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Bone Effects of Rosiglitazone in HIV-Infected Patients With Lipoatrophy

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Abstract

Objectives—Thiazolidinediones increase limb fat in HIV+ patients with lipoatrophy. However, their use in the general population has been associated with bone loss and fracture. We sought to determine the effects of rosiglitazone on bone metabolism in HIV-infected patients.

Methods—HIV+ patients with lipoatrophy were randomized to rosiglitazone versus placebo for 48 weeks in a double-blind, placebo-controlled trial. Limb fat, bone mineral density (BMD), bone formation markers (procollagen type 1 amino-terminal propeptide [P1NP], osteocalcin [OC]) and bone resorption markers (C-terminal telopeptide of type I collagen [CTX]) were measured, along with receptor activator for nuclear factor kappa β ligand (RANKL), osteoprotegerin (OPG), and inflammatory cytokines.

Results—Seventy-one subjects were randomized to rosiglitazone or placebo: 17% female and 51% white. Total BMD did not change significantly in either group. In the rosiglitazone group, P1NP showed statistically significant decreases at 24 and 48 weeks; however, changes compared to placebo were only significant at 24 weeks. OC decreased significantly in the rosiglitazone group at 24 weeks, but there were no between-group differences. CTX, RANKL, or OPG did not change for either group. Multivariable regression within the rosiglitazone arm showed P1NP changes were inversely associated with limb fat changes, protease inhibitors, and tenofovir use.

Conclusion—Rosiglitazone use was associated with decreased bone formation, but it did not alter bone resorption or total BMD. The increase in limb fat that accompanies rosiglitazone use appears to be associated with decreased osteoblast activity. Further studies are needed to determine the effect of thiazolidinediones on bone health in HIV-infected persons.

Keywords

bone mineral density; bone turnover markers; HIV; rosiglitazone; thiazolidinediones

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Age-related complications, such as osteoporosis, have increased among HIV-infected individuals. Even among younger HIV-infected patients, osteoporosis is 3 to 4 times more prevalent compared to HIV-uninfected controls,¹ and they have an increased fracture risk.^{2,3} The pathophysiologic mechanisms underlying these bone changes are poorly understood; however, traditional risk factors, HIV infection, and antiretroviral therapy (ART) (particularly protease-inhibitors [PIs] and tenofovir [TDF]) all appear to play a role.⁴⁻¹⁰

In the general population, inflammation has been implicated in the pathogenesis of osteoporosis and bone fractures, and interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) are potent stimulators of osteoclast activity, leading to uncoupled bone resorption in some people with osteoporosis.¹¹ However, the role of inflammation in HIV-related bone loss is not clear. In treatment-naïve subjects, TNF- α activity is associated with decreased bone formation and increased bone resorption.¹² With ART initiation, there are decreases in IL-6 and TNF- α activity,^{12,13} but there is still an acceleration of bone turnover and a net loss in bone.¹⁴

Bone resorption and formation are normally coupled through the interaction of RANK (receptor activator for nuclear factor kappa β), a receptor on osteoclasts, and RANK ligand (RANKL), an osteoblast-secreted factor. Osteoblasts also secrete osteoprotegerin (OPG), which binds to RANKL to prevent osteoclast activation. HIV viral components can upregulate RANKL,¹⁵ and certain antiretrovirals may enhance this effect.¹⁶ Serum RANKL and OPG concentrations are associated with lower bone mineral density (BMD) in ART-treated HIV-infected patients¹⁷ and are higher than in HIV-seronegative controls,¹⁸ despite some conflicting data.¹⁷

Medications commonly used to treat comorbidities in HIV-infected patients may also contribute to low BMD, potentially further increasing their risk of osteoporosis and fractures. For example, thiazolidinediones (TZDs) have been approved for the treatment of diabetes mellitus. They have also been investigated for the treatment of HIV-related lipoatrophy, which manifests as subcutaneous fat wasting of the face and/or extremities, caused by the use of thymidine nucleoside analogue reverse transcriptase inhibitors (tNRTIs). Thymidine NRTIs have a toxic effect on adipocyte mitochondria¹⁹ and also downregulate the nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR- γ), which inhibits adipogenesis.²⁰ Thiazolidinediones are potent agonists of PPAR- γ and increase adipogenesis.²¹ We have previously shown that rosiglitazone, a commonly used TZD, increases limb fat in HIV-infected patients with lipoatrophy,²² although not all studies support this finding.²³

