Adverse bone health among children and adolescents growing up with HIV

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Abstract

Adverse bone health is one of the important non-communicable conditions during the course of life-long HIV treatment. Adolescence is the critical period of bone mineral acquisition for attaining adult peak bone mass. With traditional and HIV–related risk factors, adolescents growing with HIV have a greater chance of having impaired bone mineral density (BMD). Prevalence of low BMD has been reported in 16–32% of HIV-infected adolescents from middle-income countries. The deep interaction between the immune and skeletal systems, called the immunoskeletal interface, is proposed as one of the underlying mechanisms of adverse bone health in HIV-infected individuals. Dual-energy X-ray absorptiometry (DXA) is a standard tool to assess BMD among HIV-infected adolescents. Non-invasive imaging techniques such as quantitative computed tomography (QCT) and quantitative magnetic resonance imaging (QMRI) provide more information on true volumetric density and bone microarchitecture. To date, there are no paediatric recommendations on the treatment and prevention of adverse bone health. Having a healthy lifestyle, routine weight-bearing exercises and adequate dietary intake are the standard approaches to optimise bone health. There are several ongoing randomised clinical trials using pharmacological treatment options, for example vitamin D, calcium and alendronate to improve bone health among this population.

Keywords: adverse bone health, bone mineral density (BMD), dual-energy X-ray absorptiometry (DXA), HIV-infected adolescents, immunoskeletal interface, non-communicable diseases (NCDs)

Introduction

The success of antiretroviral therapy (ART) has turned HIV/AIDS from a disease with a high mortality rate to a manageable chronic life-long illness. Treatment with ART can lead to restoration of immune function and sustained viral suppression, which in turn increases life expectancy for perinatally HIV-infected children and adolescents [1,2]. Currently, HIV-infected children are able to grow up and enter adolescence and young adulthood similarly to their healthy peers [3]. Yet, these individuals do experience several long-term, non-AIDS-related complications that are obstacles to the goal of normal life expectancy and quality of life. The emerging non-communicable diseases (NCDs) associated with HIV infection and antiretroviral treatment include adverse bone health, cardiovascular, liver and renal diseases, and other metabolic and endocrinological disorders [3–5]. Among these complications, adverse bone health has been recognised as an important area of investigation during the past decade in children and adolescents [6]. Since the maximum bone mineral accrual occurs during the first two decades of life [7], reduced bone deposition and increased bone resorption during these critical periods can lead to serious consequences, in particular, osteoporosis and bone fragility later in life [8,9]. This review focuses on current knowledge regarding adverse bone health among children and adolescents living with HIV, including the magnitude of the problem, immunopathogenesis and factors responsible for reduced bone mass, assessment of bone health in clinical practices and up-to-date research studies of management strategies to prevent bone loss and optimise bone health.

Prevalence of low bone mineral density among perinatally HIV-infected children and adolescents

Dual energy X-ray absorptiometry (DXA) is a commonly used technique to assess total body and lumbar spine bone mineral density (BMD) in children and adolescents. Because adults have already reached peak bone mass (PBM) [7], their BMD is assessed using T-scores. In contrast, BMD measurements in growing children need to be compared to healthy age-, sex- and race–matched population norms and are reported as Z-scores. BMD Z-scores less than or equal to -2 are regarded as low bone mass [10]. The prevalence of low BMD among perinatally HIV–infected children and adolescents is much higher in studies conducted in middle-income countries than that observed in resource-rich countries. The variation in prevalence might be explained by the differences in HIV clinical staging at time of ART initiation, duration of ART, lifestyles, nutritional status, food intake and dietary supplements across studies (Figure 1) [11–16].

Resource-rich countries

In a longitudinal study of 66 HIV-infected Dutch children with a median age of 6.7 years, almost all children (96%) were on ART for a median duration of 3.4 years and 58% had undetectable plasma viral load. The prevalence of lumbar spine and femoral neck BMD Z-scores below -2 were 8% and 4%, respectively. The median BMD Z-scores were 0.9 [interquartile range (IQR) –1.6 to 0.1] for lumbar spine and 0.5 (IQR –0.2 to 1.2) for femoral neck [11]. A large cross-sectional study was conducted among 350 HIV-infected adolescents living in the United States and Puerto Rico, with a median age of 12.6 years, median duration of ART 9.5 years and 55% with plasma HIV RNA <400 copies/mL. Similarly to the Dutch study, the prevalence of low BMD was 7% for total body and 4% for lumbar spine. This was higher than the low BMD prevalence in their uninfected peers of 2% for total body and 1% for lumbar spine [12]. Likewise, the Pediatric AIDS Clinical Trial Group (PACTG) 1045 found that post-pubertal HIV-infected adolescent males had significantly lower total body BMD [adjusted difference –0.10 g/cm^2, 95% confidence interval (CI) –0.16 to –0.04 g/cm^2] and lumbar spine BMD (adjusted difference –0.13 g/cm^2, 95%CI –0.23 to –0.04 g/cm^2), compared with HIV–uninfected males at similar Tanner stage [17].

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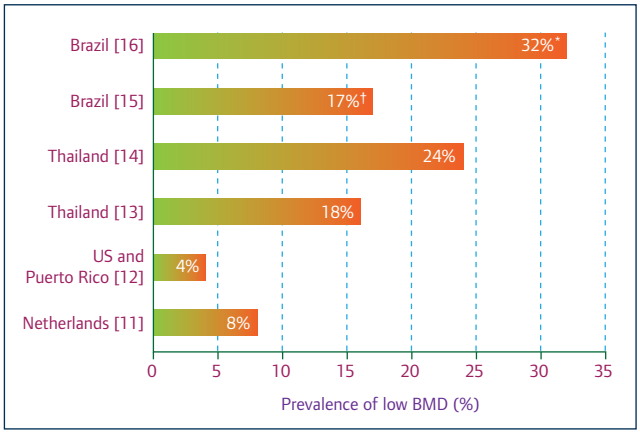

Figure 1. The prevalence of low bone mineral density among perinatally HIV-infected children and adolescents.

Low bone mineral density (BMD) is defined as BMD Z-score ≤–2.
The BMD measurements shown are taken at lumbar spine, except where otherwise indicated.
* Total body BMD and/or lumbar spine BMD; subtotal BMD

Middle-income countries

There are four reports from Thailand [13,14] and Brazil [15,16]. The prevalence of low lumbar spine BMD among HIV-infected Thais was 16–24%. The median ages of Thai participants in these studies were 14.3–15.0 years. All of them were receiving stable ART for a median duration of 7.0–9.3 years; of whom 90–96% had virological suppression [13,14]. In a cross-sectional study among 48 HIV-infected Brazilian adolescents with a mean age of 12.7 years, the prevalence of low BMD was 17% for total body less head. Almost all participants (96%) were on ART, of whom 58% had plasma HIV RNA <50 copies/mL [15]. Another cross–sectional Brazilian study found a 32% prevalence of low BMD (total body and/or lumbar spine) among 74 HIV-infected adolescents with a mean age of 17.3 years. Approximately 91% of them received ART with a mean duration of 11 years, of whom 48% had undetectable virus [16].

Pathogenesis of adverse bone health among HIV-infected population

Bone is a specialised supporting and protecting structure of the body. It contains two major components: calcium phosphate, a mineral compound that gives bone strength and rigidity; and collagen, a protein that provides a flexible framework [18,19]. Bone is not a static structure, but one that constantly undergoes longitudinal and radial growth, rebuilding and remodelling throughout life [18,20].

Bone remodelling involves bone resorption, by osteoclasts, and bone formation, by osteoblasts [18,20]. In general, bone formation predominates over bone resorption during childhood and young adulthood [20]. Thus, bone mass increases over time and reaches its peak during the third decade of life. Subsequently, bone remodelling becomes balanced between bone formation and resorption, resulting in stable bone mass with small variations [20]. Around 50 years of age, the rate of bone resorption begins to outpace that of bone formation, and thus bone mass declines [20].

Changes in bone mass with the course of HIV disease

In HIV-infected individuals, the physiological regulation of bone remodelling can be disrupted by several factors: HIV itself, ART and other HIV-related factors [21–23]. Bone mass changes with the course of HIV disease. During the pre-treatment period, many conditions, for example wasting syndrome, disrupt immune system function through loss of CD4+ T and B cells [24,25]. Together with chronic systemic inflammation [26], this disturbs bone homeostasis. Untreated individuals tend to have raised inflammatory markers and dysregulated bone turnover compared with healthy individuals [27–29]. This finding supports the linkage between systemic inflammation and bone turnover imbalance, a likely cause of bone demineralisation in HIV-infected persons.

