopause at an earlier age (Schoenbaum et al., 2005) and with greater symptom severity (Looby et al., 2014). Notably, as with HIV, the menopause transition also increases the risk of CVD and osteoporosis (Atsma, Bartelink, Grobbee, & van der Schouw, 2006; Finkelstein et al., 2008).

Cardiovascular Disease

Every year, over 400,000 women die of CVD in the U.S (CDC, 2013b). Heart disease accounts for approximately 12% of deaths in women aged 35 to 44 years; this estimate rises to 25% among women aged ≥ 65 years (CDC, 2013b). There is evidence to suggest that endothelial function progressively worsens with reproductive aging (Moreau, Hildreth, Meditz, Deane, & Kohrt, 2012), eventually surpassing that of men (Go et al., 2013). The prevalence of atherosclerosis is higher in postmenopausal women compared to premenopausal women, independent of age (Witteman, Kok, van Saase, & Valkenburg, 1986). A rise in CVD risk following menopause was evident in the longitudinal, multi-site Study of Women Across the

Nation (SWAN), which found an elevation in both triglycerides and low-density lipoprotein levels 1-3 years after the last menstrual period (Woodard et al., 2011).

A recent analysis among HIV-infected women found that older age was independently associated with increased risk of CVD, but the impact of menopause-related factors was not explored (Womack et al., 2014). It has been proposed that the potentially additive inflammatory effect of HIV and estrogen deficiency may underlie the greater risk of CVD in older women with HIV (Deeks, 2011; Fan, Maslow, Santoro, & Schoenbaum, 2008; Kojic, Wang, & Cu-Uvin, 2007). Moreover, sex-based risk factors common at menopause (surgical menopause and depression) have been linked to higher rates of both CVD (Howard et al., 2005; Matthews, Chang, Sutton-Tyrrell, Edmundowicz, & Bromberger, 2010; Matthews, Gibson, El Khoudary, & Thurston, 2013) and bone loss (Cizza, Primma, Coyle, Gourgiotis, & Csako, 2010; Wu, Magnus, Liu, Bencaz, & Hentz, 2009; Yoshida, Takahashi, Yamatani, Takata, & Kurachi, 2011); yet, few studies to date of HIV-infected women have considered these potential confounds on health outcomes.

Osteoporosis

Beginning around age 30, bone mass declines at a comparable rate in men and women until menopause, a period of more rapid bone loss in women (O'Flaherty, 2000). Among individuals age 50 and older, lifetime risk of osteoporotic fractures is 40–50% in women and 25% in men (Johnell & Kanis, 2005). In addition, bone loss accelerates in late perimenopause (Finkelstein et al., 2008), a period characterized by highly variable menstrual cycle length and amenorrhea of 60 days or longer (Harlow et al., 2012). Bone turnover markers significantly increase after menopause (Lofman, Magnusson, Toss, & Larsson, 2005) and remain elevated through late postmenopause (Garnero, Sornay-Rendu, Chapuy, & Delmas, 1996).

Risk of osteoporosis may be even higher in postmenopausal women with HIV. Among premenopausal women, previous studies found that fracture incidence and rate of change in BMD did not differ in HIV-infected women compared to uninfected controls (Dolan et al., 2006; Yin, Lu, et al., 2010). In contrast, HIV-infected postmenopausal women have a reported 2 to 6 times higher odds of low BMD compared with HIV-uninfected (Yin et al., 2005) or premenopausal controls (Anastos et al., 2007), suggesting an important influence of menopause itself on the course of HIV sequellae. A detailed evaluation of the evidence that menopause is an additional risk predictor for osteoporosis in HIV-infected women is provided in Chapter 2.

Menopause-related Factors of Cardiovascular Disease and Osteoporosis

Menopause, the cessation of menstruation, results in the loss of ovarian estrogen production, which negatively affects the cardiovascular system (Cauley et al., 2003) and bone (Garnero et al., 1996). Previous studies of CVD and osteoporosis in women have focused on markers of ovarian function including age at menarche (Canoy et al., 2015), age at menopause, parity (Murphy, Khaw, May, & Compston, 1994; Parikh et al., 2010), menopausal symptoms ((Salamone et al., 1998; Thurston, Sutton-Tyrrell, Everson-Rose, Hess, & Matthews, 2008), and natural versus surgically induced menopause (Parker, Jacoby, Shoupe, & Rocca, 2009). While HIV-infected women are now living longer and transitioning through menopause, the impact of menopause-related factors on CVD and bone loss has not been well established. This section focuses on two important risk factors for CVD and bone loss in HIV-infected older women: surgical menopause and depression.

Surgical Menopause

Surgical menopause, amenorrhea induced after the surgical removal of ovaries, may further compound the risk of CVD and osteoporosis (Hunter, 2012; Ozkaya et al., 2011).

Following surgical menopause, women have a greater risk of atherosclerosis compared to those after natural (spontaneous) menopause (Witteman, Grobbee, Kok, Hofman, & Valkenburg, 1989). In a meta-analysis of 18 studies, the risk of CVD was 2.62 times higher in women with bilateral oophorectomy, and 1.14 times higher in women with spontaneous menopause, when compared to premenopausal women (Atsma et al., 2006). At the same time, this excess risk of CVD has been reported regardless of hysterectomy with or without oophorectomy, possibly due to deficient estrogen levels brought on by impaired ovarian blood flow (Harlow & Ephross, 1995; Ritterband, Jaffe, Densen, Magagna, & Reed, 1963). Two large prospective studies, one in premenopausal women (Matthews et al., 2013) and a second in postmenopausal women (Howard et al., 2005), reported an association between adverse lipid profiles and hysterectomy with or without oophorectomy. However, as menopause stage was not confirmed with hormone evaluations in either study, this relationship remains unclear.

Low BMD and elevated levels of bone turnover markers have also been reported following surgical menopause (Bahar et al., 2011; Cheng et al., 2003; Duraes Simoes et al., 1995; Yoshida et al., 2011). While some studies suggest that hysterectomy with oophorectomy (Bahar et al., 2011; Yoshida et al., 2011) or without oophorectomy (Cheng et al., 2003; Duraes Simoes et al., 1995) effects bone metabolism and BMD, others report no such effect (Larcos, 1998; Vesco et al., 2012). Conclusions drawn from many of these studies are limited by a small sample size (approximately ≤ 50), cross-sectional study design, or the lack of medical records to confirm postmenopausal status and type of surgical procedures.

Depression

In the general population, depression differentially affects women more than men (Kessler et al., 1994; Oquendo et al., 2013). The menopausal transition has been associated with

increased risk for depression (Cohen, Soares, Vitonis, Otto, & Harlow, 2006; Freeman, 2010). In postmenopausal women, depression is associated with CVD death and all-cause mortality (Wassertheil-Smoller et al., 2004). Women with recurrent major depression are more likely to have coronary or aortic calcification, an indicator of CVD progression (Agatisa et al., 2005; Matthews et al., 2010), possibly due to the elevated levels of inflammatory markers (e.g., CRP, IL-6, TNFα) associated with depression (Baune et al., 2012; Dowlati et al., 2010). Current recommendations for prevention of CVD in women include a depression screening and an evaluation of Framingham Risk Score (FRS) (Mosca, 2007). In women with HIV, chronic depressive symptoms are significantly correlated with FRS (Schwartz et al., 2012), a CVD risk prediction model widely used in the general population to estimate the risk of developing CVD over a 10-year period (D'Agostino et al., 2008). While depression is a prevalent mental health disorder in HIV-infected individuals (Bing et al., 2001), and older women in particular (Cohen et al., 2006), the impact of depression on CVD risk exclusively in postmenopausal women with HIV has not been explored.

Depression is also an important predictor of osteoporosis. In a sample of perimenopausal women, depression was associated with lower BMD, although menopausal status was defined by self-report (Jacka et al., 2005). A meta-analysis of 14 studies conducted in men and women of all ages found a greater BMD decrease at the spine and hip in those with depression, but again, among studies in postmenopausal women, menopause was not carefully defined (Wu et al., 2009). Two review articles suggest that the discovery of a functional serotonin transporter in bone may help explain the link between depression and bone metabolism (Haney & Warden, 2008; Warden, Bliziotes, Wiren, Eshleman, & Turner, 2005). Higher cortisol serum concentrations and the sympathetic activation of pro-inflammatory cytokines may be other

important mediating factors of bone loss in depression (Altindag et al., 2007; Anderson et al., 2013). While estrogen is as an established regulator of both these neuroendocrine systems (Amin, Canli, & Epperson, 2005; Otte et al., 2005), how estrogen changes at menopause may influence bone loss via these mechanisms is not known.

Despite the prevalence of depression among HIV-infected individuals, and its recognition by the National Osteoporosis Foundation as a risk factor of osteoporosis (National Osteoporosis Foundation., 2013), current evidence is lacking on the role of depression on bone loss in people with HIV. A literature search revealed that just four articles on bone health in HIV-infected individuals reported prevalence of depression (Arnsten et al., 2006; Sharma et al., 2011; Sharma, Flom, Weedon, & Klein, 2010; Womack et al., 2013). Of these, two studies found no association between depression and bone (Sharma et al., 2011; Womack et al., 2013); however, self-reported depressive symptoms and menopause status were not confirmed by clinical criteria.

Overview of the Parent Study

This dissertation builds on a longitudinal comparison of bone loss in HIV-infected and uninfected postmenopausal women, 40 years of age and older, who were studied at two time points (baseline and follow-up). In the parent study, a number of demographic (race/ethnicity, age), anthropomorphic (body mass index) and lifestyle factors (alcohol use, smoking) were evaluated as moderators of disease burden (Yin, Zhang, et al., 2012). The prevalence of osteopenia at baseline was significantly higher in HIV-infected women compared to uninfected controls (Yin, McMahon, et al., 2010; Yin, Zhang, et al., 2012). The authors concluded that HIV was an independent predictor of bone loss in postmenopausal women (Yin, Zhang, et al., 2012).

HIV-infected subjects in the parent study carried significantly greater health burdens versus control participants. Approximately one-fifth of HIV-infected women were co-infected

with hepatitis C, 36% had a history of hypertension, and 20% reported a diagnosis of CVD. The impact of surgical menopause and depression risk factors on bone loss was not examined in the parent study. In addition, hepatitis C co-infection, which has been shown to elevate risk of CVD (Kakinami et al., 2013) and osteoporosis (Lawson-Ayayi et al., 2013; Lo Re et al., 2012; Maalouf et al., 2013), was not controlled for in the investigation. This dissertation research will address these gaps by examining the impact of HIV and menopause-related factors on bone. This dissertation will also better characterize CVD risk among HIV-infected postmenopausal women in the parent study.

Dissertation Aims and Hypotheses

Non-AIDS-defining illnesses, such as CVD and osteoporosis, are increasingly common among HIV-infected individuals. Given the growing population of older HIV-infected women, and the potential additive effects of HIV and menopause, there is a need to understand the etiology of CVD and bone loss in postmenopausal woman with HIV. Accordingly, to better characterize determinants of CVD and bone loss in older women with HIV, this dissertation carried out the following specific aims in HIV-infected and uninfected postmenopausal women:

Aim 1: To characterize and compare CVD risk, including the impact of menopause-related factors (surgical menopause and clinical depression), using the Framingham Risk Score as an assessment measure.

Hypothesis 1a: Surgical menopause will be associated with a higher (worse) Framingham Risk Score vs. natural menopause after adjusting for age, race/ethnicity, body mass index, and hepatitis C infection.

Hypothesis 1b: Diagnosis of depression will be associated with a higher (worse) Framingham Risk Score vs. no history after adjusting for age, race/ethnicity, body mass index, and hepatitis C infection.

Hypothesis 1c: Women with HIV will have higher (worse) CVD risk profiles, as assessed by the Framingham Risk Score, compared to women without HIV after adjusting for age, race/ethnicity, body mass index, HCV infection, cause of menopause, and diagnosis of depression.

Aim 2: To examine the impact of menopause-related risk factors (surgical menopause and clinical depression) on change in bone mineral density.

Hypothesis 2a: Surgical menopause will be associated with a greater decline in BMD vs. natural menopause after adjusting for age, race/ethnicity, body mass index, and HCV infection.

Hypothesis 2b: A diagnosis of depression will be associated with a greater decline in BMD vs. no history after adjusting for age, race/ethnicity, body mass index, and HCV infection.

Hypothesis 2c: HIV infection will remain an independent predictor of change in BMD after adjusting for age, race/ethnicity, body mass index, HCV infection, cause of menopause, and diagnosis of depression.

Theoretical/Conceptual Framework

This study was informed by the Women's Health Initiative (WHI) conceptual framework (Matthews et al., 1997) and the Strengthening the Reporting of Observational Studies in Molecular Epidemiology (STROBE-ME) guidelines presented by Gallo et al. (2011). The WHI framework is an explanatory model of disease outcomes specific to postmenopausal women.

According to the WHI, socio-demographic characteristics, individual differences, and the environment, influence health behaviors and intermediate biological outcomes. "Individual differences" include biological and psychological risk factors such as age at menarche, cause of menopause, BMI, depression, and chronic co-morbidities. Intermediate biological outcomes consist of documented biological markers of health (e.g. blood pressure and markers of pathogenesis or normal responses to therapy)**.** The biomarkers of interest in this study were HIV viral load, BMD, bone turnover markers, and reproductive hormone levels. The STROBE-ME guidelines describe the use of biomarkers along a disease pathway to identify individuals susceptible to disease, improve diagnosis, early detection of disease, or to predict clinical outcomes (Gallo et al., 2011). **Figure 1.1** presents an explanatory model for CVD and low BMD in HIV-infected postmenopausal women adapted from the WHI and STROBE-ME frameworks. Environmental factors will not be assessed in this study, due to the limitations of the dataset.

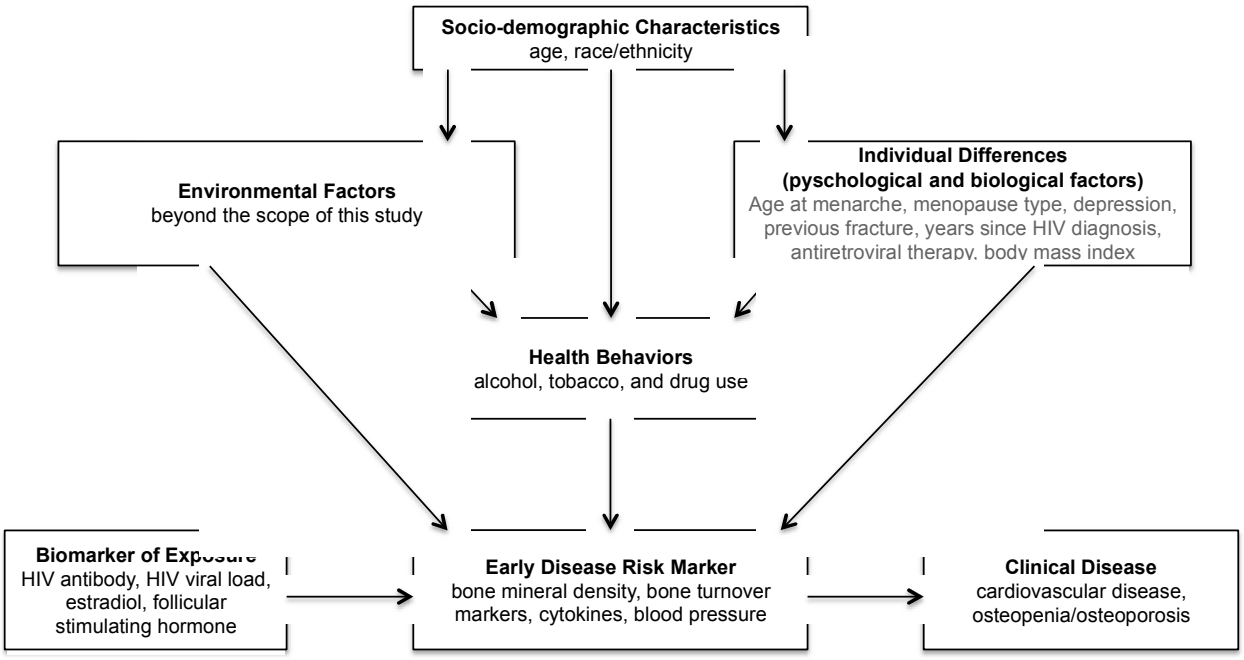

Figure 3.1. Explanatory model of sociodemographic and biobehavioral predictors of cardiovascular disease and osteoporosis in postmenopausal women with HIV. Model adapted from Matthews et al. (1997) and Gallo et al. (2011).

Potential Contributions

While HIV-infected older minority women account for approximately one-fifth of the growing HIV population age 50 years or older (CDC, 2013a), women as a group, and minority women in particular, continue to be underrepresented in HIV research. Few studies investigating the impact of HIV on bone health have been conducted exclusively among women, especially targeting the menopausal transition. Moreover, few studies have examined depression as a risk factor for low BMD and CVD in HIV-infected women, possibly due to the underdiagnosis of depression in persons with HIV (Asch et al., 2003). Limited research on older women with HIV may be due to the lack of multidisciplinary collaborations with women's health researchers, the recent growth of this HIV population, and the long follow-up necessary to understand the impact of the menopause transition on HIV. This dissertation fills the gap in the literature by focusing on postmenopausal minority women with HIV, guided by a multidisciplinary team of senior investigators.

The profound effect of HIV and menopause on metabolic and endothelial function provides compelling rationale for early identification and management of CVD and osteoporosis in this high-risk population. Despite a growing number of older women affected by HIV, sex-specific risk factors for HIV complications remain understudied. Whether additive effects of HIV and menopause contribute to aging-related comorbidities is unknown. This study has the potential to improve patient outcomes by identifying additional predictors of the impact of HIV on aging women's health.

Summary

In aging women, osteoporosis and cardiovascular disease are major public health problems, which begin to emerge at menopause. Certain subgroups of postmenopausal women,

including those with surgically induced menopause or depression, carry special risks for both diseases; however, these relationships are not well understood. Although aging-related comorbidities occur earlier in life in HIV-infected persons, sex-specific risk factors for HIV complications remain understudied. Minority women comprise a growing segment of the HIV-infected patient population. With the development of antiretroviral therapy, HIV-infected women are living longer and transitioning through menopause, which may result in accelerated disease burden. The overall goal of this dissertation is to better characterize the etiology of accelerated CVD and low bone density in HIV-infected postmenopausal women.

CHAPTER 2: BONE DENSITY AND FRACTURES IN HIV-INFECTED POSTMENOPAUSAL WOMEN: A SYSTEMATIC REVIEW

This chapter presents findings from a systematic review of the literature on bone density and fractures in HIV-infected postmenopausal women. The purpose of the review was to synthesize and critique the evidence that menopause is an additional risk predictor for osteoporosis and fractures in HIV-infected women. This manuscript has been accepted for publication in the *Journal of the Association of Nurses in AIDS Care*.

Abstract

With the development of effective antiretroviral therapy (ART), HIV-infected women are living longer and transitioning through menopause. The purpose of our study was to systematically examine the evidence that HIV-infected postmenopausal women are at increased risk for osteoporosis and fractures. Electronic databases were searched for studies of low bone density or fractures in HIV-infected postmenopausal women. Studies that met the inclusion criteria (n = 10) were appraised using a validated quality assessment tool. The majority of studies were rated as *good* quality and the remaining were *fair*. The prevalence of low bone density reported in these studies ranged from 20% to 84% and 5% to 64% in HIV-infected and uninfected postmenopausal women respectively. In the two qualifying studies, postmenopausal status was not a predictor of fractures in HIV-infected women. Findings suggest HIV care providers should accurately assess postmenopausal status and modifiable risk factors for osteoporosis in all older HIV-infected women.

Key words. bone mineral density (BMD), fractures, HIV, menopause

Introduction

Access to antiretroviral therapy (ART) has increased life expectancy among HIV-infected individuals. By 2020 roughly 70% of all people living with HIV (PLWH) will be ages 50 years and older (Karpiak, 2014). Not only is the aging HIV population at greater risk of developing comorbidities such as osteopenia and osteoporosis, but these conditions may also occur at a younger age (Guaraldi et al., 2011). Accelerated bone loss is a common complication of HIV infection and ART (McComsey et al., 2011). Studies have estimated an osteoporosis prevalence rate of 15% in PLWH (Bonjoch et al., 2010; Brown & Qaqish, 2006), and a 58% higher fracture rate compared to the general population (Shiau, Broun, Arpadi, & Yin, 2013). With the growing prevalence of HIV among people in middle and old age, there is an urgent need to better characterize the impact of HIV on osteoporosis.

From 2007 to 2010, the estimated number of adults ages 50 years or older living with HIV in the United States increased by nearly 47,000, with a growing proportion of women represented in this older group (Centers for Disease Control and Prevention, 2013). HIV-infected women face the additional consequences of estrogen withdrawal and deficiency during menopause transition, which give rise to some of the same metabolic alterations as HIV such as insulin resistance, elevated waist circumference, low high-density lipoprotein levels, and bone loss (Adeyemi, Rezai, Bahk, Badri, & Thomas-Gossain, 2008). Accordingly, menopause may accelerate HIV-related bone loss, and in turn, place HIV-infected postmenopausal women at greater risk for osteoporosis and associated fractures. While the National Osteoporosis Foundation (Cosman et al., 2013) added HIV to their list of conditions that cause or contribute to osteoporosis and fractures, the quality of the evidence on bone loss specifically in HIV-infected older women has not been previously examined.

Over the past several years, a number of reviews have been published on menopause-associated metabolic manifestations in HIV-infected women (Imai, Sutton, Mdodo, & Del Rio, 2013; Kojic, Wang, & Cu-Uvin, 2007; Looby, 2012). The evidence on the effect of ART on bone density in HIV-infected women has also been explored (Carvalho, Gelenske, Bandeira, & Albuquerque, 2010). However, to date, the quality of the literature on menopause-associated changes involving bone in HIV-infected women has not been systematically appraised. Therefore, to evaluate the evidence that HIV-infected postmenopausal women are at increased risk for accelerated bone pathology, we carried out a systematic review of the current literature.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement was used as a guideline (Moher, Liberati, Tetzlaff, & Altman, 2009). The PRISMA Statement consists of a 27-item checklist to ensure a standard method of transparent reporting of systematic reviews and meta-analyses **(Appendix A)**.

Search Strategy

The following electronic databases were thoroughly searched for all studies published through September 2014 on low bone density or fractures in HIV-infected postmenopausal women: Medline, Scopus, and Cumulative Index to Nursing and Allied Health Literature (CINAHL). A combination of the search terms “HIV infections”, “osteoporosis”, “bone density”, and “fractures” were used. The search was limited to English language peer-reviewed articles, and studies with postmenopausal women. A detailed outline of the search is provided in **Table 2.1**. Reference lists of retrieved articles were reviewed to identify additional relevant studies.

Table 2.1. Databases and search terms used in the search strategy.

Database	Years	Search Terms
Medline	Up to September 2014	exp "HIV infections" (MeSH Terms) AND exp "Bone density" (MeSH Terms) OR exp "Fractures, Bone" (MeSH Terms) OR exp "Osteoporosis, Postmenopausal" (MeSH Terms)
Scopus	Up to September 2014	Bone density OR Osteoporosis OR Fractures AND HIV infections
CINAHL	Up to September 2014	Bone density (MW) OR Osteoporosis (MW) OR Fractures (MW) AND HIV infections (MW)

Note. CINAHL = Cumulative Index to Nursing and Allied Health Literature; Exp = exploded; MeSH = Medical Subject Heading; MW = Word in Subject Heading

Eligibility Criteria

The titles and abstracts of all studies retrieved in the literature search were reviewed. No time limit was imposed and the last search was performed on September 17, 2014. Studies were eligible for analysis if they included a sample of HIV-infected postmenopausal women, and reported BMD or incident fracture data for HIV-infected postmenopausal women. Studies were included regardless of how postmenopausal was defined (e.g., amenorrhea for ≥ 12 consecutive months with out without confirmational hormone measures). Study selection was limited to BMD data obtained using dual-energy X-ray absorptiometry (DXA). DXA is a validated measure of BMD and meets the requirements of the World Health Organization diagnostic classification of osteopenia and osteoporosis (World Health Organization, 2004). Studies were excluded if they did not report any BMD or incident fracture data specifically for HIV-infected postmenopausal women.

Data Extraction

The following categories were used for data extraction: authors, year of publication, study design, sample size, number of HIV-infected participants, number of controls (if

applicable), and findings. When available, sample characteristics such as age, race/ethnicity, BMI, use of hormone replacement therapy (HRT), and ART exposure were also abstracted. Given that bone density and fractures were the outcomes of interest, information on DXA scan body site and fracture type were collected from each study.

