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Antiretroviral Therapy and Bone Loss in HIV-Infected Individuals

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Antiretroviral Therapy and Bone Loss in HIV-Infected Individuals

By

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B.A. Emory University, 2008

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James T. Laney School of Graduate Studies of Emory University
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Abstract

Antiretroviral Therapy and Bone Loss in HIV-Infected Individuals
By Emily McIntosh

Background: Close to 6% loss in bone mineral density (BMD) is observed early on (1-2 years) with antiretroviral therapy (ART) – a loss that has recently been proposed to stem from ART-induced disease reversal and immune reconstitution. To validate this hypothesis we investigated the effect on the skeleton of an ART-regimen switch at the later phase of therapy in virologically suppressed patients with stable CD4 T-cell counts. The new regimen contained raltegravir (RAL), a new generation antiretroviral. We further investigated the direct actions on the skeleton of RAL in a murine model.

Methods: Longitudinal skeletal profiling was performed on a cohort of chronically treated virologically suppressed HIV-infected patients undergoing a regimen switch to lopinivir/ritonavir (LPV/r)+RAL. Plasma levels of C-terminal telopetide of collagen (CTx), a marker of bone resorption, osteocalcin (OCN), a marker of bone formation, receptor activator of NF-κB ligand (RANKL), the key osteoclastogenic cytokine, and osteoprotegerin (OPG), a RANKL moderator were quantified using ELISAs. BMD was quantified by dual energy X-ray absorptiometry (DXA). In a complimentary animal study, C57BL6 female mice were randomized to treatment with RAL or control group. BMD was measured every 4 weeks for 12 weeks at which time the mice were sacrificed for plasma CTx and OCN quantitation using ELISA.

Results: 29 patients were evaluated, median time on ART was 3.9 years, 69% male, 79% African Americans, 21% whites, and pre-study ART consisted of 2 nucleoside reserve transcriptase inhibitors (NRTI) + either a non-NRTI (38%) or a protease inhibitor (62%). Plasma levels of resorption, CD4 T-cell counts, and BMD were stable during the study. However, there was 26.2%↓ ($p=0.002$) from baseline in plasma OCN. When RAL was administered to mice (n=15/group), BMD was markedly reduced by week 8 (5.3%↓ lumbar spine, $p=0.040$; 2.6%↓ femur, $p=0.0002$). These differences were consistent with a decline in bone formation (OCN 82%↓, $p=0.001$).

Conclusions: These results suggest that bone resorption is stable beyond the early T cell reconstitution period. However, an incidental suppression of bone formation was identified. Interestingly, data from the animal study suggest that this suppression may be related to the RAL component of the switch regimen. Further corroborative studies in human are warranted.

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INTRODUCTION

In the pre-antiretroviral therapy (ART) era, acquired immune deficiency syndrome (AIDS) defining illnesses were the leading cause of morbidity and mortality associated with the human immunodeficiency virus (HIV). With the introduction of ART in the mid-90s, the tide was turned [1]. Life expectancy for persons living with HIV/AIDS has extended due to the remarkable success of ART, and now approaches that of the general population [1]. In addition, the prevalence of AIDS-related co-morbidities has dropped sharply [2]. Non-AIDS pathologies such as renal impairment, cardiovascular disease, osteoporosis, and neurocognitive disorders now account for the majority of co-morbidities in this group [3].

Furthermore, emerging data suggest that in addition to the pathologic injury inflicted by persistent low-grade viral replication in treated patients, long-term toxicities of ART contribute to the growing prevalence of non-AIDS disorders [4].

With regards to the skeleton, it is now clear that loss in bone mineral density (BMD) results from viral skeletal assault as well as from ART-induced enhanced demineralization. Because fragility bone disease is associated with the natural aging process, there is growing concern that synergy between HIV/ART-induced assault and age-related bone loss could precipitate an epidemic of osteoporosis and fragility bone fracture as the HIV/AIDS population ages on therapy [5]. A better understanding of the mechanisms underlying these processes is needed to guide the fashioning of effective preventive and therapeutic strategies. In this regard, this work is relevant as it explores the contribution of ART-induced disease reversal and immune reconstitution to bone loss in the setting of treated HIV-infection and

additionally reveals a previously unreported skeletal effect of a specific antiretroviral drug, raltegravir.

