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CO-INFECTIONS AND COMORBIDITY (D BHATTACHARYA, SECTION EDITOR)

Bone Quality in Relation to HIV and Antiretroviral Drugs

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Abstract

Purpose of Review People living with HIV (PLWH) are at an increased risk for osteoporosis, a disease defined by the loss of bone mineral density (BMD) and deterioration of bone quality, both of which independently contribute to an increased risk of skeletal fractures. While there is an emerging body of literature focusing on the factors that contribute to BMD loss in PLWH, the contribution of these factors to bone quality changes are less understood. The current review summarizes and critically reviews the data describing the effects of HIV, HIV disease-related factors, and antiretroviral drugs (ARVs) on bone quality.

Recent Findings The increased availability of high-resolution peripheral quantitative computed tomography has confirmed that both HIV infection and ARVs negatively affect bone architecture. There is considerably less data on their effects on bone remodeling or the composition of bone matrix. Whether changes in bone quality independently predict fracture risk, as seen in HIV-uninfected populations, is largely unknown.

Summary The available data suggests that bone quality deterioration occurs in PLWH. Future studies are needed to define which factors, viral or ARVs, contribute to loss of bone quality and which bone quality factors are most associated with increased fracture risk.

Keywords Bone mineral density · Bone quality · Remodeling · Microarchitecture · Matrix composition

Introduction

The success of combination antiretroviral therapy (cART) has reduced HIV-associated death and thus increased the life expectancy of people living with HIV (PLWH) [1]. Early onset of age-related comorbidities has been observed among PLWH. Osteoporosis and the resulting osteoporotic fractures occur at nearly three times the rate in PLWH when compared to demographically matched uninfected individuals [2, 3]. Osteoporosis is defined by a loss of bone mineral density (BMD) and deterioration of bone quality, which contribute to an increased risk of fracture. Low BMD is consistently reported in PLWH when compared to demographically matched uninfected individuals [4, 5] and is likely driven by a combination of HIV, HIV viral proteins [6–8], and antiretroviral (ARV) drugs [9–11]. While there have been several reviews focused on the factors that contribute to loss of BMD and increased fracture risk in PLWH on ARVs [3, 9, 12–16], to our knowledge, no reviews have critically evaluated the state of knowledge regarding the impact of HIV and/or ARVs on bone quality.

Bone quality describes the factors that contribute to bone strength independent of bone mass or BMD, assessed by dual-energy X-ray absorptiometry (DXA, Fig. 1). Bone quality factors parameters include bone remodeling dynamics, or the activity of bone resorbing osteoclasts and bone forming osteoblasts, the spatial distribution of bone tissue in space (cortical geometry and trabecular microarchitecture), chemical composition of the bone extracellular matrix [17], all of which contribute to fracture risk independent of BMD [18–20]. A large number of review articles have been written on the importance of [19, 21–23] and measurement

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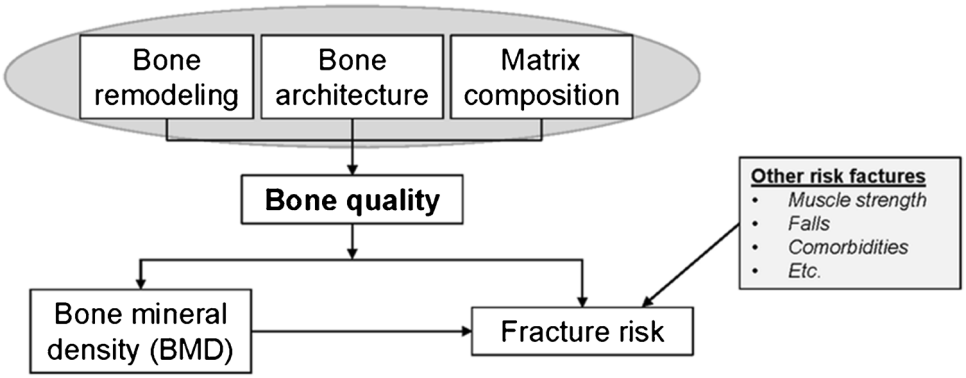

Fig. 1 Bone quality schematic. Bone quality is defined as factors, such as bone remodeling rates, bone architecture, and matrix composition, that can affect dual-energy X-ray absorptiometry measures of bone mineral density (BMD) and independently contribute to skeletal fracture risk. The current review focuses on the effects of HIV and ARVs on the factors that make up bone quality. A variety of other non-skeletal risk factors also contribute to fracture risk, such as muscle strength and falls, that are outside of the scope of the current review

strategies to assess bone quality [24, 25]; therefore, we will refer the reader to these articles for a formal description of these concepts. Instead, this review focuses on the effects of HIV and the antiretroviral drugs on bone quality. This review is timely as recent data suggests that decreased BMD does not fully explain the increased fracture risk noted in PLWH [26], and that loss of bone quality occurs coincident with the loss of DXA-defined BMD.

HIV and ARV Effects on Bone Cell Activity

The skeleton undergoes continuous self-renewal through a process known as bone remodeling. Bone remodeling is defined by the coordinated actions of bone resorbing osteoclasts and bone forming osteoblasts [27]. Due to the complexity of bone remodeling and the yet fully defined coupling factors linking osteoclasts with osteoblasts, it is common practice to investigate the effects of outside stimuli directly on primary osteoclasts and osteoblasts separately in vitro. Additionally, various studies have assessed osteoclast or osteoblast activity in response to the HIV, HIV viral products, or ARVs in preclinical animal models and human subjects. Below we provide a narrative review of the published data and include summary tables of the effects of the HIV virus and HIV viral products (Table 1) or ARVs (Table 2) on osteoclasts and osteoblasts.