Quality Assessment

The methodological quality of each study was assessed using a validated checklist developed by Downs and Black (1998). The checklist consists of 27 items, with a total possible score of 28 for randomized and 25 for nonrandomized health care interventions **(Appendix B)**. The checklist assesses quality across five domains: (a) how well study aims and procedures are reported in the paper, (b) external validity, (c) bias, (d) confounding, and (e) power. With the exception of confounding and power, each item is scored as "0" or "1", with a score of "1" indicating that the quality criterion is satisfied. One of the items on confounding is scored from "0" to "2", indicating the criterion is not satisfied (= 0), partially satisfied (=1), or fully satisfied (=2).

As in previously published reviews using the Downs and Black quality assessment tool (Gaynes et al., 2005; Samoocha et al., 2010; Uchida et al., 2013), the checklist was slightly modified for this review given that none of our included articles were interventional studies. Specifically, the item on statistical power was simplified to a score from "0" to "2" (0 = no power analysis conducted, 1 = power analysis conducted for one outcome measure, 2 = power analysis conducted for two or more outcome measures, if applicable). A quality score was calculated based on the number of quality assessment items satisfied. Items that did not pertain to the study were rated as "not applicable" and were not included in the overall score. Quality assessment scores were grouped into four quality ratings: 18 to 20 (*excellent*), 14 to 17 (*good),*

10 to 13 (*fair*), and less than 10 (*poor*).

Initially, two articles were independently assessed by two reviewers (YC, NR) and quality scores discussed. Any discrepancies were resolved by consensus. As there was no disagreement in scores, one reviewer (YC) assessed the remaining articles.

Results

Study Selection

The literature search identified 1,093 potentially relevant articles for review. After excluding duplicate records from the electronic databases, 646 titles and abstracts were screened for eligibility. Of these, 618 studies were excluded for two main reasons: postmenopausal women were not included in the sample, or the studies did not provide fracture or BMD data. This resulted in the retrieval of 28 articles for full-text review. Upon full-text review, 18 articles were excluded: 7 did not include HIV-infected postmenopausal women in the sample, 4 did not assess BMD or fractures, 3 studies did not specifically report BMD or fracture data for postmenopausal women, and 4 articles were duplicate reports. In total, 10 studies were included in the systematic review. **Figure 2.1** provides detailed results of the literature search and study selection process.

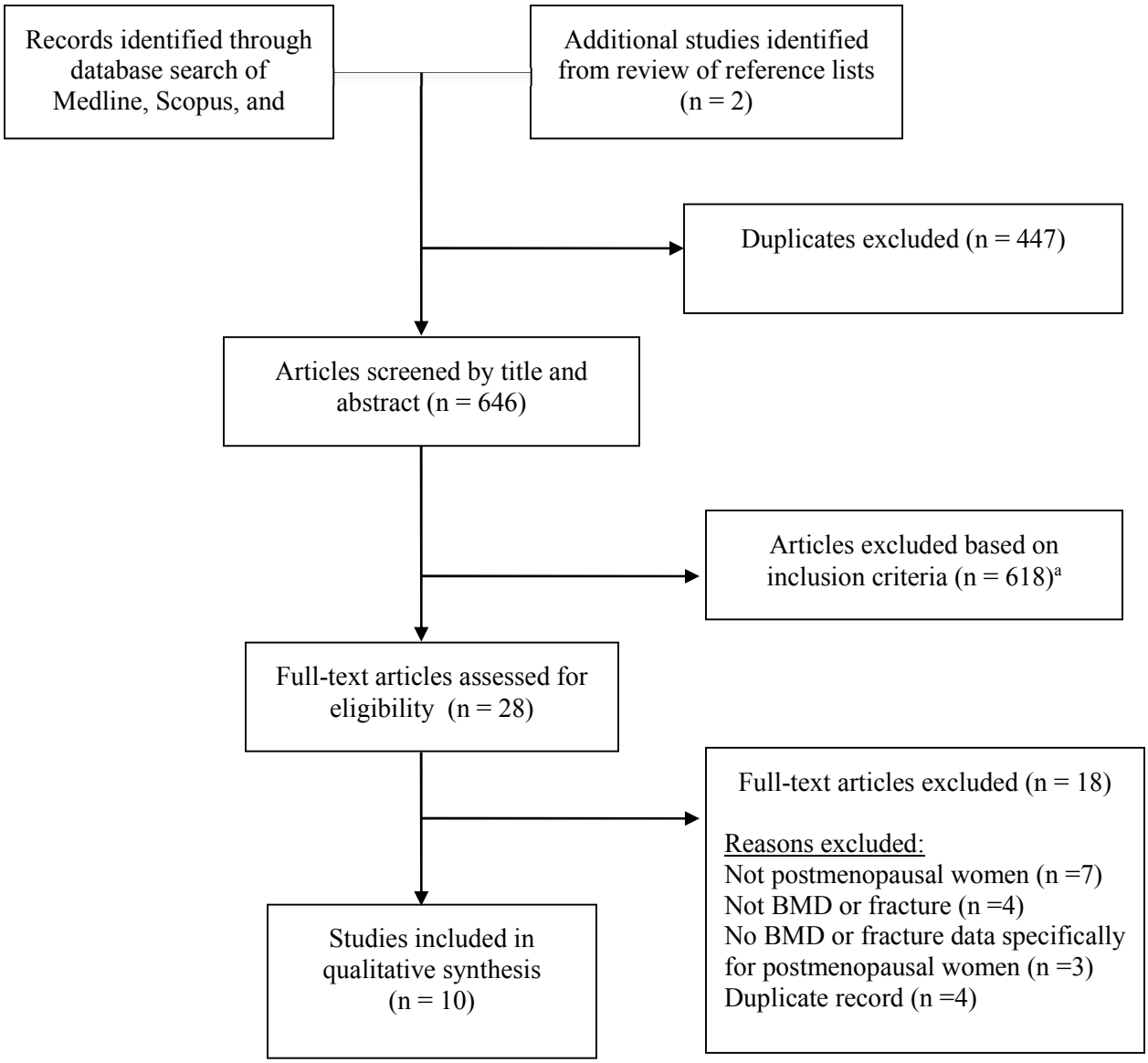

Figure 4.1. Flow diagram of study selection process. AMED = Allied and Complementary Medicine; CINAHL = Cumulative Index to Nursing and Allied Health Literature; BMD = bone mineral density.[a]Inclusion criteria: a) peer-reviewed English language articles, b) includes a sample of HIV-infected postmenopausal women, c) reports BMD or fracture incidence data for HIV-infected postmenopausal women, d) BMD data obtained using dual-energy X-ray absorptiometry.

The study characteristics of the 10 investigations included in the systematic review are presented in **Table 2.2**. Of these, six were conducted in the US, two in Brazil and one each in Italy and France. The majority of articles were cross-sectional in design (n = 6); the remaining were longitudinal cohort studies (n = 4). Eight of the 10 analyses of BMD or fractures compared HIV-infected postmenopausal women with either HIV-infected premenopausal (Cazanave et al., 2008; Jacobson, Spiegelman, Knox, & Wilson, 2008; Yin et al., 2012a) or HIV-uninfected postmenopausal women (Anastos et al., 2007; Li Vecchi et al., 2012; Sharma, Cohen, Freeman, Santoro, & Schoenbaum, 2011; Yin et al., 2010b; Yin et al., 2010a). Comparison groups in the remaining analyses included: HIV-infected pre- and perimenopausal women (Gomes et al., 2014), and HIV-infected men and premenopausal women (Pinto Neto et al., 2011). Five reports described sub-studies of larger investigations, including the Women's Interagency HIV Study, (Anastos et al., 2007; Yin et al., 2010a) the Menopause Study Cohort (Sharma et al., 2011), the ACTG Longitudinal-Linked Randomized Trial database (Yin et al., 2012a), and the ANRS CO3 Aquitaine Cohort (Cazanave et al., 2008). The primary outcome in three of the studies was incident fractures. Seven studies reported on BMD features.

One or more of the following sample characteristics were reported in each study: age, race/ethnicity, BMI, and ART exposure. Two studies reported current or past use of HRT (Yin et al., 2005; Yin et al., 2010a). Mean or median ages ranged from 38 to 59 years. Five studies included samples of both men and women (Cazanave et al., 2008; Jacobson et al., 2008; Li Vecchi et al., 2012; Pinto Neto et al., 2011; Yin et al., 2012a). Overall, nine studies reported data on race/ethnicity; in four cases, the majority of participants identified as 'Black' or 'African-

American' (Anastos et al., 2007; Jacobson et al., 2008; Sharma et al., 2011; Yin et al., 2010a). Participants were mostly overweight across studies (e.g., mean BMI between 25 to 28.5 kg/m^2).

The majority of the subjects in each report were on ART, ranging from 61% (Jacobson et al., 2008) to 100% (Yin et al., 2012a). In the single study that reported current or past use of HRT, rates were low: 7% (Yin et al., 2010a). While one of the selected investigations purposefully enrolled postmenopausal women with HIV (Yin et al., 2010b), the remaining studies included subsets of subjects meeting these criteria. The proportion of subjects meeting these criteria ranged from 5% (20/379) in the study by Jacobsen et al. (2008) to 15% (45/300) in Pinto Neto et al. (2011). Four studies defined postmenopause status as self-reported amenorrhea for at least 12 consecutive months (Anastos et al., 2007; Gomes et al., 2014; Sharma et al., 2011; Yin et al., 2010a). One study confirmed postmenopausal status using serum estradiol and FSH levels (Yin et al., 2010b).

Methodological Quality of Studies

Table 2.3 summarizes the methodological quality of studies using the checklist by Downs and Black (1998). The mean quality assessment score for included studies was 13.9 out of a possible score of 20. None of the studies fulfilled all of the quality criteria. The majority of studies (n= 6) were rated as 'good' and remainder of studies had 'fair' quality. Of the studies with the highest quality scores, two were cohort studies (Sharma et al., 2011; Yin et al., 2010a) and one was a cross-sectional analysis of a prospective cohort (Yin et al., 2010b). All studies scored well on the reporting criteria (7 to 9 out of a possible 9).

Table 2.2. Characteristics of studies included in the review (n = 10).

Author (Year)	Study Design	N (Total)	N (HIV+ PoM)	N[b] (Comparison)	Age (years)	Race/ Ethnicity (%)	BMI (kg/m^2)	% ART Exposure
Anastos et al. (2007)	Cross-sectional	426	73	125 (HIV+/PrM)	42.8 (mean)	61.3 B; 19.7 W; 19 L/H	28.0 (mean)	72.3
Cazanave et al. (2008)[a]	Cross-sectional	492	31	102 (HIV+/PrM)	41.0 (median)	--	38.8% with BMI < 20	93.1
Gomes et al. (2014)	Cross-sectional	273	111	162 (HIV+/PrM and PeriM)	47.7 (mean)	60.1 NW	48.3% with BMI ≥ 25	92
Pinto Neto et al. (2011)[a]	Cross-sectional	300	45	255 (HIV+/PrM and HIV+ men)	--	70.7 W	41.1% with BMI > 25	88.3
Yin et al. (2010b)	Cross-sectional	187	92	95 (HIV-/PoM)	57.8 (mean)	32 AA; 68 L/H	29 (mean)	79.3
Jacobson et al. (2008)[a]	Longitudinal cohort	379	20	76 (HIV+/PrM)	43 (median)	51 AA; 34.4 W; 14.6 O	29.2% with BMI ≥ 30	60.6
Li Vecchi et al. (2012)[a]	Cross-sectional	188	18	26 (HIV-/PoM)	47 (mean)	100 C	31.2% with BMI ≥ 25	93
Sharma et al. (2011)	Longitudinal cohort	620	46	41 (HIV-/PoM)	45 (mean)	57.6 B; 5.7 W; 31.4 L/H; 5.3 O	28.2 (mean)	87.8
Yin et al. (2010a)	Longitudinal cohort	2391	338	74 (HIV-/PoM)	40.4 (mean)	56.3 B; 13.3 W; 27.2 L/H; 3.2 O	28.5 (mean)	65.6
Yin et al. (2012a)[a]	Longitudinal cohort	4640	185 (PoM or PeriM)	477 HIV+/PrM	39 (mean)	28.7 B; 48 W; 20.4 L/H; 1.8 A; 1.2 O	25 (mean)	99.7

Note. A = Asian; AA = African-American; ART = antiretroviral therapy; B = Black; BMD = bone mineral density; BMI = body mass index; C = Caucasian; FN = femoral neck, L/H = Latino/Hispanic; HRT = hormone replacement therapy; LS = lumbar spine, NW = Non-white; O = Other; PeriM = perimenopausal; PoM = postmenopausal; PrM = premenopausal; W = White.

[a]Total study sample included men and women.

[b]Comparison group considered in this review to compare BMD and fractures.

Table 2.3. Methodological quality of studies included in the review.

Author (Year)	Reporting[a]	External Validity[b]	Bias[c]	Confounding[d]	Power[e]	Quality Score	Rating
Anastos et al. (2007)	8	1	3	2	0	14	Good
Cazanave et al. (2008)	8	1	3	1	0	13	Fair
Gomes et al. (2014)	8	0	3	1	2	12	Fair
Pinto Neto et al. (2011)	8	1	3	2	0	14	Good
Yin et al., (2010b)	8	1	3	3	1	16	Good
Jacobson et al. (2008)[a]	7	0	4	1	0	12	Fair
Li Vecchi et al. (2012)[a]	7	0	3	1	0	11	Fair
Sharma et al. (2011)	8	1	4	3	0	16	Good
Yin et al. (2010)	9	1	4	3	0	17	Good
Yin et al., (2012)	7	1	4	2	0	14	Good

Note: Methodological quality was assessed using a checklist by Downs and Black (1998). Each criterion was scored from 0 to 1 (0 = no, 1 = yes, n/a = not applicable), with the exception of confounding and power, which were scored from 0 to 2. Ratings were as follows: excellent (18-20), good (14 to 17) fair (10 to 13), poor (less than 10).
[a]Reporting: how well study aims and procedures are reported in the paper.
[b]External validity: generalizability to study findings to the population from which the study subjects were derived.
[c]Bias: examines biases in measurement of the intervention and the outcome.
[d]Confounding: assesses selection bias and comparability of groups.
[e]Power: whether a power analysis was conducted.

External validity was limited across studies because the source population was not adequately described and representativeness of the sample could not be determined. As all bone density studies assessed BMD using DXA, a well-established approach for the classification of osteopenia and osteoporosis (World Health Organization, 2004), outcome misclassification bias was low across these studies. In both investigations of fractures, the incidence of fractures was determined by self-report (Yin et al., 2012a; Yin et al., 2010a). Higher quality studies scored better on the confounding criteria, having sufficiently described sample selection (Sharma et al., 2011; Yin et al., 2010b; Yin et al., 2010a). Potential confounders examined across studies included: age, BMI, ART use, tobacco use/smoking history, and CD4 count. The most common

weakness among studies was the failure to report a power analysis, with only two studies having addressed power (Yin et al., 2010b; Gomes et al., 2014).

Outcomes

Individual study findings are summarized in **Table 2.4**. Five studies reported on low BMD or osteoporosis. Two of these investigations reported that the odds of osteoporosis were significantly higher among HIV-infected postmenopausal women compared with uninfected controls. Studies comparing low BMD and osteoporosis in HIV-infected postmenopausal versus premenopausal women also found a greater likelihood of low BMD in postmenopausal women (Cazanave et al., 2008; Gomes et al., 2014). Odds of low BMD were significantly higher in postmenopausal women with HIV compared to HIV-infected men and premenopausal women (Pinto Neto et al., 2011). The prevalence of low BMD reported in these studies ranged from 20% to 84% among HIV-infected postmenopausal women (Anastos et al., 2007; Cazanave et al., 2008; Gomes et al., 2014; Pinto Neto et al., 2011; Yin et al., 2010b) and 5% to 64% (Anastos et al., 2007; Yin et al., 2010b) in uninfected controls.

Table 2.4. Individual study findings of postmenopausal status and low BMD or fractures in HIV infection.

Author	Outcome Variable	Findings (95% CI)	Significant predictors in multivariate models
OR/PR of Low BMD or Osteoporosis			
Anastos et al. (2007)	Low BMD (T-score < -1.0) and osteoporosis (T-score <- 2.5) at LS or FN in HIV+ PoM versus HIV+ PrM	OR low BMD: 3.2 (1.6, 6.2) OR osteoporosis: 4.8 (1.2,18.9)	ART-naïve, HIV on ART with PI, postmenopause
Cazanave et al. (2008)	Low BMD (T-score < -1.0) and osteoporosis (T-score <-2.5) at LS or FN in HIV+ PoM versus HIV+ PrM	OR low BMD: 1.2 (0.54, 2.7)[b] OR osteoporosis: 7.1 (1.9, 26.4)[b]	Older age, nadir CD4 cell count
Gomes et al. (2014)[d]	Low BMD (T-score < -1.0) at LS and FN in HIV+ PoM versus HIV+ PrM or perimenopausal	PR low BMD LS: 23.3 (7.3, 74.2) PR low BMD FN: 56.7 (7.9, 409.4)	Postmenopause
Pinto Neto et al. (2011)[d]	Odds of low BMD (T-score < -1.0) at LS, FN, or total hip in HIV+/PoM versus HIV+ men and women not PoM	OR: 13.4 (2.5, 71.1)	Male, lower BMI, postmenopause, undetectable viral load
Yin et al. (2010b)[c]	Low BMD (T-score < -1.0) at LS, FN, and non-dominant radius in HIV+ PoM versus HIV- PoM	OR low BMD LS: 2.0 (1.0, 3.8)[b] OR low BMD TH: 1.9 (1.1, 3.5) OR low BMD FN: 2.1 (1.2, 3.7)	Older age, lower BMI, Hispanic ethnicity, HIV infection
Change in BMD			
Jacobson et al. (2008)	Annual percent change in total body BMD in HIV+ PoM v versus HIV+ PrM	PoM: -1.0 (-1.7, -0.34)[a] PrM: 0.12 (-0.16,0.41)[a]	Postmenopause, low albumin (mg/dL), lower BMI, no strength training, prednisone/hydrocortisone use, time on ART (d4T, ddi, tenofovir, saquinavir)
Li Vecchi et al. (2012)	Correlation between postmenopause and BMD T-score at LS and FN	Correlation coefficient LS= -0.17[a] Correlation coefficient FN= -0.17[a]	HIV/HCV co-infection, older age, low yogurt intake, nadir CD4, drug addiction
Sharma et al. (2011)	Annual change in BMD (g/cm^2) at LS, FN, and total hip associated with PoM in HIV+	LS: -0.010 (-0.019, -0.001) FN: -0.007 (-0.014, 0.000) TH: - 0.016 (-0.024, -0.008)	Postmenopause, methadone use, baseline BMD, lower BMI, no protease inhibitor use
HR of Fractures			
Yin et al. (2010a)	Self-reported fragility and nonfragility fractures in HIV+ PoM versus HIV- PoM	HR: 1.5 (1.1, 2.2)[a]	Older age, white race, HIV/HCV co-infection, higher serum creatinine
Yin et al. (2012a)[d]	Self-reported fragility and non-fragility fractures in HIV+ PoM and perimenopausal versus HIV+ PrM	HR: 5.8 (1.4, 23.3)[a]	Bisphosphonate use, HIV/HCV co-infection, current smoking, glucocorticoid use

Note. ART= antiretroviral therapy; BMD= bone mineral density; BMI= body mass index; FN= femoral neck; HCV= hepatitis C virus; HIV+ = HIV-infected; HIV- = HIV uninfected; HR= hazard ratio; LS= lumbar spine; OR= odds ratio; PoM= postmenopausal; PeriM= perimenopausal; PrM= premenopausal; PR= prevalence ratio

[a]Unadjusted model.

[b] Calculated by reviewer (YC) from information available in article.

[c] Study population limited to postmenopausal women.

[d] Study population limited to HIV-infected ART-naïve women.

Three studies in this review reported on bone loss in HIV infection (Jacobson et al., 2008; Li Vecchi et al., 2012; Sharma et al., 2011). Annual change in BMD was significantly higher among HIV-infected postmenopausal women compared to premenopausal women (Jacobson et al., 2008) and uninfected postmenopausal women (Sharma et al., 2011). Similarly, in a study of HIV-infected men and women, postmenopausal status was correlated with lower DXA T-scores (Li Vecchi et al., 2012).

Of the two studies reporting on fractures, one found that the likelihood of fractures was 1.5 times higher in HIV-infected postmenopausal women compared to uninfected controls (Yin et al., 2010a). In the second study, among 614 ART-naïve women, the likelihood of fractures was significantly higher in peri- and postmenopausal women with HIV than the HIV-infected premenopausal comparison group.

Predictors of Low BMD and Fractures

Predictors of low BMD and fractures for the entire sample in each study are presented in **Table 2.4**. Several traditional risk factors were independently associated with low BMD: older age (Cazanave et al., 2008; Li Vecchi et al., 2012; Yin et al., 2010b), lower BMI (Jacobson et al., 2008; Pinto Neto et al., 2011; Sharma et al., 2011; Yin et al., 2010b), and corticosteroid use (Jacobson et al., 2008). Behavioral predictors of low BMD included strength training (Jacobson et al., 2008) and substance abuse (Li Vecchi et al., 2012; Sharma et al., 2011). HIV characteristics independently associated with low BMD included: ART with (Anastos et al., 2007) or without (Sharma et al., 2011) a protease-inhibitor, time on ART (Jacobson et al., 2008), low nadir CD4 count (Cazanave et al., 2008; Li Vecchi et al., 2012), and higher viral load (Pinto Neto et al., 2011). HIV/HCV co-infection was associated with low BMD in a single report (Li Vecchi et al., 2012). In six (75%) of the eight BMD studies, postmenopause was independently associated with low BMD or bone loss in HIV infection (Anastos et al., 2007; Gomes et al., 2014; Jacobson et al., 2008; Pinto Neto et al., 2011;

Sharma et al., 2011). In the single study among postmenopausal HIV-infected and uninfected women, HIV infection was a determinant of low BMD (Yin et al., 2010b).

In fracture studies, older age (Yin et al., 2010a), white race (Yin et al., 2010a) corticosteroid use (Yin et al., 2012a), and current smoking (Yin et al., 2012a) were independent predictors of incident fractures. While HIV characteristics were not independently associated with incident fractures, both studies found that HIV/HCV co-infection was a predictor of incident fractures among HIV-infected men and women (Yin et al., 2012a; Yin et al., 2010a). Postmenopause was not independently associated with fractures in multivariate models (Yin et al., 2012a; Yin et al., 2010a).

Discussion

In the general population, BMD screening is recommended for women age 65 or older, postmenopausal women of any age, and those in the menopause transition with one or more of the following clinical risk factors for fractures: lower BMI, a prior osteoporotic fracture, use of glucocorticoids, current smoking, and alcohol intake (National Osteoporosis Foundation, 2013). Similarly, in this review of studies of HIV-infected women, older age (Cazanave et al., 2008; Li Vecchi et al., 2012; Yin et al., 2010a), lower BMI (Jacobson et al., 2008; Pinto Neto et al., 2011; Sharma et al., 2011), postmenopausal status (Anastos et al., 2007; Gomes et al., 2014; Jacobson et al., 2008; Pinto Neto et al., 2011; Sharma et al., 2011; Yin et al., 2005), and glucocorticoid use (Jacobson et al., 2008; Yin et al., 2010a) were associated with lower BMD. Strength training had a protective effect on bone (Jacobson et al., 2008), which is consistent with evidence from studies of healthy women (Wallace & Ballard, 2002). HIV/HCV co-infection was independently related to fractures in both of the fracture studies (Yin et al., 2012a; Yin et al., 2010a), a finding previously reported for the general HIV patient population (Lo Re et al., 2012; Maalouf et al., 2013). Studies in this

review also confirmed that ART exposure is associated with reduced BMD and osteoporosis (Anastos et al., 2007; Jacobson et al., 2008; Sharma et al., 2011).