BACKGROUND

The immune and skeletal systems are intricately linked with common cell types and cytokines such that impairment in one system impacts the other and vice-versa in what is increasingly being referred to as the "immuno-skeletal interface" [6]. The clinical significance of this is evident in diseases such as rheumatoid arthritis [7], periodontal disease [8], and inflammatory bowel disease [9] where immune dysregulation and chronic inflammation leads to a decrease in BMD. It is therefore not surprising that in HIV, a disease of profound immunodeficiency concomitant with and chronic immune activation, skeletal deterioration is common. Up to 67% of antiretroviral treatment naïve HIV-infected patients have osteopenia and about 15% have frank osteoporosis [10]. This underlying skeletal deterioration is further worsened following antiretroviral therapy (ART) initiation with up to 6% decrease in BMD at the hip and spine [11-13]. Recent observational cohort studies suggest that the observed loss in BMD associated with HIV-infection and ART have clinical consequences with fracture prevalence increased as high as 4-fold compared with the general population [14, 15]. Equally worrisome is the concern that HIV/ART-induced bone demise could connive with age-related bone loss to precipitate an epidemic of fragility bone disease and fracture in the aging HIV/AIDS population [16].

Thus, a clearer understanding of the mechanisms underlying bone loss in the setting of HIV-infection is warranted to guide the fashioning of effective preventive and therapeutic measures in this relatively young, but rapidly aging at-risk HIV/AIDS population.

Historically, it was thought that the effects of individual antiretroviral medications were the primary cause of ART-associated bone loss. More recent analyses show that bone loss is a property of virtually all effective ART regimens. It is unlikely that that so many individual agents, which target completely different pathways, could all directly affect bone cells. In addition, bone loss is most pronounced early (1-2 years) in the course of ART. Taken together, it has been proposed that bone loss may be aligned with the events of ART-induced HIV disease reversal and immune reconstitution [5, 13, 17, 18]. Should this hypothesis be valid, bone resorption would be expected to be stable in long-term treated patients who have gone beyond the early events of immune reconstitution; and a change in regimen at this delayed phase of ART when the immune profile is presumably stable should not further affect bone mineralization and skeletal integrity.

To test this hypothesis, in Part A of this study, bone turnover in a cohort of patients who had been on ART for a considerable length of time (median = 3.9 years) was studied. The primary aim was to demonstrate that in this setting bone turnover, particularly bone resorption, does not change over time (48 weeks) when there is a change in ART regimen. This would demonstrate that the dominant effect of ART on the skeleton is independent of specific ART class and individual components, and is more likely to be related to HIV disease reversal/immune regeneration. Our secondary aim was to test whether bone formation was also stable in the delayed-phase of ART and whether bone formation is also independent of ART regimen.

Based on the identification of a suppressive effect of the RAL-containing regimen in the human study, in Part B, we hypothesized that RAL is a potent direct inhibitor of osteoblastic differentiation and/or activity, leading to diminished bone formation. Our primary aim in this study was to confirm that RAL suppresses bone formation *in vivo in the absence of other confounding antiretroviral drugs*. We investigated the effect of RAL on bone formation in C57BL6 mice, a commonly used murine model ideally suited to the study of bone turnover and structure.

MATERIALS AND METHODS

A. Clinical Study

Patient Selection: This study was approved by the local institutional review board at Emory University. HIV-infected subjects who had received ART containing two nucleoside reverse transcriptase inhibitors (NRTI) and either a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) for ≥ 6 months with undetectable plasma HIV-1 RNA (<50 copies/mL) were recruited. Subjects were ≥ 18 years, and had hemoglobin > 18 g/dL, creatinine < 2 mg/dL, and AST/ALT less than two times the upper limit of normal. Subjects were excluded if they had previous intolerance to lopinivir or ritonavir, a history of noncompliance with medications, HBV co-infection and receiving a nucleoside analogue for both HIV and HBV suppression, drug or alcohol dependence, serious illness requiring systemic treatment and/or hospitalization prior to the screening visit or were pregnant or breast feeding.