HIV and ARV Effects on Osteoclasts

Bone resorbing osteoclasts differentiate from the monocyte/macrophage lineage [28], which are known to be infected by HIV [29]. Therefore, it is not surprising that osteoclasts themselves are also targets for HIV infection [30, 31]. Raynaud-Messina et al. confirmed the presence of HIV-infected osteoclasts in bones derived from HIV-1–infected BLT-humanized mice and in human synovial explant tissues [31]. HIV infection promotes osteoclast differentiation, expression of pro-resorptive genes, and osteolytic activity in vitro [30, 31].

Osteoclast survival, proliferation, differentiation, and activation are driven by interactions between soluble receptor activator of nuclear factor-κB ligand (RANKL) and its receptor, the receptor activator of nuclear factor κB (RANK). Osteoprotegerin (OPG) is a soluble antagonist to RANKL [32, 33] and the RANKL/OPG ratio is commonly used to monitor bone remodeling activity [34]. The OPG/RANKL ratio is significantly lower in cART naïve PLWH with low BMD and RANKL levels are positively associated with HIV viral load [35]. However, circulating OPG levels and not the OPG/RANKL ratio have been reported to be associated with BMD [36, 37]. Changes in the expression of OPG and RANKL in PLWH appear to be due to aberrant expression by HIV-infected immune cells. For example, macrophages [38], B-cells [7], and T-cells [39] are all reported to have increased RANKL and decreased OPG expression in response to HIV infection. Mechanistic studies using cultured human T-cells have shown that exposure to HIV viral proteins such as Vpr [40] or gp120 [41] is sufficient to upregulate the expression of RANKL. Collectively, these findings demonstrate that elevated bone resorption in PLWH is likely a combination of both direct HIV infection and indirect activation of HIV-infected immune cells. A more detailed description of the role of RANKL and OPG in HIV can be found here [42].

The initiation of cART therapy can also promote osteoclast differentiation and activation either directly or indirectly through immune cell-mediated effects. The initiation of cART is associated with suppression of HIV replication, resulting in immune reconstitution including repopulation of the CD4 + T-cells [43]. Despite the importance

Table 1 HIV effects on osteoclast and osteoblast activity

Citation	Virus/Viral Product	Model System	Findings
Osteoclasts			
Gohda et al. [30]	• HIV-1	• Human $CD14^+$ monocytes	• HIV-1 infects and replicates in osteoclasts • HIV infection enhances osteoclast differentiation and increases resorption activity
Raynaud-Messina et al. [31]	• HIV-1	• Human monocytes	• Osteoclasts are permissive to HIV infection via cell free virus throughout differentiation • Infection is more robust at the later stages of osteoclast differentiation • Infection is more effective via direct contact of osteoclasts with infected T-cells than with cell free virus • Infection enhances osteoclast precursor migration and resorption activity
Mascarau et al. [38]	• HIV-1	• Human monocytes	• HIV infection induces macrophage multinucleation and the expression of osteoclastic genes • HIV infection promotes the formation of a sealing zone like structure • Infection of macrophages does not enhance bone degradation activity but does promote osteoclast migration via RANKL production
Fakruddin et al. [41]	• HIV-gp120	• Human PBMCs • Raw 264.7	• HIV-gp120 induced RANKL expression in CD3 + T-cells • Conditioned media from HIV-gp120 treated PBMCs increased osteoclast differentiation, as evidenced by increased TRAP expression and activity and increased formation of multinucleated cells
Osteoblasts			
Nacher et al. [56]	• HIV-1	• Primary human osteoblasts	• HIV DNA was undetectable in osteoblasts obtained from PLWH • Osteocalcin secretion is elevated in cultured osteoblasts derived from uninfected subjects when compared to those from PLWH
Gibellini et al. [55]	• HIV-1 • HIV-gp120	• Primary human osteoblasts • Human osteoblast-like SV-40 T antigen immortalized cell line (HOBIT)	• Cultured osteoblasts are not infected by HIV • Gp120 induced osteoblast apoptosis • HIV-1 and HIV-gp12- upregulated TNFα expression
Cummins et al. [71]	• HIV-gp120	• Primary human osteoblasts (hOBs) • Fetal osteoblasts • HOBIT • U2OS osteosarcoma cells	• Gp120 did not induce apoptosis in osteoblast cell lines or primary human osteoblasts • Gp120 did not affect osteoblast proliferation
Cotter et al. [57]	• HIV-gp120 • HIV-Rev • HIV-p55-gag	• Human mesenchymal stem cells (MSCs)	• Rev increased calcium deposition, Runx2 activity, and BMP2 secretion • p55-gag decreased calcium deposition, Runx2 activity and BMP2 secretion
Beaupere et al. [58]	• HIV-Tat • HIV-Nef	• Human MSCs	• Tat and Nef inhibited MSC proliferation • Tat and Nef induced senescence, oxidative stress and mitochondrial dysfunction in MSCs • Tat and Nef inhibited MSC differentiation into osteoblasts evidenced by reduced calcium deposition and Runx2 and osteocalcin gene expression

of ARV-induced immune reconstitution in prolonging the lifespan of PLWH, immune reconstitution is also associated with an increased release of pro-inflammatory cytokines [44], which may contribute to bone loss. For example, immune reconstitution following cART initiation is associated with an increase in circulating RANKL