This is the first systematic review to assess the quality of the evidence on low BMD and fractures in HIV-infected postmenopausal women. Our review found moderately strong evidence that HIV-infected postmenopausal women are at heightened risk for osteoporosis, beyond that normally experienced by uninfected women after menopause or HIV-infected premenopausal women. The majority of studies in this review observed that postmenopausal status, or years since menopause, was independently associated with lower BMD. These findings suggest that the additive effects of HIV infection and menopause may worsen bone loss, perhaps due to an increase in inflammatory markers such as tumor necrosis factor-alpha (TNFα) and interleukin-6 (IL6). TNFα and IL6 levels have been shown to increase after menopause (Pfeilschifter, Koditz, Pfohl, & Schatz, 2002) and HIV infection (Deeks, 2011). Higher levels of TNFα and IL6 are associated with increased activation and differentiation of osteoclasts, which lead to greater bone resorption (Fouda et al., 2012).

The literature search resulted in only two studies of incident fractures in postmenopausal women with HIV. While these studies found a modest increase in fracture risk among postmenopausal women compared to HIV-uninfected or premenopausal controls, postmenopausal status was not an independent predictor of fractures in women with HIV, despite the higher risk of low BMD. These findings may be attributed to the relatively young age of study participants. Although the incidence of fractures in uninfected individuals increases after age 50 (Kanis et al., 2001), the mean ages of HIV-infected individuals across fracture studies in this review were 39-40 years (Yin et al., 2012a; Yin et al., 2010a). At the same time, a recent meta-analysis of 13 studies, conducted primarily in men younger than 50 years, found that HIV infection was associated with a modest increase in fractures. To better

address the risk of fractures in HIV-infected women, longitudinal studies assessing age, menopause status and time since menopause will be needed.

This review identified several gaps in the current research on bone density and fractures in HIV-infected postmenopausal women. First, the literature on postmenopausal women with HIV is limited. Five of the 10 studies in this review were conducted solely in women, with the remaining studies including a large sample of men. Comparison groups varied from uninfected postmenopausal women to HIV-infected premenopausal women and men. Studies with a larger sample of HIV-infected postmenopausal women would strengthen overall validity of findings in this review.

Next, the majority of studies classified women as postmenopausal based on the exclusive criteria of self-reported amenorrhea for at least 12 consecutive months (Anastos et al., 2007; Gomes et al., 2014; Jacobson et al., 2008; Sharma et al., 2011; Yin et al., 2010a). The Stages of Reproductive Aging Workshop (STRAW) defines postmenopause as the cessation of menses for >12 consecutive months with confirmational changes in FSH, estradiol, and antimüllerian hormone concentrations (Harlow et al., 2012). The importance of reproductive hormone measures was evident in a recent study of low BMD in HIV-infected individuals, where higher levels of estradiol were significantly related to higher BMD measures (El-Maouche, Xu, Cofrancesco, Dobs, & Brown, 2011). Studies have also shown that HIV-infected women experience prolonged amenorrhea and early menopause more frequently than women in the general population (Cejtin et al., 2006; Prior et al., 2007), indicating the need for more reliable endocrine measures to avoid misclassification of reproductive stage. Thus, the application of the STRAW guidelines for staging menopause may be especially useful in research with middle-aged HIV-infected women.

Another gap in the current literature is the lack of research on emerging risk factors for osteoporosis and fractures in HIV-infected women. Recent studies in otherwise healthy

individuals have shown that both depression (Cizza, Primma, Coyle, Gourgiotis, & Csako, 2010) and surgical menopause (Yoshida, Takahashi, Yamatani, Takata, & Kurachi, 2011) are independently associated with lower BMD and fractures. A meta-analysis of 20 studies found that BMD was up to 7.32% lower among individuals of both genders with major or minor depression (Cizza et al., 2010). The sympathetic activation of pro-inflammatory cytokines in depression, such as interleukin-6 (Anderson et al., 2013), is one potential explanation for the association between bone metabolism and depression. The odds of depressive symptoms are significantly higher among HIV-infected women in early perimenopause (Maki et al., 2012), suggesting its potential role as an important but understudied confound of bone loss among HIV-infected postmenopausal women. One study in this review assessed depression, but did not include this variable in its analyses of BMD (Sharma et al., 2011).

Similarly, the role of hysterectomy as a contributing risk factor to the elevated bone loss in HIV-infected postmenopausal women remains understudied. In healthy women, serum bone turnover markers rapidly increase after bilateral oophorectomy (Bahar et al., 2011). Women with HIV are more likely than uninfected women to require a hysterectomy, most often due to a higher prevalence of cervical neoplasia (Massad et al., 2007). Although information on hysterectomy was collected at baseline in several of the reports reviewed here (Anastos et al., 2007; Jacobson et al., 2008; Yin et al., 2010b; Yin et al., 2012a), none of these included surgical menopause in their analyses of bone loss predictors (Anastos et al., 2007; Jacobson et al., 2008; Yin et al., 2012a; Yin et al., 2010b).

This systematic review highlights areas for strengthening design and methodologies in future research of reduced BMD and fractures in HIV-infected postmenopausal women. Going forward, studies should plan for an adequate sample size of postmenopausal women, sufficient to determine the potential additive effects of menopause and HIV infection on bone. Serial endocrine biomarkers should be incorporated into study designs to confirm

menopause status, as well as confirmatory records of surgical procedures to avoid the misclassification of postmenopausal women. Finally, given the well-established influence of depression on bone in uninfected individuals, and the high rates of depression in HIV-infected patients, mental health status should also be adjusted for when calculating bone loss and fracture risk in HIV-infected postmenopausal women.

This review has several limitations. Included studies were restricted to English language articles published in peer-reviewed journals. Only studies that assessed BMD by DXA were included, so it is possible that other less rigorous studies were missed. Given the significant heterogeneity, a meta-analysis was not conducted.

Conclusions

Although there is sufficient evidence to support that HIV-infected postmenopausal women are at increased risk for bone loss and low BMD, there is still inadequate evidence to suggest a role in fracture incidence at this time. Future studies that follow women across the menopause transition, using confirmatory endocrine biomarkers are needed to gain a better understanding of the impact of menopause on HIV disease progression and bone loss. Longer than usual follow-up of HIV-infected women may be necessary to determine risk for fractures in this population, given their earlier age of menopause and age at which fractures commonly occur. In addition, a greater understanding of the effect of reproductive hormones on HIV-associated non-AIDS conditions, such as low BMD, is important to prevent and treat these conditions more effectively among the growing population of older women with HIV (High et al., 2012). Findings in this review are relevant to healthcare providers with an HIV patient population because they highlight the need to accurately assess postmenopausal status and modifiable risk factors for osteoporosis in all older HIV-infected women.

CHAPTER 3: CARDIOVASCULAR DISEASE IN HIV-INFECTED POSTMENOPAUSAL WOMEN: CHARACTERIZING RISK AND PERFORMANCE OF THE FRAMINGHAM RISK SCORE

This chapter presents findings from a cross-sectional study of cardiovascular disease (CVD) risk in HIV-infected and uninfected women. The overall goal of the study was to better characterize CVD risk in HIV-infected postmenopausal women using the Framingham Risk Score, and assess the impact of menopause-related factors on CVD risk. The History of CVD in HIV-infected and uninfected postmenopausal women was also compared. This has been prepared for submission to *Menopause*.

Abstract

Objective: To characterize CVD risk in HIV-infected older women using the Framingham Risk Score (FRS) as an assessment measure.

Methods: We used a subset of data from 152 female subjects (109 HIV+, 43 HIV-) from two existing longitudinal cohorts of HIV-infected and uninfected women. All subjects were either Hispanic or African-American and had experienced either spontaneous or surgical menopause. Variables not available from the existing dataset were retrieved from medical records. The 2013 ACC/AHA guidelines were used to determine eligibility for statin therapy. The performance of the FRS in HIV-infected women was evaluated using a ROC curve and predictive factors were determined by linear regression.

Results: The HIV-infected group was younger, had lower rates of diabetes and surgical menopause, but higher rates of depression versus controls. Over 50% of the HIV-infected group was judged as low risk for CVD according to the FRS (FRS<10%), compared to 28% of the uninfected controls. In a subset of participants matched by age, median FRS did not differ between groups (14.6. (IQR=9.1, 21.6) vs. 15.5 (IQR=12.3, 22.1); p=0.73). Nearly twice as many HIV-infected women with FRS<10% had a history of CVD compared to controls. The ROC curve for the FRS demonstrated limited discriminative potential between HIV-infected women with and without CVD (area under curve=0.665). Older age at HIV diagnosis was associated with higher (worse) FRS. According to 2013 guidelines, 59% of the HIV-infected group was eligible for statin therapy, but less than half of these were prescribed treatment.

Conclusions: Performance of the FRS may be compromised in older HIV-infected women. Older age at HIV diagnosis may be a risk factor for CVD. Future studies should address low administration of statins in HIV-infected individuals.

Key words: HIV, menopause, Framingham Risk Score

Introduction

The development of antiretroviral therapy (ART) has substantially reduced AIDS-related mortality and increased life expectancy among HIV-infected individuals (Effros et al., 2008; High et al., 2012). An aging HIV population is at greater risk for chronic comorbidities and the premature onset of age-related conditions such as cardiovascular disease (CVD) (High et al., 2012). CVD is a leading cause of morbidity and mortality in HIV-infected patients.(Panos et al., 2008) Prior studies found higher rates of CVD in HIV-infected individuals compared to uninfected controls (Chow et al., 2012; D.A.D. Study Group et al., 2007). However, these studies were conducted mostly in men with few participants over age 50 years, or of minority race/ethnicity.

In the United States, CVD results in the death of approximately 400,000 women annually (Go et al., 2014). Risk of CVD increases profoundly after menopause, eventually surpassing that of men (Go et al., 2013). Older HIV-infected women are at special risk for CVD, possibly due to the additive effect of menopause and existing risk factors associated with HIV and ART (Friis-Moller et al., 2003; Kotler, 2008). HIV and menopause are associated with similar metabolic manifestations including increased visceral fat, dyslipidemia, and insulin resistance, which are traditional risk factors for CVD (Looby, 2012; Mesch et al., 2008). In addition, menopause is associated with elevated levels of inflammatory mediators such as tumor necrosis factor-alpha (TNFα) and interleukin-6 (IL-6) (Kim et al., 2012), which are thought to play a role in the development of atherosclerosis (Hansson & Libby, 2006). These menopause-related effects are presumed to be the result of a decline in estrogen associated with the spontaneous loss of ovarian function (Lenfant, Tremollieres, Gourdy, & Arnal, 2011; Mesch et al., 2008). In those with surgically induced menopause, the risk of earlier onset of CVD is heightened, though evidence is conflicting

(Jacoby, Grady, & Sawaya, 2009; Verhoeven et al., 2009; Yoshida, Takahashi, Yamatani, Takata, & Kurachi, 2011).

Statin therapy is the recommended treatment to prevent CVD events (Stone et al., 2014). Statins have been associated with a decline in the inflammatory markers often elevated with HIV infection and menopause (Calza et al., 2012). Despite the potential benefits of statin therapy, observational studies have reported the undertreatment of patients at increased risk for CVD (Pearson, 2000; Zafrir & Cohen, 2006). In a previous cross-sectional analysis, the proportion of HIV-infected and uninfected men not receiving statin therapy was similar (Monroe et al., 2015). However, the use of statins among HIV-infected older women, a population that may be at elevated CVD risk, has not been examined.

Although older women comprise a growing proportion of HIV-infected individuals, few studies have addressed the pathogenesis or potential risk determinants of CVD exclusively in this high-risk group. In the general population, the Framingham Risk Score (FRS) is recommended to assess CVD risk in women (Mosca, 2007). The FRS is a multivariable algorithm widely used to predict the risk of developing CVD over a 10-year period (D'Agostino et al., 2008). Previous studies have found that the FRS may underestimate CVD risk in people living with HIV (De Socio et al., 2007; Law et al., 2006; Parra et al., 2010). At the same time, these studies have not examined its use in HIV-infected postmenopausal women, where CVD may be pronounced given the potential additive effects of HIV and menopause. Accordingly, the overall goal of this study was to characterize CVD risk in HIV-infected older women. To do this, we: 1) compared CVD risk factors in an HIV-infected group to those in an uninfected group of similar postmenopausal status and race/ethnicity; and 2) tested the performance of the FRS as a CVD risk measure in the HIV-infected group. We also compared the percentage of subjects eligible for statin therapy against actual rates of statin prescription orders in the HIV-infected group.

Methods

Study design and sample

Prior to data collection, the Institutional Review Board at Columbia University Medical Center approved the study. Cross-sectional data were extracted from longitudinal cohorts of two existing datasets (R01AI065200, PI: Shane): 187 (92 HIV+, 95 HIV-) from previously published longitudinal cohort (Yin et al., 2012b) and 248 (148 HIV+, 100 HIV-) from an ongoing study that has not been published (**Appendix C**). In brief, between 2002 and 2014, postmenopausal African-American and Hispanic women age ≥40 years were recruited consecutively from infectious disease clinics and general internal medicine clinics at four medical centers in New York City. Blood samples were collected to determine study eligibility (confirm negative HIV testing in the control group and postmenopausal status). Postmenopausal status (either spontaneous or surgically induced) was defined as amenorrhea for ≥12 consecutive months associated with either a single serum value for follicular stimulating hormone (FSH) of >30mIU/ml, or an FSH value >20mIU/ml combined with an estradiol value <30 pg/ml. Women with metabolic bone disease, cancer, renal insufficiency (serum creatinine >1.5 mg/dl), osteoporosis, current glucocorticoids or menopause hormone therapy (HT) use, or past treatment for osteoporosis were excluded.

For the purposes of this study, additional data on menopause features and variables needed to calculate the FRS were collected from the medical records of patient-volunteers enrolled in the parent study. A chart review within the 6-month period preceding or subsequent to the screening visit was performed. Subject records were excluded from this analysis if data necessary to determine the FRS were missing. As in a previous report (Knobel et al., 2007), subjects were not excluded from the study if they had a history of CVD; instead, this was used as an endpoint to assess the performance of the FRS as a CVD

risk measure in HIV-infected women. After exclusions, the final sample included 152 (109 HIV+, 43 HIV-) subject records.

Independent variables

The primary independent variable, HIV infection, was confirmed by diagnostic criteria including the detection of HIV-1 RNA in the serum using an ELISA assay (Yin et al., 2012) (see dissertation variables in (**Appendix D**). To assess the impact of menopause-related factors, information on last menstrual period, age at menopause, years since menopause, and past use of menopause HT was also obtained from the parent study's dataset (**Appendix E**). Natural menopause was distinguished from surgical menopause based on whether amenorrhea resulted from the spontaneous cessation of menses after 12 months (natural) versus a surgical induction (hysterectomy with bilateral oophorectomy) (Hunter, 2012).

Secondary independent variables included: race/ethnicity, body mass index (BMI), chronic comorbidities (e.g., Hepatitis C infection, dyslipidemia, diabetes), alcohol consumption, and injection drug use, and were available from the parent study's dataset (Yin et al., 2010b; Yin et al., 2012b). In addition, HIV-1 RNA, current and nadir CD4, ART use (ever), age at HIV diagnosis, and years since HIV diagnosis were retrieved from the parent dataset. Other covariates, including lipid values and CVD diagnoses, were assessed by retrospective chart review. History of CVD (i.e. myocardial infarction, coronary artery disease, cerebrovascular disease, heart failure, and peripheral vascular disease) was determined using the International Classification of Diseases, Ninth Revision (ICD-9) codes for these diagnoses.

Since depression is a common correlate of HIV-infection (Ciesla & Roberts, 2001), and CVD (Baune et al., 2012), and increases in prevalence in healthy women during the early postmenopause years (Freeman, 2010), it was also assessed as a covariate. Depression was

defined using ICD-9 codes for depressed mood and depressive disorders (296.20-296.26, 296.30-296.36, 300.4, 311). In the case of medical records where no ICD-9 code for depression was present, evidence of antidepressant medications prescribed by a HIV provider, primary care provider, or mental health specialist were considered confirmatory.

CVD risk ascertainment

The primary dependent variable was CVD risk, which was assessed using the FRS, a sex-based risk prediction model that estimates the 10-year risk of a cardiovascular event (e.g. myocardial infarction, coronary insufficiency, angina, stroke, transient ischemic attack, peripheral artery disease, heart failure) (Wilson et al., 1998). The FRS ranges from 0% to 100% and scores are typically categorized as low (FRS<10%), moderate (10-20%), or high (FRS>20%) risk (Greenland et al., 2010). Here, we further categorized the FRS into low risk (FRS<10%) and moderate/high risk (FRS ≥10%), as in previous investigations evaluating the use of the FRS in HIV-infected individuals (Falcone et al., 2011; Pirs et al., 2014; Serrano-Villar et al., 2012). This risk stratification is reasonable, given the high sensitivity (approximately 80%) of the FRS at a "cut-off" value of 10% in HIV-uninfected individuals (Arts et al., 2015; Murphy, Dhangana, Pencina, Zafar, & D'Agostino, 2011).

The FRS was determined with the following set of variables: sex (male/female), age (years), systolic blood pressure (mmHg), treatment for hypertension (yes/no), current smoker (yes/no), diabetes (yes/no), total cholesterol (mg/dL), and high-density lipoprotein (mg/dL) (D'Agostino et al., 2008) (**Appendix F**). Data on age and current smoking were available from the parent study's dataset. Information on blood pressure, hypertensive treatment, lipid profile, and diabetes was gathered by chart review of clinical and laboratory data closest to the screening visit. In the majority of cases, the blood pressure measurement was calculated as the average of two blood pressure readings closest to the screening visit. In 71 cases,

where two blood pressure readings were not available, a single reading closest to the screening date was used as previously described (De Socio et al., 2007; Falcone et al., 2011).

Statin therapy

The secondary outcome was whether participants were receiving statin therapy as recommended by the 2013 American College of Cardiology/American Heart Association (ACC/AHA) guidelines (Stone et al., 2014). According to these guidelines, statin therapy is recommended for the following types of patients: age ≥21 years with low-density lipoprotein (LDL) levels ≥190 mg/dl, age 40 to 75 years with diabetes and LDL 70-189 mg/dl, and individuals at risk for or with the clinical presence of atherosclerotic CVD events (i.e. myocardial infarction, coronary artery disease, nonfatal and fatal stroke). Information on statin therapy prescription was available from the parent dataset and confirmed by medical record review.

Statistical analysis

Data were analyzed using SPSS 22.0 software. Comparisons between the HIV-infected and uninfected group were exploratory. Continuous variables were examined for normality with the Shapiro-Wilk normality test. Between-group differences were performed using the Student's t-test or the Mann Whitney U test for continuous variables, and χ^2 or Fisher's exact tests for categorical variables.

To gain a better understanding of the performance of the FRS as a risk prediction measure in HIV-infected postmenopausal women, a Receiver Operating Characteristic (ROC) curve was generated using SPSS software. We did not have an adequate sample size in the uninfected group (n=43) to compute a ROC curve. The ROC curve is used to measure a tool's sensitivity and determine the optimal "cut-off" value to equally balance sensitivity and specificity (Metz, 1978). In general, a value of 70% or higher is considered adequate or better for both performance attributes (Cantor & Kattan, 2000; Weinstein, 1980). The area under the

ROC curve (AUC) is a measurement of how accurate the tool correctly differentiates between individuals with and without disease (Faraggi & Reiser, 2002). Here, how accurately the FRS differentiates between individuals with and without a history of CVD was assessed. AUC values range from 0 to 1, with values of 0.5 or less indicating the tool has no discriminative ability (i.e., the test is as good as a random guess), and 1 indicating the tool has perfect discriminative ability (Cantor & Kattan, 2000).

Using the approach of prior studies of this population, HIV-infected women were divided into two CVD risk groups based on their FRS: low (<10%), moderate/high (≥10%). Using chi-square tests, we compared the ratio of HIV-infected women with and without a history of CVD across these CVD risk groups. To determine predictors of the FRS in the HIV-infected group, variables with $p<0.20$ in between-group comparisons among HIV-infected women were entered into a linear regression model. Collinearity was assessed and redundant variables removed from the final model. With a sample size of 152 (109 HIV+, 43 HIV-), the observed power of our FRS comparison was 37%, and the power of our regression model was 99%. All statistical tests were performed at a level of significance of $p<0.05$.

Results

Sample characteristics

Sample characteristics are presented according to study group (n=109 HIV+,43 HIV-) in **Table 3.1**. Both groups of women were predominantly Hispanic and overweight-obese according to BMI criteria. Compared to the uninfected group, HIV-infected women were younger, more likely to be African-American, had lower BMI, and were earlier in postmenopause. Age at menopause in both groups was less than 50 years. The HIV-infected group also had significantly lower rates of surgical menopause (4% vs. 19%; $p<0.01$). Mean concentrations of serum FSH levels did not differ between groups, but the HIV-infected group had significantly lower levels of serum estrone ($p<0.01$). In terms of medical history,

HIV-infected women demonstrated higher rates of alcohol use (≥1 drink/day) and clinical depression, but a lower rate of diabetes (26% vs. 49%; p<0.01). While rates of current smoking and dyslipidemia were similar between groups, median triglyceride and total cholesterol levels were higher in HIV-infected women. Among HIV-infected women, approximately 79% had previous ART exposure, with the majority (58%) receiving a protease inhibitor (PI)-based therapy.

History of CVD was similar between groups, (25% vs. 33%; p=0.33). Median (IQR) FRS was lower in HIV-infected than uninfected women (9.2 (IQR=5.9, 16.3) vs. 13.5 (IQR=8.3, 17.7); p=0.03). Given the significant group differences across several CVD risk factors (e.g., age, BMI, diabetes), a *post hoc* analysis was conducted to compare the FRS in an age-matched sample of HIV-infected and uninfected women who were >55 years of age (40 HIV+; 40 HIV-). In the age-matched sample, mean age (HIV+ = 60.1±5.5 vs. HIV- = 60.3±6.4; p = 0.76) and mean BMI (HIV+ = 28.3±5.6 vs. HIV- = 30.9 ±7.0; p = 0.07) were similar between the two groups, and rates of diabetes (HIV+=32.6% vs. HIV-=47.5%; p=0.17) were no longer different. In the *post hoc* analysis, median (IQR) FRS was similar between the HIV-infected and uninfected groups (13.4. (IQR=8.1, 17.1) vs. 14.0 (IQR=8.3, 22.5); p=0.49).

Performance of the FRS in HIV-infected postmenopausal women

Using the standard FRS cut-off of 10% to classify women into low (FRS<10%) versus moderate/high (FRS≥10%) risk groups (Falcone et al., 2011), we compared the proportion of HIV-infected and uninfected women with a history of CVD in each FRS category (**Figure 3.1**). Over 50% of the HIV-infected group was judged as low risk according to the FRS, compared to 28% of the uninfected controls. However, nearly twice as many HIV-infected women with FRS<10% had a history of CVD compared to controls (**Figure 3.1**). In a *post-hoc* analysis among women >55 years, the proportion of HIV-infected and

uninfected women judged as low risk was similar (**Figure 3.2**); of these, 13% had a history of CVD (**Figure 3.2**). Using the ROC curve, the sensitivity and specificity of the FRS at the standard cut-off value of 10% was 67% and 62%, respectively; the AUC was 0.665 (**Appendix G**).

Factors associated with FRS in HIV-infected women

Table 3.2 presents characteristics of HIV-infected women when grouped according to the low and moderate/high FRS categories. HIV-infected women in the moderate/high risk group were significantly older, and later in postmenopause. Rates of surgical menopause and exposure to HT did not differ by FRS category. Current CD4 cell counts were significantly lower among women with FRS<10%, but HIV-1 RNA and ART exposure was similar between groups. The moderate/high risk group was significantly older at the time of HIV diagnosis, but years since diagnosis did not differ between groups. Variables with $p<0.20$ in bivariate analyses (**Table 3.2)** were entered into linear regression analyses. After examining removing redundant variables, and those strongly correlated with variables used to calculate FRS, older age at HIV diagnosis and higher CD4 cell counts were independently associated with worse FRS (**Table 3.3**). When time on ART was forced into the model, it attenuated the association between CD4 and FRS. Past HT approached significance ($p=0.08$) in the multivariable model.

Statin therapy

Table 3.4 reports the percentage of women who met the recommendations for statin therapy according to the 2013 ACC/AHA guidelines (Stone et al., 2014). There was no difference in the rate of dyslipidemia between groups. However, 31% of the HIV-infected group was prescribed a statin compared to 58% of the uninfected group ($p<0.01$). Of those not on current statin treatment, 52% (n=39) were eligible for treatment in the HIV-infected

group compared to 67% (n=12) in the uninfected group according to the ACC/AHA guidelines.

