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# Bone abnormalities in HIV-infected patients on HAART

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**Abstract**

**Recently, a high incidence of osteopenia and osteoporosis has been observed in HIV-infected individuals. This problem seems to be more frequent in patients receiving potent antiretroviral therapy, although a specific contribution (if any) of the drugs used in combination regimens has yet to be established. There have also been several reports of other bone-related complications in HIV-infected individuals including avascular necrosis of the hip and compression fracture of the lumbar spine. People living with HIV have significant alterations in bone metabolism regardless of whether or not they are receiving potent antiretroviral therapy. The underlying mechanisms to account for these observations remain unknown, although studies are underway to examine the relationship between the bone abnormalities and other complications associated with HIV and antiretroviral therapy. HIV-infected patients with osteopenia or osteoporosis should be treated similarly to seronegative patients with appropriate use of nutritional supplements (Calcium and Vitamin D) and exercise. Hormone replacement and anti-resorptive therapies might be also indicated.**

**Key words**

**Bone. Mitochondrial toxicity. Lactic acidosis. Avascular osteonecrosis. Osteoporosis.**

## Introduction

The use of potent combinations of antiretroviral therapies has resulted in dramatic decreases in HIV-related morbidity and mortality in recent years[1,2]. The success of such therapies, however, has resulted in new problems including the emergence of multi-drug resistant HIV strains and the development of numerous acute and long-term toxicities of antiretroviral medications[3-10].

These toxicities include the development of insulin resistance and diabetes, hyperlipidemia, body fat redistribution (lipoatrophy and lipodystrophy), and lactic acidosis[4-10].

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Another toxicity potentially linked to potent antiretroviral therapy is bone demineralization, leading to osteoporosis and increased risk of fracture. We recently reported a high prevalence of osteopenia and osteoporosis in HIV-infected individuals treated with protease inhibitor (PI)-containing highly active antiretroviral therapy (HAART)[11]. Other groups have further confirmed these findings, although a specific contribution (if any) of the drugs used in combination regimens has yet to be established[12,13]. There have also been several reports of other bone-related complications in HIV-infected individuals (both on or off antiretroviral therapy) including avascular necrosis of the hip and compression fracture of the lumbar spine[14-20]. People living with HIV appear to have significant alterations in bone metabolism regardless of whether or not they are receiving potent antiretroviral therapy. The underlying mechanisms to account for these observed effects remain unknown, although studies are underway to exam-

ine possible relationships between the bone abnormalities and other complications associated with antiretroviral therapy such as lactic acidosis, lipodystrophy, and hypertriglyceridemia. In addition, other factors such as immune reconstitution, nutrition and wasting, and HIV itself may have significant effects on bone metabolism.

## Osteoporosis

Osteoporosis is a common disease resulting in substantial morbidity and mortality for millions of people. It is a skeletal disease characterized by significant loss of bone mass and strength which leads to an increased risk of fracture. Most individuals experience gradual bone demineralization as part of the normal aging process. Men and women naturally begin to lose bone around age 35, at a rate of 0.5-1% per year. Women are at an increased risk for osteopenia/osteoporosis because they also lose bone at an accelerated rate after menopause. Additional risk factors for accelerated bone loss include white race, low weight, smoking, excessive alcohol use, and history of prior fracture. A number of secondary causes of osteoporosis are also well established and may be particularly relevant to the HIV-infected population. These include corticosteroid use, chronic illness, prolonged immobility, nutritional deficiencies or malabsorption, hypogonadism, hyperthyroidism, and concurrent medications such as anticonvulsants, ketoconazole, pentamidine, and anticoagulants.

The diagnosis of osteopenia or osteoporosis is generally made by measuring an individual's bone mineral density (BMD). In a consensus statement from 1993 the World Health Organization (WHO) agreed to standard definitions of osteoporosis according to normalized measurements of BMD. By WHO criteria, a *t-score* is defined as the number of SDs above or below the average BMD for race and sex-adjusted population norms determined at peak bone mass (which occurs at approximately 30 years of age). A *z-score* compares the patient to population norms adjusted for age, race, and sex. Currently WHO defines osteoporosis as a t-score ≥ –2.5 SDs. Osteopenia is defined as a BMD between –1 and –2.5 SDs. Osteoporosis without a history of fracture carries a 4 to 5-fold increase in fracture risk compared to individuals with normal BMD. A history of fracture and diagnosis of osteoporosis carries a 20-fold increased risk, whereas osteopenia alone still carries a 2-fold increase in fracture risk[21-23].