Table 2 ARV effects on osteoclast and osteoblast activity

Citation	ARV Treatment	Model System	Findings
Osteoclasts			
Jain et al. [49]	• Nelfinavir (NFV) • Indinavir (IDV) • Saquinavir (SQV) • Ritonavir (RTV) • Lopinavir (LPV) • Amprenavir (APV)	• Excised rat calvaria	• NFV, RTV, SQV, IDV increased osteoclast resorptive activity measured via calcium release from bone organ culture • LPV and APV had no effect on osteoclast activity
Yin et al. [51]	• RTV	• Human peripheral blood mononuclear cells (PBMCs)	• Sera from women receiving RTV increased osteoclast differentiation when compared to sera from women receiving cART without RTV
Modarressi et al. [50]	• RTV • IDV • NFV	• Human PBMCs	• RTV increased osteoclast differentiation • RTV blocked nuclear translocation of β-catenin and upregulated the expression of the Wnt antagonist, Wnt5B • IDV and NFV did not affect osteoblast differentiation
Osteoblasts			
Jain et al. [49]	• NFV • IDV • SQV • RTV • LPV • APV	• Human MSCs	• NFV, LV, RTV, and SQV inhibited alkaline phosphatase activity • NFV, LV, and RTV inhibit alkaline phosphatase gene expression • APV and IDV had no effect on osteoblasts
Hernandez-Vallejo et al. [61]	• RTV • LPV • Atazanavir (ATV)	• Human MSCs	• ATV and LPV induced senescence and oxidative stress with or without RTV • ATV and LPV reduced osteoblast differentiation, as evidenced by lower matrix calcification and reduced alkaline phosphatase activity
Malizia et al. [63]	• NFV • IDV • SQV • RTV • IDV	• Primary hOBs	• NFV and IDV reduced matrix calcification, alkaline phosphate activity, and Runx2 gene expression • NFV and IDV increased TIMP3 gene expression
Malizia et al. [64]	• NFV • RTV	• Primary hOBs	• NFV and RTV increased monocyte chemoattractant protein (MCP)-1 and interleukin-8 (IL-8) gene expression
Barbieri et al. [68]	• Tenofovir disoproxil fumarate (TDF)	• Primary hOBs	• TDF impaired cell viability at high concentrations • TDF reduced matrix calcification but did not affect alkaline phosphate activity • TDF decreased Col1A1 and ATF4 expression
Cazzaniga et al. [62]	• ATV • Dolutegravir (DTG) • Darunavir (DRV)	• Human MSCs • Normal Human Osteoblasts (NHOst)	• ATV and DTG inhibited osteoblast differentiation as evidenced by reduced gene expression and matrix calcification • DRV inhibited Runx2 and Col1A1 expression, but had no effect on matrix calcification • ATV and DTG inhibited osteoblast differentiation into osteocytes as evidenced by reduced SOST and DMP1 expression • DRV had no effect on osteoblast differentiation into osteocytes

and TNFα [45], which promote osteoclastogenesis [46]. Ofotokun et al. reported that the degree of bone loss in PLWH is positively associated with the magnitude of immune reconstition. PLWH with the greatest increase in CD4 + cell count following cART initaition experience the greatest BMD loss [45].

ARVs can also directly influence osteoclast activity. While one study found that an association between tenofovir

disoproxil fumarate (TDF) exposure and increased circulating RANKL levels after cART initiation [47], others have failed to find an association between RANKL or the OPG/RANKL ratio and bone loss regardless of the cART regimen initiated [47, 48]. While some of the cART initiation data suggest that direct effects of ARVs on bone cells may contribute to the observed decrease in BMD, because regimens are given as combinations of 2 to 3 ARVs it is difficult to sort out independent effects. Relatively few studies have investigated the direct effects of ARVs on osteoclasts in vitro. The three studies that have been performed have primarily focused on protease inhibitors (PI) which are no longer used in the management of HIV. Jain et al. noted that osteoclast activity is elevated in response to treatment with nelfinavir (NFV), indinavir (IDV), saquinavir (SQV), or ritonavir (RTV) [49]. In contrast, Fakruddin et al. reported that RTV and SQV, but not IDV or NFV enhanced osteoclast differentiation [41]. The same group further reported that these effects are likely driven by the suppression of Wnt/β-catenin signaling in osteoclast precursors [50]. The increased osteoclast activity following PI exposure was confirmed by Yin et al. who demonstrated greater osteoclast differentiation potential of PBMCs isolated from PLWH treated with RTV when compared to other ARVs [51]. However, at least one study using murine bone marrow macrophages reported that RTV inhibits osteoclast differentiation and function [52]. These contradictory findings may be due to differences in human versus mouse cells or the significant differences in the concentrations of ARVs investigated, which ranged from 0.7–3.6 μg/mL [41, 50] to 10–20 μg/mL [52], stressing the importance of future experiments to determine the concentration of ARVs that reach the bone microenvironment. Further, it is important to determine the effects of newer, more widely used ARVs on osteoclast function to fully appreciate their effects on bone.

HIV and ARV Effects on Osteoblasts

Bone matrix is formed by osteoblasts, which differentiate from mesenchymal stem cells (MSCs). After synthesizing the new bone matrix, a small fraction of osteoblasts become trapped within the bone and terminally differentiate into osteocytes [53], a long-lived cell responsible for mediating the skeletal response to mechanical loading and producing bone-derived hormones [54]. Despite detectable mRNA expression of HIV binding receptors CD4 and CCR5 [55], neither cultured osteoblasts nor osteoblasts isolated from PLWH show evidence of HIV infection [55, 56]. However, viral products, including gp120 [55], gag p55 [57], Tat and Nef [58] all of which continue to circulate even in well controlled PLWH [59, 60], have all been reported to impair osteoblast differentiation or function in vitro (summarized in Table 1).