Discussion

In this cross-sectional, secondary analysis, the performance of the FRS was assessed for the first time in a group of older women infected with HIV. We hypothesized that, as in other studies conducted mostly with HIV-positive, younger men, the FRS would underestimate CVD risk. Median FRS scores in our study were higher than in previous investigations comparing HIV-infected and uninfected individuals, perhaps due to the older age and higher BMI of our study population (De Socio et al., 2007; Kakinami et al., 2013). In earlier studies, investigators compared subclinical markers for CVD (e.g., carotid intima-media thickness) across FRS category (low vs. moderate/high) and found that nearly half of HIV-infected individuals in the low risk group had evidence of subclinical atherosclerosis (Falcone et al., 2011; Parra et al., 2010), concluding that the FRS may underestimate presence of subclinical atherosclerosis and CVD risk in HIV-infected individuals.

Although the majority of the HIV-infected group was judged as low risk according to the FRS, nearly one-sixth of these women had a history of a cardiovascular condition. When the analysis was limited to an age-matched sample of women >55 years, FRS was similar, but the CVD rates held. Additionally, the AUC measure of 0.665, fell below the acceptable level of 0.7 (Weinstein, 1980), indicating that the FRS may be a relatively poor measure of the FRS in our sample. These findings are consistent with earlier reports, mostly in younger subjects (Law et al., 2006; Parra et al., 2010), that the FRS underestimates CVD risk in HIV-infected individuals. In one such study, 56% of HIV-infected patients were considered at low risk for CVD according to the FRS (FRS<10%), but met criteria for subclinical atherosclerosis (Parra et al., 2010).

A limitation of this study is that it was a cross-sectional analysis and the incidence

rate of CVD events could not be assessed. Also, we did not exclude individuals with a history of CVD, which may have attributed to the higher FRS observed in our sample. However, even with this limitation, our AUC (=0.665) was similar to that reported previously for the FRS in HIV-infected individuals with and without atherosclerosis measured by the carotid intima-media thickness (AUC=0.686) (Serrano-Villar et al., 2012).

A potential explanation for the failure of the FRS to better identify those with CVD in this sample is that the FRS does not incorporate factors associated with HIV pathogenesis (e.g., HIV-1 RNA, CD4 cell count) and treatment. Early studies of CVD in HIV-infected individuals suggested that excess risk of CVD was associated with ART (Bergersen, Sandvik, Bruun, & Tonstad, 2004; D.A.D. Study Group et al., 2007), possibly due to its adverse metabolic effects, such as lipodystrophy (Hadigan et al., 2001). More recent evidence on the relationship between CVD and ART is conflicting. While several observational studies have reported an increased risk of myocardial infarction with ART exposure (D.A.D Study Group et al., 2008; Obel et al., 2010), others, such as an 8-year retrospective study of cardiovascular and cerebrovascular events in HIV-infected men and women, have found no such link (Bozzette, Ake, Tam, Chang, & Louis, 2003). This is consistent with our analysis where ART was not associated with history of CVD. Conflicting evidence of the association between CVD and ART may in part be due to many studies in this field being short-term and underpowered (Bavinger et al., 2013), as in our analysis. Additionally, because the majority of HIV-infected women in this study were ART-experienced, it may have been difficult to assess the role of ART on CVD.

Immunologic and virologic factors (e.g., IL-6, C-reactive protein, CD4 cell count) related to HIV infection have also been associated with CVD (Triant, Meigs, & Grinspoon, 2009; Triant et al., 2010). Among HIV-infected women (mean age 44 years) in the Veterans Aging Cohort Study, a recent analysis found that lower CD4 cell counts and higher HIV-1

RNA were associated with incident CVD (Womack et al., 2014). Surprisingly, in our analysis, higher CD4 cell counts were associated with higher (worse) FRS. This relationship was attenuated when time on ART was entered into the model, suggesting that current CD4 was a surrogate marker for longer time on ART. Longer duration on ART has been positively correlated with coronary atherosclerotic plaques in HIV-infected men (Zanni et al., 2013). These findings indicate that while the evidence on the role of ART regimen in CVD remains unclear, longer duration on ART is associated increased risk for CVD. Further clinical trials and longer observational studies are warranted to better characterize the impact of particular ART regimens and duration of use on CVD, particularly in older women.

In our study, older age at HIV diagnosis was associated with higher CVD risk as measured by the FRS. Because this analysis was limited to cross-sectional data, we cannot determine whether women in the moderate/high CVD risk group were already at elevated risk for CVD prior to HIV infection, or whether HIV infection itself accelerated risk of CVD. Older age is a predictor of hypertension in the general population and in HIV-infected individuals (Antonello et al., 2015), suggesting that age is an important CVD risk factor regardless of HIV status. However, in HIV-infected individuals, hypertension and diabetes have been observed at an earlier age than uninfected controls (Guaraldi et al., 2011), perhaps resulting in earlier CVD events in this population. Findings from this study are consistent with those of an earlier investigation, which found that older age and duration of diabetes were associated with coronary heart disease in HIV-infected individuals (Worm et al., 2009). Taken together, these results support the view that the additive effects of aging and HIV infection heighten CVD risk, particularly among individuals with chronic comorbidities that are associated with CVD.

Independent of advancing age, menopause-related factors are also associated with risk of CVD events in the general population. In our analysis, HIV-infected women with FRS

≥10%, were older at the time of HIV diagnosis, and spent twice as long in the postmenopausal years while infected with HIV. These findings may suggest that fewer years of estrogen exposure together with older age at HIV infection may increase risk of CVD. The negative effects of estrogen withdrawal on the heart have been well established in the general population (Lenfant et al., 2011), but less is understood about the menopause transition in HIV-infected women and the interaction between estrogen and HIV infection. Moreover, in uninfected women, years since menopause and the use of menopausal HT are linked to higher CVD risk (Baer et al., 2011). In our multivariable analysis, past use of HT approached significance as an independent predictor of FRS in HIV-infected women. Previous evidence from the Women's Health Initiative demonstrated that HT use increases CVD risk in older women (Rossouw et al., 2002). Moreover, early menopause (before age 50) is also an independent risk factor (Lobo, 2007). Some studies report that menopause occurs earlier in HIV-infected women (age 47-48 vs. 49-51) (Ortiz, Harlow, Sowers, Nan, & Romaguera, 2006; Schoenbaum et al., 2005). In healthy women, evidence suggests that hysterectomy with or without bilateral oophorectomy may increase risk for CVD (Jacoby et al., 2009; Verhoeven et al., 2009). In the current study, after controlling for age, surgical menopause did not predict FRS in HIV-infected women. It is important to note that few HIV-infected women in this study had a bilateral oophorectomy (4%), which limits our ability to speculate about the significance of these findings.

Statins are widely used in the treatment of dyslipidemia and prevention of atherosclerotic CVD events in the general population. In addition to lowering lipid levels, studies have found that statin therapy has anti-inflammatory properties (Nezic et al., 2009; Qadir, Alam, Siddiqi, & Kamran, 2014). Among HIV-infected individuals, rosuvastatin, atorvastatin, and pravastatin have been associated with a significant reduction of inflammatory markers (i.e., C-reactive protein and TNFα) (Calza et al., 2012). Therefore,

statin therapy may attenuate CVD risk in older HIV-infected women by suppressing chronic inflammation associated with HIV infection and estrogen deficiency (Pfeilschifter, Koditz, Pfhol, & Schatz, 2002). Using the 2013 ACC/AHA guidelines, we found that over half of subjects who were not prescribed a statin, were eligible for treatment. These findings suggest undertreatment with statin therapy in HIV-infected and uninfected individuals. Our findings are consistent with a recent observation that the majority of HIV-infected individuals with subclinical atherosclerosis who would benefit from statin therapy do not receive treatment (Zanni et al., 2014). Although there have been reports of potential drug-drug interactions between statins and ART regimens, specifically protease inhibitors (Ray, 2009; Feinstein, Achenbach, Stone, & Lloyd-Jones, 2015), whether this concern is associated with low rates of statin prescription is unclear. In this study, we could not ascertain the reasons for undertreatment with statins. Among HIV-uninfected individuals, qualitative investigations have identified several barriers to statin prescribing including, provider concerns about cost, increased workload, and patient adherence to treatment (Kedward & Dakin, 2003; Ab, Dening, van Vliet, & Dekker, 2009).

There are several limitations to this study. First, given the cross-sectional nature of the study design, causality cannot be determined. We were also limited by the scope of the existing dataset and sample size, which prevented a more thorough assessment of differences in CVD risk between HIV-infected and uninfected women, including the presence of subclinical atherosclerosis. Another limitation was that we did not have data on incident CVD events. Women with a history of CVD were included in our sample, even though the FRS is recommended for use among individuals without a prior CVD event. Since we did not have data available for incident CVD events, or subclinical markers of atherosclerosis (e.g, C-reactive protein, carotid intima-media thickness), we relied on the history of CVD to assess the performance of the FRS.

A particular strength of our design is that the comparison group of uninfected women was in some ways, a positive "high-risk" control for the simultaneous co-morbidities often seen in HIV patients that potentially confound results. While HIV-infected women in previous investigations had higher rates of smoking and substance abuse (Schwartz et al., 2012; Womack et al., 2014), rates of these CVD risk factors were similar in our study groups. Another strength was that eligibility in the parent study was restricted to postmenopausal women and confirmed with reproductive hormone levels. A sample exclusively of postmenopausal women eliminated potential confounding due to variable estrogen exposure and enabled us to better characterize CVD risk associated with postmenopause.

Although the European AIDS Clinical Society suggests that a FRS assessment should be performed for HIV-infected women >50 years without CVD (Lundgren, 2013), our results suggest that the FRS may underestimate cardiovascular risk in HIV-infected postmenopausal women and that CVD may occur earlier in life in this population. Primary care guidelines issued by the HIV Medicine Association and Infectious Disease Society of America for the management of HIV-infected individuals do not include recommendations for evaluating CVD in this population, but recommend fasting blood glucose and lipid levels prior to and after initiating ART (Aberg et al., 2014). Further research is necessary to determine how aggressively to treat chronic comorbidities in HIV-infected individuals to prevent CVD events. The use of a HIV-tailored CVD risk-prediction tool and recommendations for the administration of statins in HIV-infected individuals may help reduce risk of CVD in postmenopausal women with HIV.

Conclusions

This study offers novel findings on performance of the FRS in HIV-infected postmenopausal women. In an age-matched subsample, FRS did not differ significantly by HIV status. However, nearly 15% of HIV-infected women judged as low risk for CVD

according to the FRS, had a history of CVD. Study findings suggest the FRS may underestimate CVD risk in HIV-infected postmenopausal women. Older age at HIV diagnosis was associated with worse FRS. Higher CD4 cell counts were associated with worse FRS, but the relationship was attenuated when time on ART was included in the model, suggesting that CD4 cell counts were a surrogate marker for duration of ART. This study also found that HIV-infected postmenopausal women were undertreated with statins, a therapy that may reduce CVD risk. Future studies of CVD in this population would benefit from large longitudinal analyses of HIV-infected and uninfected women, as well as the use of inflammatory and immunologic markers to better characterize CVD risk and its association with HIV infection.

Table 3.1. Characteristics of study participants (n = 152)

Variable Total Sample	HIV+ n = 109	HIV- n = 43	p-value[a]
Demographics and anthropomorphic features			
Age (mean ± SD)	56.2 ± 6.2	60.0 ± 6.5	**<0.001**
Race/ethnicity, n(%)			**0.048**
Hispanic	66 (60.6)	34 (79.1)	
African-American	43 (39.4)	9 (20.9)	
BMI (kg/m^2) (median [IQR])	27.8 [24.8, 32.2]	30.2 [27.4, 33.2]	**0.045**
Menopause features			
Age at menopause (mean ± SD)	46.2 ± 6.3	46.6 ± 6.1	0.69
Years since menopause (mean ± SD)	10.2 ± 7.7	14.3 ± 8.7	**<0.01**
Surgical menopause, n (%)	4 (3.7)	8 (18.6)	**<0.01**
Menopause hormone therapy use ever, n (%)	13 (11.9)	13 (30.2)	**<0.01**
FSH (mIU/ml) (median [IQR])	60.5 [41.4, 83.1]	63.9 [47.0, 81.1]	0.63
Estrone (pg/ml) (median [IQR])	12.0 [10.8, 15.5]	24.2 [20.0, 32.3]	**<0.01**
Chronic conditions and medications, n (%)			
Current smoker	29 (26.6)	16 (37.2)	0.20
Alcohol (>1 drink/day)	18 (16.6)	2 (4.7)	0.06
IVDU	12 (11.0)	3 (7.0)	0.56
Dyslipidemia	80 (73.4)	29 (67.4)	0.46
Diabetes	18 (25.7)	21 (48.8)	**<0.01**
Hepatitis C seropositive	18(16.5)	3 (7.0)	0.31
Depression	65 (60.2)	17 (39.5)	**0.02**
Treatment for hypertension	63 (57.8)	29 (67.4)	0.27
Oral hypoglycemic/insulin	27 (24.8)	21 (48.8)	**<0.01**
Antidepressants	42 (38.5)	12 (27.9)	0.22
Blood pressure and lipid profile			
Systolic blood pressure (mean ± SD)	129.2 ± 15.5	129.5 ± 16.0	0.91
Diastolic blood pressure (mean ± SD)	75.1 ± 10.5	78.7 ± 10.2	0.06
Total cholesterol (mg/dL) (median [IQR])	196.0 [169.0, 237.0]	176.0 [148.0,	**<0.01**
Triglyceride (mg/dL) (median [IQR])	161.0 [108.5, 232.0]	216.0]	**<0.001**
LDL (mg/dL) (mean ± SD)	114.0 ± 35.6	113.0 [80.0, 151.0]	0.15
HDL (mg/dL) (median [IQR])	47.0 [38.5, 57.5]	105.6 ± 39.1 50.0 [43.0, 58.0]	0.17
HIV and ART characteristics			
Years since HIV diagnosis	11.0 [6.0, 16.0]	N/A	
AIDS criteria ever, n (%)	56 (52.3)	N/A	
Current CD4 (median [IQR])	545.0 [353.5, 724.0]	N/A	
Nadir CD4 (median [IQR])	164.0 [56.0, 289.5]	N/A	
HIV RNA (copies/ml) (median [IQR])	50.0 [20.0, 75.0]	N/A	
ART use (ever), n(%)	86 (78.9)	N/A	
NRTI exposure n(%)	82 (75.2)	N/A	
NNRTI exposure n (%)	45 (41.3)	N/A	
PI exposure n(%)	63 (57.8)	N/A	
Years on ART (median [IQR])	8.0 [4.5, 12.5]	N/A	
History of any cardiovascular disease,[b] n(%)	27 (24.8)	14 (32.5)	0.33
Framingham Risk Score[c]			
Median [IQR]	9.2 [5.9, 16.3]	13.5 [8.3, 17.7]	**0.03**
Mean ± SD	12.4 ± 9.2	15.1 ± 9.2	0.09

Note. ART = antiretroviral therapy; HDL = high-density lipoprotein; IVDU = intravenous drug use; LDL = low-density lipoprotein; NRTI = nucleotide reverse transcriptase; NNRT = non-nucleotide reverse transcriptase; PI = protease inhibitor.

[a]Between-group differences were performed using the Student's t-test or the Mann Whitney U test for continuous variables, and χ^2 or Fisher's exact tests for categorical variables.

[b]History of CVD was determined using ICD-9 codes for the following diagnoses: coronary artery disease, myocardial infarction, cerebrovascular disease, peripheral vascular disease, and heart failure.

[c]Framingham Risk Scores (FRS) were calculated using the following variables: age, blood pressure, lipid profile, smoking status, diabetes status, and whether on hypertension treatment. FRS was calculated among individuals regardless of statin treatment.

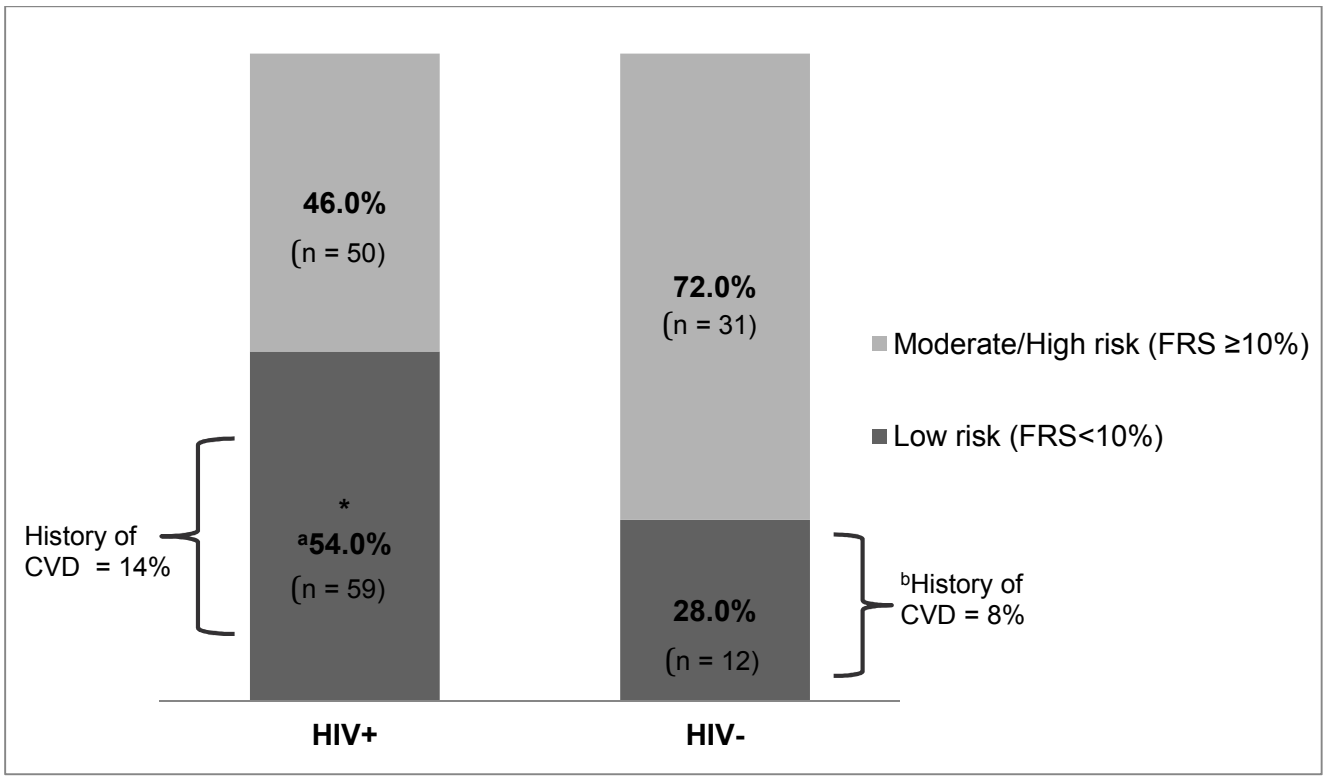

Figure 3.1. Percent of HIV-infected and uninfected study subjects in each Framingham Risk Score category (low vs. moderate/high).
[a]Based on chi-square analyses (p<0.01).
[b]Based on chi-square analyses (p=0.64).

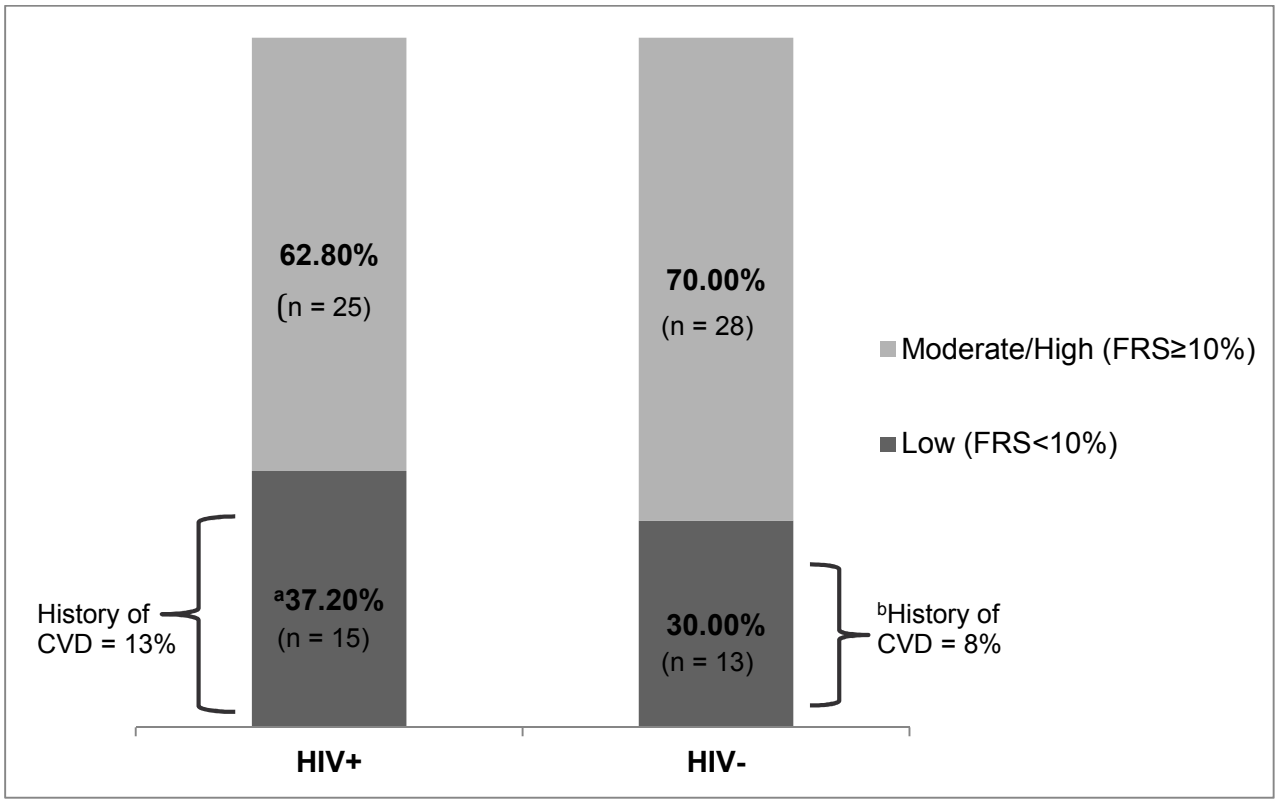

Figure 3.2. Percent of age-matched HIV-infected and uninfected study subjects in each Framingham Risk Score category (low vs. moderate/high).
[a]Based on chi-square analyses (p=0.49).
[b]Based on chi-square analyses (p=0.72).

Table 3.2. Characteristics of HIV-infected women by Framingham Risk Score category (n =109)

Variable	Low (FRS <10%) n = 59	Moderate/High (FRS ≥10%) n= 50	p-value[a]
Demographics and anthropomorphic features			
Age (mean ± SD)	53.5 ± 5.7	59.3 ± 5.3	**<0.001**
Race/ethnicity n(%)			0.78
Hispanic	35 (59.3)	31 (62.0)	
African-American	24 (40.7)	19 (38.0)	
Menopause features			
Age at menopause (mean ± SD)	45.7 ± 4.8	46.7 ± 7.7	0.15
Years since menopause (mean ± SD)	7.8 ± 6.5	12.9 ± 8.2	**<0.001**
Surgical menopause n(%)	1 (1.7)	3 (6.0)	0.33
Menopause hormone therapy use ever n(%)	4 (6.8)	9 (18.0)	0.08
FSH (mIU/ml) (median [IQR])	59.4 [39.9, 85.9]	62.0 [44.3, 82.4]	0.70
Estrone (median [IQR])	23.7 [15.2, 33.7]	22.0 [13.7, 31.1]	0.52
Chronic conditions and medications n(%)			
Alcohol (>1 drink/day)	10 (16.9)	8 (16.0)	0.89
Injection drug use	8 (13.6)	4 (8.0)	0.54
Hepatitis C seropositive	11 (18.6)	7 (14.0)	0.51
Depression	36 (61.0)	30 (60.0)	0.91
Antidepressants	24 (40.7)	18 (36.0)	0.62
HIV and ART characteristics			
Age at HIV diagnosis (mean ± SD)	40.5 ± 9.7	45.4 ± 8.8	**<0.01**
Years since HIV diagnosis (mean ± SD)	12.9 ± 7.5	13.9 ± 6.5	0.46
AIDS criteria ever, n(%)	32 (56.1)	24 (48.0)	0.40
Current CD4 (mean ± SD)	553.1 ± 272.6	747.1 ± 387.9	**0.046**
Nadir CD4 (median [IQR])	162.0 [48.0, 261.0]	204.5 [127.5, 303.8]	0.32
HIV RNA (copies/ml) (median [IQR])	50.0 [20.0, 91.8]	50.0 [20.0, 75.0]	0.78
ART use (ever), n (%)	45 (76.3)	41 (82.0)	0.47
NRTI exposure, n (%)	42 (72.4)	41 (82.0)	0.17
NNRTI exposure, n (%)	24 (41.4)	21 (42.0)	0.94
PI exposure, n (%)	34 (58.6)	29 (58.0)	0.89
Years on ART (median [IQR])	7.0 [5.0, 12.0]	10.5 [5.0, 14.0]	0.14

Note. ART = antiretroviral therapy; BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NRTI = nucleotide reverse transcriptase; NNRT = non-nucleotide reverse transcriptase; PI = protease inhibitor.
[a]Between-group differences were performed using the Student's t-test or the Mann Whitney U test for continuous variables, and χ^2 or Fisher's exact tests for categorical variables.