The most widely used technique for determining BMD is dual energy x-ray absorptiometry (DEXA). DEXA scanning measures bone mass and density in central regions of interest (hip and spine) as well as appendicular regions (wrist, forearm, heel). It has become the gold standard to which all other bone densitometry technologies are compared[24,25]. DEXA studies are also useful to define fracture risk as well as measure effectiveness of various therapies on bone mass[26]. Biochemical markers of bone turnover can provide complementary information to DEXA scans including changes in bone remodeling that can be identified prior to changes in BMD[26]. However, the use of such markers in establishing the current rate of bone loss and predicting future changes in bone mass and fracture risk remains controversial. Bone markers may be helpful in providing information on the effectiveness of various treatment interventions (such as nutritional supplements or antiresorptive therapies). Generally such markers are obtained at 3 and 6 months after a specific therapeutic intervention.

## Bone mineralization and metabolism in HIV-infected individuals

Prior to the era of HAART, studies indicated that bone mineral metabolism was only minimally affected in HIV-infected individuals. In one study, Paton *et al.*[27] reported that 45 HIV-infected patients had marginally lower BMD at the lumbar spine than seronegative controls ($p = 0.04$). The subjects and controls did not differ in total body or hip BMD. On longitudinal follow-up (15 months), a small decrease in total body BMD (–1.6%; $p = 0.02$) was observed, but there was no significant reduction in spine and hip BMD. We conducted a similar cross-sectional analysis in 20 HIV-infected subjects *naïve* to antiretroviral therapy to evaluate if osteopenia was a manifestation of HIV infection itself. Total body, hip and spine BMD were measured by DEXA with no significant differences from the general population. There was also no significant correlation between CD4 count, HIV viral load, and BMD among our cohort (Fig. 1). At this year's 8th Conference on Retroviruses and Opportunistic Infections, there were additional studies presented which showed a significant prevalence of low BMD in HAART-*naïve* patients compared to the general population[28-30]. In the larger of these studies the prevalence of osteopenia/osteoporosis was 28% compared to the 16% that one would expect in the general population. These studies suggest that HIV infection itself has a significant impact on bone metabolism and may be a contributing factor in the pathogenesis of reduced BMD.

Studies have also examined biochemical markers of bone metabolism and bone biopsy specimens of HIV-infected and seronegative individuals. Quero *et al.*[31] reported the results of a small study of bone mineral metabolism in 16 HIV-infected subjects and 27 seronegative controls. The most characteristic finding was a marked decrease in the concentration of osteocalcin (a marker of bone formation) that correlated with declining CD4 count. These findings were later confirmed in a larger study by Aukrust *et al.*[32] that included 73 HIV-infected subjects. Additional findings included enhanced activation of the tumor necrosis factor (TNF) system and an increase in C-telopeptide (a marker of bone resorption). TNF receptors were negatively correlated with osteocalcin and positively correlated with C-telopeptide levels. Subjects that subsequently started triple-drug antiretroviral therapy experienced a marked rise in serum osteocalcin and CD4 cell count along with a significant decline in