In vitro studies have demonstrated the deleterious effects of various ARVs on the differentiation and function of osteoblasts [49, 61, 62]. For instance, treatment of cultured MSCs with PIs, atazanavir (ATV) or lopinavir (LPV), induced cell senesence and inhibited osteoblast differentiation [61]. Interestingly, RTV alone had no effect on osteoblast differentiation [61]. A similar study by Jain et al. comparing the effects of several PIs on MSCs reported that NFV, SQV, LPV, and RTV were all able to inhibit osteoblast differentiation, as measured by alkaline phosphatase staining, while amprenavir (APV) and IDV had no effect [49]. While the two studies differed in their conclusions regarding the effects of RTV on osteoblast differentiation, the discrepancy likely stems from the differences in ARV concentrations employed. The former study by Hernandez-Vallejo et al. [61] used 10 μM LPV and 2 μM RTV, while the study by Jain et al. [49] used 10 or 20 μM for each PI tested; highlighting the need for future work defining the concentration of ARVs that reach the bone microenvironment in vivo.

Protease inhibitors also inhibit the function of mature osteoblasts. Malizia et al. exposed mature human osteoblasts to several PIs, including SQV, RTV, NFV and IDV, and found that NFV and IDV impaired matrix formation in vitro [63]. Subsequent microarray analysis identified that the extracellular matrix regulator TIMP-3 is upregulated in response to NFV and IDV, and suppression of TIMP-3 transcription prevented NFV and IDV induced matrix inhibition [63]. Cazzaniga et al. investigated the terminal differentiation of osteoblasts by monitoring the expression of the osteocyte markers sclerostin and dental matrix protein 1 (DMP1) and noted that ATV but not darunavir (DRV) inhibited osteocyte-related gene expression [62]. In addition to inhibiting differentiation and matrix production, NFV and RTV are also reported to promote the osteoblast expression of pro-inflammatory cytokines, such as monocyte chemoattractant protein 1 (MCP)-1 and interleukin-8 (IL-8) [64], both of which are associated with increased osteoclastogenesis and subsequent bone resorption [65, 66].

Relatively little is known about the effects of non-PI ARVs on osteoblast function. Despite the well-described bone loss associated with TDF [67], only one study has investigated its effects on osteoblast function and found that TDF inhibited matrix formation by reducing the extent of calcification and suppressing the expression of collagen I [68]. Integrase strand transfer inhibitors (INSTIs) are now recommended as part of first line cART regimens, yet, to date, only one study has investigated the effects of INSTIs on osteoblasts, reporting that dolutegravir (DTG), inhibits both the differentiation of osteoblasts from MSCs and the terminal differentiation of osteoblasts into osteocytes, as evidenced from impaired expression of sclerostin and DMP1 [62]. Further work is necessary to establish the effects of newer generation INSTIs such as bictegravir, or commonly

used nucleoside reverse-transcriptase inhibitors (NRTIs), such as emtricitabine, lamivudine or abacavir on osteoblast function. Finally, apart from the study by Cazzaniga et al. [62], the effects of ARVs on osteocytes remain largely unknown. Importantly, osteocytes constitute between 90 and 95% of bone cells and are master regulators of bone remodeling, as well as the primary producer of fibroblast growth factor 23 (FGF23), a bone-derived hormone responsible for controlling phosphate metabolism [69, 70].

Bone Remodeling

Bone Cell Activity

Bone remodeling involves the coordinated action of osteoclasts and osteoblasts. A variety of studies have investigated the effects of HIV and ARVs on osteoclast and osteoblast activity separately, and from these results, inferred the cumulative effects on bone remodeling. A more complete understanding of bone remodeling requires directly investigating bone tissues from PLWH.

Due to the inherent difficulty of obtaining bone samples from PLWH, many of the tissue-level remodeling measurement studies have been performed in preclinical animal models. Raynaud-Messina et al. [31] and Vikulina et al. [72] used the Nef overexpressing mouse and the HIV transgenic rat, respectively, to model HIV infection and demonstrated elevated tissue-level osteoclast activity in both animal models. Relatively few studies have evaluated the effects of ARVs on bone tissue and those that have are limited primarily to characterization of the effects of tenofovir, which reduces the number of osteoblasts and increases the number of osteoclasts in mice [73], zebrafish [74], and primates [75]. Although ARVs are administered in combination clinically, to our knowledge, there have been no studies evaluating the effects of cART regimens on tissue-level remodeling in these preclinical models.

At least two studies have evaluated tissue-level remodeling in human biopsies. Serrano et al. evaluated bone tissue biopsies taken from cART naïve PLWH and compared histological measures of bone remodeling according to HIV-uninfected controls [76]. Bone samples from PLWH had decreased osteoblast and osteoclast surfaces, bone formation rate, and activation frequency when compared to tissues from uninfected patients and the all parameters were further reduced in those with the most severe disease. Ramalho et al. evaluated bone tissue biopsies taken from men with HIV prior to and 12-months after initiating a fixed dose cART regimen of TDF/3TC/EFV [77]. Prior to cART initiation, PLWH have an elevated number of osteoclasts and increased eroded surface, a measure of osteoclast activity. Additionally, bone formation rate was lower and the mineralization lag time was elevated among PLWH prior to cART initiation when compared to an uninfected reference cohort. After cART initiation, osteoid volume, osteoblast surface, and osteoclast surface indices all increased, confirming the elevated bone remodeling in response to ARVs. A detailed summary of the in vivo effects of HIV and ARVs on bone remodeling is presented in Tables 3 and 4.

Bone Turnover Markers (BTMs)

In lieu of bone biopsies, bone cell activity is commonly assessed clinically using circulating bone turnover markers (BTMs), or the protein products of bone remodeling released into the circulation due to bone cell activity. BTMs are used to compliment DXA-based BMD measures in the diagnosis of osteoporosis and have been reported to predict fracture risk independent of BMD [78, 79]. Moreover, changes in BTMs have been proposed as a method to monitor the response to osteoporosis treatments [80], including in PLWH [81, 82]. In cART naïve PLWH, serum calcium, phosphate, and 25-hydroxy vitamin D are all reduced when compared to HIV-uninfected individuals, as are BTMs commonly associated with bone formation, including osteocalcin and amino-terminal propeptide of type 1 procollagen (P1NP) [7, 83–86]. In contrast, BTMs released during bone resorption, such as C-terminal telopeptide of collagen (CTX), are elevated when compared to HIV-uninfected individuals [7].