Table 3.3. Factors associated with Framingham Risk Score in HIV-infected women (n = 109)[a,b]

Variable	β (95% CI)	SE	p-value
Age at menopause	0.01 (-0.01, 0.03)	0.01	0.38
Age at HIV diagnosis	0.02 (0.01, 0.04)	0.01	**<0.01**
HRT (past use)	0.35 (-0.05, 0.75)	0.20	0.08
Current CD4 (50 cells/mL)	0.03 (0.004, 0.05)	0.01	**0.02**
NRTI exposure	0.06 (-0.23, 0.34)	0.14	0.70

Note. HRT = hormone replacement therapy.
[a] In the model, the dependent variable, Framingham Risk Score (%), was normalized using a log transformation.
[b] HIV-infected women were categorized into low FRS (<10%) versus moderate/high FRS (≥10%) and group differences determined using chi-square tests for categorical variables and t-test or Mann Whitney U for continuous variables. Variables with p<0.20 were entered into regression model. Collinearity diagnostics were assessed and redundant variables removed.

Table 3.4. HIV-infected and uninfected women prescribed statin therapy and proportion of women for whom statins are recommended (n=152).

Variable, n (%)	HIV+ (n=109)	HIV- (n=43)	[c]p-value
Dyslipidemia			
Yes	80 (73.4)	29 (67.4)	0.46
No	29 (26.6)	14 (32.6)	
Currently on statin therapy			
Yes	34 (31.2)	25 (58.1)	**<0.01**
No	75 (68.8)	18 (41.9)	
Not on statin, but met criteria for statin therapy according to guidelines[a]			0.26
Yes	39 (52.0)	12 (66.7)	
No	36 (48.0)	6 (33.3)	

[a] Recommendation based on the 2013 American College of Cardiology/American Heart Association guidelines (Stone et al., 2014).
[b] Among those for whom a statin is recommended (n= 64 HIV+, 33 HIV-), proportion prescribed a statin.
[c] Based on Chi-square analyses

CHAPTER 4: DETERMINANTS OF BONE LOSS CHANGE IN HIV-INFECTED POSTMENOPAUSAL WOMEN: A SECONDARY ANALYSIS OF REPRODUCTIVE HISTORY AND DEPRESSION-RELATED RISK FACTORS

This chapter presents findings from a longitudinal secondary analysis of clinical factors influencing change in BMD in HIV-infected and uninfected postmenopausal women. The overall goal of the study was to better characterize the role of reproductive history and depression on bone loss in older women with HIV. Well-established moderators of bone loss were compared in HIV-infected and uninfected postmenopausal women. The section below has been prepared in manuscript form for submission to *Calcified Tissue International*.

Abstract

Purpose: The purpose of this study was to assess the influence of reproductive history and depression on annualized percent change in BMD in HIV-infected postmenopausal minority women.

Methods: This secondary analysis expands on an existing longitudinal dataset accrued for a study of osteoporosis in HIV-infected and uninfected postmenopausal women. 173 women (mean age: 58 years ±6.4, mean BMI: 29.7 ±7.4) were studied at baseline and followed for approximately 15 months. All were African-American or Hispanic, age≥40 years, and postmenopausal Data on change in BMD by dual-energy x-ray absorptiometry, baseline bone turnover markers, and serum levels of inflammatory cytokines were retrieved from the existing dataset and combined with additional retrospective data on reproductive history and depression obtained from chart review of the participants' medical records.

Results: At baseline, the HIV-infected group demonstrated fewer menopause risk characteristics for osteoporosis (earlier in postmenopause, lower rates of surgical menopause and use of menopause hormone therapy), although the prevalence of depression, use of antidepressants and alcohol were higher versus controls. As in the parent study, lower BMD was evident in the HIV-infected group at the lumbar spine and total hip. In the 127 (73 HIV+, 54 HIV-) subjects with follow-up data, the HIV-infected group had a greater decline in BMD than the uninfected group at the forearm, but T-scores in both groups remained in the osteopenia range as in the baseline study. Surgical menopause was associated with bone loss at the lumbar spine. Depression was not associated with either baseline BMD or bone loss. After controlling for confounders, HIV infection remained a predictor of bone loss.

Conclusions: A diagnosis of depression does not contribute substantially to the accelerated bone loss seen in HIV-infected postmenopausal women.

Keywords: bone mineral density, HIV, menopause

Introduction

While women comprise approximately 25% of HIV-infected individuals age 50 years and older (CDC., 2013), few studies have examined determinants of bone loss in this population (Arnsten et al., 2006; Yin et al., 2010b). Studies report an estimated 2 to 7-fold higher odds of osteoporosis in postmenopausal HIV-infected women than their premenopause counterparts, or HIV-uninfected controls (Anastos et al., 2007; Cazanave et al., 2008; Yin et al., 2005). In healthy women, menopause, or the natural cessation of menses that occurs at approximately age 51, is a well-established accelerator of bone loss. The gradual loss of ovarian estrogen production and rising concentrations of follicle stimulating hormone (FSH) are associated with accelerated osteoclast formation and bone remodeling (Garnero, Sornay-Rendu, Chapuy, & Delmas, 1996). In women who undergo premature menopause due to bilateral oophorectomy, the risk of bone loss occurs earlier and the amount of bone loss is more pronounced (Bahar et al., 2011; Yoshida, Takahashi, Yamatani, Takata, & Kurachi, 2011). While HIV-infected women are now living longer and transitioning through menopause, the impact of menopause and other reproductive factors on bone loss has not been well established (Anastos et al., 2007; Sharma, Cohen, Freeman, Santoro, & Schoenbaum, 2011).

Depression is the most prevalent mental health disorder in HIV-infected individuals (Bing et al., 2001). A previous meta-analysis found that the rate of depression is nearly two times higher in people living with HIV than in uninfected controls (Ciesla & Roberts, 2001). Risk factors for depression in HIV-infected individuals include female sex and older age (Nanni, Caruso, Mitchell, Meggiolaro, & Grassi, 2015). In a recent systematic review, seven observational studies among older adults reported that depression or depressive symptoms were

associated with reduced BMD (Gebara et al., 2014). Additionally, there is growing concern over the impact of antidepressants, particularly selective serotonin reuptake inhibitors (SSRI), on bone loss, but the evidence is conflicting (Diem, Blackwell, Stone, Yaffe, Haney, et al., 2007; Diem et al., 2013). A possible mechanism for the association between bone loss and depression is the sympathetic activation of pro-inflammatory cytokines (Altindag et al., 2007; Anderson et al., 2013), which promote skeletal degradation (Barbour et al., 2014). These biomarkers are elevated in both HIV infection and menopause (Aberg, 2012)

Despite the prevalence of depression among HIV-infected individuals, and its recognition by the National Osteoporosis Foundation as a risk factor for osteoporosis (National Osteoporosis Foundation., 2013), current evidence is lacking on the role of depression on bone loss in people with HIV. A recent longitudinal study to assess the etiology of bone loss in HIV-infected postmenopausal minority women found that HIV infection was independently associated with decline in BMD at the spine and hip (Yin et al., 2012b), although underlying mechanisms were not evaluated. Taken together, these separate lines of evidence suggest that HIV-infected postmenopausal women may be at special risk for accelerated bone loss due to high rates of depression and the potential additive effects of menopause and HIV infection. Here, we expand on the original investigation to (1) examine the secondary role of reproductive history on bone loss, and (2) assess the impact of depression on annual change of BMD.

Methods

Study design and sample

This longitudinal, secondary analysis builds on data from a NIH-funded investigation of osteoporosis (R01 AI065200, PI: Shane) in HIV-infected and uninfected postmenopausal

minority women (n=187; 92 HIV+, 95 HIV-) (**Appendix C**). Details of the parent study have been provided elsewhere (Yin et al., 2005; Yin et al., 2010b; Yin et al., 2012b). In brief, the sample included postmenopausal women recruited consecutively between 2002 and 2007 from infectious disease clinics and general internal medicine clinics at Columbia University Medical Center (CUMC) and Bronx-Lebanon Hospital Center in New York City. Eligible subjects were postmenopausal African-American and Hispanic women at least 40 years of age. Postmenopausal status was defined as amenorrhea for ≥12 consecutive months associated with either a single measure of serum FSH >30mIU/ml, or a value of FSH >20mIU/ml combined with a serum estradiol value of less than 30 pg/ml. Women who were 55 years of age or older were eligible regardless of FSH and estradiol levels. Surgical menopause was defined as menopause induced after the surgical removal of ovaries. Reproductive hormone level criteria also applied to these women.

Potential subjects were excluded if they had metabolic bone disease or a disease that affects bone (i.e. Paget's disease, Cushing's syndrome, primary hyperparathyroidism), multiple myeloma, cancer, renal insufficiency (serum creatinine >1.5 mg/dl), a previous diagnosis of osteoporosis, and celiac or inflammatory bowel disease. Women were also excluded if they were currently taking glucocorticoids, menopause hormone therapy (HT), or any medication for osteoporosis at baseline, or if they initiated bisphosphonates or menopause HT between the baseline and follow-up visit. Subjects the parent study were not eligible for this analysis if data were missing on depression, or if they were diagnosed with depression in between the baseline and follow-up visit (n=14; 3 HIV+, 11 HIV-).

Bone mineral density

The dependent variable was annualized percent change in BMD at five sites. BMD

(g/cm^2) of the total hip (TH), femoral neck (FN), lumbar spine (LS; L_1–L_4), non-dominant one-third radius (DR), and ultradistal radius (UDR) were measured at two time points by dual-energy x-ray absorptiometry (DXA) using a QDR 4500 bone densitometer (Hologic, Inc., Bedford, MA) at CUMC. T-scores, which compare a subject's bone mass with that of a healthy 30-year old adult of the same sex and race, were derived from the National Health and Nutrition Examination Survey III for the hip and from Hologic normative databases for the LS and forearm. In accordance with the World Health Organization (WHO) T-score criteria (World Health Organization., 2004), osteopenia was defined as a T-score between -1 and -2.49 at any of the sites examined, and osteoporosis was defined as a T-score ≤ -2.5. Normal BMD was defined as a T-score > -1.0 at all BMD sites. Height and weight were measured by Harpenden stadiometer and balance beam scale, respectively.

Reproductive history

Reproductive health factors that have been linked to bone loss in the general population were retrieved from the parent study dataset, or by retrospective chart review. Age at menarche, age at menopause, months/years since amenorrhea, and use of menopause HT were determined by self-report in the parent study (**Appendix E**). For this secondary analysis, a retrospective chart review was performed to retrieve information on the cause of menopause (natural versus surgical). Natural menopause was defined as the report of spontaneous, permanent ending of menses for at least 12 months. Surgical menopause was defined as induced amenorrhea resulting from hysterectomy with bilateral oophorectomy (Hunter, 2012). Data regarding the type of hysterectomy (partial versus total) was also retrieved by chart review.

Depression

For this analysis, information on depression was retrieved by retrospective chart review and added to the database of the parent study (**Appendix D**). Depression was defined using the International Classification of Diseases, Ninth Revision (ICD-9) codes for depressed mood and depressive disorders (i.e. 296.2, 296.3, 296.5, 300.4, 301.12, 309.0, 309.1, and 311). ICD-9 codes for depression have been shown to be valid measures of depression, with positive predictive values ranging from 89% to 92% (Fiest et al., 2014). All problem lists and ICD-9 codes in the medical charts prior to baseline BMD were reviewed by a member of the research team. Pharmacy logs and medication history were also assessed for evidence of antidepressant medications prescribed by an HIV provider, primary care provider, or mental health specialist.

Clinical measures

Fasting blood samples were collected for medical screening to determine eligibility (complete blood count, serum calcium and phosphate, liver function tests, and creatinine) and assessment of reproductive hormones, bone metabolism, and HIV characteristics. All assays were performed at the Biomarker Core of the Irving Institute for Clinical and Translational Research, and in the CUMC Bone Marker Laboratory in the Division of Endocrinology according to well-established procedures as described in the original report (Yin et al., 2012).

Statistical analysis

Data were analyzed using SPSS 22.0 software. Normal distribution was examined with the Shapiro-Wilk normality test. Descriptive statistics were performed to assess sample characteristics. Between-group differences were examined using the Student's t-test or the Mann Whitney U test for continuous variables, and χ^2 or Fisher's exact tests for categorical variables.

Multivariable regression analyses were performed to assess factors associated with baseline BMD. First, univariate analyses were performed to determine baseline variables associated with BMD at DXA each site: age; race/ethnicity; body mass index (BMI); age at menarche; age at menopause; surgical menopause; depression; antidepressants; SSRI; previous fracture; family history of osteoporosis; current tobacco use; use of glucocorticoid ever; type 2 diabetes; hyperthyroidism; alcohol use (more than two drinks per day); injection drug use history; Hepatitis C virus infection; calcium-regulating hormones (parathyroid hormone, corrected calcium level, 25-OHD), bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, C-telopeptide type I collagen, cross-linked N-telopeptide of bone collagen type I), and cytokines (TNF-α, interleukin-6). Factors selected *a priori* for inclusion in the regression models included HIV status, depression, and surgical menopause. Covariates with $p<0.20$ were entered into multivariable regression models with the *a priori* variables. Collinearity diagnostics were assessed and redundant variables removed. Final models included only those covariates with $p<0.05$.

Using these same methods, multivariable regression models were developed to assess determinants of bone loss in the total sample. Factors selected *a priori* for inclusion in these models were time to follow-up assessment, baseline BMD, HIV status, depression, and surgical menopause. A *post-hoc* analysis was also performed among individuals without antidepressants to determine if untreated depression was associated with bone loss. Other analyses conducted for this dissertation aim are located in **Appendix G**.

With a baseline sample size of 173 (89 HIV+, 84 HIV-), we were able to detect a medium effect size (Cohen's d=0.5) with 90% power. At follow-up, with 73 HIV+ and 54 HIV-, the analysis had 79% power. Two-tailed $p<0.05$ were considered statistically significant.

Results

Sample characteristics

Baseline demographic and clinical characteristics by study group (n =173; 89 HIV+, 84 HIV-) are shown in **Table 4.1**. Race/ethnicity, current smoking status and intravenous drug use history did not differ across groups. HIV-infected women were 5 years younger (median age 55.0 yrs HIV+ vs. 60.0 yrs HIV-; $p<0.001$) and less overweight compared to the uninfected group. HIV-infected women were earlier in their postmenopause (median 8.0 yrs HIV+ vs. 11.0 yrs HIV-; $p<0.01$), had a lower rate of surgical menopause (5.6% HIV+ vs. 23.8% HIV-; $p<0.01$), and were less likely to have ever used menopause HT (18% HIV+ vs 31.6% HIV-; $p=0.04$). Levels of estrone and FSH were similar across groups. Rates of depression and alcohol use were higher in the HIV-infected group (48% HIV+ vs. 27% HIV-; $p<0.01$). A greater proportion of HIV-infected women were prescribed antidepressants (37% HIV+ vs. 20% HIV-; $p<0.01$). In terms of HIV status, the mean time since HIV diagnosis was 8.6 years and approximately half of the group had experienced a history of an AIDS-defining illness (**Table 1**). The majority of HIV+ women were on ART.

Serum calcium, 25-OHD, and 1, 25$(OH)_2$D were similar across groups, but parathyroid hormone levels were lower in the HIV-infected group. On average, women in the HIV-infected group had lower levels of parathyroid hormone. The bone turnover marker, C-terminal telopeptide (CTx), was higher in the HIV-infected group than uninfected group. Serum TNFα was also higher in the HIV-infected group.

BMD: baseline and follow-up

Table 4.2 summarizes baseline and follow-up BMD as well as change in BMD. At baseline, unadjusted BMD (g/cm^2) was significantly lower at the TH in the HIV-infected group (p=0.04) compared with controls; T-scores were lower at the TH (0.048) and LS (p=0.02), but none of the T-scores in either group at baseline or at follow-up were within the osteoporosis range.

Follow-up BMD data were available for 127 enrollees (73 HIV+, 54 HIV-; 83% HIV+, 63% HIV-). The median interval and interquartile range (IQR) between DXA scans was 15.0 months (IQR: 13.0, 18.0) for the HIV-infected group and 15.5 months (IQR: 13.0, 28.8) for the uninfected group. On average, the HIV-infected group had a greater decline in BMD compared with the uninfected group at the LS (p=0.02), DR (p=0.03), and UR (p=0.04).

Determinants of baseline BMD

In the multivariable regression analysis, older age and lower BMI at baseline were associated with lower baseline BMD at all sites **(Table 4.3)**. Hispanic race/ethnicity was associated with lower BMD at the LS, FN, and DR. Surgical menopause and depression were not independent predictors of baseline BMD. HIV status and alcohol use were associated with reduced BMD in univariate analyses, but were not significant when explored in models containing age, BMI, race/ethnicity, depression, and surgical menopause **(Table 4.3)**.

Determinants of change in BMD

Factors associated with annualized percent change in BMD at the LS, TH, DR, and UDR in the total sample (n=127; 73 HIV+, 54 HIV-) are shown in **Table 4.4**. Surgical menopause was associated with bone loss at the LS. Depression was not independently associated with bone loss. In univariate analyses (**Appendix G**), prescribed SSRI was associated with decline of BMD at

the TH ($p<0.01$), but the association was no longer significant when entered into the multivariable models. HIV infection was associated with bone loss at the LS (β= -2.44; $p<0.01$) and DR (β= -2.09; p=0.04) (**Table 4.4**). Age, race/ethnicity, BMI, past menopausal HT use, age at menarche, and current smoking were tested for inclusion in the multivariable models, but were not associated with change in BMD at any site.

In a *post hoc* analysis, when individuals prescribed antidepressants were excluded (n=33; 23 HIV+, 10 HIV-) from the total sample of 127 HIV-infected and uninfected individuals, surgical menopause was negatively associated with change in BMD at the LS (β= -3.63; p=0.02) (**Table 4.5**). HIV status was associated with change in BMD at the LS after controlling for time between DXA, baseline BMD, surgical menopause, and untreated depression (β= -2.37; p=0.02).

Discussion

In this secondary analysis, we compared menopause and depression-related risk factors for osteoporosis in a sample of HIV-infected postmenopausal minority women versus uninfected controls, and tested whether these additional characteristics would contribute to the explanatory model for accelerated bone loss associated with HIV. Both the cross-sectional comparisons and the longitudinal analyses failed to demonstrate a significant influence for either clinical parameter on prediction models, despite their well-known independent relationships to osteoporosis in the general population (Diem, Blackwell, Stone, Yaffe, Cauley, et al., 2007; Duraes Simoes et al., 1995)

At baseline, the HIV-infected group demonstrated fewer menopause risk characteristics for osteoporosis (earlier in postmenopause, lower rates of surgical menopause and use of hormone therapy), although the prevalence of depression, use of antidepressants and alcohol were higher versus controls. As in the parent study, greater bone loss was evident in the HIV-

infected group as judged by baseline biomarkers and T-scores (LS, total hip). Although serial hormone measures were not performed, the similarity in estrone concentrations between groups at baseline suggests that the more adverse profiles of TNF-alpha, C-teleopeptide and PTH seen in the HIV+ group were not the result of greater hypoestrogenic effects alone on inflammation mechanisms, as proposed in the original investigation. In that report, women with HIV demonstrated lower estrone versus controls (Yin et al., 2012b).

Only in the total sample, was surgical menopause associated with bone loss. In community-based samples, low BMD and elevated levels of bone turnover markers have been reported following surgical menopause (Bahar et al., 2011; Yoshida et al., 2011), possibly owing to the abrupt depletion of ovarian estrogen production (Vaananen & Harkonen, 1996). Approximately a quarter of subjects had a history of menopause therapy use, but less than half of these were those who had undergone surgical menopause. This is clinically relevant given that menopause hormone therapy is bone protective, but prescription of this therapy has declined since the Women's Health Initiative reported its association with possible increased risks for cardiovascular disease and breast cancer (Rossouw et al., 2002). Taken together, these findings indicate the protective effects of ovarian conservation on bone health and the importance of considering menopause hormone therapy in women who are candidates for therapy. It is important to note that rates of surgical menopause in our analysis were substantially below the national prevalence rates for HIV-infected and uninfected women. According to a recent estimate from a national survey of female veterans, over 50% of women ages 45-60 years had undergone surgical menopause (Rouen, Krein, & Reame, 2015). Among HIV-infected women in the U.S., data from the Nationwide Inpatient Sample revealed that 36% of women age 15 years and older had a hysterectomy with oophorectomy (Penman-Aguilar et al., 2012). To what extent

unique sociodemographic features of our sample may have accounted for this low rate of bilateral oophorectomy is unclear, as information was not collected in the parent study on factors such as education level or health insurance provider that may be differentially associated with rates of surgical menopause.

In this secondary analysis, we also examined for the first time the role of depression in bone loss among postmenopausal women with HIV. The prevalence of depression in our HIV-infected group, though relatively high (48%), was consistent with rates reported in the New York City-based project, *Research in Older Adults with HIV* (mean age 55 years) (Havlik, Brennan, & Karpiak, 2011). Among medically- healthy older women, depression has been associated with accelerated bone loss at the hip (Diem, Blackwell, Stone, Yaffe, Cauley, et al., 2007). Even though depression was a prevalent comorbidity in nearly half of the HIV-infected group, it was not associated with low BMD or bone loss. This finding is consistent with a previous investigation of bone loss in middle-aged HIV-infected women (Sharma et al., 2011); although in that study, self-reported depressive symptoms and menopause status were not confirmed by clinical criteria. Furthermore, we did not find an association between untreated depression and bone loss. One reason for the lack of an observed association may be that the robust influence of HIV status in the model coupled with the relatively small sample size may have masked other potential moderators.

Over one-third of HIV-infected women in this analysis were prescribed antidepressants, which is higher than the reported prevalence of antidepressant use in the general population (10%) (Mojtabai & Olfson, 2014). There is evidence to suggest a possible deleterious effect of SSRIs on bone, but the mechanism remains unclear (Diem, Blackwell, Stone, Yaffe, Haney, et al., 2007; Haney et al., 2007). The potential adverse effect of SSRIs on bone is important given

that HIV-infected individuals are at increased risk for depression (Ciesla & Roberts, 2001; Gaynes, Pence, Eron, & Miller, 2008), and SSRIs are the most commonly prescribed antidepressants (Olfson & Marcus, 2009). In our analysis, prescribed SSRI was associated with decline of BMD in univariate analyses, but was not a predictor of bone loss in the multivariable model. To what extent the relatively small sample size of this sub-group limited our ability to test for this association is unclear; previous analyses noting such an association consisted of approximately 200 subjects on SSRIs (Cauley et al., 2005; Diem et al., 2007). Moreover, our analysis was restricted to the review of medical charts to retrieve information on SSRI prescription orders and thus, the actual use of SSRIs could not be assessed. Future studies in older HIV-infected women should incorporate the use of pharmacy records, medication container review, or pill counting as approaches to confirm SSRI use behavior and its role in bone loss.

Even in the presence of relatively high rates of depression, the association was not attenuated between HIV infection and bone loss at the LS and DR, common bone sites for fractures in postmenopausal women (Schuit et al., 2004). Whether HIV infection itself contributes to reduced BMD remains unclear. However, one potential mechanism by which HIV may lead to reduced BMD in older women includes cytokine activation by the virus. Cytokine activation has been noted to predict bone loss in older adults (Ding, Parameswaran, Udayan, Burgess, & Jones, 2008). Both surgical menopause and depression are associated with persistent inflammation through different mechanisms (Miller, Maletic, & Raison, 2009; Pacifici et al., 1991; Pfeilschifter, Koditz, Pfohl, & Schatz, 2002), but to what extent either of these factors may have contributed to the underlying bone deficiencies requires further study.