After ART initiation, HIV-infected persons usually have improved health status and restored immunological system function. Weight is regained and systemic inflammation is reduced [30]. However, such individuals may experience transient reduced bone mass and worsening of bone health during the first 1–2 years of ART [31–33]. Possible explanations are poor health status and wasting before ART initiation [21], ART causing an imbalance of bone turnover [34,35], and time lag between ART initiation and improvement of BMD [21,31]. Accelerated bone loss, as much as 2–6%, has been demonstrated in HIV-infected individuals on various ART regimens, including tenofovir disoproxil fumarate (TDF) [31], efavirenz (EFV) [32], nevirapine [33] and boosted protease inhibitors (PIs) [32,33]. In HIV-infected adults, both TDF and PIs have been associated with a 33% increase in osteocalcin (OC), a bone formation biomarker [34]. Another study demonstrated that switching to TDF *vs* staying on zidovudine resulted in a significant increase in both bone resorption marker, C-terminal cross-linked telopeptide of type I collagen (CTX) and bone formation markers, OC and procollagen type I amino-terminal propeptide (PINP), which correlate with reductions in lumbar spine BMD [35].

Immunoskeletal interface and bone health

In the context of untreated HIV infection, there are two concurrent and important alterations in bone health: progressive loss of immune function and accelerated bone resorption. Emerging evidence suggests that the immune and skeletal systems are deeply intertwined as a result of a centralisation of common cell types and cytokine mediators [22]. This is called the immunoskeletal interface (Figure 2). There is well-established evidence of the association between immune and skeletal systems observed in many inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease and systemic lupus erythematosus [36–38]. These immune alterations accelerate the natural skeletal ageing process, contributing to adverse bone health.

The immunoskeletal interface can be divided into two aspects: the interactions of immune cells with osteoblasts and with osteoclasts. Osteoblastic cells, derived from osteoprogenitor mesenchymal stem cells, are able to modulate the immune system by regulating the haematopoietic stem cell microenvironment in which immune cells are derived [39]. Immune cells can produce cytokine mediators, such as tumour necrosis factor-alpha (TNF–α), that functions as a potent inhibitor of osteoblast differentiation and activity [40]. Osteoclasts are derived from mature cells of monocyte and macrophage lineage. Osteoclastogenesis requires the presence of RANK ligand (RANKL) and RANK interactions. RANKL is a receptor activator of nuclear factor κβ ligand expressed by osteoblastic lineage cells, while RANK is a receptor activator of nuclear factor κβ presented on the surface of osteoclast precursors and mature osteoclasts [41]. RANKL is recognised as a key osteoclastogenic cytokine and the final effectors of osteoclast formation and activity [42–45]. Additionally, it is considered to have important immunological functions as a mediator for T cell proliferation and dendritic cell function [46,47]. The interactions between RANKL with RANK (RANKL–RANK system) induce the formation and

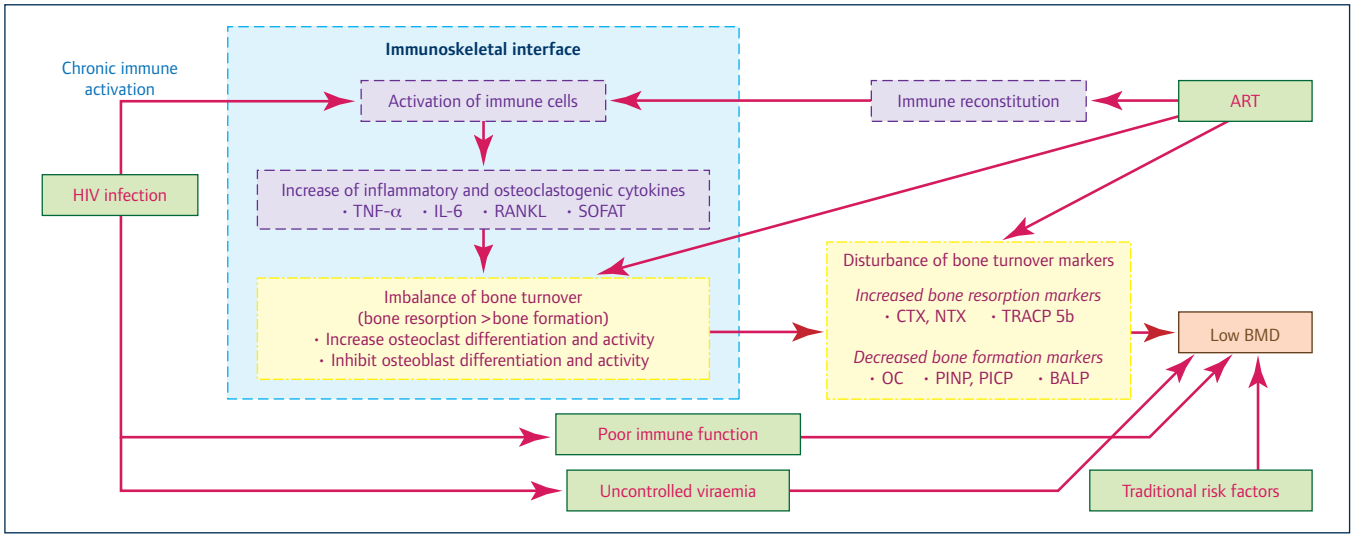

Figure 2. The immunopathogenesis and risk factors of low BMD among HIV-infected children and adolescents.
■ Represent important risk factors of low BMD. ■ Represent components of the immune system. ■ Represent components of the skeletal system.
■ Represents the immunoskeletal interface

ART, antiretroviral treatment; BALP, bone-specific alkaline phosphatase; BMD, bone mineral density; CTX, C-terminal cross-linked telopeptide of type I collagen; IL-6, interleukin-6; NTX, N-terminal cross-linked telopeptide of type I collagen; OC, osteocalcin; PICP, procollagen type I carboxy-terminal propeptide; PINP, procollagen type I amino-terminal propeptide; RANKL, receptor activator of nuclear factor κβ ligand; SOFAT, secreted osteoclastogenic factor of activated T-cells; TNF-α, tumour necrosis factor-α; TRACP 5b, tartrate-resistant acid phosphatase 5b

differentiation of osteoclast precursors into pre-osteoclasts, which then fuse together to form the mature osteoclasts [42,48]. The essential regulatory component of the RANKL–RANK system is osteoprotegerin (OPG), a member of the TNF receptor superfamily and a RANKL decoy receptor. OPG modulates RANKL activity by binding itself to RANKL and prevents the RANKL–RANK interaction, resulting in the inhibition of osteoclast formation and maturation [42–44,48,49]. A new cytokine, secreted osteoclastogenic factor of activated T cells (SOFAT), was recently identified and observed to potently induce osteoblastic IL-6 production [50], which in turn stimulates osteoclastogenesis independently of RANKL [51].

B and T cells are critical for preserving bone homeostasis [52]. B cells produce OPG in response to T cell co-stimulation [53]. Activated T cells, through the CD40 ligand and its receptor CD40 on B cells, can promote B lineage OPG production *in vivo* [52]. However, under inflammatory conditions, B cells and activated T cells turn into a significant source of RANKL and SOFAT production [50,54–57]. In addition, several inflammatory cytokines, including interleukin (IL)-1, IL-6, IL-7 and TNF-α, overexpressed by immune cells during inflammation are able to drive up RANKL-dependent osteoclastogenesis [58,59]. Taken all together, osteoclastic bone resorption is enhanced (Figure 2).

Risk factors for adverse bone health among perinatally HIV-infected children and adolescents

The causes of adverse bone health among perinatally HIV-infected children and adolescents are multifactorial. They can be classified into traditional risk factors and HIV-specific factors (Figure 2). The traditional risk factors for low bone mass include malnutrition, short stature, low body mass index, delayed puberty, vitamin D deficiency, inadequate calcium intake, physical inactivity, smoking and steroid exposure [12–14,60,61]. HIV-specific factors, including HIV itself, advanced HIV disease, poor immunological status, uncontrolled viraemia, exposure to some specific types of ART, as well as persistent immune activation and chronic systemic inflammation also play a significant role in driving bone loss among this population [11–17,62–70].