The initiation of cART is also associated with changes in the circulating levels of BTMs. Indeed, cART initiation increases osteoblast-derived, osteocalcin and P1NP, and osteoclast-derived, CTX, as early as 3 to 6 months post-cART initiation [85, 87–89]. The peak levels of circulating BTMs are generally achieved between 12–48 weeks following cART initiation before reaching a new steady state [85, 89–92], which appears to be higher than what is reported in cART naïve PLWH.

BTMs have also been used to evaluate the extent of ARV effects on bone. For example, changes in both bone resorption (CTX) and bone formation (P1NP and bone-specific alkaline phosphatase) were reported to be greater in PLWH initiating TDF/FTC when compared to abacavir (ABC)/3TC [90, 91]. The initiation of TDF-based cART increases circulating RANKL levels more than TDF-sparing cART regimens [47]. Compared to TDF-based therapy, cART containing INSTIs appear to cause less bone remodeling. For example, compared to PLWH initiating TDF-based therapy, those receiving either raltegravir (RAL) [93] or DTG-based treatment [92] exhibit smaller changes in BTM levels. Switching regimens can also affect BTM levels. For example, switching from zidovudine (AZT)-based cART onto a TDF-based regimen resulted in a significant increase in the circulating levels of CTX, P1NP, and osteocalcin [94]. Switching from TDF-based cART onto a regimen of ABC/3TC + ATV resulted in reduced circulating

Table 3 HIV and ARV effects on tissue-level bone remodeling parameters

Citation	Treatment	Model	Findings
Raynaud-Messina et al. [31]		• Nef transgenic mouse	• Mice expressing Nef protein had increased number of TRAP positive osteoclasts, coincident with decreased bone density
Vikulina et al. [72]		• HIV transgenic (Tg) rat	• HIV Tg rats have increased osteoclast surface
Ofotokun et al. [112]		• TCRβ knockout (KO) mice	• CD3 + T-cell reconstitution led to reduced osteoblast number, mineral apposition rate, and bone formation rate
Weitzmann et al. [115]		• TCRβ knockout (KO) mice	• CD3 + T-cell reconstitution increased the number of osteoclasts per bone surface, but did not affect bone formation or mineral apposition surface per bone surface
Serrano et al. [76]		• cART naïve PLWH	• PLWH had reduced osteoid volume, surface and thickness, osteoblast and osteoclast surfaces, mineralizing surface, bone formation rate, and activation frequency when compared to uninfected controls
Matuszewska et al. [116]	• Efavirenz (EFV) • Tenofovir[1]	• Wistar rat	• EFV treated rats had increased TRAP staining intensity • Tenofovir treated rats did not show any changes in TRAP staining intensity
Castillo et al. [75]	• Tenofovir with and without SIV infection	• Rhesus macaques infected with SIV	• Tenofovir treatment increased osteoid seam widths in both SIV-infected and uninfected animals • SIV infection increased resorption cavity density regardless of tenofovir treatment
Carnovali et al. [74]	• Tenofovir disoproxil fumarate (TDF)	• Danio rerio (zebrafish)	• TDF reduced alkaline phosphatase activity in a dose-dependent manner • TDF increased TRAP activity, particularly at 100 nM
Conesa-Buend et al. [73]	• Tenofovir	• C57Bl/6 mice	• Tenofovir treatment increases osteoclast number and decreases osteoblast number
Conradie et al. [117]	• TDF • Stavudine (d4T) • Lopinavir (LPV)	• Wistar rat	• d4T treatment led to increased eroded surface (%) and osteoblast numbers • TDF and d4T treatment decreased osteoblast surface • TDF, d4T, LPV treatment reduced the osteoid surface • TDF treatment decrease the bone formation rate
Ramalho et al. [77]	• cART regimen of TDF/Lamivudine (3TC)/Efavirenz (EFV)	• cART naïve PLWH initiating cART	• PLWH had higher osteoid volume, osteoclast surface, and osteoblast surface per bone surface following cART initiation

[1]The tenofovir isoform used in this study was specifically designed for preclinical studies [118]

bone-specific alkaline phosphatase, CTX, and osteocalcin [95, 96] and switching from TDF-based cART to either RAL- [97] or DTG-based cART [98] similarly reduced BTM level. For a more detailed review on the various studies that have assessed BTMs in PLWH, the reader is referred to the following review specifically addressing this topic [99].

Table 4 Summary table of in vivo findings of the effects of HIV and ARV on bone architecture