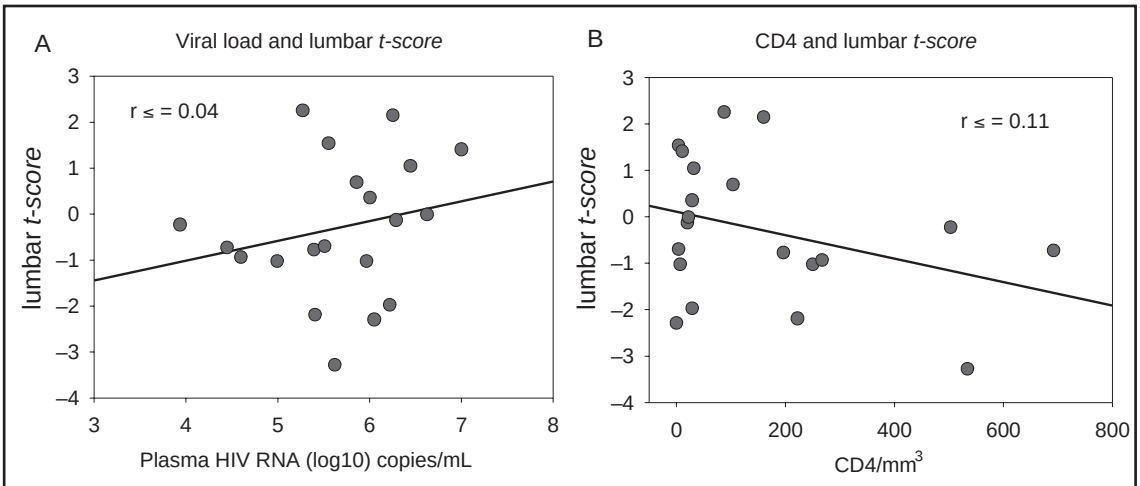


**Fig. 1.** *Relationship between CD4 cell count (A), Plasma HIV RNA (B) and BMD* t-score *in the lumbar spine in 20 antiretroviral* naïve *subjects.*

TNF components and HIV RNA level. This data thus suggests an increase in markers of bone turnover with the initiation of antiretroviral therapy. Also, since enhanced activation of the tumor necrosis factor (TNF) system and numerous cytokines are known to induce differentiation of bone marrow precursors into osteoclasts which would favor bone resorption and the development of osteoporosis, it seems that immune system activation could play a role in the development of bone abnormalities associated with HIV. Unfortunately, the results of these studies are limited due to lack of BMD data.

The only study published so far of bone biopsies of antiretroviral-*naïve* HIV-infected subjects did not show alterations in BMD or biochemical differences in bone metabolism compared to healthy controls except for decreased levels of osteocalcin in individuals with more advanced HIV infection[33]. Osteocalcin levels directly correlated with the number of CD4+ T lymphocytes. There was no correlation observed between the bone mineral content of the biopsy sites and the number of CD4+ T lymphocytes, confirming previous observations. The number of osteoclasts was found to be significantly lower in HIV-infected individuals rather than higher as earlier predicted. These findings suggest that progression of HIV may promote a decline in activity of bone metabolism but that the prevalence of low BMD observed in treated individuals is unlikely due to HIV disease alone. Although these conclusions are limited due to the cross-sectional nature of the studies, larger prospective studies are currently underway that also include bone biopsies of patients on potent antiretroviral therapy.

## Association of osteopenia with potent antiretroviral therapy

As part of our ongoing studies of HAART-related metabolic complications, we performed a cross-sectional analysis of whole-body, lumbar spine (L1-L4) and proximal femur BMD in 112 male subjects (HIV-infected patients on HAART that included a PI, HIV-infected patients not receiving a PI, and healthy seronegative adults) using DEXA scans[11]. The median exposure to PIs in the group receiving PI therapy was 104 weeks. Men receiving PIs had lower median *t-scores* for the lumbar spine BMD than the other two groups (p = 0.02). Median *z-scores* for the lumbar spine BMD were also lower in PI recipients (p = 0.04) (Table 1 and Fig. 2). Median *z-scores* for BMD in the trochanter, femoral neck, and Ward's triangle regions of the proximal femur were significantly lower in PI-treated subjects than in HIV-infected subjects not receiving PIs and in controls. Using lumbar spine BMD *t-scores*, 50% of the subjects on PIs were classified as osteopenic or osteoporotic according to the WHO classification. The relative risk for osteoporosis in these subjects was 2.19 (95% confidence interval 1.13-4.23) when compared to HIV-infected subjects not receiving PIs. Subjects with more prolonged use of PIs tended to