Study Protocol: At enrollment, demographic information, medication history, clinical and laboratory data were collected. Baseline plasma HIV-1 RNA level, CD4 T-cell counts, and DXA-scan were performed. Subjects were switched to an ART regimen containing lopinivir/ritonavir (LPV/r) 400/100 mg and raltegravir (RAL) 400 mg, both administered orally twice daily. Plasma was collected and stored at weeks 2, 24, and 48 after regimen change. All blood samples were drawn into heparinized tubes and within 1 hour of collection, plasma was separated by centrifugation at 900 x g for 10 minutes. Plasma was aliquotted into cryovials and

stored at -80ºC for study endpoints. Serum levels of CTx, OCN, RANKL, and OPG were measured using ELISA assay kits according to the manufacture's instructions. CTx and OCN kits were obtained from IDS (Fountain Hills, AZ); free RANKL and OPG were obtained from ALPCO (Salem, NH). Plasma HIV-1 RNA PCR and CD4 T-cell counts were performed at baseline and during each follow up time point. DXA-scan was performed at baseline and at week 48.

Statistical Methods: Baseline characteristics of patients are reported as mean +/- SD or number (%). Continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test, and logarithmic transformation was performed when necessary. Change over time was assessed using an analysis of repeated measures to properly account for the correlation between multiple observations from the same patient. Random effect linear models were performed for concentrations of markers of bone turnover and cytokines and CD4 T-cell counts using SAS Proc Mixed. This model took into account fixed effects (mean baseline concentrations and mean change in concentrations over time) as well as random effects (residual error for each individual). The result of interest was the estimated change in concentration over time. Tukey's method was used for the pairwise comparisons at each time point when there were statistically significant changes over time. The changes in concentration were converted to a percent change over the 48 weeks of the study period. BMD measurements were compared at baseline and week 48 using paired sample t-test. Statistical tests were 2-sided, and a p-value less than 0.05 was considered statistically significant.

Power estimation was based on preliminary data from a recent unpublished study in which CTx concentrations were examined in treatment naïve HIV/AIDS patients at baseline and prospectively at 2, 12 and 24 weeks of treatment with ART. The observed mean serum CTx level at baseline was 3.29 ug/L. The observed change of serum CTx from baseline to 24 weeks was 2.06 μg/L, approximately a 60% increase (observed SD of change = 1.37 μg/L). Because the sample was small, and as such variability around mean change is wide, a more conservative change of 20% was assumed in this study. A sample size of 31 was determined to give us 90% power to see a 20% change in CTx using a standard deviation of 1.4. All statistical analyses were done using SAS v9.3.

B. Animal Study

RAL Administration and Bone Densitometry: Emory University IACUC approval was obtained before the initiation of this study. Female C57BL6 mice 6 weeks of age were purchased from Jackson Laboratories (Bar Harbor, Maine). Animals were randomized to a control group or a RAL group. Animals in the RAL group were treated with RAL administered in water at a dose of 90mg/kg/day [20]. This is an allometrically scaled dose from the human dose of 400mg twice daily to account for higher metabolic rates in mice [19]. BMD analysis by DXA-scan was performed on a PIXImus2 bone densitometer (GE Medical Systems, Waukesha, Wisconsin) as previously described [21]. Mice were evaluated prospectively every 4 weeks over a period of 12 weeks to identify changes in BMD. At week 12, mice were sacrificed after an overnight fast. Blood was collected using cardiac puncture and transferred into a microtainer serum separator tube. The blood was left at room temperature for one hour to clot and then centrifuged for five minutes to separate serum. The serum was removed and stored at -20ºC until analysis. CTx and OCN were measured in serum using ELISA kits (Immunodiagnostics, Inc).

Statistical Analyses: Normality of data was assessed by the Kolmogorov-Smirnov test. Mixed effect modeling with SAS Proc Mixed was used to assess changes in BMD over time as explained above. To account for non-linear growth, reference cell coding for time points was used in the analysis. Simple comparisons of CTx and OCN serum levels were made using independent sample two-tailed t-test or Mann-Whitney U test depending on normality of data. Sample size was determined by a

power analysis based on histomorphometry, the technique with the greatest degree of sample deviation due to the small bone size of mice. We selected 15 mice per group in order to ensure power > 95% based on our previous published studies using the same techniques [22, 23]. All analyses were done using SAS v9.3 and p-values less than 0.05 were considered significant.