Strengths in this study included access to a dataset exclusively of postmenopausal women who were screened with confirmatory hormone measures (Yin et al., 2010b; Yin et al., 2012b),

to eliminate the potential confound of differing stages of reproductive aging and estrogen exposure (Harlow et al., 2012), Previous studies on bone loss in older women have relied heavily on self-report to assess menopausal status (Anastos et al., 2007; Arnsten et al., 2006; Sharma et al., 2011). Another strength of this study was the inclusion of age of menarche as part of the necessary reproductive history, which has not been reported in previous analyses (Anastos et al., 2007; Sharma et al., 2011). However, other important reproductive factors associated with bone health were not examined in this analysis, including parity and abortion.

Several limitations should also be acknowledged. First, the loss of 46 participants from the follow-up collection point (attrition rate of 18% HIV+, 36% HIV-) reduced our sample size and may have limited the conclusions reached in the regression analyses. With our small sample size of HIV-infected women at follow-up, we could not perform adequate analyses exclusively in the HIV-infected group. Since this study was based on a pre-existing dataset, variables were restricted to those collected in the parent study or those that could be retrieved in the medical charts. While this study included longitudinal assessments of BMD, data on covariates such as cytokines and bone turnover markers was cross-sectional and therefore a temporal relationship with reduced BMD could not be established. Another limitation was that actual use of antidepressants could not be determined and we relied on the provider's prescription of antidepressants for our analyses. Moreover, follow-up was relatively short (approximately 15 months) and a longer follow-up interval may have revealed more remarkable changes in bone loss in older women with HIV. In studies examining the relationship between depression and bone loss, average frame for follow-up was 3 to 4 years (Diem et al., 2007; Diem et al., 2013). Finally, without additional data on socio-demographic characteristics or physical activity, we

were also unable examine their relationship with other covariates, or to account for their role in bone loss.

Conclusions

In summary, we found lower rates of surgical menopause and higher rates of depression in HIV-infected postmenopausal women compared to postmenopausal, HIV-uninfected controls. In the total sample, surgical menopause was associated with bone loss at the lumbar spine. Depression diagnosis did not predict BMD changes either in the cross-sectional comparison or in the longitudinal analysis. On average, the HIV-infected group had a greater decrease in BMD than the uninfected group. Moreover, HIV infection contributed substantially to accelerated bone loss in postmenopausal women after adjusting for important covariates, including surgical menopause and depression.

Table 4.1. Baseline demographic and clinical characteristics of study population (n = 173)

Variable	HIV+ (n = 89)	HIV- (n = 84)	p-value[a]
Demographic and anthropomorphic features			
Age (median [IQR])	55.0 [52.0, 59.5]	60.0 [55.0, 65.0]	**<0.001**
Race/ethnicity, n (%)			0.23
Hispanic	57 (64.0)	61 (72.0)	
African-American	32 (36.0)	23 (27.4)	
BMI (kg/m^2) (median [IQR])	27.1 [24.0, 31.2]	29.9 [25.5, 33.2]	**<0.01**
Reproductive history			
Age at menarche (yr) (median, IQR)	13.0 [12.0, 14.0]	13.0 [12.0, 14.0]	0.77
Age at menopause (yr) (median, IQR)	47.0 [43.5, 50.0]	48.0 [45.0, 50.0]	0.57
Years since menopause (median, IQR)	8.0 [3.5, 14.0]	11.0 [7.0, 18.8]	**<0.01**
Surgical menopause, n (%)	5 (5.6)	20 (23.8)	**<0.01**
Menopause hormone therapy ever (%)	16 (18.0)	26 (31.7)	**0.04**
Osteoporosis risk factors, n (%)			
Depression	43 (48.3)	23 (27.4)	**0.01**
Antidepressants	33 (37.1)	17 (20.2)	**<0.01**
Current smoker	25 (28.1)	17 (20.2)	0.23
Alcohol (>1 drink/day)	11 (12.4)	3 (3.6)	**0.03**
Intravenous drug use (ever)	11 (12.4)	6 (7.1)	0.25
Family history of osteoporosis	18 (20.2)	17 (20.2)	0.99
Previous incident fracture	8 (9.0)	4 (4.8)	0.37
Chronic conditions and medications, n (%)			
Hepatitis C	18 (20.2)	6 (7.1)	**0.01**
Hyperthyroidism	4 (4.5)	4 (4.8)	1.0
Diabetes	21 (23.6)	22 (26.2)	0.69
Glucocorticoids (ever)	15 (16.9)	7 (8.4)	0.06
Calcium supplements	21 (23.6)	31 (36.9)	**<0.01**
Multivitamins	46 (51.7)	27 (32.1)	**<0.01**
HIV and ART characteristics			
Years since HIV diagnosis (mean ± SD)	8.6 ± 4.2	N/A	
AIDS criteria (%)	47 (52.8)	N/A	
Current CD4 (median [IQR])	420.0 [288.0, 632.0]	N/A	
Nadir CD4 (median [IQR])	198.0 [86.0, 315.0]	N/A	
Current ART (%)	71 (79.8)	N/A	
NRTI-only ART (%)	8 (9.0)	N/A	
NNRTI-based ART (%)	16 (18.0)	N/A	
PI-based ART (%)	31 (34.8)	N/A	
Hormones, bone turnover markers, and cytokines			
FSH (mIU/ml) (median [IQR])	60.8 [44.0, 81.6]	63.9 [47.2, 84.2]	0.48
Estrone (pg/ml) (median [IQR])	23.3 [16.2, 30.4]	25.9 [20.1, 35.0]	0.06
PTH (pg/ml) (median [IQR])	35.4 [25.9, 45.0]	42.3 [31.0, 51.1]	**0.01**
25-OHD (ng/ml) (median [IQR])	41.5 [29.7, 54.2]	41.4 [31.7, 50.0]	0.97
1,25(OH)2D (pg/ml)	21.1 [11.9, 32.4]	20.4 [13.0, 27.4]	0.84
BSAP (U/liter) (median [IQR])	31.0 [23.0, 41.9]	28.6 [23.5, 37.5]	0.35
Osteocalcin (ng/ml) (median [IQR])	5.9 [4.5, 8.2]	5.5 [4.4, 7.0]	0.15
NTx (nmol/BCE/liter) (median [IQR])	17.4 [12.5, 22.8]	15.6 [12.6, 18.2]	0.08
CTx (ng/ml) (median [IQR])	0.58 [0.38, 0.75]	0.45 [0.32, 0.54]	**<0.01**
TNF-α (pg/ml) (median [IQR])	38.7 [27.5, 49.4]	26.1 [19.6, 43.4]	**<0.001**
IL-6 (pg/ml) (median [IQR])	1.6 [1.0, 2.9]	1.7 [0.98, 2.7]	0.92

Note. ART = antiretroviral therapy; BSAP = bone-specific alkaline phosphatase; CTx = C-terminal telopeptide; FSH = follicular stimulating hormone; NRTI = nucleoside reverse transcriptase inhibitor; NNRT = non-nucleoside reverse transcriptase inhibitor; NTx = N-terminal telopeptide; PTH = parathyroid hormone; TNF-α = tumor necrosis factor alpha; IL-6 = interleukin-6.

[a] Between-group differences were performed using the Student's t-test or the Mann Whitney U test for continuous variables, and χ^2 or Fisher's exact tests for categorical variables.

Table 4.2. Bone mineral density: Baseline, Follow-up and BMD Change (g/cm^2)

Variable	HIV+ (n = 89)	HIV- (n = 84)	p-value[a]
Baseline unadjusted BMD (mean ± SD)			
Lumbar spine	0.89 ± 0.13	0.92 ± 0.16	0.21
Total hip	0.87 ± 0.14	0.91 ± 0.14	**0.04**
Femoral neck	0.75 ± 0.14	0.78 ± 0.15	0.27
Distal radius	0.66 ± 0.09	0.66 ± 0.08	0.99
Ultradistal radius	0.40 ± 0.06	0.40 ± 0.07	0.80
Baseline T-score (mean ± SD)			
Lumbar spine	-1.8 ± 1.1	-1.4 ± 1.3	**0.048**
Total hip	-0.86 ± 0.96	-0.52 ± 0.96	**0.02**
Femoral neck	-1.2 ± 1.0	-0.98 ± 1.2	0.18
Distal radius	-0.52 ± 1.4	-0.52 ± 1.4	0.99
Ultradistal radius	-0.78 ± 1.1	-0.74 ± 1.2	0.80
	(n = 73)	**(n = 54)**	
Follow-up unadjusted BMD (mean ± SD)			
Lumbar spine	0.88 ± 0.12	0.89 ± 0.14	0.69
Total hip	0.86 ± 0.15	0.89 ± 0.14	0.28
Femoral neck	0.75 ± 0.13	0.76 ± 0.15	0.67
Distal radius	0.65 ± 0.09	0.66 ± 0.09	0.66
Ultradistal radius	0.38 ± 0.06	0.39 ± 0.07	0.65
Follow-up T-score (mean ± SD)			
Lumbar spine	-1.8 ± 1.0	-1.6 ± 1.2	0.32
Total hip	-0.89 ± 1.0	-0.61 ± 1.0	0.17
Femoral neck	-1.2 ± 0.97	-1.1 ± 1.2	0.48
Distal radius	-0.77 ± 1.4	-0.65 ± 1.5	0.66
Ultradistal radius	-1.0 ± 1.0	-0.92 ± 1.3	0.65
	(n = 73)	**(n = 54)**	
Unadjusted change in BMD (mean ± SD)			
Lumbar spine	-0.02 ± 0.04	-0.001 ± 0.03	**0.02**
Total hip	-0.02 ± 0.06	-0.01 ± 0.03	0.36
Femoral neck	-0.02 ± 0.06	-0.01 ± 0.04	0.50
Distal radius	-0.02 ± 0.03	-0.01 ± 0.02	**0.03**
Ultradistal radius	-0.02 ± 0.02	-0.01 ± 0.02	**0.04**

Note. BMD = bone mineral density; SD = standard deviation

[a]Between-group differences were performed using the Student's t-test.

Table 4.3. Multivariable models of factors associated with baseline BMD (g/cm^2) at each bone site for the total sample (n=173)[a,b]

	Lumbar Spine $R^2 = 0.290$		Total Hip $R^2 = 0.342$		Femoral Neck $R^2 = 0.360$		Distal Radius $R^2 = 0.364$		Ultradistal Radius $R^2 = 0.199$	
Characteristics	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***
Age (10 yr)	-3.64 (-6.57, -0.71)	**0.02**	-3.20 (-5.92, -0.47)	**0.02**	-3.92 (-6.66, -1.18)	**<0.01**	-5.97 (-7.57, -4.36)	**<0.001**	-2.75 (-4.18, -1.33)	**<0.001**
BMI (5 kg/m^2)	2.96 (1.44, 4.47)	**<0.001**	5.20 (3.79, 6.61)	**<0.001**	5.13 (3.71, 6.55)	**<0.001**	1.15 (0.32, 1.98)	**<0.01**	1.62 (0.89, 2.35)	**<0.001**
Race/ethnicity (AA:Hispanic)	11.08 (6.86, 15.30)	**<0.001**	3.68 (-0.25, 7.61)	0.07	5.85 (1.90, 9.80)	**<0.01**	4.08 (1.77, 6.40)	**<0.01**		
Depression	0.66 (-3.38, 4.70)	0.75	0.25 (-2.89, 4.63)	0.89	-1.11 (-4.89, 2.68)	0.56	0.29 (-1.92, 2.51)	0.79	0.02 (-1.91, 1.95)	0.98
BSO	2.71 (-2.83, 8.25)	0.34	-1.38 (-6.05, 4.26)	0.60	-0.48 (-5.67, 4.70)	0.86	0.30 (-2.73, 3.34)	0.85	0.18 (-2.56, 2.91)	0.90
HIV status +/-	-3.17 (-7.52, 1.18)	0.15	-3.56 (-7.37, 0.72)	0.08	-1.39 (-5.46, 2.69)	0.50	-2.13 (-4.51, 0.26)	0.08	-0.48 (-2.58, 1.62)	0.65

Note. BMD = bone mineral density; BMI= body mass index; BSO = bilateral salpingo-oophorectomy (surgical menopause); CI = confidence interval.

[a]Univariate analyses were performed to determine baseline variables associated with BMD at DXA each site. Variables tested in univariate analyses included age; race/ethnicity; body mass index (BMI); age at menarche; surgical menopause; depression; antidepressants; SSRI; previous fracture; family history of osteoporosis; current tobacco use; use of glucocorticoid ever; diabetes; hyperthyroidism; alcohol use (more than two drinks per day); injection drug use history; Hepatitis C virus infection; calcium-regulating hormones (parathyroid hormone, corrected calcium level, 25-OHD), bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, C-telopeptide type I collagen, cross-linked N-telopeptide of bone collagen type I), and cytokines (TNF-α, interleukin-6). Factors selected *a priori* for inclusion in the models were HIV status, depression, and surgical menopause. Covariates with p<0.20 in univariate analysis were entered into multivariable regression models with *a priori* variables. Covariates with p<0.05 were retained.

[b] BMD unit here was 0.01 g/cm^2

Table 4.4. Multivariable models of factors associated with change in BMD at each bone site for the total sample (n=127)[a,b]

	Lumbar Spine $R^2 = 0.144$		**Total Hip $R^2 = 0.065$**		**Femoral Neck $R^2 = 0.125$**		**Distal Radius $R^2 = 0.085$**		**Ultradistal Radius $R^2 = 0.095$**	
Characteristics	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***
IDI	-0.04 (-0.13,0.05)	0.63	-0.14 (-0.29, -0.001)	0.05	-0.17 (-0.32, -0.03)	**0.02**	-0.10 (-0.20, 0.00)	0.05	-0.09 (-0.22, 0.04)	0.16
Baseline BMD	-7.64 (-13.72, -1.56)	**0.01**	-0.81 (-9.75, 8.14)	0.86	-11.12 (-20.07, -2.17)	**0.02**	-3.18 (-13.50, 7.15)	0.79	-3.95 (-21.51, 13.61)	0.66
Depression	0.57 (-1.10, 2.25)	0.68	1.69 (-0.98, 4.36)	0.21	0.73 (-1.93, 3.38)	0.59	-0.37 (-2.21, 1.47)	0.69	-1.89 (-4.20, 0.41)	0.11
BSO	-3.63 (-6.77, -0.49)	**0.02**	0.15 (-5.22, 5.51)	0.96	-4.84 (-10.16, 0.47)	0.07	0.10 (-2.91, 3.11)	0.95	1.52 (-2.25, 5.29)	0.43
HIV status +/-	-2.44 (-4.23, -0.64)	**<0.01**	-2.22 (-5.13, 0.68)	0.13	-1.81 (-4.68, 1.06)	0.21	-2.09 (-4.07, -0.11)	**0.04**	-2.05 (-4.53, 0.43)	0.10

Note. BMD = bone mineral density; BSO = bilateral salpingo-oophorectomy; FSH = follicular stimulating hormone; IDI = standardized inter-DXA duration (time to follow-up assessment);
[a] Multiple regression β equals change in BMD (grams per square centimeter per unit change in predictor);Time between DXA (IDI), baseline BMD, HIV status (being HIV+), depression (0, no; 1, yes), and BSO (0, no; 1, yes) were forced into a model with variables significant in univariate analyses ($p<0.20$). Covariates with $p<0.05$ were retained.
[b]Variables tested in univariate analyses included age; race/ethnicity; body mass index (BMI); age at menarche; age at menopause; surgical menopause; years since menopause, past use of menopause hormone therapy; depression; antidepressants; SSRI; previous fracture; family history of osteoporosis; current tobacco use; use of glucocorticoid ever; diabetes; hyperthyroidism; alcohol use (more than two drinks per day); injection drug use history; Hepatitis C virus infection; calcium-regulating hormones (parathyroid hormone, corrected calcium level, 25-OHD), bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, C-telopeptide type I collagen, cross-linked N-telopeptide of bone collagen type I), and cytokines (TNF-α, interleukin-6). Factors selected *a priori* for inclusion in the models were HIV status, depression, and surgical menopause. Covariates with $p<0.20$ in univariate analysis were entered into multivariable regression models with *a priori* variables.

Table 4.5. Multivariable models of factors associated with change in BMD at each bone site for individuals not on antidepressants (n=94)[a,b,c]

	Lumbar Spine $R^2 = 0.162$		Total Hip $R^2 = 0.093$		Femoral Neck $R^2 = 0.088$		Distal Radius $R^2 = 0.082$		Ultradistal Radius $R^2 = 0.036$	
Characteristics	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***
IDI	0.01 (-0.09,0.11)	0.86	-0.10 (-0.23, 0.02)	0.11	-0.15 (-0.34, 0.04)	0.11	-0.07 (-0.17, 0.02)	0.13	-0.002 (-0.15, 0.14)	0.98
Baseline BMD	-3.68 (-10.12, 2.76)	0.26	0.35 (-8.45, 9.15)	0.94	-11.74 (-25.86, 2.38)	0.10	-2.09 (-14.15, 5.62)	0.39	-3.64 (-24.99, 17.71)	0.74
Untreated depression	0.15 (-2.06, 2.36)	0.89	0.52 (-2.32, 3.36)	0.72	-0.12 (-4.43, 4.20)	0.96	0.89 (-1.25, 3.03)	0.41	-0.37 (-3.62, 2.88)	0.82
BSO	-4.01 (-7.26, -0.75)	**0.02**	0.39 (-4.23, 5.02)	0.87	-4.88 (-11.81, 2.05)	0.16	0.06 (-2.52, 2.65)	0.96	1.46 (-2.45, 5.38)	0.46
HIV status +/-	-2.37 (-4.27, -0.47)	**0.02**	-2.46 (-4.96, 0.05)	0.05	-2.22 (-5.95, 1.51)	0.24	-1.56 (-3.34, 0.22)	0.09	-1.45 (-4.16, 1.25)	0.29

Note. BMD = bone mineral density; BSO = bilateral salpingo-oophorectomy; FSH = follicular stimulating hormone; IDI = standardized inter-DXA duration (time to follow-up assessment);

[a] Multiple regression β equals change in BMD (grams per square centimeter per unit change in predictor);Time between DXA (IDI), baseline BMD, HIV status (being HIV+), depression (0, no; 1, yes), and BSO (0, no; 1, yes) were forced into a model with variables significant in univariate analyses ($p<0.20$). Covariates with $p<0.05$ were retained.

[b]Variables tested in univariate analyses included age; race/ethnicity; body mass index (BMI); age at menarche; age at menopause; surgical menopause; years since menopause, past use of menopause hormone therapy; depression; antidepressants; SSRI; previous fracture; family history of osteoporosis; current tobacco use; use of glucocorticoid ever; diabetes; hyperthyroidism; alcohol use (more than two drinks per day); injection drug use history; Hepatitis C virus infection; calcium-regulating hormones (parathyroid hormone, corrected calcium level, 25-OHD), bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, C-telopeptide type I collagen, cross-linked N-telopeptide of bone collagen type I), and cytokines (TNF-α, interleukin-6). Factors selected *a priori* for inclusion in the models were HIV status, depression, and surgical menopause. Covariates with $p<0.20$ in univariate analysis were entered into multivariable regression models with *a priori* variables.

CHAPTER 5: CONCLUSIONS

This chapter summarizes the findings of the dissertation. The chapter begins with a synopsis of the systematic review. Then, the findings from each of the dissertations aims are discussed. The strengths and limitations of the entire dissertation are presented and the chapter ends with recommendations for practice and future research.

Summary of Results

Systematic Review

Although women comprise a growing proportion of older adults with HIV, the literature on the potential additive effects of HIV and menopause on age-related comorbidities, such as osteoporosis, is scant. Based on the findings of a systematic review of 10 studies, there is sufficient evidence to support menopause as an important predictor of bone loss and low bone mineral density (BMD) in HIV-infected older women, but the evidence on fracture rates is inadequate. Limitations of the current research include small sample size and the lack of biomarkers to confirm menopause status. Also, studies did not account for the potential impact of other menopause-related risk factors, such as surgical menopause and depression, which are associated with reduced bone density in the general population (Diem, Blackwell, Stone, Yaffe, Cauley, et al., 2007; Duraes Simoes et al., 1995). Research designs were also problematic; the majority of studies in this review were cross-sectional and causality could not be inferred. Longitudinal studies did not extend beyond two years (Sharma, Cohen, Freeman, Santoro, & Schoenbaum, 2011; Yin et al., 2012a), which may not be sufficient to observe the impact of depression or surgical menopause on bone loss.

The existing evidence suggests that HIV-infected postmenopausal may be at increased risk for lower BMD. However, there were only two studies that examined the impact of HIV and menopause on fractures. Findings in this review highlight the need for clinicians to accurately assess postmenopausal status and modifiable risk factors for osteoporosis in all older HIV-infected women. To address the gaps in the current literature, one of the dissertation aims was to assess the impact of menopause-related risk factors (e.g., depression and surgical menopause) on BMD in postmenopausal women with and without HIV.

Aim 1: To characterize and compare CVD risk, including the impact of menopause-related factors (surgical menopause and clinical depression), using the Framingham Risk Score as an assessment measure.

Heart disease accounts for approximately 12% of deaths in women aged 35 to 44 years; this estimate rises to 25% among women aged ≥ 65 years (CDC., 2013b). Previous studies have found that HIV is associated with CVD in women (Triant et al., 2007; Womack), but the impact of menopause-related factors on CVD risk in HIV-infected women has not been previously investigated. For this portion of the dissertation, a cross-sectional study was conducted to compare and characterize CVD risk in HIV-infected and uninfected postmenopausal women. Study findings revealed that FRS did not differ significantly in an age-matched sub-sample of HIV-infected and uninfected women. While history of CVD also did not differ between groups, nearly twice as many HIV-infected subjects judged as low risk for CVD according to the FRS had a history of CVD compared to controls.

Earlier studies have reported that the FRS may underestimate CVD risk in HIV-infected individuals, possibly due to the failure to include to HIV-related characteristics in the algorithm (i.e. HIV-1 RNA, CD4 cell count, antiretroviral therapy) (Knobel et al., 2007; Law et al., 2006). In this dissertation, performance of the FRS in older HIV-infected women was evaluated using the Receiving Operating Characteristic (ROC) curve. These analyses demonstrated that the performance of the FRS was compromised. In the multivariable analysis, older age at HIV diagnosis was associated with worse (higher) FRS. Since our analysis was limited to cross-sectional data, we cannot determine CVD risk prior to HIV infection. However, older age is an important predictor of hypertension in the general population and in HIV-infected individuals (Antonello et al., 2015), suggesting that individuals infected with HIV later in life may already be at increased risk for CVD. Our multivariable model also demonstrated that higher current CD4 cell counts were associated

with worse FRS. After adjusting for time on ART, current CD4 was no longer associated with FRS, suggesting that higher CD4 cell counts may be a surrogate marker for longer time on ART. While evidence on the relationship between CVD and ART is conflicting, ART has been associated with worse FRS (Bergersen et al., 2004), perhaps due to the adverse metabolic effects of ART.

The potential benefits of statin therapy as an anti-inflammatory (Calza et al., 2012) may help reduce CVD risk in HIV-infected postmenopausal women. However, this study also found that over half of subjects not receiving a statin, were eligible for this medication. Statins are recommended for the treatment of dyslipidemia and prevention of CVD events in the general population. Low rates of statin prescription in HIV-infected individuals may contribute to CVD risk and the earlier occurrence of CVD events in this population (Feinstein, Achenbach, Stone, & Lloyd-Jones, 2015; Rasmussen et al., 2013). While we were unable to ascertain the reasons for undertreatment with statins in this study population, studies in the general population have identified several barriers to statin prescribing including, provider concerns about cost, increased workload, and concern over patient adherence to treatment (Kedward & Dakin, 2003; Ab, Dening, van Vliet, & Dekker, 2009). Whether there are similar barriers to statin prescription in HIV-infected individuals remains unclear.

Increased risk of CVD in aging HIV-infected women is a growing concern. Clinicians treating HIV-infected older women should carefully consider all CVD risk factors, including of CD4 count and age at HIV diagnosis, which may improve CVD risk assessment. Administration of statin therapy when indicated may be important to reduce risk of CVD among HIV-infected postmenopausal.

Aim 2: To examine the impact of menopause-related risk factors (surgical menopause and clinical depression) on change in bone mineral density.