**Table 1.** *Characteristics of the 112 men included in our original description[11]. HIV+ PI+ = Subjects on protease inhibitors, HIV+PI- = Subjects not taking protease inhibitors, Control= Healthy, uninfected subjects.*

| | HIV+ PI+ (N = 60) | HIV+ PI– (N = 35) | Controls (N = 17) | P values |
|---|---|---|---|---|
| Age (yr) | 41 ± 8 | 37 ± 7 | 33 ± 9 | 0.001 |
| BMI (kg/m$^2$) | 24 ± 4 | 22 ± 6 | 23 ± 4 | 0.706 |
| Median lumbar spine BMD | 0.9860 | 1.0690 | 1.0660 | 0.002 |
| Median *t-score* | –1.005 | –0.382 | –0.227 | 0.02 |
| % of subjects with (*t-score* < –1) | 50 | 23 | 29 | 0.02 |

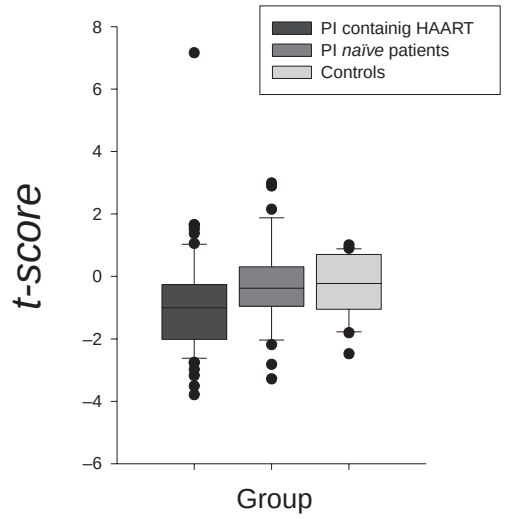


**Fig. 2.** *Lumbar* t-scores *in three groups of patients.*

have more negative *t-scores* in the lumbar spine (Pearson correlation coefficient = –0.19, p = 0.14), but this correlation did not reach statistical significance.

To confirm these results we have performed additional preliminary studies of multiple bone metabolic parameters in subjects taking PI-containing potent antiretroviral therapy. We performed DEXA scans and evaluated multiple bone metabolic parameters in a different cohort of 73 HIV-infected subjects on PI-containing HAART[34]. Ninety-five percent of the cohort had an undetectable viral load and the median CD4 cell count was over 540 cells/mm$^3$, thus representing a group that had responded remarkably well to HAART. Forty-three percent of the subjects were osteopenic or osteoporotic according to the WHO definition, confirming our previous observation in the earlier cohort. There was no association of osteopenia with regards to specific PIs. A significant proportion of subjects had both increased markers of bone resorption and bone formation including elevations in urine pyridinolines, bone alkaline phosphatase, and osteocalcin. This is in sharp contrast with the previous observations of low osteocalcin levels in subjects with advanced disease. The levels of bone alkaline phosphatase and pyridinolines in urine correlated with BMD. Testosterone levels and TSH levels were normal in this population. More than 50% of the patients had urinary calcium levels greater than 200 mg per 24 h, and 25% had greater than 300 mg/24 h. Another more recent study looking at similar bone metabolic parameters in HIV-infected children also found HAART-associated losses in BMD that were associated with an increased rate of bone turnover[35]. These findings suggest that subjects receiving PI-containing HAART may have low BMD secondary to increased bone remodeling and/or an alteration of bone mineralization that leads to increased urinary calcium loss.