RESULTS

A: Clinical Study

Baseline Characteristics: There were 29 subjects included in the clinical study (median age 47.7, 69% male, 79% black and 21% white). The median BMI was 29.0, and median time on ART therapy before study began was 3.9 years (**Table 1**). All patients were virologically suppressed at baseline and remained suppressed throughout the study.

Immunologic Stability: CD4 T-cell counts were 487, 524, and 545 cells/μL at baseline, week 24, and week 48 respectively. There were no significant changes in CD4 T-cell counts during the study (**p-value = 0.14**).

Bone Resorption: Plasma levels of CTx, the biochemical marker of bone resorption, and osteoclast-regulating cytokines RANKL and OPG, are shown in **Figure 1**. Repeated-measures analyses, using mixed linear models, were performed. Estimates of the percent change in concentration over the course of the study and corresponding p-values are shown in **Table 2**. None of these biomarkers changed significantly with time following the switch in regimen.

Bone Formation: OCN, a marker of bone formation, significantly decreased over the 48 weeks of ART regimen switch (**Figure 2**). The mean plasma OCN concentration decreased by 26.2% at week 48 (**Table 2**).

Effect of previous LPV/r treatment: 14 patients had received treatment with LPV/r before the start of the study. The patients who had previously received treatment with LPV/r had similar decreases in osteocalcin to those who had never received LVP/r (p=0.85).

Bone Mineral Density: There were no changes in BMD based on DXA scan at the spine (1.12 g/cm^3 at baseline vs. 1.26 g/cm^3 at week 48; p=0.51) or at the pelvis (1.18 g/cm^3 at baseline vs. 1.19 g/cm^3 at week 48; p=0.61) over the course of the study.

B: Animal Study

Baseline Characteristics: Part B included 30 C57BL6 female 6-week-old mice, of which 15 received no active drugs (placebo) and 15 were treated with an allometrically scaled dose of RAL (90mg/kg). Baseline weight and BMD at lumbar spine, femur, and tibia were similar in the two groups (**Table 3**).

BMD: Significant differences between the two groups were seen as early as week 2 at the femur (**Figure 3**). By week eight, there were significant differences in BMD at the femur, tibia, and lumbar spine (**Figure 4**). The rate of weight gain was however not different between the two groups.

Bone Resorption: Plasma levels of CTx were similar in the two groups (15.1 +/- SD in control group versus 13.0 +/- SD in treated group, p=.580) (**Figure 5**).

Bone Formation: Consistent with the human data, plasma concentrations of OCN were significantly lower in the RAL-treated group compared to the control group (18.7 +/- SD ng/mL in treated group versus 34.0 +/- SD ng/mL in control group, p=.001), shown in **Figure 5**.

DISCUSSION

Our goal was to determine whether bone turnover is stable among chronically treated HIV-infected patients beyond the early period (1-2 years) of ART initiation where the observed accelerated bone loss is attributed in part to HIV disease reversal. We speculated that if ART-induced bone loss is indeed driven by events aligned with immune reconstitution, such loss should be minimal or non-existent at the later phase of HIV therapy when virologic suppression has been achieved and CD4 T-cell counts have become relatively stable. Furthermore, we argued that a change in ART regimen at this delayed phase of therapy should have little or no effect on bone turnover. By contrast, if any of the previous regimens contained drugs that directly altered bone resorption, changing the regimen should lead to an increase or decrease in resorption depending on its effect on basal bone turnover. To test these hypotheses, we performed detailed skeletal profiling in a cohort of virologically suppressed patients who have been on long standing therapy (median treatment time = 3.9 years) and were undergoing a pre-study ART regimen switch to LPV/r + RAL. All subjects maintained undetectable plasma HIV-1 viral loads during the follow up period, and had no significant change in CD4 T-cell counts with the new regimen. We demonstrated that indeed, bone resorption over time as measured by plasma CTx this late in the course of therapy is unchanged. Consistent with this finding, RANKL and OPG, the key protagonist and inhibitor of osteoclastogenesis respectively were unchanged during the one year of follow-up supporting the notion that accelerated bone resorption observed early in the course of ART may be due in part to HIV disease reversal and immune reconstitution and