Traditional risk factors

Weight and height are independently associated with bone mass in healthy individuals; however, the effects may be exaggerated in the HIV-infected population because they usually have significantly delayed linear growth and poor weight gains [71–74]. Although low vitamin D has been considered a traditional risk factor for poor bone health, there is no well-established evidence demonstrating the significant direct association between vitamin D deficiency or inadequate vitamin D intake and adverse bone health in HIV-infected adolescents [13,14,60]. Similarly, the evidence for inadequate calcium intake as a determinant of reduced bone mass is limited and controversial in this population [14,60,61]. A cross-sectional study among 19 HIV-infected girls whose calcium intake was 20–50% lower than the recommended daily allowance hypothesised that suboptimal calcium intake might lead to the increased bone resorption and impaired bone mineral acquisition [61]. In contrast, two cross-sectional studies carried out in Brazil and the United States did not find such an association [15,60]. Calcium supplementation may be only beneficial to individuals with insufficient calcium intake.

The relationship between weight-bearing physical activity and bone health among HIV-infected youth was demonstrated in a few studies [12,15,62]. The challenge of these studies is the best measure for physical activity level. Self-report and questionnaires are used by some [12,62], while an accelerometer is used by others [15].

HIV-specific factors

HIV *per se* is one factor for bone abnormalities. Higher prevalence of bone demineralisation was reported among treatment-naïve HIV-infected adults compared with HIV-uninfected controls [75]. HIV infection is related to a high bone turnover state, as demonstrated by the disturbances of histomorphometric parameters and/or the dysregulation of biochemical markers of bone formation and bone resorption, for example significant increases in levels of RANKL and OPG [28,29,76].

Advanced HIV disease, uncontrolled viraemia and poor immunological status are predictors of adverse bone health among HIV-infected children and adolescents [11–16,63,64].

Individuals with severe clinical symptoms of HIV had a significant impairment of BMD, evaluated by DXA [14] and quantitative high-frequency ultrasound (QUS) techniques [63]. High plasma HIV RNA also correlated with low bone mass [11,12]. A positive correlation between current CD4 percentage and BMD/bone mineral content (BMC) was observed in HIV-infected youth [11,64].

Several studies demonstrated a significant linkage between ART exposure and reduced bone mass [12,13,15–17,62,66–70]. These were either performed in treatment-naïve patients [31–33,62,65] or treatment-experienced patients who switch treatment because of poorly controlled viraemia [69]. Although early loss of bone mass within the first 1–2 years of ART initiation has been observed in treatment-naïve adults and adolescents [31–33,62,65], there are no data in young children. Significant bone abnormalities after ART switching have also been demonstrated in children and adolescents [69]. Early bone loss is observed during the first 24–48 weeks of TDF but remained stable thereafter. Such an effect is, however, not detected among treatment-experienced children with stable clinical status and controlled viraemia prior to switch to TDF [77]. Tenofovir alafenamide (TAF), a prodrug of TDF, has a more favourable bone safety profile. Two randomised clinical trials in HIV-infected adults demonstrated significantly less bone toxicity (mean change of lumbar spine BMD: –1.3% *vs* -2.9%, $P<0.0001$; total hip BMD: –0.7% *vs* –3.0%, $P<0.0001$) among adults receiving TAF *vs* TDF in combination with elvitegravir/cobicistat/emtricitabine [78].

Consequences of adverse bone health in perinatally HIV-infected children and adolescents

Childhood and adolescence represent the critical periods for bone mineralisation and maturation, and at least 90% of final adult bone mass is achieved during these periods [79]. Any factor impairing bone mineral acquisition may diminish bone gain, induce bone loss and compromise adult PBM [7,80]. From two meta-analyses, the prevalence of osteopenia and osteoporosis among HIV-infected adults was 52–67% and 15%, respectively [81,82]. A study conducted in a large United States cohort reported higher prevalence of overall fracture in the HIV–infected group compared with uninfected controls (2.87 *vs* 1.77 patients with fractures per 100 persons) [8]. Furthermore, the HIV Outpatient Study revealed that the adjusted fracture rate among HIV-infected adults increased from 57.7 to 89.9 per 10,000 population between the years 2000 and 2008, respectively, and that it was 49.6–72.9% higher than in the uninfected population over the study period [9]. However, an increased risk of fracture events among HIV-infected children and adolescents has not been documented. A prospective cohort PACTG219/219C study, which followed more than 1,000 HIV–infected children for a median of 5 years showed a similar incidence rate of fracture among HIV-infected compared with HIV-exposed but uninfected children (1.2 *vs* 1.1 per 1,000 person-years) [83]. Since fracture is a long–term complication of adverse bone health, these populations may be too young to demonstrate the increase in the incidence of this condition.

Measurements to assess bone health in HIV-infected children and adolescents

Standard measurement in clinical practice

DXA is a non-invasive imaging technique that uses two X-ray beams with different photon energy levels aimed at the bone to be measured. The BMD can be determined from the absorption of each beam by bone once soft tissue absorption is subtracted. DXA is the most commonly used bone densitometric technique for children and adolescents throughout the world [10]. The measurements provided include bone mineral content (BMC, grams) and areal bone mineral density (BMD, g/cm^2). The most appropriate skeletal sites for performing densitometry in children and adolescents are posterior–anterior (PA) lumbar spine and total body less head [10]. Compared with conventional radiographs, measurement of the spine provides more information about the trabecular bone status, while total body measurement focuses more on cortical bone status [84]. In children with short stature or delayed growth, the areal BMD and BMC should be adjusted in order to eliminate the influences of bone size and skeletal dimension. For total body less head, adjustment should be performed using the height Z-score. For the spine, adjustment can be made by using either bone mineral apparent density (BMAD) or the height Z-score [10]. According to the 2013 International Society for Clinical Densitometry (ISCD) Pediatric Official Positions, an areal BMD Z-score less than or equal to –2.0 SD is described as low bone mass or BMD [10,85].

Advanced measurements in research settings

During the past decade, the concept of bone strength has encompassed a number of bone characteristics, including trabecular and cortical architectures, bone turnover, mineralisation and cellularity, aspects of bone quality [86–88]. Summary of advanced techniques for assessing bone health are shown in Table 1.

Bone histomorphometry is a histological examination of bone biopsy specimens to obtain qualitative and quantitative information on *in vivo* bone structure and remodelling. It is the gold standard for bone metabolic and mineralisation evaluation [89,90]. Currently, there are non-invasive imaging techniques using three-dimensional reconstruction for bone microstructure and microarchitecture analysis [91]; therefore bone biopsy is frequently avoided.

Quantitative ultrasonography (QUS) is a non-invasive method using high-frequency sound waves that are transmitted through bone to assess the bone quality and strength. The longitudinal sound wave transmitted through the calcaneus is the accepted measurement to determine bone health status [92].

Quantitative computed tomography (QCT) is a three-dimensional imaging technique to assess true volumetric density (mg/cm^3) without the overlapping of other tissues [93]. This technique ranges from a volumetric QCT to advanced imaging modalities such as high-resolution CT (hrCT) and microCT. Currently, there is no preferred QCT method for clinical evaluation in children and adolescents [94]. Most QCT studies in children investigated peripheral sites, primarily the radius and tibia, because of radiation exposure concerns [94]. The advantage of QCT over DXA is that it provides a separate analysis of trabecular or cortical components of bone.

Quantitative magnetic resonance imaging (QMRI) is a non–invasive, non-ionising radiation technique that provides three-dimensional imaging of trabecular bone architecture. Magnetic resonance is based on the application of a strong magnetic fields and a series of radiofrequency waves to generate three-dimensional images of the hydrogen protons in the water within skeletal tissues [88].