## Effects of protease inhibitors on bone metabolism

Since PIs are potent inhibitors of p450 enzymes including multiple hydroxylases involved in vitamin D metabolism, we performed further studies to determine whether our findings could be a direct effect of PIs on bone, mediated through interferences in the metabolism of vitamin D. We examined the *in vitro* effects of three different PIs (indinavir, ritonavir and nelfinavir) on the conversion of 25 (OH) vitamin $D_3$ to 1,25 $(OH)_2$ vitamin $D_3$ in isolated human macrophages[36]. We used efavirenz (a non-nucleoside reverse transcriptase inhibitor) as a control in this experiment. Monocyte-macrophage 1-hydroxylase is a P450 monooxygenase, identical to the renal enzyme[37]. We found that concentrations of PIs in the incubation media, equivalent to maximal levels of PI's in plasma after administration (11 µg/mL Ritonavir, 10 µM Indinavir or 6 µg/mL Nelfinavir), caused an inhibition of the conversion of tritiated 25(OH)D to tritiated $1,25(OH)_2D_3$ by human macrophages of 79.6%, 66% or 31.7%, respectively. When cells were incubated with efavirenz we found no inhibition of vitamin D conversion when compared to the control cells.

Other studies suggest that different PIs may have different effects on bone activity. Wang *et al.*[38] recently tested ritonavir and indinavir for alterations in osteoblast and osteoclast differentiation. Indinavir was found to inhibit *in vitro* bone formation through an inhibitory effect on osteoblast function, whereas ritonavir and nelfinavir exhibited no such inhibition. The effect of indinavir occurred during the first half only of the culture period, suggesting its action during early osteoblastogenesis. A murine osteoblastic cell line treated with indinavir also exhibited a dose-dependent decrease in alkaline phosphatase activity, an early marker of osteoblast differentiation. These *in vitro* effects were further studied in an ex vivo calvarial organ culture system and similar re-

sults were obtained. There was a tenfold decrease in osteoblast colony forming units with indinavir.

To explore the possibility that an inhibition of osteoblast differentiation diverts the stromal cell precursors to an adipocyte lineage, an *in vitro* culturing system was used that promotes differentiation of murine calvarial stromal cells into both osteoblasts and adipocytes. Both adipocytic and osteoblastic differentiation were inhibited by indinavir, suggesting inhibition of stromal cell differentiation at an early stage. These effects were not observed with ritonavir or nelfinavir. Opposite inhibitory effects were observed when the drugs were tested with osteoclasts. Ritonavir but not indinavir reversibly suppressed osteoclast differentiation with an inhibitory dose 50% of 10 µg/mL. When ritonavir was applied to mature osteoclasts on bone slices, osteoclasts failed to resorb bone even though osteoclast number remained unchanged. Indinavir appeared to have no effect on osteoclast differentiation or function. These studies show that even though indinavir and ritonavir target the same HIV protease, they have different effects on osteoclast and osteoblast differentiation and function *in vitro* and *in vivo*. Further studies are needed to determine the clinical significance of the opposing effects of these drugs.

## Other potential causes of osteopenia

While other studies have confirmed our earlier findings of a more accelerated loss of BMD in patients receiving potent antiretroviral therapy, an association with PI use remains speculative. Patients on successful HAART regimens experience immune reconstitution with significant changes in circulating cytokine levels, HIV viremia, and immune cell turnover. Multiple cytokines exert a variety of regulatory effects on bone formation and resorption, thus it is possible that a relationship exists between the development of bone loss and the altered immunity due to HAART and HIV infection.

Most patients on PI therapy have also been on concurrent nucleoside analogue (NRTI) therapy. The use of NRTIs has been associated with mitochondrial toxicity and lactic acidosis, thus it has been suggested that lactic acidosis and possibly mitochondrial toxicity could be directly related to the development of osteoporosis. Preliminary data has been presented correlating osteopenia/osteoporosis, total duration of NRTI therapy, and magnitude of NRTI-related lactic acidemia[39]. Unfortunately, given the cross-sectional nature of these recent studies, it is impossible to attribute cause and effect and to measure the cumulative effects of other important but common risk factors for BMD loss in HIV-infected individuals. In our smaller series we could not confirm an association between lactic acid levels and spinal *t-scores*[40] but the sample size was too small to rule out that an association exists. Further prospective, longitudinal studies are needed to evaluate the possible associations between BMD loss and the numerous long-term effects of antiretroviral therapy.