hence such ART-induced bone effects, if any, should be minimal later in the course of therapy. The fact that stability in bone resorption was maintained despite the change in regimen from a variety of different drugs and drug classes to a single uniform regimen, further suggests that the component antiretroviral drugs in the original regimens were not impacting bone resorption at the study onset and that neither the original drugs, nor the drugs associated with the regimen switch are overtly toxic to the osteoclastic cells. Taken together, these findings are corroborative of the near universal observation of enhanced BMD loss (regardless of regimen type) during the first year or two of ART that tappers off thereafter [5, 13, 17, 18].

Although bone resorption and osteoclastogenesis were stable, the same was not true for bone formation in our cohort. Plasma OCN, a sensitive marker of bone formation was markedly suppressed. Mean OCN concentration decreased by 19.4% and 25.8% from baseline to week 24 and week 48 respectively – changes comparable to those seen with high dose steroid therapy in cancer patients [24]. The observed suppression in bone formation did not translate into loss in BMD. The likely explanation for this is the relatively short duration of the study, and the fact that high density cortical bone, the compartment that makes up over 80% of total bone mass, is slower to remodel than cancellous bone. Consequently, dramatic changes in trabecular bone can be significant under-represented by DXA-based estimates of BMD. For this reason BMD only partly explains bone strength [24]. The low sensitivity of DXA based BMD determinations and the inability of BMD to address bone microarchitecture and load bearing capacity is reflected in part, by the

fact that 50% of postmenopausal women who sustain a fragility fracture are not clinically defined as being osteoporotic based on BMD measurements. Consequently, our data demonstrating a significant decline in bone formation with RAL use needs to be carefully considered and should not be summarily dismissed based on an apparent failure to impact BMD in the short term.

Mechanistically, this observation was intriguing and raises concerns as whether the component drugs in the switch regimen (LPV/r and RAL) are inhibitory to the osteoblastic bone forming cells. The challenge to such interpretation however is the diversity of the pre-study ART regimens that subjects were treated with and the difficulty in resolving whether it was the removal of a medication, rather than the addition of RAL or LPV/r that was the cause of OCN decline. Of note, 14/29 patients (52%) already were on LPV/r-based regimens prior to study entry and ART switch, and none were on RAL. Patients who were previously treated with LPV/r had similar levels of osteocalcin declines to those who had never been treated with LPV/r ($p=0.85$). Thus, if a component of the switch regimen were responsible for the observed suppression in bone formation, we speculated it would likely be related to the RAL.

To explore this further, we employed a murine model to examine the skeletal effect of RAL when administered as a single agent, without the confounding effect of other antiretroviral drugs. BMD growth was decreased in the mice treated with RAL compared to control mice. Consistent with the findings from our clinical study, CTx was unaffected by RAL monotherapy, while OCN, on the other hand was markedly decreased in the RAL treated mice compared to the control group (82%↓, $p=0.001$).

Unlike in the human study, in the mice, the decrease in OCN was associated with delay in the rate of bone mineralization as evident by statistically significant differences in BMD between the treated and the control groups at week-2, week-4 and week-8. In young mice the rate of bone formation and accretion of bone mass and BMD is very high compared to adult humans who have passed the stage of peak bone mass acquisition and consequently this is a very sensitive model for testing the effect of drugs on bone formation. However, in C57BL6 mice peak BMD is reached by 5 months of age [25] and the high rate of bone formation subsides. As a likely consequence of the control mice reaching peak BMD and bone formation declining, RAL treated mice were able to partially catch up by 12 weeks of treatment (18 weeks of age), suggesting that RAL-induced bone formation suppression had significantly delayed the acquisition of peak BMD but did not prevent it. Importantly, serum OCN measurements at 12 weeks of treatment continued to reflect a significant imbalance in the rate of bone formation between control and RAL treated mice. These data suggest that an ongoing deficit in bone formation was still in effect despite the apparent catch up in BMD, which may only be temporary. The effects of RAL on mature mice beyond the onset of peak BMD remain to be examined.