Biochemical markers of bone turnover are helpful research tools to reflect the ongoing bone remodelling processes. Currently available markers are classified into biochemical markers of bone

Table 1. Methods for assessment of bone health in HIV-infected children and adolescents

Method of assessment	Advantages	Disadvantages	Clinical data from HIV-infected children and adolescents
Bone mineral density (BMD) and bone mineral content (BMC)			
Dual-energy X-ray absorptiometry (DXA)	• Widely available • Safe • Excellent precision • High reproducibility • Examination time 5 minutes	• Subject to systematic errors • Cannot differentiate cortical and trabecular bones • Limited paediatric normative data references	• HIV-infected adolescents had high prevalence of BMD Z-score ≤–2 (16–32%) in middle-income countries [13–16] • BMD and BMC of HIV-infected adolescents is significantly lower than healthy controls [12,14]
Speed of sound (SOS) and broadband ultrasound attenuation (BUA)			
Quantitative ultrasonography (QUS)	• Radiation free • Portable and simple to operate • Correlates well with DXA • Cost-effective	• Limited skeletal site of measurement • Lack of paediatric normative data for interpretation	• HIV-infected children with severe clinical symptoms had lower calcaneal BUA Z-score [63] and phalangeal SOS [99] compared with healthy controls • Tibial and radial SOS were associated with lumbar spine BMC and BMD, and total body BMC and BMD [100]
True volumetric bone density and bone microarchitecture			
Quantitative computed tomography (QCT)	• More accurate assessment of BMD than DXA • Provides separate analysis of cortical and trabecular bones • Not susceptible to degenerative changes of bone calcifications	• High radiation dose • High cost • Hard to access • Lack of paediatric normative data for interpretation	• Similar vertebral volumetric bone density in HIV-infected children compared with controls [101] • DXA Z-scores were significantly lower than QCT Z-scores in HIV-infected children [101] • Cortical BMD (peripheral QCT) was positively associated with NNRTI use, but negatively associated with PI use [64]
Quantitative magnetic resonance imaging (QMRI)	• Lack of ionising radiation • Ability to investigate marrow fat content, marrow diffusion and marrow perfusion	• Long acquisition time • Requires specialised machine • High cost • Lack of paediatric reference data	• None
Bone turnover rate, osteoclast and osteoblast activity			
Bone biochemical markers	• Non-invasive • Can be performed from blood and urine specimens • Helpful tools in diagnosis and treatment assessment of bone health and diseases	• Diurnal variation • Limited paediatric normative data and cut-off levels	• Higher serum BALP and urine NTX in ART-experienced HIV-infected children compared with untreated children and healthy controls [102] • Significantly reduced osteocalcin and urinary deoxypyridinoline in HIV-infected children with severe clinical symptoms compared with healthy controls [63] • CTX and PINP levels were not different between HIV-infected adolescents with and without low BMD, but PINP was significantly inversely correlated with BMD Z-score [13]

BALP: bone-specific alkaline phosphatase; CTX: C-terminal cross-linked telopeptide of type I collagen; NNRTI: non-nucleoside reverse transcriptase inhibitors; NTX: N-terminal cross-linked telopeptide of type I collagen; PI: protease inhibitor; PINP: procollagen type I amino-terminal propeptide

formation and resorption [95–98]. Biomarkers of bone formation are products of active osteoblasts expressed during different developmental stages, including: (i) osteoblast-specific enzymes such as bone-specific alkaline phosphatase (BALP); (ii) osteoblast-related proteins such as OC; and (iii) by-products of collagen synthesis such as PINP. All can be measured in serum or plasma [95–98]. The biomarkers for bone resorption are classified into: (i) osteoclast-specific enzymes such as tartrate-resistant acid phosphatase (TRACP) 5b; and (ii) collagen degradation products such as hydroxyproline, pyridinoline, deoxypyridinoline, CTX, and N-terminal cross-linked telopeptide of type I collagen (NTX) [95–98]. The measurements can be performed from blood or urine.

Bone-health assessment in HIV-infected children and adolescents in clinical practice

The recommendations for the evaluation and management of bone disease in HIV-infected adults have recently been developed by HIV specialists from 16 countries [103]. Screening for adverse bone health depends on an individual's risk for fragility fracture. For individuals with major risk factors, including a previous history of fragility fracture; receipt of glucocorticoid treatment for more than 3 months; and at high risk for falls; BMD assessed by DXA should be performed. Additionally, DXA, if available, is recommended for all men aged >50 years, postmenopausal women and individuals with a 10-year risk of major osteoporotic fracture >10% by the Fracture Risk Assessment Tool (FRAX) score. For those without major risk factors, including men aged 40–49 years and premenopausal women aged ≥40 years, FRAX (without DXA) is the recommended assessment [103]. FRAX is a prediction tool for assessing an individual's fracture risk and is applicable for people aged between 40 and 90 years. The model incorporates several components such as age, race, sex, body mass index, smoking, alcohol consumption, long-term use of glucocorticoids, vitamin D deficiency, prior fragility fracture and parenteral history of hip fracture into the calculation [103]. Therapeutic management guidelines vary by country and are based on the availability, as well as cost of diagnostic tools and medications. In the United States, anti-osteoporosis treatments are prescribed in individuals presenting with hip or vertebral fracture, osteoporosis (T-score no more than –2.5), or osteopenia (T-score between –1.0 and –2.5) with a 10-year probability of hip fracture ≥3% or major osteoporosis-related fracture ≥20% based on FRAX [104]. The follow-up interval of DXA should be

adjusted according to degree of bone demineralisation, repeated after 1–2 years for those with advanced osteopenia (T-score between –2.0 and –2.5) and after 5 years for those with mild to moderate osteopenia (T-score between –1 and –2) [103].

The objective of bone health assessment in the paediatric population is to screen children who fail to achieve the expected gains in bone size, mass and strength, and which leaves them vulnerable to fracture as they age. The ISCD 2013 Statement recommends that DXA should be considered only in children and adolescents who may benefit from interventions and those whose DXA results will influence management [105]. Among chronic diseases, cystic fibrosis has a well-established recommendation for bone health assessment and monitoring. The European Cystic Fibrosis Mineralisation Guidelines recommend the first routine bone density scans at age around 8–10 years, to be repeated every 5 years if the BMD Z-score is above -1; every 2 years if the Z-score is between –1 and –2; and every year if the Z-score is below –2 [106]. However, to date, there is no specific recommendation for DXA screening among HIV-infected children and adolescents in any national or international guidelines. In settings where DXA is available and accessible, bone density scans may be performed at 6–12 months after ART initiation since transient reductions in bone mass may be occurring, with repeat measurements every year if BMD Z-score is less than or equal to –2. In settings where access to DXA is limited, one may consider performing bone density scans only in individuals who have a combination of multiple risk factors for bone demineralisation, for example history of wasting or stunting, advanced HIV disease, use of TDF with ritonavir–boosted PIs and vitamin D deficiency.

Management of adverse bone health in perinatally HIV-infected children and adolescents

As adverse bone health during childhood and adolescence may result in adult osteoporosis and bone fragility, several approaches, primarily to prevent bone loss and optimise bone health, should be implemented during these critical periods.

General management

Promoting a healthy lifestyle

Healthy lifestyle choices include avoiding smoking and heavy alcohol consumption. Smoking is a major lifestyle risk factor for osteoporosis. Studies in twins have provided a powerful study design by controlling for age, sex and genetic background to identify the effects of smoking on bone health [107–109]. A cross-sectional study of 41 pairs of female twins found that smoking one pack of cigarettes per day throughout adulthood would reduce BMD by approximately 5–10%, thus increasing the risk for osteoporosis by the time of menopause [107]. Similarly, a study of 146 female twin pairs showed that a discordance of 10 pack-years smoking was related to a 2.3–3.3% decrease in BMD at the lumbar spine, proximal femur and total body [108]. Furthermore, meta-analyses indicated that smoking substantially increased hip fracture risk by 31–60% when comparing current smokers with non-smokers [110–112].

Alcohol consumption negatively impacts bone health in several ways. First, excessive alcohol consumption causes hypo-vitaminosis D, which in turn reduces calcium reserves [113,114]. Secondly, chronic heavy alcohol consumption can disturb testosterone production, a male hormone linked to the production of osteoblasts [115], while, cortisol, a hormone that arrests osteoblast differentiation, is increased [116].

Exercise

Weight-bearing and muscle strengthening exercises are important for building and maintaining bone density. Weight-bearing exercise can be either high impact, such as dancing, running, jumping, gymnastics, soccer, basketball or low impact, such as fast walking or low-impact aerobics. Muscle strengthening exercises include weight lifting, using elastic exercise bands or weight machines, or functional movements. Previous studies showed that children who usually participate in high-impact activities have higher bone mass compared with individuals who are less active or frequently engage in non-weight bearing exercises [117–120]. The American College of Sports Medicine recommends exercising for 10–20 minutes per day, at least 3 days per week [117]. These exercise prescriptions could improve bone strength in children and adolescents.

Nutrition

Key bone nutrition includes calcium and vitamin D [121]. The Institute of Medicine (IOM) recommendation for daily calcium intake for children and adolescents age 9–18 years is 1,300 mg per day [122]. In clinical practice, diet should be the primary source for calcium. Calcium supplementation should be provided to individuals who are unable to obtain adequate calcium from their diet and who are at high risk for adverse bone health. Common dietary sources of calcium are dairy products, soymilk, soybeans, dark leaf greens and sardines. According to the IOM, the recommended vitamin D intake for children and adolescents age 9–25 years, is 600 IU per day [122]. The most common source of vitamin D is sunlight. However, in countries without year-round sunlight, foods containing vitamin D such as fatty fish (e.g. salmon, tuna and mackerel) and fish oils are among the best sources. Vitamin D-fortified foods may be available in resource–rich countries [123], but they are not in resource-limited settings.