In this aim, a longitudinal analysis was conducted to examine clinical factors influencing change in BMD in HIV-infected and uninfected women. At baseline, the HIV-infected group demonstrated fewer menopause risk characteristics for osteoporosis (earlier in postmenopause, lower rates of surgical menopause and use of hormone therapy), although the prevalence of depression, use of antidepressants and alcohol were higher versus controls. As in the parent study, greater bone loss was evident in the HIV-infected group as judged by baseline biomarkers and T-scores (LS, total hip).

In the total sample, where surgical menopause rates approach 15%, surgical menopause was independently associated with bone loss at the lumbar spine. This is consistent with current evidence in the general population that bilateral oophorectomy is a risk factor for accelerated bone loss (Yoshida et al., 2011), perhaps due to the abrupt depletion of estrogen. Contrary to our hypothesis, diagnosis of depression, based on ICD-9 codes, was not associated with baseline BMD or annualized percent change in BMD. Furthermore, in a *post hoc* analysis among women without prescribed antidepressants, untreated depression was not associated with bone loss at any site.

There is evidence to suggest a possible adverse effect of SSRIs on bone (Diem et al., 2007; Haney et al., 2007). In our analysis, prescribed SSRI was associated with decline of BMD in univariate analyses, but was not a predictor of bone loss in the multivariable model. Previous studies observing a higher rate of bone loss with SSRI use were large (n>2,000) prospective cohort studies (Diem et al., 2007; Diem et al., 2013); to what extent the relatively small sample size of this sub-group limited our ability to test for this in the model is unclear.

Even in the presence of relatively high rates of depression, HIV infection was an independent predictor bone loss at the LS and DR. The proposed mechanism by which HIV infection may lead to reduced BMD in older women includes cytokine activation by HIV infection itself together with the impact of modifiable risk factors for bone loss common in

HIV-infected individuals (e.g., alcohol and substance abuse, smoking, and physical activity). Both surgical menopause and depression have been associated with persistent inflammation through different mechanisms (Miller, Maletic, & Raison, 2009; Pacifici et al., 1991; Pfeilschifter, Koditz, Pfohl, & Schatz, 2002), but to what extent either of these factors may contribute to underlying bone deficiencies requires further study. One reason for the lack of an observed association may be that the robust influence of HIV status in the model coupled with the relatively small sample size may have masked other potential moderators.

In summary, while surgical menopause was associated with bone loss in the total sample at the LS, depression did not predict BMD changes either in the cross-sectional comparison or in the longitudinal analysis. When entered *a priori* into regression models, untreated depression was not significantly associated with bone loss. Further analyses are necessary to assess the influence of antidepressants, particularly SSRIs on bone loss in older HIV-infected women.

Dissertation Strengths and Limitations

Strengths

There were several strengths of this dissertation. First, in the systematic review, 28 full-text articles were reviews from various disciplines. The search was conducted over several months and using three electronic databases. The methodological quality of each study was assessed using a validated checklist developed by Downs and Black (1998). The review was comprehensive, including studies on low BMD as well as fractures. Moreover, this was the first systematic review to of the current evidence on menopause and bone health in HIV-infected individuals.

Other strengths of the dissertation include its focus on HIV-infected postmenopausal minority women, a population at high risk for cardiovascular disease and bone loss, but that

has been underrepresented in previous research. This is the first study to assess CVD risk exclusively in HIV-infected postmenopausal women. To our knowledge, it is also the first study to focus on the role of menopause-related factors (i.e. depression and surgical menopause) on bone loss in postmenopausal women with HIV.

Another strength of this dissertation is that the control group of uninfected women was in some ways, a positive "high-risk" control for the simultaneous co-morbidities often seen in HIV patients that potentially confound results. In prior studies focusing on HIV-infected women, the uninfected group had lower rates of smoking and substance abuse (Schwartz et al., 2012; Womack et al., 2014), traditional risk factors for CVD and low BMD.

This dissertation expanded on a previous study of bone loss in HIV-infected and uninfected postmenopausal women, which measured bone turnover markers and cytokines in the Biomarkers Core Laboratory and Metabolic Bone Unit at Columbia University Medical Center. The availability of these biomarkers was important in **Aim 2** because they provided further information on early changes in bone, which could help elucidate the pathogenesis of osteoporosis in the study sample. Additionally, postmenopausal status was confirmed with reproductive hormone levels, which helped ensure that participants met eligibility criteria for study inclusion.

Limitations

The findings of this dissertation are subject to some limitations in terms of the research design, sample, and measurement of variables:

Design: This dissertation expanded on an existing dataset and was limited by the data collected in the parent study. Because **Aim 1** was a cross-sectional study, causality could not be inferred. In **Aim** 2, a longitudinal analysis was conducted with a mean follow-up time of approximately 15 months, which may not have been long enough to observe significant changes in bone.

Sample: In the systematic review, only studies that assessed BMD by DXA were included so it is possible that other less rigorous studies were missed. **Aim 1** included a small sample size of HIV-uninfected women, limiting the inferences that can be drawn regarding the differences in Framingham risk between HIV-infected and uninfected women. Similarly, attrition in **Aim 2,** particularly among the HIV-uninfected group, was problematic. Different rates of losses to follow-up between groups may result in attrition bias.

Measurement: The use of a retrospective chart review was a limitation because some participants had to be excluded from the study for lack of access to medical records. In **Aim 1**, rates of CVD prevalence, based on any previous ICD-9 codes of a CVD event (i.e. myocardial infarction, coronary artery disease, stroke, peripheral artery disease, heart failure), we used to evaluate the FRS. This was a limitation since the FRS is a measure of incident CVD. However, since individuals with prevalent CVD are at higher risk for a subsequent CVD event (Miller, Seidler, Kwiterovich, & Pearson, 1992), it is expected that the FRS would be able to differentiate these individuals from those at low risk for CVD. Depression diagnosis based on ICD-9 codes was another limitation, particularly in **Aim 2**. Individuals with depressive symptoms, a risk factor for bone loss (Diem et al., 2013), may have been missed in the analysis. Additionally, different levels of depression may have varying effects on bone, but severity of depression could not be assessed in this study.

Recommendations

Based on the overall findings of this dissertation, several recommendations can be made for clinical practice and future research. While a FRS assessment has been recommended for HIV-infected women >50 years without CVD (Lundgren, 2013), results of this dissertation suggest that the FRS may underestimate cardiovascular risk in HIV-infected postmenopausal women. Additionally, CVD may occur earlier in this population. The

dissertation findings suggest the importance of CVD risk assessment in HIV-infected postmenopausal women. Assessment of CVD risk in this population should consider the role of HIV-related characteristics such as ART and CD4 cell counts. Administration of statin therapy is important to reduce CVD risk in this high-risk population. Future studies of CVD in older women with HIV would benefit from the inclusion of subclinical markers for atherosclerosis to better characterize CVD risk and its association with menopause and HIV infection.

Moreover, our finding that HIV infection is a determinant of bone loss after adjusting for menopause-related characteristics (i.e. surgical menopause and depression) support recent recommendations to perform a bone density scan on all postmenopausal women with HIV (Brown et al., 2015). Findings also suggest that cause of menopause (natural versus surgical) and use of menopausal HT may be additional factors to consider during risk assessment for bone loss. Future studies that follow women across the menopause transition, using confirmatory reproductive biomarkers are needed to gain a better understanding of the impact of menopause on HIV disease progression and bone loss. Longer than usual follow-up of HIV-infected women may be necessary to determine risk for fractures in this population, given their earlier age of menopause and age at which fractures commonly occur.

Conclusions

In summary, this dissertation found lower rates of surgical menopause and higher rates of depression in HIV-infected postmenopausal women compared to uninfected controls. Although several of the dissertation hypotheses were not supported, some unexpected findings arose in the analyses. First, the performance of FRS as a CVD risk prediction tool may be compromised in older HIV-infected women. Older age at HIV diagnosis and CD4 cell counts may be important HIV-related factors contributing to CVD risk in this population. In terms of change in BMD, depression does not contribute substantially to the accelerated

bone loss seen in HIV-infected postmenopausal women.

This is the first study to assess the impact of menopause-related factors on CVD and bone loss in postmenopausal HIV-infected minority women. With emerging evidence that HIV infection and its treatment are associated with similar metabolic complications to reproductive aging, a greater understanding of the effect of sex hormones and menopause-related factors on HIV disease progression is necessary. In addition, future research is necessary to develop guidelines to both prevent and treat these conditions more effectively in older HIV-infected women as they transition through the stages of reproductive aging.

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LIST OF APPENDICES

Appendix A: PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	20
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	21
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	22-23
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	23
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	23-24
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	24
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	24
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	23-25
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	23-25
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	23-25

Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	25-26
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	23-25, 32
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	23-26, 32
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	38
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A

RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	26-27
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	28-29
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	29, 32
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	29, 32
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	30-34
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	34-37
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	38
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	37-38
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	See publication

Appendix B: Quality Assessment Tool Adapted from Downs and Black (1998)

Article Identifier (Author, Year):

REPORTING

1. Is the hypothesis/aim/objective of the study clearly described?

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

2. Are the main outcomes to be measured clearly described in the Introduction or Methods section? *If the main outcomes are first mentioned in the Results section, the question should be answered no.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

3. Are the characteristics of the patients included in the study clearly described? *In cohort studies and trials, inclusion and/or exclusion criteria should be given. In case-control studies, a case-definition and the source for controls should be given.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

4. Are the interventions of interest clearly described*? Treatments and placebo (where relevant) that are to be compared should be clearly described.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

5. Are the distributions of principal confounders in each group of subjects to be compared clearly described? *A list of principal confounders is provided.*

☐ Yes = 2 ☐ Partially = 1 ☐ No = 0 ☐ Not Applicable

6. Are the main findings of the study clearly described? *Simple outcome data (including denominators and numerators) should be reported for all major findings so that the reader can check the major analyses and conclusions. (This question does not cover statistical tests, which are considered below).*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

7. Does the study provide estimates of the random variability in the data for the main outcomes? *In non-normally distributed data the inter-quartile range of results should be reported. In normally distributed data the standard error, standard deviation or confidence intervals should be reported. If the distribution of the data is not described, it must be assumed that the estimates used were appropriate and the question should be answered yes.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

8. Have all important adverse events that may be a consequence of the intervention been reported? *This should be answered yes if the study demonstrates that there was a comprehensive attempt to measure adverse events. (A list of possible adverse events is provided).*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

9. Have the characteristics of patients lost to follow-up been described? *This should be answered yes where there were no losses to follow-up or where losses to follow-up were so small that findings would be unaffected by their inclusion. This should be answered no where a study does not report the number of patients lost to follow-up.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

Total Reporting Score: ____

EXTERNAL VALIDITY

11. Were the subjects asked to participate in the study representative of the entire population from which they were recruited? *The study must identify the source population for patients and describe how the patients were selected. Patients would be representative if they comprised the entire source population, an unselected sample of consecutive patients, or a random sample. Random sampling is only feasible where a list of all members of the relevant population exists. Where a study does not report the proportion of the source population from which the patients are derived, the question should be answered as unable to determine.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

12. Were those subjects who were prepared to participate representative of the entire population from which they were recruited? *The proportion of those asked who agreed should be stated. Validation that the sample was representative would include demonstrating that the distribution of the main confounding factors was the same in the study sample and the source population.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

13. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive? *For the question to be answered yes the study should demonstrate that the intervention was representative of that in use in the source population. The question should be answered no if, for example, the intervention was undertaken in a specialist center unrepresentative of the hospitals most of the source population would attend.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

Total External Validity Score: ____

INTERNAL VALIDITY

14. Was an attempt made to blind study subjects to the intervention they have received? *For studies where the patients would have no way of knowing which intervention they received, this should be answered yes.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

15. Was an attempt made to blind those measuring the main outcomes of the intervention?

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

16. If any of the results of the study were based on "data dredging", was this made clear? *Any analyses that had not been planned at the outset of the study should be clearly indicated. If no retrospective unplanned subgroup analyses were reported, then answer yes.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

17. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case-control studies, is the time period between the intervention and outcome the same for cases and controls? *Where follow-up was the same for all study patients the answer should yes. If different lengths of follow-up were adjusted for by, for example, survival analysis the answer should be yes. Studies where differences in follow-up are ignored should be answered no.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

18. Were the statistical tests used to assess the main outcomes appropriate? *The statistical techniques used must be appropriate to the data. For example nonparametric methods should be used for small sample sizes. Where little statistical analysis has been undertaken but where there is no evidence of bias, the question should be answered yes. If the distribution of the data (normal or not) is not described*
it must be assumed that the estimates used were appropriate and the question should be answered yes.

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

19. Was compliance with the intervention/s reliable? *Where there was non compliance with the allocated treatment or where there was contamination of one group, the question should be answered no. For studies where the effect of any misclassification was likely to bias any association to the null, the question should be answered yes.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

20. Were the main outcome measures used accurate (valid and reliable)?
For studies where the outcome measures are clearly described, the question should be answered yes. For studies which refer to other work or that demonstrates the outcome measures are accurate, the question should be answered as yes.

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

Total Internal Validity Score: ____

INTERNAL VALIDITY - CONFOUNDING

21. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population? *For example, patients for all comparison groups should be selected from the same hospital. The question should be answered unable to determine for cohort and case-control studies where there is no information concerning the source of patients included in the study.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

22. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same period of time? *For a study which does not specify the time period over which patients were recruited, the question should be answered as unable to determine.*

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

23. Were study subjects randomized to intervention groups?
Studies which state that subjects were randomized should be answered yes except where method of randomization would not ensure random allocation. For example alternate allocation would score no because it is predictable.

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

24. Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?
All non-randomised studies should be answered no. If assignment was concealed from patients but not from staff, it should be answered no.

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

25. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?
This question should be answered no for trials if: the main conclusions of the study were based on analyses of treatment rather than intention to treat; the distribution of known confounders in the different treatment groups was not described; or the distribution of known confounders differed between the treatment groups but was not taken into account in the analyses. In nonrandomized studies if the effect of the main confounders was not investigated or confounding was demonstrated but no adjustment was made in the final analyses the question should be answered as no.

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

26. Were losses of patients to follow-up taken into account?
If the numbers of patients lost to follow-up are not reported, the question should be answered as unable to determine. If the proportion lost to follow-up was too small to affect the main findings, the question should be answered yes.

☐ Yes = 1 ☐ No = 0 ☐ Not Applicable

Total Confounding Score: ____

POWER
27. Did the study mention having conducted a power analysis to determine the sample size needed to detect a significant difference in effect size for one or more outcome measures?

☐ Yes, two or more measures = 2 ☐ Yes, one measure = 1 ☐ No = 0

Total Power Score: ____

TOTAL QUALITY SCORE: _____

Appendix C: Dissertation Sample Sources

Data from longitudinal cohorts of two existing datasets (R01AI065200, PI: Shane) were considered for the dissertation project. The dissertation sample sources are summarized in **Figure 1.** In Aim 1, 283 (131 HIV+, 152 HIV-) women were excluded because of missing data needed to calculate the Framingham Risk Score. Overall, 152 (109 HIV+, 43 HIV-) women were included in Aim 1. Of 187 (92 HIV+, 95 HIV-) individuals from previously a published longitudinal cohort (Yin et al., 2012b), 14 (3 HIV+, 11 HIV-) subject records were excluded from Aim 1 due to missing data on depression. For Aim 2, baseline data were available for 173 (89 HIV+, 84 HIV-) women and at follow-up 127 (74 HIV+, 53 HIV-) women were included.

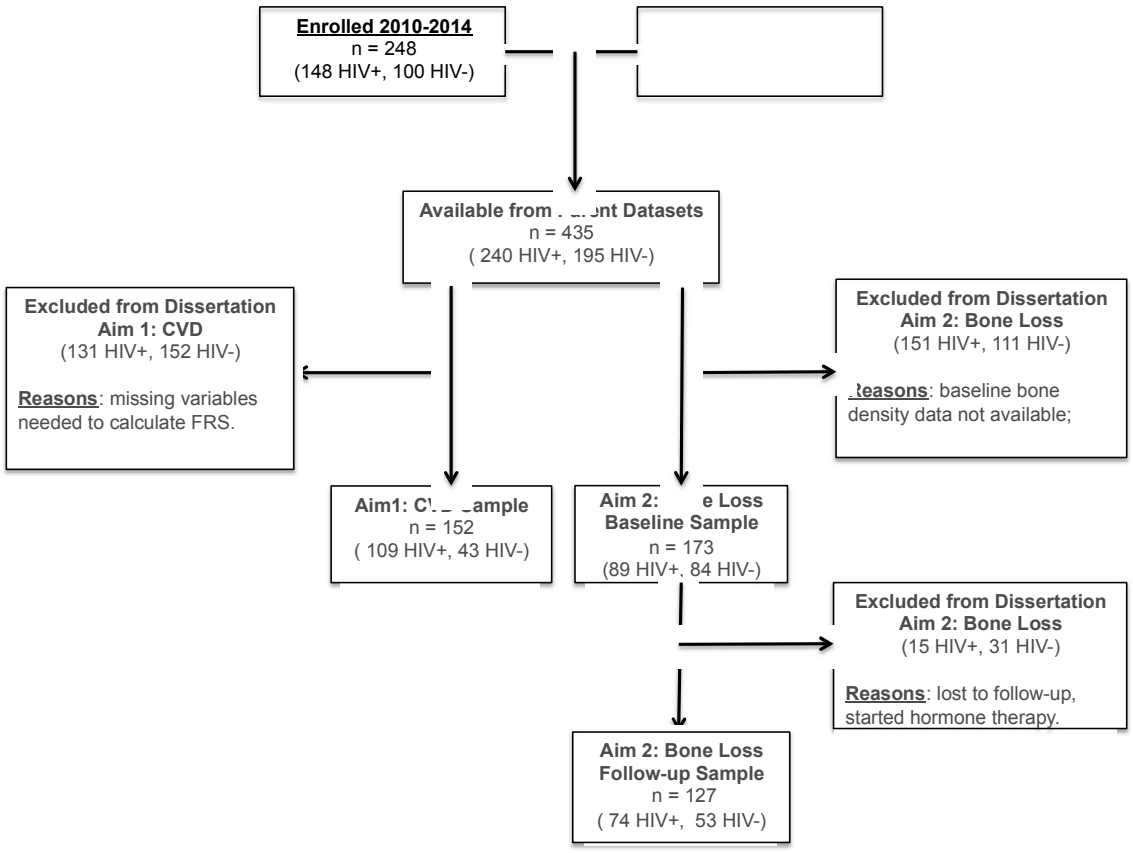


Figure 1. Dissertation sample sources.

Appendix D: Summary of Study Variables and Measurements

Variable	Data Source	Units of Measure
Socio-demographic characteristics		
Age[a]	Interview with study questionnaire	Years
Race/Ethnicity	Interview with study questionnaire	White (non Hispanic); Hispanic white; Hispanic black; African-American; Black (not American); Asian
Reproductive History		
Age at menarche	Interview with study questionnaire	Years
Age at menopause	Interview with study questionnaire	Years
Years since menopause	Interview with study questionnaire	Years
Surgical menopause[b]	Interview with study questionnaire; Chart review	Hysterectomy (yes/no); Bilateral salpingo-oophorectomy (yes/no)
Menopause hormone therapy (HT)	Interview with study questionnaire	Yes/No
Disease Risk Biomarkers		
Estradiol	Blood sample by venipuncture	pg/mL
Follicular stimulating hormone (FSH)	Blood sample by venipuncture	mIU/mL
Glucose	Chart review	mg/dL
25-OHD[d]	Blood sample by venipuncture	ng/mL
$1,25(OH)_2D$[d]	Blood sample by venipuncture	pg/mL
Bone alkaline phosphatase[d]	Blood sample by venipuncture	U/liter
Osteocalcin[d]	Blood sample by venipuncture	ng/mL
N-telopeptide[d]	Blood sample by venipuncture	nmol/BCE/liter
C-telopeptide[d]	Blood sample by venipuncture	ng/mL
Tumor Necrosis Factor-α[d]	Blood sample by venipuncture	pg/mL
Interkleukin-6[d]	Blood sample by venipuncture	pg/mL
Systolic blood pressure[a,b,c]	Chart review	mmHg
Diastolic blood pressure[b,c]	Chart review	mmHg
Total cholesterol[a,b,c]	Chart review	mg/dL
Low-density lipoprotein[b,c]	Chart review	mg/dL
High-density lipoprotein[a,b,c]	Chart review	mg/dL
Triglyceride[b,c]	Chart review	mg/dL
HIV Characteristics		
HIV status	Blood sample by venipuncture	Yes/No
Years since HIV diagnosis	Interview with study questionnaire	Years
Nadir CD4	Chart review	cells/mm^3
Current CD4	Blood sample by venipuncture; Chart review	cells/mm^3

Current HIV viral load	Blood sample by venipuncture; Chart review	log_{10} copies/mL
AIDS diagnosis	Interview with study questionnaire	Yes/No
ART exposure ever	Interview with study questionnaire	Yes/No
Current ART	Interview with study questionnaire	Yes/No
Months on ART	Interview with study questionnaire	Months
PI-based ART	Interview with study questionnaire	Yes/No
NNRTI-based ART	Interview with study questionnaire	Yes/No
NRTI-only ART	Interview with study questionnaire	Yes/No
Medical History		
Asthma[d]	Interview with study questionnaire	Yes/No
Depression[b]	Chart review (ICD9: 296.2, 296.3, 296.5, 300.4, 301.12,	Yes/No
Diabetes[a]	309.0, 309.1, 311)	Yes/No
Family history of osteoporosis[d]	Chart review	Yes/No
History of atraumatic fractures[d]	Interview with study questionnaire	Yes/No
Hepatitis C seropositive	Interview with study questionnaire	Yes/No
History of myocardial infarction[b,c]	Interview with study questionnaire	Yes/No
	Chart review (ICD9: 410.00-410.02, 410.10-410.12, 410.20-410.22, 410.30-410.32, 410.40-410.42, 410.50-410.52, 410.60-410.62, 410.70-410.72, 410.80-410.82, 410.90-410.92, 411.0, 412)	
History of coronary artery disease[b,c]	Chart review (ICD9: 411.1, 411.81, 414.00-414.07, 414.3, 414.4, 440.0, 411.89, 414.8, 414.9)	Yes/No
History of cerebrovascular disease[b,c]	Chart review (ICD9: 430, 431, 432.0-432.9, 433.01, 433.11, 433.21, 433.31, 433.81, 433.91, 434.01, 434.11, 434.91, 435, 435.8, 453.9)	Yes/No
History of peripheral vascular disease[b,c]	Chart review (ICD9: 440.21, 443.89, 443.9, 459.81)	Yes/No
History of heart failure[b,c]	Chart review (ICD9: 402.01, 402.11, 402.91, 404.01, 404.03, 404.11, 404.13, 404.91, 404.93, 428.0, 428.1, 428.20-428.23, 428.30-428.33, 428.40-428.43, and 428.9)	Yes/No
	Interview with study questionnaire	Yes/No
Hyperlipidemia	Interview with study questionnaire	Yes/No
Hyper/hypothyroidism[d]	Interview with study questionnaire; Chart review	Yes/No
Hypertension	Interview with study questionnaire	Yes/No
Seizures[d]		
Medications (not HRT or ART)		
Antidepressants[b]	Chart review	Yes/No
Calcium supplementation	Interview with study questionnaire	Yes/No
Glucocorticoids	Interview with study questionnaire	Yes/No
Hypertensive treatment[a,b]	Chart review	Yes/No

Levothyroxine[d]	Interview with study questionnaire	Yes/No
Dilantin/phenoparbitol/carbamazepine[d]	Interview with study questionnaire	Yes/No
Statins	Interview with study questionnaire	Yes/No
Oral hypoglycemic	Interview with study questionnaire	Yes/No
Insulin	Interview with study questionnaire	Yes/No
Multivitamin	Interview with study questionnaire	Yes/No
Behavioral Factors		
Alcohol consumption	Interview with study questionnaire	None; ≤ 1 drink/day; >1 drink/day
Current tobacco use[a]	Chart review	Yes/No
Daily calcium intake	Dietary rapid assessment	mg
Daily vitamin D intake	Dietary rapid assessment	IUs
Injection drug use	Interview with study questionnaire	Yes/No
Body Composition		
Body Mass Index[a]	Physical examination	Weight in kg divided by height in m^2
Body Fat	Dual energy X-ray Absorptiometry (DXA)	%
Truncal Fat	DXA	%
Bone Mineral Density[d]	DXA	g/cm^2

Note. All variables, unless otherwise indicated, were available from the parent study dataset.
[a] Used to determine Framingham Risk Score for cardiovascular disease risk profile.
[b] Additional variable for the purpose of this dissertation project, not available from parent study dataset.