Taken together, the data from our human study and the corroborative animal study strongly suggest that RAL suppresses osteoblastic bone formation. The clinical significance of this finding however remains unclear as no change in BMD was observed in the current cohort. More translational work will be needed to validate this finding in large clinical cohort and to tease out the impact of mono vis-à-vis

combination antiretroviral therapy on the osteoblastic inhibitory effect of antiretroviral drugs.

Some limitations of the current work include lack of control arm in the clinical study making it difficult to ascertain whether discontinuation rather than the addition of new agents was responsible of the observed bone effect. The diversity of the pre-study ART regimens although allowing the baseline effect on bone turnover of a wide sampling of antiretroviral agents to be assessed, was also a confounding factor in understanding the specific effect of RAL on bone cells. It is possible that removal of a drug, rather than the introduction of RAL, could have caused the observed bone effects.

Furthermore, BMD interpretation was limited to total body DXA-scans rather than dedicated scans directed at key fracture prone anatomical sites (hip, spine, and femur).

The animal study was limited to mono-therapy with RAL rather than a combination of mono-therapy and dual-therapy with LPV/r + RAL as used in humans. Future studies designed to address these limitations will be informative to the field.

CONCLUSION

These limitations notwithstanding, the observed stability in bone resorption and osteoclastogenic profile late in the course of ART when virologic suppression had been achieved and CD4 T-cell counts were stable lend additional support to the notion that bone resorption does not continue to increase in the late phase of ART. The incidental finding of a decrease in bone formation as assessed by a decline in plasma OCN level in our clinical cohort and corroborated in our animal study associated with RAL exposure is intriguing. Additional studies to elucidate the mechanism underlying this phenomenon and to understand its clinical implications are warranted.

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Table 1: Baseline characteristics of subjects in clinical study.	
Characteristic	Median (IQR) or Frequency (%)
Age	47.7 (43.2-50.3)
Gender	
Male	20 (69)
Female	9 (31)
Race	
White	6 (21)
Black	23 (79)
BMI	29.0 (25.83 – 34.64)
Time on ART (Years)	3.94 (1.74-8.27)
Age: Years of subject at enrollment of study BMI: Body mass index, kg/m^2 Time on ART: Years since initiation of antiretroviral therapy	

Table 2: Changes in markers of bone turnover after initiation of raltegravir		
Markers of bone turnover and osteoclastogenic cytokines	Estimated % change over 48 weeks of study	P-value[a]
CTx[b] (ng/ml)	-3.5	0.77
RANKL[c] (pmol/l)	-4.6	0.86
OPG[d] (pg/ml)	-2.0	0.65
OCN[e] (ng/ml)	-26.2	**0.002**

[a]Analysis done using logarithmic transformation of data and mixed effect model
[b]C-terminal telopetide of collagen
[c]Receptor activator of NF-κB ligand
[d]Osteoprotegerin
[e]Osteocalcin

Table 3: Baseline characteristics of 6 week old C57BL6 female mice in control group and raltegravir-treated group.

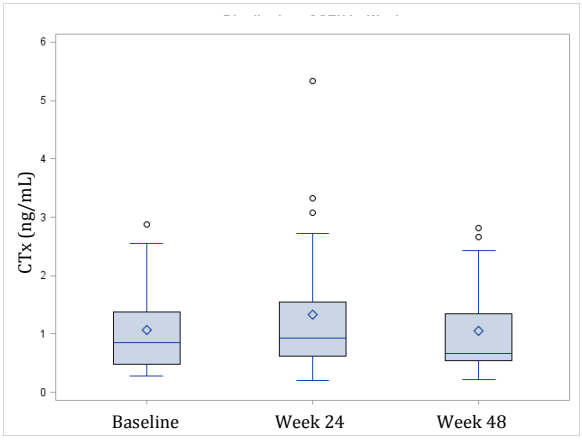
Characteristic	Control Group[a] (mean +/- SD)	Raltegravir Group[b] (mean +/- SD)
Lumbar spine BMD (g/cm^3)	0.0596 +/- 0.0042	0.0595 +/- 0.0042
Mean Femur BMD (g/cm^3)	0.0613 +/- 0.0018	0.0625 +/- 0.0018
Mean Tibia BMD (g/cm^3)	0.0475 +/- 0.0023	0.0477 +/- 0.0022
Weight (g)	17.97 +/- 0.94	17.99 +/- 1.26