A high prevalence of vitamin D deficiency and insufficiency among HIV-infected children and adolescents has been reported, ranging from 71% to 96% [124–127]. Vitamin D deficiency may diminish calcium absorption in the gastrointestinal tract. Therefore, if 25-hydroxyvitamin D (25-OHD), a surrogate for vitamin D levels, is lower than 30 ng/mL, supplementation should be initiated. However, in settings where 25-OHD measurement is not available, supplementation should be considered based on history of vitamin D intake and the clinician decision. However, evidence demonstrating the benefit of calcium and vitamin D supplementation on bone health among HIV-infected children and adolescents is limited and controversial [128,129]. A randomised clinical trial assessing the effects of calcium (1 g per day) and vitamin D3 (1,600 IU per day) supplementation for 2 years on bone mineral accrual among HIV-infected children and adolescents with normal baseline BMD in the United States found no significant difference in BMD when compared with placebo groups [128]. In contrast, a recent small observational study supplementing 1,200 mg calcium and 400 IU vitamin D3 daily for 6 months showed a significant improvement of lumbar spine BMD and BMD Z-scores among HIV-infected Thai adolescents with low BMD at baseline [129]. Currently, there are ongoing randomised clinical trials determining the impact of calcium and/or vitamin D supplementation on BMD among HIV–infected adolescents (Table 2).

Pharmacological interventions

There are several agents used in the treatment of low BMD in HIV-infected populations, including anti-resorptive therapies (bisphosphonates, serum oestrogen receptor modulators or

SERMs, and monoclonal antibodies to RANKL), strontium ranelate and peptides of the parathyroid hormone family [130]. Among all treatment options, bisphosphonates are the longest established therapy for osteoporosis. Bisphosphonates are derivatives of inorganic pyrophosphate that have a high affinity for bone minerals. These agents are preferentially incorporated into sites of active bone remodelling and accelerated bone turnover and inhibit hydroxyapatite breakdown, which in turn suppresses bone resorption [131]. This property results in their utility as clinical agents for osteoporosis treatment. Alendronate and zolendronate are the only two agents recommended for HIV–infected adults with osteoporosis [103]. However, clinical evidence for efficacy of these medications in HIV-infected individuals is scarce and no studies in children and adolescents have taken place. Previous randomised clinical trials of the bisphosphonates, alendronate (weekly) or zolendronate (annually), in HIV-infected adults with low bone density demonstrated significant improvement of BMD compared to placebo groups [132–137]. Before bisphosphonates can be recommended as an anti-osteoporosis treatment, larger studies with longer follow-up periods should be performed. Currently, there are several ongoing randomised clinical trials that aim to determine the efficacy of alendronate on bone density among HIV-infected adolescents and young adults (Table 2).

Conclusions

Adverse bone health is common in perinatally HIV-infected children and adolescents, particularly those living in middle–income countries. The pathogenesis of low BMD is complex, and is related to HIV disease course and systemic inflammation (immunoskeletal interface). Many factors, both traditional and HIV specific, can lead to adverse bone health. An important consequence of low bone density during childhood and adolescence is compromised PBM, which may result in osteoporosis and bone fracture later in life. Refraining from smoking and heavy alcohol consumption, performing regular weight–bearing exercises and adequate dietary intake of calcium are basic health education messages for patients to optimise bone health. More data are required to support the efficacy of calcium and vitamin D supplementation, and bisphosphonates in restoring bone mineralisation and preventing bone loss among HIV–infected children and adolescents.

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Conflicts of interest

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References

1. Lee GM, Gortmaker SL, McIntosh K *et al*. Quality of life for children and adolescents: impact of HIV infection and antiretroviral treatment. *Pediatrics* 2006; **117**: 273–283.
2. McConnell MS, Byers RH, Frederick T *et al*. Trends in antiretroviral therapy use and survival rates for a large cohort of HIV-infected children and adolescents in the United States, 1989–2001. *J Acquir Immune Defic Syndr* 2005; **38**: 488–494.
3. Hazra R, Siberry GK, Mofenson LM. Growing up with HIV: children, adolescents, and young adults with perinatally acquired HIV infection. *Annu Rev Med* 2010; **61**: 169–185.
4. Puthanakit T, Siberry GK. Bone health in children and adolescents with perinatal HIV infection. *J Int AIDS Soc* 2013; **16**: 18575.
5. Aldrovandi GM, Lindsey JC, Jacobson DL *et al*. Morphologic and metabolic abnormalities in vertically HIV-infected children and youth. *AIDS* 2009; **23**: 661–672.

Table 2. Ongoing randomised clinical trials on the interventions for improving bone health in HIV-infected adolescents and young adults

Investigator, study name, (Trials identifier)	Study location	Study population	Intervention	Primary outcome
Siberry GK *et al*. IMPAACT P1076 (NCT00921557)	United States, Brazil, Puerto Rico	Age 11–24 years Lumbar spine BMD Z-score <–1.5 or history of fragility fracture	ARM 1: Oral alendronate 70 mg weekly for 96 weeks ARM 2: Oral alendronate 70 mg weekly for 48 weeks plus placebo for 48 weeks ARM 3: Placebo for 96 weeks	Changes of LS BMD after 24 and 48 weeks of alendronate treatment versus placebo
Havens P *et al*. ATN109 (NCT01751646)	United States, Puerto Rico	Age 16–24 years receiving TDF-containing ART	ARM 1: Vitamin D3 50,000 IU orally every 4 weeks for 48 weeks ARM 2: Placebo for 48 weeks	Changes in LS BMD after 48 weeks of supplementation
Sudjaritruk T *et al*. CAL-D (NCT02426840)	Thailand	Adolescents age 10–20 years receiving stable ART	ARM 1: Co-formulated oral calcium (600 mg elemental calcium)/vitamin D3 (200 IU) twice daily for 48 weeks ARM 2: Co-formulated oral calcium/vitamin D3 twice daily plus vitamin D2 (20,000 IU/cap) once weekly for 48 weeks	Changes in LS BMD after 48 weeks of supplementation
Tan D *et al*. BATARI (NCT01968850)	Canada	ART-naïve age >18 years with low fracture risk (FRAX 10-year fracture risk scores <10%)	ARM 1: Standard of care ARM 2: Co-formulated oral alendronate (70 mg)/vitamin D3 (5,600 IU) weekly started at time of ART initiation for 24 weeks ARM 3: Co-formulated oral alendronate weekly started at week 24–48 of ART	Changes in LS and proximal femur BMD at week 48
Mallon PW *et al*. APART (NCT02322099)	Ireland	ART-naïve adults age >30 years initiated with TDF/FTC	ARM 1: Oral alendronate (70 mg) weekly plus daily calcium (500 mg elemental calcium)/vitamin D3 (400 IU) for 14 weeks ARM 2: Placebo plus calcium (500 mg elemental calcium)/vitamin D3 (400 IU) for 14 weeks	Between-group differences in the change in total hip, LS, femoral neck BMD and body composition to week 50

ART: antiretroviral therapy; BMD: bone mineral density; FRAX: fracture risk assessment tool; FTC: emtricitabine; LS: lumbar spine; TDF: tenofovir disoproxil fumarate