Citation	Treatment	Preclinical Animal Model	Bone Tissue	Findings
Ofotokun et al. [112]		• TCRβ knockout (KO) mice	• Femur	• CD3 + T-cell reconstitution reduced the trabecular bone volume fraction, trabecular thickness, and number and increased trabecular spacing when compared to non-reconstituted mice • CD3 + T-cell reconstitution reduced cortical thickness and area
Weitzmann et al. [122]		• TCRβ knockout (KO) mice	• L3 vertebrae • Femur	• CD4 + but not CD8 + T-cell reconstitution reduced vertebral trabecular bone volume fraction, trabecular thickness, cortical area, and cortical thickness when compared to non-reconstituted controls • Both CD4 + and CD8 + T-cell reconstitution reduced femoral BV/TV and trabecular number and increased trabecular separation • CD4 + but not CD8 + T-cell reconstitution reduced femoral cortical area and thickness
Weitzmann et al. [115]		• TCRβ knockout (KO) mice	• L3 vertebrae • Femur	• CD3 + T-cell reconstitution reduced vertebral trabecular bone volume fraction and trabecular number and increased trabecular when compared to non-reconstituted controls • CD3 + T-cell reconstitution accelerated the age-related loss of trabecular thickness in the vertebrae • CD3 + T-cell reconstitution reduced femoral trabecular bone volume fraction and trabecular number, and increased trabecular spacing • CD3 + T-cell reconstitution accelerated the age-related loss of trabecular number and age-related increase in trabecular spacing in the femoral metaphysis
Vikulina et al. [72]		• HIV transgenic (Tg) rat	• Femur	• HIV Tg rats had lower femoral trabecular bone volume fraction, trabecular thickness and number, and trabecular connective density, and higher trabecular spacing compared to wild-type controls • HIV Tg rats had lower cortical bone volume
Matuszewska et al. [116]	• EFV • Tenofovir[1]	• Male Wistar rats	• L2 vertebrae • Femur • Tibia	• EFV treated rats had lower vertebral trabecular total bone surface bone volume area and trabecular number when compared to vehicle treated rats • Tenofovir treated rats had lower femoral trabecular number and higher trabecular spacing when compared to vehicle treated rats
Conesa-Buend et al. [73]	• TDF • d4T • LPV	• C57Bl/6 Mice	• Femur	• MicroCT analysis showed Tenofovir treated mice had lower cortical total volume and bone volume fraction when compared to control • MicroCT analysis Tenofovir treated mice had lower trabecular total volume, bone volume fraction, trabecular spacing, trabecular number when compared to control mice • Histomorphometric analysis of long bone showed tenofovir treated mice has lower bone volume, bone volume fraction, and trabecular number

Table 4 (continued)

Citation	Treatment	Preclinical Animal Model	Bone Tissue	Findings
Szelag et al. [123]	• ABC • Etravirine (ETR)	• Wistar rat	• Tibia • Femur	• MicroCT analysis showed ABC treated mice had higher tibial trabecular bone volume fraction, trabecular number, connectivity density, and lower total porosity compared to control mice • ETR did not affect tibial trabecular bone • No differences were detected at the tibial cortical bone • No differences were detected at the femora bone across treatment groups

[1]The tenofovir isoform used in this study was specifically designed for preclinical studies [118]

Circulating bone-related proteins have also been measured to assess the mechanistic drivers of HIV and ARV-associated bone loss. Sclerostin is an osteocyte-derived regulator of bone remodeling that is reported to decrease with the initiation of cART [84]. Switching from more bone-toxic TDF-containing cART regimens to those containing ABC led to increased sclerostin levels [95]. Importantly, higher sclerostin levels have been shown to be positively associated with increased BMD in PLWH [100, 101] and findings from Ross et al. [100] suggest that the relationship between sclerostin and BMD is influenced by HIV serostatus [100]. Parathyroid hormone (PTH) is another regulator of bone remodeling that is reported to be elevated in both cART naïve PLWH and following cART initiation [85, 89]. The extent of PTH increase following cART is greater in PLWH receiving TDF-containing cART [102], which may be due to low circulating vitamin D levels [103] or TDF-induced alterations the PTH and vitamin D relationship responsible for regulating circulating mineral homeostasis [104]. While vitamin D is a critical regulator of calcium homeostasis and bone health, a formal review of the potential role of vitamin D in HIV is outside of the scope of the current review and the reader is instead pointed to a recent summary [105]. Finally, novel studies evaluating potential mechanistic drivers of bone loss have reported that extracellular vesicles expressing markers of bone activity [106] and bone remodeling regulating microRNAs, 23a-3p, 24–2-5p, and 21-5p [107], are associated with BMD in PLWH.

The circulating levels of pro-inflammatory cytokines have also been evaluated as a potential mechanism of bone loss. Markers of immune activation remain elevated in PLWH on cART when compared to uninfected people [108, 109]. Higher baseline levels of pro-inflammatory cytokines known to affect bone cell function [110] including IL-6 are associated with greater BMD loss after cART initiation [111]. Soluble CD14 levels, a marker of immune activation, found on the surface of monocytes and macrophages, are also associated with greater BMD loss [111], confirming that immune reconstitution contributes to HIV and ARV-induced bone loss. Indeed, immune reconstitution suppresses histomorphometric indices of osteoblast activity in a mouse model of T-cell reconstitution [112]. Additionally, both Gazzola et al. [113] and Manavalan et al. [114] showed that immune activation in PLWH is associated with lower BMD and Manavalan et al. further implicated a reduction in circulating osteoblast precursor cells as a probable mechanism of bone loss.

Bone Architecture

Osteoporosis is also characterized by a deterioration of bone architecture, which can negatively affect bone strength and fracture resistance independently of BMD [93, 119–121]. A variety of preclinical rodent studies have leveraged the widespread availability of micro-computed tomography (μCT) to investigate the effects of HIV and ARVs on bone architecture. For example, μCT measurements have only been performed in the HIV transgenic rat model, which confirmed the negative effects of HIV viral proteins on bone architecture in addition to the negative effects on bone mass [72]. Several well-designed studies have utilized the TCRβ knockout (KO) mouse model to simulate immune reconstitution following cART initiation in PLWH [112, 122]. These studies have demonstrated the negative effects of immune reconstitution on bone architecture [112], confirmed the importance of CD4 + T-cells on these negative effects [122] and more recently confirmed that the effects of immune reconstitution accelerate age-related bone architectural deterioration [115].