The adipogenesis associated with TZDs occurs at the expense of bone formation. In the general population, TZDs decrease osteoblast formation and increase osteoblast apoptosis,²⁴⁻²⁶ thereby increasing fracture risk.²⁷⁻³⁰ In contrast, TZDs possess anti-inflammatory effects,^{31,32} which have been shown to improve periodontal bone loss in mouse models.³³ In mouse and in vitro models, some studies have shown that TZDs decrease RANKL expression and inhibit osteoclast differentiation,³³⁻³⁵ while others have shown an increase.³⁶ There are little data on the effects of TZDs on bone health in HIV³⁷ and none specifically on rosiglitazone.

Thus, the purpose of this study was to investigate the effect of rosiglitazone on bone metabolism in HIV-infected subjects. Our primary objective was to evaluate changes in bone turnover markers, as a more sensitive measure of bone change than BMD. The secondary objectives were (1) to determine the relationship between changes in BMD and those in bone turnover markers, (2) to investigate whether the OPG/RANKL system and systemic inflammation contribute to the BMD changes associated with rosiglitazone in HIV,

and (3) to investigate predictive factors for changes in bone turnover markers, including limb fat and insulin resistance.

METHODS

Subject Selection

This was a double-blinded, placebo-controlled study designed to evaluate the effect of rosiglitazone on bone density and bone turnover markers in HIV-infected individuals with lipoatrophy. HIV-infected subjects were enrolled at Case Western Reserve University and the Cleveland Clinic in Cleveland, Ohio. As previously described,²² participants had to be at least 18 years old with clinical lipoatrophy, defined as fat loss of at least moderate severity in at least 2 different areas of the given body areas such as face, arms, legs, or buttocks. Inclusion criteria included a past history of tNRTI for at least 12 cumulative months, discontinuation of tNRTI therapy and receipt of a stable tNRTI-sparing regimen for at least 24 weeks prior to study entry, HIV-1 RNA <5000 copies/mL, and no intent on the part of the subject or provider to alter ART over the study period.

Individuals were excluded if they had liver cirrhosis, heart failure of New York Heart Association class 3 or 4, or diabetes mellitus or were receiving metformin or TZDs. Individuals who were pregnant or breastfeeding or who were receiving any hormonal supplementation with recombinant growth hormone, anabolic steroids, or estrogen or testosterone (except at replacement doses) were excluded. Additionally, individuals were excluded if they had serum transaminases >2 the upper limit of normal (ULN), lipase >2.5 ULN, creatinine >3 ULN, PT/PTT >1.2 ULN, absolute neutrophil <750/mm³, hemoglobin <9.0 g/dL, platelet count <75,000/mm³, or glucose <70 mg/dL.

Intervention

Subjects were centrally randomized 1:1 in a double-blinded fashion to receive either rosiglitazone or matching placebo for 48 weeks. In the dose-escalation period, individuals received rosiglitazone 4 mg daily for 4 weeks. The dose was then increased to 4 mg twice daily for the remainder of the study. All ART was continued unchanged, and individuals were asked to maintain their current diet and exercise regimens.

Clinical and Laboratory Assessments

Study evaluations included physical examination, fasting metabolic assessments, clinical lipoatrophy questionnaires filled out independently by physicians and individuals, and dietary questionnaires. Whole body dual-energy X-ray absorptiometry (DXA) scanning was obtained at study entry, week 24, and week 48 for measurements of overall and regional body fat and total body BMD. All DXA scans were performed at a single site (Case) on all study individuals using a Hologic QDR-4500A (Hologic Inc, Bedford, Massachusetts, USA). The scans were assessed with a dedicated scanner and a technologist who was blinded to treatment allocation.