6. Arpadi SM, Shiau S, Arpadi CM, Yin MT. Bone health in HIV-infected children, adolescents and young adults: a systematic review. *J AIDS Clin Res* 2014; **5**: 374.
7. Heaney RP, Abrams S, Dawson-Hughes B *et al*. Peak bone mass. *Osteoporos Int* 2000; **11**: 985–1009.
8. Triant VA, Brown TT, Lee H, Grinspoon SK. Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non-HIV-infected patients in a large U.S. healthcare system. *J Clin Endocrinol Metab* 2008; **93**: 3499–3504.
9. Young B, Dao CN, Buchacz K *et al*.; HIV Outpatient Study (HOPS) Investigators. Increased rates of bone fracture among HIV-infected persons in the HIV Outpatient Study (HOPS) compared with the US general population, 2000–2006. *Clin Infect Dis* 2011; **52**: 1061–1068.
10. Crabtree NJ, Arabi A, Bachrach LK *et al*. Dual-energy X-ray absorptiometry interpretation and reporting in children and adolescents: the revised 2013 ISCD Pediatric Official Positions. *J Clin Densitom* 2014; **17**: 225–242.
11. Bunders MJ, Frinking O, Scherpbier HJ *et al*. Bone mineral density increases in HIV–infected children treated with long-term combination antiretroviral therapy. *Clin Infect Dis* 2013; **56**: 583–586.
12. Dimegio LA, Wang J, Siberry GK *et al*. Bone mineral density in children and adolescents with perinatal HIV infection. *AIDS* 2013; **27**: 211–220.
13. Sudjaritruk T, Bunupuradah T, Aurpibul L *et al*. Adverse bone health among perinatally HIV-infected Asian adolescents with virological suppression. *7th International Workshop on HIV Pediatrics*. July 2015. Vancouver, Canada.
14. Puthanakit T, Saksawad R, Bunupuradah T *et al*. Prevalence and risk factors of low bone mineral density among perinatally HIV-infected Thai adolescents receiving antiretroviral therapy. *J Acquir Immune Defic Syndr* 2012; **61**: 477–483.
15. Lima LR, Silva RC, Giuliano Ide C, Sakuno T, Brincas SM, Carvalho AP. Bone mass in children and adolescents infected with human immunodeficiency virus. *J Pediatr* 2013; **89**: 91–99.
16. Schtschrbyna A, Pinheiro MF, Mendonça LM *et al*. Factors associated with low bone mineral density in a Brazilian cohort of vertically HIV-infected adolescents. *Int J Infect Dis* 2012; **16**: e872–878.
17. Jacobson DL, Lindsey JC, Gordon CM *et al*. Total body and spinal bone mineral density across Tanner stage in perinatally HIV-infected and uninfected children and youth in PACTG 1045. *AIDS* 2010; **24**: 687–696.
18. Clarke B. Normal bone anatomy and physiology. *Clin J Am Soc Nephrol* 2008; **3** Suppl 3: S131–139.
19. Taichman RS. Blood and bone: two tissues whose fates are intertwined to create the hematopoietic stem-cell niche. *Blood* 2005; **105**: 2631–2639.
20. Kini U, Nandeesh BN. Physiology of bone formation, remodeling, and metabolism. In: Fogelman I, Gnanasegaran G, Wall H, eds. *Radionuclide and Hybrid Bone Imaging*. Springer, Berlin, 2012; pp 29–57.
21. Bolland MJ, Grey A, Reid IR. Skeletal health in adults with HIV infection. *Lancet Diabetes Endocrinol* 2015; **3**: 63–74.
22. Ofotokun I, Weitzmann MN. HIV and bone metabolism. *Discov Med* 2011; **11**: 385–393.
23. Sharp M. HIV and bone health. *BETA* 2011; **23**: 28–37.
24. Douek DC. Disrupting T-cell homeostasis: how HIV-1 infection causes disease. *AIDS Rev* 2003; **5**: 172–177.
25. Moir S, Ho J, Malaspina A *et al*. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J Exp Med* 2008; **205**: 1797–1805.
26. Brenchley JM, Price DA, Schacker TW *et al*. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006; **12**: 1365–1371.
27. Aukrust P, Liabakk NB, Müller F, Lien E, Espevik T, Frøland SS. Serum levels of tumor necrosis factor-alpha (TNF alpha) and soluble TNF receptors in human immunodeficiency virus type 1 infection: correlations to clinical, immunologic, and virologic parameters. *J Infect Dis* 1994; **169**: 420–424.
28. Gibellini D, Borderi M, De Crignis E *et al*. RANKL/OPG/TRAIL plasma levels and bone mass loss evaluation in antiretroviral naive HIV-1-positive men. *J Med Virol* 2007; **79**: 1446–1454.
29. Serrano S, Mariñoso ML, Soriano JC *et al*. Bone remodeling in human immunodeficiency virus-1-infected patients. A histomorphometric study. *Bone* 1995; **16**: 185–191.
30. Brown TT, Tassiopoulos K, Bosch RJ, Shikuma C, McComsey GA. Association between systemic inflammation and incident diabetes in HIV-infected patients after initiation of antiretroviral therapy. *Diabetes Care* 2010; **33**: 2244–2249.
31. Cassetti I, Madruga JV, Suleiman JM *et al*. The safety and efficacy of tenofovir DF in combination with lamivudine and efavirenz through 6 years in antiretroviral-naïve HIV-1-infected patients. *HIV Clin Trials* 2007; **8**: 164–172.
32. Brown TT, McComsey GA, King MS, Qaqish RB, Bernstein BM, da Silva BA. Loss of bone mineral density after antiretroviral therapy initiation, independent of antiretroviral regimen. *J Acquir Immune Defic Syndr* 2009; **51**: 554–561.
33. Duvivier C, Kolta S, Assoumou L *et al*. Greater decrease in bone mineral density with protease inhibitor regimens compared with nonnucleoside reverse transcriptase inhibitor regimens in HIV-1 infected naïve patients. *AIDS* 2009; **23**: 817–824.
34. Brown TT, Ross AC, Storer N, Labbato D, McComsey GA. Bone turnover, osteoprotegerin/RANKL and inflammation with antiretroviral initiation: tenofovir versus non-tenofovir regimens. *Antivir Ther* 2011; **16**: 1063–1072.
35. Cotter AG, Vrouenraets SM, Brady JJ *et al*. Impact of switching from zidovudine to tenofovir disoproxil fumarate on bone mineral density and markers of bone metabolism in virologically suppressed HIV-1 infected patients; a substudy of the PREPARE study. *J Clin Endocrinol Metab* 2013; **98**: 1659–1666.
36. Kong YY, Yoshida H, Sarosi I *et al*. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 1999; **397**: 315–323.
37. Abitbol V, Roux C, Chaussade S *et al*. Metabolic bone assessment in patients with inflammatory bowel disease. *Gastroenterology* 1995; **108**: 417–422.
38. Schett G, David JP. The multiple faces of autoimmune-mediated bone loss. *Nat Rev Endocrinol* 2010; **6**: 698–706.
39. Calvi LM, Adams GB, Weibrecht KW *et al*. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 2003; **425**: 841–846.
40. Nanes MS. Tumor necrosis factor-alpha: molecular and cellular mechanisms in skeletal pathology. *Gene* 2003; **321**: 1–15.
41. Burgess TL, Qian Y, Kaufman S *et al*. The ligand for osteoprotogerin (OPGL) directly activates mature osteoclasts. *J Cell Biol* 1999; **145**: 527–538.
42. Lacey DL, Timms E, Tan HL *et al*. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998; **93**: 165–176.
43. Teitelbaum SL. Bone resorption by osteoclasts. *Science* 2000; **289**: 1504–1508.
44. Khosla S. Minireview: the OPG/RANKL/RANK system. *Endocrinology* 2001; **142**: 5050–5055.
45. Matsuzaki K, Udagawa N, Takahashi N *et al*. Osteoclast differentiation factor (ODF) induces osteoclast-like cell formation in human peripheral blood mononuclear cell cultures. *Biochem Biophys Res Commun* 1998; **246**: 199–204.
46. Anderson DM, Maraskovsky E, Billingsley WL *et al*. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature* 1997; **390**: 175–179.
47. Leibbrandt A, Penninger JM. Novel functions of RANK(L) signaling in the immune system. *Adv Exp Med Biol* 2010; **658**: 77–94.
48. Yasuda H, Shima N, Nakagawa N *et al*. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A* 1998; **95**: 3597–3602.
49. Simonet WS, Lacey DL, Dunstan CR *et al*. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997; **89**: 309–319.
50. Rifas L, Weitzmann MN. A novel T cell cytokine, secreted osteoclastogenic factor of activated T cells, induces osteoclast formation in a RANKL-independent manner. *Arthritis Rheum* 2009; **60**: 3324–3335.
51. Hofbauer LC, Heufelder AE. Intercellular chatter: osteoblasts, osteoclasts and interleukin 6. *Eur J Endocrinol* 1996; **134**: 425–426.
52. Li Y, Toraldo G, Li A *et al*. B cells and T cells are critical for the preservation of bone homeostasis and attainment of peak bone mass *in vivo*. *Blood* 2007; **109**: 3839–3848.
53. Yun TJ, Chaudhary PM, Shu GL *et al*. OPG/FDCR-1, a TNF receptor family member, is expressed in lymphoid cells and is up-regulated by ligating CD40. *J Immunol* 1998; **161**: 6113–6121.
54. Vikulina T, Fan X, Yamaguchi M *et al*. Alterations in the immuno-skeletal interface drive bone destruction in HIV-1 transgenic rats. *Proc Natl Acad Sci U S A* 2010; **107**: 13848–13853.
55. Weitzmann MN, Cenci S, Rifas L, Haug J, Dipersio J, Pacifici R. T cell activation induces human osteoclast formation via receptor activator of nuclear factor kappaB ligand-dependent and -independent mechanisms. *J Bone Miner Res* 2001; **16**: 328–337.
56. Kong YY, Feige U, Sarosi I *et al*. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature* 1999; **402**: 304–309.
57. Kawai T, Matsuyama T, Hosokawa Y *et al*. B and T lymphocytes are the primary sources of RANKL in the bone resorptive lesion of periodontal disease. *Am J Pathol* 2006; **169**: 987–998.
58. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. *J Clin Invest* 2006; **116**: 1186–1194.
59. Cenci S, Weitzmann MN, Roggia C *et al*. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. *J Clin Invest* 2000; **106**: 1229–1237.
60. Jacobson DL, Spiegelman D, Duggan C *et al*. Predictors of bone mineral density in human immunodeficiency virus-1 infected children. *J Pediatr Gastroenterol Nutr* 2005; **41**: 339–346.
61. O'Brien KO, Razavi M, Henderson RA, Caballero B, Ellis KJ. Bone mineral content in girls perinatally infected with HIV. *Am J Clin Nutr* 2001; **73**: 821–826.
62. Mulligan K, Harris DR, Emmanuel P *et al*. Low bone mass in behaviorally HIV–infected young men on antiretroviral therapy: Adolescent Trials Network Study 021B. *Clin Infect Dis* 2012; **55**: 461–468.
63. Stagi S, Bindi G, Galluzzi F, Galli L, Salti R, de Martino M. Changed bone status in human immunodeficiency virus type 1 (HIV-1) perinatally infected children is related to low serum free IGF-I. *Clin Endocrinol* 2004; **61**: 692–699.
64. Macdonald HM, Chu J, Nettlefold L *et al*. Bone geometry and strength are adapted to muscle force in children and adolescents perinatally infected with HIV. *J Musculoskelet Neuronal Interact* 2013; **13**: 53–65.
65. Bonnet E, Ruidavets JB, Genoux A *et al*. Early loss of bone mineral density is correlated with a gain of fat mass in patients starting a protease inhibitor containing regimen: the prospective Lipotrip study. *BMC Infect Dis* 2013; **13**: 293.
66. Stellbrink HJ, Orkin C, Arribas JR *et al*. Comparison of changes in bone density and turnover with abacavir-lamivudine versus tenofovir-emtricitabine in HIV-infected adults: 48-week results from the ASSERT study. *Clin Infect Dis* 2010; **51**: 963–972.
67. McComsey GA, Kitch D, Daar ES *et al*. Bone mineral density and fractures in antiretroviral-naïve persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202. *J Infect Dis* 2011; **203**: 1791–1801.
68. Bedimo R, Maalouf NM, Zhang S, Drechsler H, Tebas P. Osteoporotic fracture risk associated with cumulative exposure to tenofovir and other antiretroviral agents. *AIDS* 2012; **26**: 825–831.
69. Gafni RI, Hazra R, Reynolds JC *et al*. Tenofovir disoproxil fumarate and an optimized background regimen of antiretroviral agents as salvage therapy: impact on bone mineral density in HIV-infected children. *Pediatrics* 2006; **118**: e711–718.
70. Zuccotti G, Viganò A, Gabiano C *et al*. Antiretroviral therapy and bone mineral measurements in HIV-infected youths. *Bone* 2010; **46**: 1633–1638.