Appendix E: Parent Study Questionnaire

Study Title: Bone Disease in Patients with HIV Infection (IRB#AAAA5450)

(R01AI065200, PI: Shane)

Date:_____ Primary Care MD: _____

Name: _____ MRN: _____

DOB:_____ AGE: _____

Contact telephone number: _____

Address: _____

Ethnicity (please circle):

White (non Hispanic)

Hispanic white Country of Origin: _____

Hispanic black Country of Origin:_____

African-American

Black (not American) Country of Origin: _____

Asian

Exclusion Criteria (please circle if patient has any of the following conditions)

Multiple Myeloma

Primary Hyperparathyroidism

Paget's Disease

Cushing's Syndrome

Renal insufficiency (serum CR > 2.5)

History of bisphosphonate or calcitonin treatment

Last period < 1 year ago

If yes for any of above, please page Michael Yin: 917-899-0412

Reproductive History (please circle)

Age at Menarche: _____

Menses: **Regular** **Irregular**

Infertility: **Yes** **No**

Hysterectomy: **Yes** **No** Removal of Ovaries: **Yes** **No** **Year** _____

Pre-menopausal amenorrhea (amennorhea > 3 months) **Yes** **No**

If yes, at what age and for how long? _____

Age at Menopause: _____ FSH (if known): _____

Date of last LMP: _____ Years since menopause: _____

Did you have menopausal symptoms: **Yes** **No**

Hormone Replacement Therapy: **Yes** **No**

For symptoms or for prevention: _____

Medication/Dose: _____

Date started and Date stopped: _____

Osteoporosis Related Factors (please circle)

Tobacco: **Current** **Past** **Never** Pack yrs: _____

Alcohol: **None** **≤ 1 drink per day** **>1 drink per day**

Family history of Osteoporosis: **Yes** **No** Relation: _____

Lowest adult weight: _____(year) _____

Lowest weight after diagnosis of HIV _____ (year) _____

Weight one year ago: _____

Current weight: _____

History of *Atraumatic* Fracture (fall from standing height or less) **Yes** **No**

Spine **Yes** **No**

Hip **Yes** **No**

Forearms **Yes** **No**

Ribs **Yes** **No**

Others sites_____

Medical History (please circle and detail)

Diabetes If yes, list meds_____

Hyperlipidemia If yes, list meds _____

Hyper/Hypothyroidism If yes, list treatment_____

Asthma If yes, list meds_____

Seizures If yes, list meds_____

Malignancy If yes, list type and treatment_____

HTN If yes, list meds _____

Heart Disease If yes, list treatment _____

Renal Insufficiency If yes, list treatment_____

Chronic Hepatitis C or B If yes, list diagnosis date and treatment

_____

Medication History (please circle, if yes, please give dose and duration)

L- thyroxine	**Yes**	**No**	_____
Dilantin/phenobarbitol/carbamazepine	**Yes**	**No**	_____
Statins	**Yes**	**No**	_____
Oral hypoglycemics	**Yes**	**No**	_____
Prednisone	**Yes**	**No**	_____
Calcium supplementation	**Yes**	**No**	_____
Multivitamin	**Yes**	**No**	_____

HIV History

Date of HIV diagnosis: _____

History of IVDU **Yes** **No**

Mode of HIV transmission:

CD4 Nadir: _____Date: _____

Most recent CD4 _____ _____Date: _____

Most recent Viral load _____ Date: _____

AIDS diagnosis: **Yes** **No** AIDS defining DX:_____

OI's (list all diagnosis and dates): _____

_____

Antiretroviral treatment (ART) exposure ever: **Yes** **No**

Date beginning ART:

Currently on ART: **Yes** **No**

If no, when stopped and why?_____

If yes, please list current regimen below

Total ART exposure in months:

Nucleosides: _____

Non-nucleosides:_____

Protease Inhibitors:_____

The following is a worksheet for calculating total ART exposure by class:

First Regimen:

_____*Date:* _____

_____

_____

Second Regimen

_____ *Date:* _____

_____

_____

Third Regimen

_____ *Date:* _____

_____

_____

_____

Current Regimen

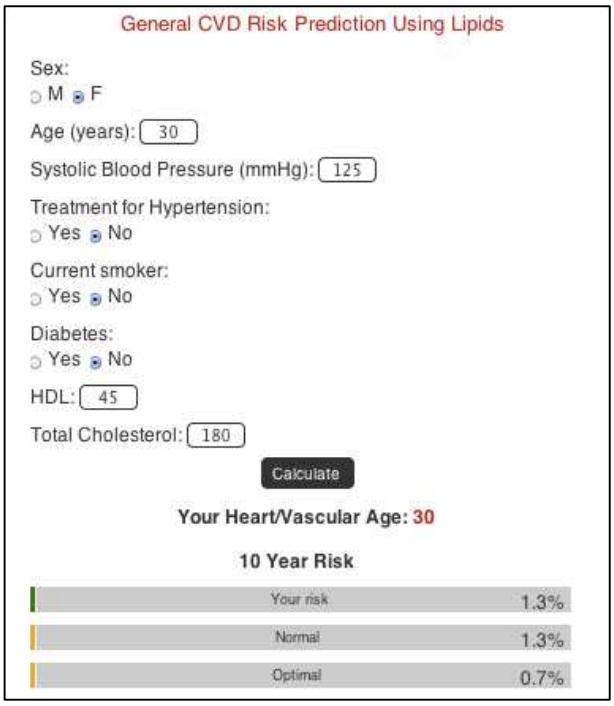
_____ *Date:* _____

_____

_____

_____

Appendix F: Framingham Risk Score (10-year Cardiovascular Disease)



Regression Coefficients and Hazard Ratios - Primary Model

Men* (10-year Baseline Survival: So(10) = 0.88936)				
Variable	Beta**	p-value	Hazard Ratio	95% CI
Log of Age	3.06117	<.0001	21.35	(14.03, 32.48)
Log of Total Cholesterol	1.12370	<.0001	3.08	(2.05, 4.62)
Log of HDL Cholesterol	-0.93263	<.0001	0.40	(0.30, 0.52)
Log of SBP if not treated	1.93303	<.0001	6.91	(3.91, 12.20)
Log of SBP if treated	1.99881	<.0001	7.38	(4.22, 12.92)
Smoking	0.65451	<.0001	1.92	(1.65, 2.24)
Diabetes	0.57367	<.0001	1.78	(1.43, 2.20)

Women* (10-year Baseline Survival: So(10) = 0.95012)				
Variable	Beta**	p-value	Hazard Ratio	95% CI
Log of Age	2.32888	<.0001	10.27	(5.65, 18.64)
Log of Total Cholesterol	1.20904	<.0001	3.35	(2.00, 5.62)
Log of HDL Cholesterol	-0.70833	<.0001	0.49	(0.351, 0.691)
Log of SBP if not treated	2.76157	<.0001	15.82	(7.86, 31.87)
Log of SBP if treated	2.82263	<.0001	16.82	(8.46, 33.46)
Smoking	0.52873	<.0001	1.70	(1.40, 2.06)
Diabetes	0.69154	<.0001	2.00	(1.49, 2.67)

Appendix G: Receiver Operating Characteristic Curve

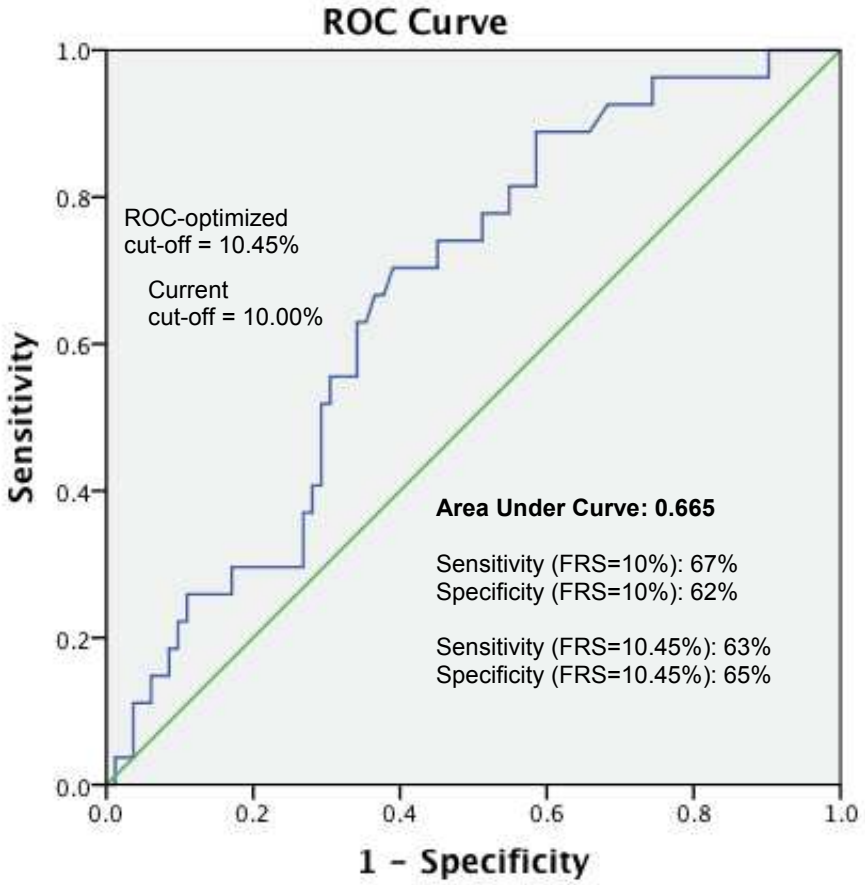


Figure 3.2. Receiver operating characteristic (ROC) curve, area under the curve, and ROC-optimized cut-off for the Framingham Risk Score in HIV-infected postmenopaual women.

Note. How accurately the FRS differentiates between HIV-infected older women with and without a history of CVD was assessed using the area under the ROC curve (AUC) (Faraggi & Reiser, 2002). AUC values range from 0 to 1, with values of 0.5 or less indicating the tool has no discriminative ability (i.e., the test is as good as a random guess), and 1 indicating the tool has perfect discriminative ability (Cantor & Kattan, 2000). Here, the standard FRS cut-off score of 10% is compared to the optimal cut-off score in the ROC curve.

Appendix H: Univariate Analyses in Aim 2 (Bone)

Table 1. Univariate regression analyses of factors independently associated with BMD (0.01 g/cm^2) in the total sample (n = 173)[a,b]

	Lumbar Spine			Total Hip			Femoral Neck			Distal Radius			Ultradistal Radius		
Characteristics	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***
Age (10 yr)	-4.45	1.59	<0.01	-3.94	1.54	0.01	-5.15	1.55	<0.01	-6.10	0.82	<0.001	-0.03	0.01	<0.01
BMI (5 kg/m^2)	4.11	0.79	<0.001	5.86	0.68	<0.001	5.80	0.70	<0.001	1.90	0.47	<0.001	0.02	0.01	<0.01
Race/ethnicity (AA:Hispanic)	12.79	2.19	<0.001	6.32	2.26	<0.01	9.11	2.25	<0.001	5.55	1.32	<0.001			
Family history of osteoporosis													0.03	0.02	0.19
Corrected calcium													0.04	0.03	0.10
Alcohol use	4.78	1.72	<0.01	3.15	1.67	0.06	2.71	1.71	0.12	1.41	1.01	0.16			
Years since menopause	-0.01	0.001	<0.001	-0.01	0.002	0.01	-0.01	0.003	0.03	-0.01	0.001	<0.001	-0.003	0.001	<0.01
Age at menarche													-0.01	0.004	0.09
FSH (mIU/ml)	-0.10	0.04	0.09	-0.12	0.04	<0.01	-0.11	0.04	<0.01	-0.04	0.03	0.10			
Estrone (pg/ml)													0.002	0.001	0.01
Osteocalcin (ng/ml)	-1.10	0.42	<0.01	-1.63	0.39	<0.001	-1.22	0.41	<0.01	-0.80	0.24	<0.01	-0.01	0.003	0.006
BSAP (10 U/L)	-0.10	0.08	0.17	-0.21	0.07	<0.01	-0.14	0.07	0.05	-0.12	0.04	<0.01	-0.01	0.01	0.11
NTx (10 nmol/BCE/L)				-0.34	0.15	0.03	-0.20	0.16	0.19	-0.15	0.09	0.09			
CTx (ng/ml)	-12.59	4.93	0.01	-16.38	4.45	<0.001	-16.74	4.64	<0.001	-6.47	2.70	0.02	-0.17	0.05	0.01
IL-6 (pg/mL)	1.21	0.49	0.02				0.73	0.49	0.14						
HIV status +/-				-4.41	2.12	0.04									

Note. BSAP = bone specific alkaline phosphatase; BMD = bone mineral density; BMI = body mass index; CTx = C-terminal telopeptide; FSH = follicular stimulating hormone; IL-6 = interleukin-6; NTx = N-terminal telopeptide; OC =osteocalcin

[a]Variables tested in univariate analyses included age; race/ethnicity; body mass index (BMI); age at menarche; age at menopause; years since menopause; use of menopausal hormone therapy (ever); surgical menopause; depression; antidepressants; SSRI; previous fracture; family history of osteoporosis; current tobacco use; use of glucocorticoid ever; diabetes; hyperthyroidism; alcohol use (more than two drinks per day); injection drug use history; Hepatitis C virus infection; calcium-regulating hormones (parathyroid hormone, corrected calcium level, 25-OHD), bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, C-telopeptide type I collagen, cross-linked N-telopeptide of bone collagen type I), and cytokines (TNF-α, interleukin-6).

[b]Only variables with p<0.20 are reported here.

Table 2. Univariate regression analyses of factors independently associated with BMD (0.01 g/cm^2) in HIV-infected postmenopausal women (n = 89)[a,b]

	Lumbar Spine			Total Hip			Femoral Neck			Distal Radius			Ultradistal Radius		
Characteristics	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***
Age (10 yr)	-6.14	2.08	<0.01	-6.17	2.20	<0.01	-6.14	2.12	<0.01	-6.60	1.22	<0.001	-0.03	0.02	0.17
BMI (5 kg/m^2)	3.14	1.11	<0.01	5.52	1.06	<0.001	5.02	1.05	<0.001	1.98	0.72	<0.01	0.02	0.01	0.03
Race/ethnicity (AA:Hispanic)	9.98	2.75	<0.001	5.19	3.05	0.09	6.75	2.91	0.02	6.01	1.80	<0.01	0.04	0.02	0.12
Family history of osteoporosis													0.05	0.03	0.04
25-OHD (ng/ml)													0.001	0.001	0.11
Alcohol use	7.23	1.85	<0.001	3.78	2.07	0.07	4.98	1.97	0.01	2.58	1.27	0.045			
IVDU	8.82	4.20	0.04										0.04	0.03	0.19
Age at menarche													-0.01	0.01	0.01
FSH (mIU/ml)	-0.09	0.06	0.14	-0.13	0.06	0.03	-0.13	0.06	0.03	-0.06	0.04	0.17			
Osteocalcin (ng/ml)	-0.96	0.47	0.046	-1.18	0.49	0.02	-0.70	0.49	0.15	-0.92	0.30	<0.01			
BSAP (10 U/L)				-0.16	0.09	0.09				-0.13	0.06	0.02			
CTx (ng/ml)				-11.01	6.40	0.09	-8.65	6.35	0.18	-8.28	3.41	0.02			
BSO (yes/no)	15.78	5.91	<0.01	9.64	6.39	0.14									
Nadir CD4 cell count	-0.02	0.01	0.03							-0.01	0.01	0.20			
HCV+/-													0.04	0.03	0.18

Note. BSAP = bone specific alkaline phosphatase; BMD = bone mineral density; BMI = body mass index; CTx = C-terminal telopeptide; FSH = follicular stimulating hormone; NTx = N-terminal telopeptide; OC =osteocalcin

[a]Variables tested in univariate analyses included age; race/ethnicity; body mass index (BMI); age at menarche; age at menopause years since menopause; use of menopausal hormone therapy (ever); surgical menopause; depression; antidepressants; SSRI; previous fracture; family history of osteoporosis; current tobacco use; use of glucocorticoid ever; diabetes; hyperthyroidism; alcohol use (more than two drinks per day); injection drug use history; Hepatitis C virus infection; calcium-regulating hormones (parathyroid hormone, corrected calcium level, 25-OHD), bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, C-telopeptide type I collagen, cross-linked N-telopeptide of bone collagen type I), and cytokines (TNF-α, interleukin-6).

[b]Only variables with p<0.20 are reported here.

Table 3. Univariate regression of factors independently associated with change in BMD in the total sample (n=127)[a,b]

	Lumbar Spine			Total Hip			Femoral Neck			Distal Radius			Ultradistal Radius		
Characteristics	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***
IDI				-0.12	0.06	0.06	-0.08	0.05	0.09				-0.08	0.06	0.19
Baseline BMD				-0.13	0.04	<0.01									
Age at menarche				-0.01	0.004	<0.01	-0.01	0.01	0.17	0.003	0.002	0.15			
25-OHD (ng/ml)	0.001	0.001	0.16												
Alcohol use	0.03	0.02	0.09	-0.03	0.02	0.10				-0.02	0.01	<0.01			
Steroids ever	0.03	0.01	0.04										-0.01	0.01	0.17
IVDU	-0.04	0.02	0.03				0.03	0.02	0.16	-0.04	0.01	0.01	-0.02	0.01	0.06
Depression													1.76	1.08	0.11
SSRI				-0.03	0.01	0.02									
BSO													3.83	2.26	0.10
TNF-alpha	-0.01	0.01	0.08	-0.01	0.01	0.07									
IL-6	-0.01	0.004	0.09												
CTx (ng/ml)										-0.04	0.02	0.02	0.03	0.02	0.09
NTx (nmol/BCE/ml)													0.001	0.001	0.04
Osteocalcin (ng/ml)													0.002	0.001	0.11
BSAP (U/liter)				0.001	0.001	0.03	0.001	0.001	0.13				0.001	0.001	0.17
Corrected calcium	-0.02	0.01	0.07												
HCV status +/-	-0.03	0.02	0.11	0.06	0.03	0.04				-0.03	0.01	0.02	-0.02	0.01	0.07
HIV status +/-	-0.02	0.01	0.02	-0.08	0.02	<0.01				-0.01	0.01	0.03	0.01	0.004	0.04

Note. BMD = bone mineral density; BSO = bilateral salpingo-oophorectomy; BSAP = bone-specific alkaline phosphatase; CTx = C-telopeptide; IDI = standardized inter-DXA duration; IL-6 = interleukin-6; IDU = injection drug use; NTx = N-terminal telopeptide; SSRI = serotonin reuptake inhibitor; TNF-alpha = tumor necrosis factor alpha.

[a]Variables tested in univariate analyses included age; race/ethnicity; body mass index (BMI); age at menarche; age at menopause years since menopause; use of menopausal hormone therapy (ever); surgical menopause; depression; antidepressants; SSRI; previous fracture; family history of osteoporosis; current tobacco use; use of glucocorticoid ever; diabetes; hyperthyroidism; alcohol use (more than two drinks per day); injection drug use history; Hepatitis C virus infection; calcium-regulating hormones (parathyroid hormone, corrected calcium level, 25-OHD), bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, C-telopeptide type I collagen, cross-linked N-telopeptide of bone collagen type I), and cytokines (TNF-α, interleukin-6).

[b]Only variables with p<0.20 are reported here.

Table 4. Univariate regression of factors independently associated with change in BMD in HIV-infected postmenopausal women (n=127)[a,b]

	Lumbar Spine			Total Hip			Femoral Neck			Distal Radius			Ultradistal Radius		
Characteristics	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***	**β**	**SE**	***p***
IDI				-0.001	0.001	0.08							-0.001	0.001	0.15
Baseline BMD				-0.09	0.05	0.06				-0.07	0.03	0.04			
Age at menarche				-3.56	1.25	0.01	-0.01	0.01	0.17	0.003	0.002	0.15			
FSH (mIU/ml)	0.05	0.03	0.13												
Alcohol use										-0.01	0.01	0.19			
Tobacco use	-3.40	2.12	0.16												
IVDU	-0.04	0.02	0.03				0.03	0.02	0.16	-0.04	0.01	0.01	-0.02	0.01	0.06
Depression				-0.04	0.02	0.03				-0.01	0.01	0.19	0.02	0.01	0.07
SSRI				-3.73	2.08	0.08									
TNF-alpha				-0.09	0.04	0.06									
IL-6	-1.15	0.64	0.08												
CTx (ng/ml)										-0.05	0.02	0.01			
HCV status +/-	-5.59	3.36	0.13	23.03	8.38	0.02				-8.41	3.77	0.05	-5.59	3.36	0.13

Note. BMD = bone mineral density; BSO = bilateral salpingo-oophorectomy; BSAP = bone-specific alkaline phosphatase; CTx = C-telopeptide; FSH = follicular stimulating hormone; IDI = standardized inter-DXA duration; IL-6 = interleukin-6; IVDU = intravenous drug use; NTx = N-terminal telopeptide; SSRI = serotonin reuptake inhibitor; TNF-alpha = tumor necrosis factor alpha;

[a]Variables tested in univariate analyses included age; race/ethnicity; body mass index (BMI); age at menarche; age at menopause; years since menopause; use of menopausal hormone therapy (ever); surgical menopause; depression; antidepressants; SSRI; previous fracture; family history of osteoporosis; current tobacco use; use of glucocorticoid ever; diabetes; hyperthyroidism; alcohol use (more than two drinks per day); injection drug use history; Hepatitis C virus infection; calcium-regulating hormones (parathyroid hormone, corrected calcium level, 25-OHD), bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, C-telopeptide type I collagen, cross-linked N-telopeptide of bone collagen type I), and cytokines (TNF-α, interleukin-6).

[b]Only variables with p<0.20 are reported here.

Table 5. Multivariable regression of factors associated with change in BMD in HIV-infected postmenopausal women (n=73)[a,b]

	Lumbar Spine $R^2 = 0.211$		Total Hip $R^2 = 0.359$		Femoral Neck $R^2 = 0.180$		Distal Radius $R^2 = 0.101$		Ultradistal Radius $R^2 = 0.144$	
Characteristics	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***	**β (95% CI)**	***p***
IDI	-0.16 (-0.29, -0.04)	**0.01**	-0.17 (-0.43, 0.08)	0.17	-0.32 (-0.54, -0.10)	**<0.01**	-0.19 (-0.35, -0.03)	**0.02**	-0.23 (-0.41, -0.06)	**<0.01**
Baseline BMD	-11.91 (-19.64, -4.19)	**<0.01**	3.88 (-10.35, 18.11)	0.61	-11.71 (-24.25, 0.83)	0.07	-1.58 (-16.54, 13.37)	0.83	-7.53 (-30.53, 15.48)	0.52
Depression	0.28 (-1.65, 2.22)	0.77	1.54 (-2.46, 5.53)	0.44	1.06 (-2.30, 4.43)	0.53	-1.45 (-3.90, 1.01)	0.24	-2.39 (-5.00, 0.23)	0.07
HCV status +/-			-5.48 (0.77, 10.18)	**0.02**						

Note. BMD = bone mineral density; BSO = bilateral salpingo-oophorectomy; HCV = Hepatitis C; IDI = standardized inter-DXA duration (time to follow-up assessment).

[a]Multiple regression β equals change in BMD (grams per square centimeter per unit change in predictor);Time between DXA (IDI), baseline BMD, HIV status (being HIV+), depression (0, no; 1, yes), and BSO (0, no; 1, yes) were forced into a model with variables significant in univariate analyses (p<0.20). Covariates with p<0.05 were retained.

[b]Variables tested in univariate analyses included age; race/ethnicity; body mass index (BMI); age at menarche; age at menopause; surgical menopause; years since menopause, past use of menopause hormone therapy; depression; antidepressants; SSRI; previous fracture; family history of osteoporosis; current tobacco use; use of glucocorticoid ever; diabetes; hyperthyroidism; alcohol use (more than two drinks per day); injection drug use history; Hepatitis C virus infection; calcium-regulating hormones (parathyroid hormone, corrected calcium level, 25-OHD), bone turnover markers (bone-specific alkaline phosphatase, osteocalcin, C-telopeptide type I collagen, cross-linked N-telopeptide of bone collagen type I), and cytokines (TNF-α, interleukin-6), and HIV characteristics (years since HIV diagnosis, current CD4 cell count, nadir CD4 cell count, HIV-1 RNA, antiretroviral therapy, years on antiretroviral therapy). Factors selected *a priori* for inclusion in the models were IDI, baseline BMD, and depression. Covariates with p<0.20 in univariate analysis were entered into multivariable regression models with *a priori* variables.