[a]Control Group: Treated with no active drugs
[b]Raltegravir Group: Treated with 90mg/kg raltegravir in drinking water
SD: Standard Deviation
BMD: Bone Mineral Density

Figure 1: Plasma levels of (a) CTx, (b) RANKL, and (c) OPG after initation of raltegravir. 29 chronicially treated and virologically suppressed HIV-infected individuals underwent an ART regimen switch to raltegravir and lopinivir/ritonivir. Plasma levels of CTx, RANKL, and OPG were measured at baseline and 24 and 48 weeks after regimen switch. No significant changes were observed.

CTx: C-terminal telopetide of collagen, a marker of bone resorption
RANKL: receptor activator of NF-κB ligand, the key osteoclastogenic cytokine
OPG: Osteoprotegerin, a RANKL moderator
All p-values non significant

a.



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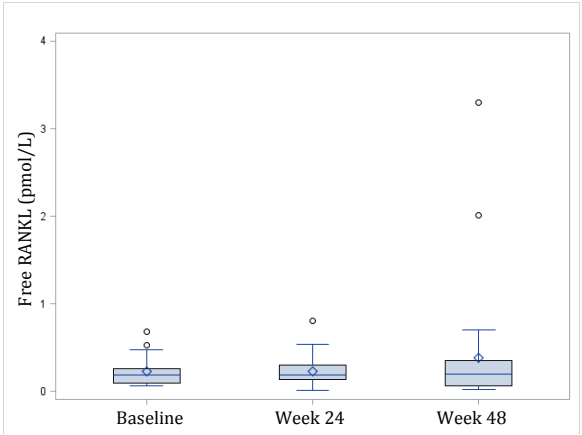


c.

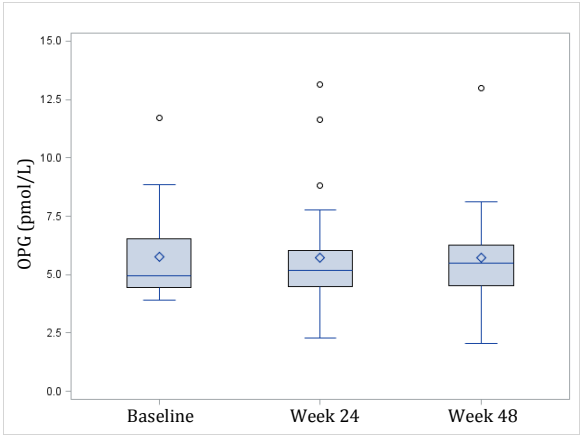


Figure 2: Plasma levels of OCN after initiation of raltegravir. 29 chronically treated and virologically suppressed HIV-infected individuals underwent an ART regimen switch to raltegravir and lopinivir/ritonivir. Plasma levels of OCN were measured at baseline and 24 and 48 weeks after regimen switch. A significant decrease in levels of OCN was observed.
OCN: Osteocalcin, a marker of bone formation
** p<0.01

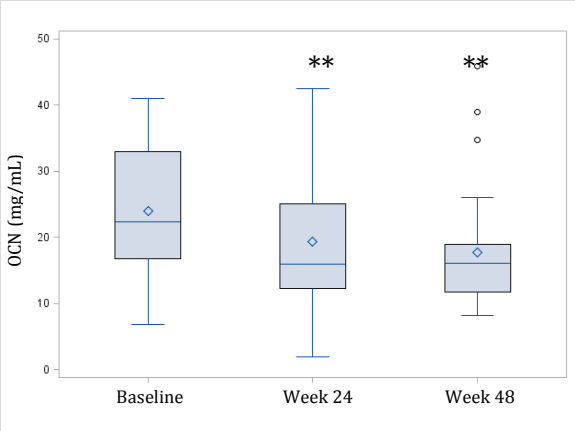


Figure 3: Femur BMD percent change from baseline. Female C57BL6 mice 6 weeks of age were treated with raltegravir (90 mg/kg/day) or no active drug (n=15/group). BMD analysis by DXA-scan was performed at baseline and every 2-4 weeks until week 12. There was a significant decrease in femur BMD in raltegravir-treated mice compared with control mice at weeks 2, 4, and 8.