71. Gilsanz V, Nelson DA. Primer on the Metabolic Bone Disorders and Disorders of Mineral Metabolism. Favus, M.J. 5th ed. Washington: American Society for Bone and Mineral Research 2003: pp. 72–77.
72. Saavedra JM, Henderson RA, Perman JA, Hutton N, Livingston RA, Yolken RH. Longitudinal assessment of growth in children born to mothers with human immunodeficiency virus infection. *Arch Pediatr Adolesc Med* 1995; **149**: 497–502.
73. Moye J, Rich KC, Kalish LA *et al*. Natural history of somatic growth in infants born to women infected by human immunodeficiency virus. Women and Infants Transmission Study Group. *J Pediatr* 1996; **128**: 58–69.
74. McKinney RE, Robertson JW. Effect of human immunodeficiency virus infection on the growth of young children. The Duke Pediatric AIDS Clinical Trials Unit. *J Pediatr* 1993; **123**: 579–582.
75. Bruera D, Luna N, David DO, Bergoglio LM, Zamudio J. Decreased bone mineral density in HIV-infected patients is independent of antiretroviral therapy. *AIDS* 2003; **17**: 1917–1923.
76. Haskelberg H, Carr A, Emery S. Bone turnover markers in HIV disease. *AIDS Rev* 2011; **13**: 240–250.
77. Aurpibul L, Puthanakit T. Review of tenofovir use in HIV-infected children. *Pediatr Infect Dis J* 2015; **34**: 383–391.
78. Sax PE, Wohl D, Yin MT *et al*. Tenofovir alafenamide versus tenofovir disoproxil fumarate, coformulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: two randomised, double-blind, phase 3, non–inferiority trials. *Lancet*. 2015. [Epub ahead of print]
79. Bonjour JP, Theintz G, Law F, Slosman D, Rizzoli R. Peak bone mass. *Osteoporos Int* 1994; **4** Suppl 1: 7–13.
80. Davies JH, Evans BA, Gregory JW. Bone mass acquisition in healthy children. *Arch Dis Child* 2005; **90**: 373–378.
81. Paccou J, Viget N, Legrout-Gérot I, Yazdanpanah Y, Cortet B. Bone loss in patients with HIV infection. *Joint Bone Spine* 2009; **76**: 637–641.
82. Brown TT, Qaqish RB. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. *AIDS* 2006; **20**: 2165–2174.
83. Siberry GK, Li H, Jacobson D; Pediatric AIDS Clinical Trials Group (PACTG) 219/219C Study. Fracture risk by HIV infection status in perinatally HIV-exposed children. *AIDS Res Hum Retroviruses* 2012; **28**: 247–250.
84. Rauch F, Neu C, Manz F, Schoenau E. The development of metaphyseal cortex–implications for distal radius fractures during growth. *J Bone Miner Res* 2001; **16**: 1547–1555.
85. Shaw NJ. Management of osteoporosis in children. *Eur J Endocrinol* 2008; **159** Suppl 1: S33–9.
86. Griffith JF, Engelke K, Genant HK. Looking beyond bone mineral density: Imaging assessment of bone quality. *Ann N Y Acad Sci* 2010; **1192**: 45–56.
87. Licata A. Bone density vs bone quality: what's a clinician to do? *Cleve Clin J Med* 2009; **76**: 331–336.
88. Donnelly E. Methods for assessing bone quality: a review. *Clin Orthop Relat Res* 2011; **469**: 2128–2138.
89. Kulak CA, Dempster DW. Bone histomorphometry: a concise review for endocrinologists and clinicians. *Arq Bras Endocrinol Metabol* 2010; **54**: 87–98.
90. Weinsten R. Clinical use of bone biopsy. In: Marcus R, Feldman D, Nelson D, Rosen CJ eds. *Osteoporosis*. 3rd edn. Academic Press, 2008; pp 448–465.
91. Genant HK, Jiang Y. Advanced imaging assessment of bone quality. *Ann N Y Acad Sci* 2006; **1068**: 410–28.
92. Krieg MA, Barkmann R, Gonnelli S *et al*. Quantitative ultrasound in the management of osteoporosis: the 2007 ISCD Official Positions. *J Clin Densitom* 2008; **11**: 163–187.
93. Adams JE. Quantitative computed tomography. *Eur J Radiol* 2009; **71**: 415–424.
94. Adams JE, Engelke K, Zemel BS, Ward KA; International Society of Clinical Densitometry. Quantitative computer tomography in children and adolescents: the 2013 ISCD Pediatric Official Positions. *J Clin Densitom* 2014; **17**: 258–274.
95. Seibel MJ. Clinical application of biochemical markers of bone turnover. *Arq Bras Endocrinol Metabol* 2006; **50**: 603–620.
96. Seibel MJ. Biochemical markers of bone turnover: part I: biochemistry and variability. *Clin Biochem Rev* 2005; **26**: 97–122.
97. Seibel MJ. Biochemical markers of bone remodeling. *Endocrinol Metab Clin North Am* 2003; **32**: 83–113.
98. Russell RG. The assessment of bone metabolism *in vivo* using biochemical approaches. *Horm Metab Res* 1997; **29**: 138–144.
99. Rosso R, Vignolo M, Parodi A *et al*. Bone quality in perinatally HIV-infected children: role of age, sex, growth, HIV infection, and antiretroviral therapy. *AIDS Res Hum Retroviruses* 2005; **21**: 927–932.
100. Mora S, Viganò A, Cafarelli L *et al*. Applicability of quantitative ultrasonography of the radius and tibia in HIV-infected children and adolescents. *J Acquir Immune Defic Syndr* 2009; **51**: 588–592.
101. Pitukcheewanont P, Safani D, Church J, Gilsanz V. Bone measures in HIV-1 infected children and adolescents: disparity between quantitative computed tomography and dual-energy X-ray absorptiometry measurements. *Osteoporos Int* 2005; **16**: 1393–1396.
102. Mora S, Sala N, Bricalli D, Zuin G, Chiumello G, Viganò A. Bone mineral loss through increased bone turnover in HIV-infected children treated with highly active antiretroviral therapy. *AIDS* 2001; **15**: 1823–1829.
103. Brown TT, Hoy J, Borderi M *et al*. Recommendations for evaluation and management of bone disease in HIV. *Clin Infect Dis* 2015; **60**: 1242–1251.
104. Cosman F, de Beur SJ, LeBoff MS *et al*. Clinician's guide to prevention and treatment of osteoporosis. *Osteoporos Int* 2014; **25**: 2359–2381.
105. Bianchi ML, Leonard MB, Bechtold S *et al*. Bone health in children and adolescents with chronic diseases that may affect the skeleton: the 2013 ISCD Pediatric Official Positions. *J Clin Densitom* 2014; **17**: 281–294.