The individual effects of ARVs have been tested in uninfected rodents. These studies have included confirmation that tenofovir negatively affect bone architecture in both mouse and rat models, in addition to its negative effects on bone mass [73, 116]. Interestingly, while femoral architecture is affected in both models, Matuszewska et al. [116] reported that tenofovir had no effects in the lumbar vertebra or tibia, which may suggest site specificity. In contrast, ABC may have positive effects on bone architecture, with increased

bone volume fraction and trabecular number noted in the tibiae following long-term ABC administration in growing rats [123].

The first studies evaluating bone architecture in PLWH used DXA data from the lumbar spine to assess the trabecular bone score (TBS), which has been reported to be a risk factor for osteoporotic fracture independent of BMD [124]. In further support of the clinical relevance of TBS, Ciullini et al. demonstrated that TBS is significantly reduced in PLWH with vertebral fractures compared to those without, while BMD was not [125]. Additionally, McGinty et al. reported that HIV serostatus significantly reduces TBS independently of lumbar spine bone mineral content (BMC) [126]. Interestingly, while several studies have confirmed that HIV serostatus reduces TBS [125–127], the age-related change in TBS does not appear to vary according to HIV serostatus [128]. TBS relies on DXA-based imaging to obtain an indirect measure of bone architecture, which are relatively well correlated with more direct architecture measures [129] but can be negatively impacted by imaging noise [130].

The increasing availability of high-resolution peripheral quantitative computed tomography (HRpQCT) has made it possible to perform high-resolution imaging of bone architecture in PLWH (Table 5). Kazakia et al. showed that despite having comparable BMD, HIV-infected men on long-term cART have reduced bone architecture when compared to age-matched HIV-uninfected men [131]. Biver et al. [132] reported that HIV serostatus negatively affects bone architecture independently of classical clinical risk factors, such as age, BMI, and low vitamin D levels. Instead, HIV-induced architectural degradation was primarily associated with CTX levels, suggesting that increased resorption with HIV infection was the driver of architectural loss [132]. In addition to HIV infection-induced impairment of bone architecture [133–135], cART use, particularly TDF, negatively affects bone architecture [133, 136, 137]. Additional risk factors, such as poor nutrition and low physical activity have been reported to negatively affect bone architecture in PLWH [138]. To date, HRpQCT studies in PLWH have been cross-sectional; therefore, future studies are needed to determine whether the age-related change in architectural parameters differ as a function of HIV serostatus.

Combining HRpQCT measurements with finite element (FE) analysis is a powerful tool to noninvasively and non-descriptively assess mechanical properties of bone tissue. Importantly, FE-based estimates of bone strength have been shown to be significant predictors of fracture risk after adjusting for BMD in HIV-uninfected people [139, 140]. Macdonald et al. [133] noted decreased FE-based bone strength measures in HIV-infected women when compared to uninfected women that remained significant after adjusting for a variety of characteristics and risk factors. Foreman et al. [138] further noted that longer-term HIV infection and the combination of TDF and PI use were associated with decreased FE-based estimates of bone strength. It is yet to be determined whether these FE-based bone strength measures are similarly predictive of fracture risk as has been established in the uninfected population.

Additional studies by Guerri-Fernandez et al. [141, 142] used micro-indentation to directly measure bone strength. Their results have shown that after adjusting for sex, age, BMI and vitamin D status, bone material strength index (BMSi) was reduced in PLWH independently of BMD [142] and that TDF treatment significantly reduced BMSi when compared to ABC-based cART [141]. Importantly, BMSi assesses changes in the material properties of bone, suggesting that both HIV infection and cART treatments negatively affect the matrix composition of the bone material.

Matrix Composition

Bone as a structure derives its strength from the amount of bone, bone mass, the organization of bone, architecture, and the chemical makeup of the bone tissue, matrix composition. Changes to matrix composition can significantly affect bone strength even with minimal changes in BMD [147]. Although micro-indentation studies by Guerri-Fernandez et al. [141, 142] suggest that HIV serostatus and cART treatment can negatively affect matrix composition, there have been no direct measures in either preclinical models or in human samples. Histology-based measurements of mineralization are the best proxy to evaluate matrix composition in PLWH. As such, the study by Ramalho et al. evaluating biopsies from PLWH found no difference prior to and after cART initiation in either osteoid thickness or mineralization lag time measures [77]. However, it is worth noting that both prior to and post-cART, the mineralization lag time values were nearly double that of the reference population [77], which may suggest delayed skeletal mineralization occurs in PLWH. This measure, combined with the data presented in Tables 1 and 2, showing impaired osteoblast differentiation and function provide further motivation for future studies to directly compare the effects of HIV and cART on bone matrix composition.

Conclusion and Persective

While osteoporosis is diagnosed clinically by low BMD, there is a growing appreciation for the BMD-independent factors, termed bone quality, that contribute to increase fracture risk. Additional investigation is necessary to fully define the contribution of HIV-related factors to bone quality changes as PLWH age. For example, while BTMs have become more commonly evaluated in PLWH, the direct

Table 5 Summary table of clinical findings of the effects of HIV and ARV on bone architecture