## Treatment of osteopenia/osteoporosis in HIV-infected individuals

Measurement of BMD as a routine test in HIV-infected patients is not recommended. A detailed history and physical to assess individual risk for osteopenia is important and should include an evaluation of nutritional status and potential secondary causes of osteoporosis. Abnormal laboratory values obtained during the course of HIV treatment (i.e. an elevated alkaline phosphatase or low testosterone) should also prompt consideration of further testing for osteoporosis. No studies have been completed that specifically address osteoporosis treatment in HIV-infected individuals and unfortunately the underlying mechanism(s) to account for these effects remain poorly understood. At this time it is reasonable to follow osteoporosis treatment strategies that have been proven effective in the general population. A careful search for reversible causes of secondary osteoporosis should be performed and vitamin D and calcium intake should be optimized to meet recommended dietary levels. Moderate physical activity is also helpful to help preserve bone mass. For more severe cases, the use of hormone replacement therapy (if otherwise indicated), bisphosphonates, calcitonin, or raloxifene is recommended[26]. No significant interactions are expected with PIs or other antiretroviral therapies (John Gerber, personal communication), although studies utilizing these drugs in HIV-infected individuals have yet to be completed.

## Osteonecrosis

Another bone-related complication seen with increasing frequency in HIV-infected individuals is osteonecrosis. This is generally a rare disease that is felt to be due to an inadequate blood supply to bone. Although the femoral head is most often affected, osteonecrosis in HIV-infected patients has frequently involved multiple sites[19,41]. Patients typically present with pain and the diagnosis is made using radiologic techniques such as CT or MRI. The pathogenesis of this condition is not well known but factors associated with osteonecrosis have included prolonged steroid use, chronic alcoholism or injection drug use, hypertriglyceridemia, antiphospholipid antibodies, sickle cell anemia, and radiation exposure.

Although osteonecrosis has been reported in HIV-infected individuals prior to the development of antiretroviral therapy, there have been suggestions of a possible association between osteonecrosis and potent antiretroviral therapy[14,41,42]. Hypotheses have included loss of blood supply to the bone through PI-related hypertriglyceridemia, fat redistribution, and increased antiphospholipid production due to enhanced humoral immunity. However, most reports have represented small case reports and in one recent review it was determined that HIV was the sole risk factor in 33% of the cases[41]. In a large case-control study by Judy Fallon and Henry Masur at the National Institute of Health, 339 asymptomatic

patients were followed along with 118 HIV-negative age and sex-matched controls[20]. They found 15 cases of osteonecrosis of the hip in the HIV-infected patients and none in the controls (p = 0.015). Factors that did not seem to be associated with osteonecrosis were CD4 cell count, HIV RNA level, or use of a PI. Factors that did seem associated with this complication included previous corticosteroid use, lipid lowering drugs, use of testosterone, and body building/weight lifting. Notable was the finding that cases of osteonecrosis were mostly asymptomatic and did not seem to progress as expected to a symptomatic state.

Treatment for osteonecrosis usually requires surgery, as there are no good medical therapies available at this time. However, therapy is not necessary until an individual becomes symptomatic and in the meantime it may be useful to eliminate any other known risk factors for osteonecrosis including hypertriglyceridemia, steroid use, or heavy alcohol use. There is no evidence at this time to advocate a change or a discontinuation of an individual's antiretroviral regimen.

## Conclusion

Additional long-term, prospective studies are clearly needed to further elucidate the complex effects of antiretroviral therapies, immune reconstitution, and HIV infection itself on bone metabolism. Studies of the individual effects of various antiretrovirals (using *in vitro* methods, animal models, or healthy volunteers) may be helpful in determining a more precise mechanism for the potential effects of antiretroviral therapy on bone metabolism. Future and ongoing clinical trials involving HIV-infected patients should also integrate BMD, hormonal, and bone marker measurements into study protocols in order to gain more prospective data.

In the meantime, HIV-infected patients with low BMD should be treated similarly to seronegative patients with the appropriate use of nutritional supplements, exercise, hormone replacement, and antiresorptive therapies. Data is lacking with regards to the bone effects of switching or discontinuing antiretroviral medications, but these approaches may also be reasonable in the patient with relatively high CD4 counts or with additional metabolic complications on their current antiretroviral regimen.

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