106. Sermet-Gaudelus I, Bianchi ML, Garabédian M *et al*. European cystic fibrosis bone mineralisation guidelines. *J Cyst Fibros* 2011; **10** Suppl 2: S16–23.
107. Hopper JL, Seeman E. The bone density of female twins discordant for tobacco use. *N Engl J Med* 1994; **330**: 387–392.
108. MacInnis RJ, Cassar C, Nowson CA *et al*. Determinants of bone density in 30- to 65-year-old women: a co-twin study. *J Bone Miner Res* 2003; **18**: 1650–1656.
109. Flicker L, Hopper JL, Rodgers L, Kaymakci B, Green RM, Wark JD. Bone density determinants in elderly women: a twin study. *J Bone Miner Res* 1995; **10**: 1607–1613.
110. Kanis JA, Johnell O, Oden A *et al*. Smoking and fracture risk: a meta-analysis. *Osteoporos Int* 2005; **16**: 155–162.
111. Ward KD, Klesges RC. A meta-analysis of the effects of cigarette smoking on bone mineral density. *Calcif Tissue Int* 2001; **68**: 259–270.
112. Law MR, Hackshaw AK. A meta-analysis of cigarette smoking, bone mineral density and risk of hip fracture: recognition of a major effect. *BMJ* 1997; **315**: 841–846.
113. Turner RT. Skeletal response to alcohol. *Alcohol Clin Exp Res* 2000; **24**: 1693–1701.
114. Laitinen K, Välimäki M, Lamberg-Allardt C *et al*. Deranged vitamin D metabolism but normal bone mineral density in Finnish noncirrhotic male alcoholics. *Alcohol Clin Exp Res* 1990; **14**: 551–556.
115. Emanuele MA, Emanuele N. Alcohol and the male reproductive system. *Alcohol Res Health* 2001; **25**: 282–287.
116. Badrick E, Bobak M, Britton A, Kirschbaum C, Marmot M, Kumari M. The relationship between alcohol consumption and cortisol secretion in an aging cohort. *J Clin Endocrinol Metab* 2008; **93**: 750–757.
117. Kohrt WM, Bloomfield SA, Little KD *et al*. American College of Sports Medicine Position Stand: physical activity and bone health. *Med Sci Sports Exerc* 2004; **36**: 1985–1996.
118. Slemenda CW, Miller JZ, Hui SL, Reister TK, Johnston CC Jr. Role of physical activity in the development of skeletal mass in children. *J Bone Miner Res* 1991; **6**: 1227–1233.
119. Cassell C, Benedict M, Specker B. Bone mineral density in elite 7- to 9-yr-old female gymnasts and swimmers. *Med Sci Sports Exerc* 1996; **28**: 1243–1246.
120. Courteix D, Lespessailles E, Peres SL, Obert P, Germain P, Benhamou CL. Effect of physical training on bone mineral density in prepubertal girls: a comparative study between impact-loading and non-impact-loading sports. *Osteoporos Int* 1998; **8**: 152–158.
121. Rizzoli R. Nutrition: its role in bone health. *Best Pract Res Clin Endocrinol Metab* 2008; **22**: 813–829.
122. Ross AC, Manson JE, Abrams SA *et al*. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2011; **96**: 53–58.
123. Calvo MS, Whiting SJ, Barton CN. Vitamin D fortification in the United States and Canada: current status and data needs. *Am J Clin Nutr* 2004; **80** (6 Suppl): 1710S–1716S.
124. Stephensen CB, Marquis GS, Kruzich LA, Douglas SD, Aldrovandi GM, Wilson CM. Vitamin D status in adolescents and young adults with HIV infection. *Am J Clin Nutr* 2006; **83**: 1135–1141.
125. Rutstein R, Downes A, Zemel B, Schall J, Stallings V. Vitamin D status in children and young adults with perinatally acquired HIV infection. *Clin Nutr* 2011; **30**: 624–628.
126. Meyzer C, Frange P, Chappuy H *et al*. Vitamin D deficiency and insufficiency in HIV–infected children and young adults. *Pediatr Infect Dis J* 2013; **32**: 1240–1244.
127. Chokephaibulkit K, Saksawad R, Bunupuradah T *et al*. Prevalence of vitamin D deficiency among perinatally HIV-infected Thai adolescents receiving antiretroviral therapy. *Pediatr Infect Dis J* 2013; **32**: 1237–1239.
128. Arpadi SM, McMahon DJ, Abrams EJ *et al*. Effect of supplementation with cholecalciferol and calcium on 2-y bone mass accrual in HIV-infected children and adolescents: a randomized clinical trial. *Am J Clin Nutr* 2012; **95**: 678–685.
129. Puthanakit T, Brukesawan C, Poomlek V *et al*. Effect of calcium and cholecalciferol supplement on bone mass accrual among perinatally HIV-infected adolescents with osteopenia. *6th International Workshop on HIV Pediatrics*. July 2014. Melbourne, Australia.
130. Cotter AG, Mallon PW. Therapeutic options for low bone mineral density in HIV–infected subjects. *Curr HIV/AIDS Rep* 2012; **9**: 148–159.
131. Drake MT, Clarke BL, Khosla S. Bisphosphonates: mechanism of action and role in clinical practice. *Mayo Clin Proc* 2008; **83**: 1032–1045.
132. Mondy K, Powderly WG, Claxton SA *et al*. Alendronate, vitamin D, and calcium for the treatment of osteopenia/osteoporosis associated with HIV infection. *J Acquir Immune Defic Syndr* 2005; **38**: 426–431.
133. Negredo E, Martinez-Lopez E, Paredes R *et al*. Reversal of HIV-1-associated osteoporosis with once-weekly alendronate. *AIDS* 2005; **19**: 343–345.
134. Bolland MJ, Grey AB, Horne AM *et al*. Annual zoledronate increases bone density in highly active antiretroviral therapy-treated human immunodeficiency virus–infected men: a randomized controlled trial. *J Clin Endocrinol Metab* 2007; **92**: 1283–1288.
135. McComsey GA, Kendall MA, Tebas P *et al*. Alendronate with calcium and vitamin D supplementation is safe and effective for the treatment of decreased bone mineral density in HIV. *AIDS* 2007; **21**: 2473–2482.
136. Huang J, Meixner L, Fernandez S, McCutchan JA. A double-blinded, randomized controlled trial of zoledronate therapy for HIV-associated osteopenia and osteoporosis. *AIDS* 2009; **23**: 51–57.
137. Rozenberg S, Lanoy E, Bentata M *et al*. Effect of alendronate on HIV-associated osteoporosis: a randomized, double-blind, placebo-controlled, 96-week trial (ANRS 120). *AIDS Res Hum Retroviruses* 2012; **28**: 972–980.