Citation	Study Design	Imaging Strategy	Primary Findings
McGinty et al. [126]	• Prospective cohort study comparing PLWH and unmatched uninfected controls	• Trabecular bone score (TBS)[1]	• PLWH had reduced TBS compared to uninfected controls • HIV serostatus was a significant predictor of reduced TBS and independent of lumbar spine BMC
Kim et al. [127]	• Cross-sectional case–control comparison of HIV-infected men compared to HIV-uninfected controls	• Trabecular bone score (TBS)	• HIV-infected men had reduced trabecular bone scores (TBS) compared to uninfected controls
Sharma et al. [128]	• Longitudinal case–control comparison of HIV-infected women and uninfected controls	• Trabecular bone score (TBS)	• HIV-infected women had reduced baseline TBS compared to uninfected controls • HIV-infected women and uninfected controls had no difference in TBS change over the time
Guerri-Fernandez et al. [141]	• Cross-sectional case–control comparison of PLWH treated with either TDF-FTC or ABC-3TC	• Trabecular bone score (TBS)	• TBS was not different in TDF or ABC treated PLWH
Guerri-Fernandez et al. [143]	• Longitudinal study of cART naïve PLWH initiating TDF/FTC + elvitegravir/cobicist	• Trabecular bone score (TBS)	• TBS decreased 48 weeks post-cART initiation compared to baseline
Ciullini et al. [125]	• Cross-sectional case–control comparison of PLWH with and without vertebral fractures	• Trabecular bone score (TBS)	• PLWH with vertebral fractures had reduced TBS
Pitukcheewanont et al. [144]	• Cross-sectional case–control comparison of perinatally HIV-infected children and adolescents and uninfected controls	• HRpQCT	• HIV-infected children and adolescents had decreased vertebral cross-sectional area compared to uninfected controls
Kazakia et al. [131]	• Cross-sectional case–control comparison of HIV-infected men on long-term stable cART and uninfected controls	• MRI • HRpQCT	• HIV-infected men had decrease bone volume fracture, trabecular thickness and number and increased trabecular spacing in the femoral compared to uninfected controls • HIV-infected men had decreased trabecular number and increased trabecular spacing in the ultra-distal tibia compared to uninfected controls
Biver et al. [132]	• Cross-sectional case–control comparison of HIV-infected men on long-term stable cART and uninfected controls	• HRpQCT	• HIV-infected men had decreased trabecular, decreased trabecular number, increased trabecular spacing, decreased cortical bone volume and decreased cortical thickness at the distal radius compared to uninfected controls • HIV-infected men had decreased trabecular, increased trabecular spacing, decreased cortical bone volume and decreased cortical thickness at the distal radius compared to uninfected controls
Yin et al. [136]	• Cross-sectional case–control comparison of HIV-infected postmenopausal women and uninfected controls	• HRpQCT	• HIV-infected and uninfected postmenopausal women had similar trabecular architectural parameters at both the distal radius and tibia • HIV-infected postmenopausal women had reduced cortical area and thickness at the distal tibia, but not the distal radius
Macdonald et al. [133]	• Cross-sectional case–control comparison of HIV-infected women and uninfected controls	• HRpQCT	• HIV-infected women had reduced trabecular number, trabecular thickness and failure load at the distal radius • HIV-infected women had reduced trabecular thickness and failure load at the distal tibia

Table 5 (continued)

Citation	Study Design	Imaging Strategy	Primary Findings
Shiau et al. [134]	• Cross-sectional case–control comparison of HIV-infected children and uninfected controls	• HRpQCT	• HIV-infected boys and girls had reduced trabecular and total area at the 4% distal radius and tibial sites compared to uninfected controls • HIV-infected boys and girls had reduced cortical area and thickness and reduced periosteal cortical circumference compared to uninfected controls at the 38% tibial site
Sellier et al. [137]	• Cross-sectional case–control comparison of HIV-infected men treated with TDF-containing cART and uninfected controls	• HRpQCT	• HIV-infected and TDF treated men had reduced trabecular density and trabecular number, and increased trabecular separation in the tibia • HIV-infected and TDF treated men had reduced trabecular density and trabecular thickness in the radius
Tan et al. [145]	• Cross-sectional case–control comparison of HIV-infected adults with and without history of fracture	• HRpQCT	• HIV-infected adults with prior fracture had lower cortical thickness and reduced trabecular bone mass at the tibia • HIV-infected adults with prior fracture had higher cortical porosity at the radius
Calmy et al. [135]	• Cross-sectional case–control of HIV-infected premenopausal women and uninfected controls	• HRpQCT	• HIV-infected women had reduced cortical density at the distal radius • HIV-infected women had reduced trabecular density and number and greater trabecular separation at the distal tibia
Foreman et al. [138]	• Cross-sectional study of the effects of nutrition status in PLWH	• HRpQCT	• PLWH infected for greater than 20 years had lower trabecular BMD, cortical thickness, and estimated bone stiffness and failure load of the distal radius
Abraham et al. [146]	• Cross-sectional case–control comparison of HIV-infected men and women and uninfected controls	• HRpQCT	• HIV-infected men did not differ with cortical and trabecular architecture compared to HIV-uninfected men • HIV-infected women had reduced cortical thickness when compared to HIV-uninfected women

[1]Failure load was estimated using finite element analysis

effect of HIV on bone cells is limited to in vitro culture systems studying each bone cell separately. Although these in vitro studies can separately investigate the contribution of individual ARVs or specific HIV-associated viral factors, they have not investigated the potential synergistic effects of the multitude of factors experienced by PLWH. Further, it is difficult to study osteocytes using in vitro systems, and therefore, the effects of HIV, HIV-related factors, and antiretrovirals on the longest lived and most abundant bone cells are largely unknown. Direct investigation of bone tissue samples from PLWH would overcome many of the current limitations in the published studies, including establishing the consequences of altered osteoblast and osteoclast function, determining the response of osteocytes, determining whether these cellular effects lead to changes in the bone matrix composition or mechanical properties of bone. However, the difficulty in obtaining bone tissue for PLWH makes these studies complicated, and therefore, efforts should be made to establish preclinical HIV models of bone mass and quality loss. An effective model would help to disentangle the individual and combined contributions of HIV, HIV-related factors, and antiretrovirals to bone quality changes, establish the mechanisms of action, and potentially identify disease modifying treatment strategies.

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Declarations

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