BMD: Bone mineral density
DXA: Dual energy X-ray absorptiometry
** p<0.01
*** p<0.001

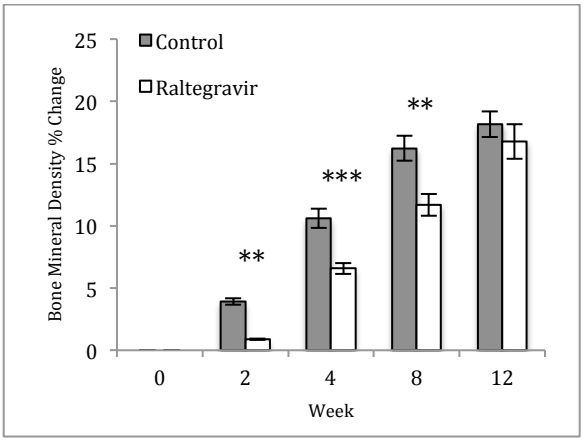
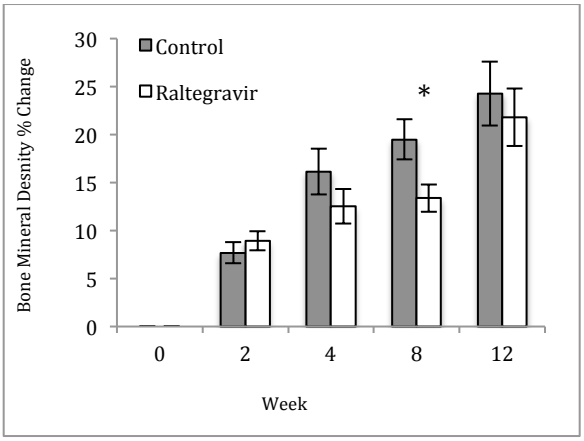


Figure 4: a) Lumbar spine BMD and b) tibia BMD percent change from baseline. Female C57BL6 mice 6 weeks of age were treated with raltegravir (90 mg/kg/day) or no active drug (n=15/group). BMD analysis by DXA-scan was performed at baseline and every 2-4 weeks until week 12. There was a significant decrease in lumbar spine BMD and tibia BMD in raltegravir-treated mice compared with control mice at week 8.

BMD: Bone mineral density
DXA: Dual energy X-ray absorptiometry
*p<0.05

a.



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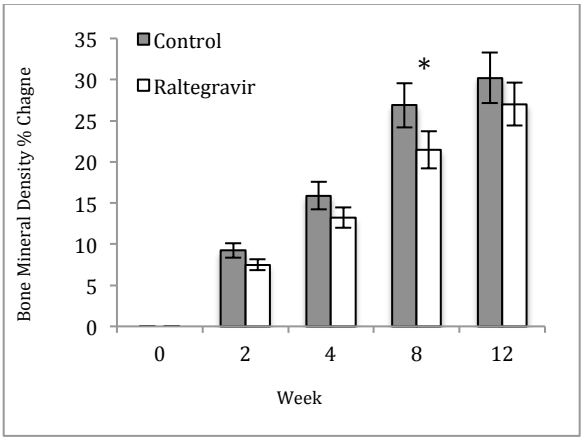

Figure 5: Bone resorption and formation in raltegravir-treated mice. Female C57BL6 mice 6 weeks of age were treated with raltegravir (90 mg/kg/day) or no active drug (n=15/group). Serum levels of CTx and OCN were measured after 12 weeks of treated. Levels of CTx were similar in the two groups, but there was a significant decrease in OCN in mice treated with raltegravir compared to controls.
CTx: C-terminal telopetide of collagen, a marker of bone resorption
OCN: Osteocalcin, a marker of bone formation
NS: P-value non-significant
** p<0.01