Fasting glucose, insulin, CD4 cell counts, and HIV-1 RNA levels were measured at each time point. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: $\text{HOMA-IR} = (\text{fasting glucose (mg/dL)} \times \text{fasting insulin (mU/mL)}) / 405$.³⁸

Biomarker Assays

Plasma samples were stored at -80°C until analysis without prior thawing. All assays were performed at Dr. Neal Fedarko's laboratory at Johns Hopkins Bayview Advanced Chemistry Laboratory, Baltimore, Maryland, USA. Bone turnover markers included the bone formation

markers, osteocalcin (OC) and procollagen type 1 amino-terminal propeptide (P1NP), and the bone resorption marker, C-terminal telopeptide of type I collagen (CTX). CTX was measured using an enzyme-immunosorbent assay (Osteometer BioTech, Herlev, Denmark), OC was measured using an immuno-radiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, California, USA), and P1NP was measured by radio-immunoassay (IDS Inc., Fountain Hills, Arizona). Median intra-assay coefficients of variation for OC, P1NP, and CTX were 2.97%, 2.74%, and 8.15%, respectively, and the median inter-assay coefficients of variation were 6.30%, 2.74%, and 0.01%, respectively. Expected normal range was 3.4-11.7 ng/mL (men) and 2.4-10.0 ng/mL (women) for OC; 22-87 µg/L (men), 19-83 µg/L (premenopausal women), and 16-96 µg/L (postmenopausal women) for P1NP; and 0.115-0.748 ng/mL (men), 0.112-0.738 ng/mL (premenopausal women), and 0.142-1.351 ng/mL (postmenopausal women) for CTX.

Plasma concentrations of OPG and total soluble RANKL (sRANKL) were determined by enzyme immunoassays (ALPCO, Salem, New Hampshire, USA) with coefficients of variations of 4.1% and 3.5% (intra-assay) and 6.2% and 9.3% (inter-assay), respectively. OPG to sRANKL ratio was then calculated. Plasma levels of inflammatory markers, including high-sensitivity C-reactive protein, IL-6, and the soluble receptors of TNF-α (sTNFR-I, -II) were measured via an enzyme-labeled immunosorbent sandwich assay (Aushon Biosystems, Billerica, Massachusetts, USA).

Statistical Analysis

Continuous variables were summarized as medians and interquartile ranges, and categorical variables were summarized as frequencies and percentages. Within-group percent changes of DXA, bone turnover markers, and OPG/sRANKL were evaluated with paired *t* tests or Wilcoxon signed rank tests at week 24 and week 48. Unpaired *t* tests or Wilcoxon rank sum tests were used to test between-group changes for each time point.

To investigate the relationships between changes in bone turnover markers, BMD, OPG/sRANKL, and inflammation, Spearman correlation coefficients (*R*) were determined for both groups separately at baseline, at week 24, and at week 48 with the various variables. Spearman correlation coefficients were also determined between bone turnover markers and BMD with changes in limb fat at the 24 and 48 week time points.

After the primary results were known, we conducted a post hoc exploratory multivariable linear regression in the treatment group to determine variables associated with percent change in P1NP at 48 weeks. Percent change in P1NP was log-transformed prior to analysis. Variables were considered for inclusion in the regression based on clinical significance and the results of the uni-variable analysis and included age, sex, race, BMI, smoking, CD4 cell count, CD4 cell count nadir, ART duration, current efavirenz (EFV) use, current PI use, current TDF use, and percent changes in limb fat, HOMA-IR, and each inflammatory marker over 48 weeks. The combination of variables left in the final model was that which produced the best adjusted *R*².

The sample size calculation for the randomized controlled trial was based on having >80% power to detect a significant change in limb fat between the rosiglitazone and placebo groups. With this sample size, we had 80% power to detect a 2.2% difference in mean change in BMD between groups. The evaluation of the effect of rosiglitazone on bone turnover markers was conducted in order to generate observational data.

P values <.05 were considered statistically significant. All analyses were carried out using SAS, v.9.2 (SAS Institute, Cary, North Carolina, USA).

RESULTS

Study Population

Baseline characteristics of study participants for both groups are shown in **Table 1**. There were no statistically significant differences between groups. Overall, 17% were female and 51% were white. The majority of subjects had HIV-1 RNA levels <50 copies/mL with approximately half on a PI-based regimen.

Bone Mineral Density Changes

Effects on lipotrophy and safety assessments, including changes in BMD, are published elsewhere.²² Briefly, there were no significant within- or between-group BMD changes.

Bone Marker and Inflammation Changes

Percent changes for the bone turnover markers are shown in **Figure 1**. The bone formation marker, P1NP, had significant percent ($P < .01$) and absolute ($P < .01$) decreases within the rosiglitazone group from baseline to the 24 week time point without any significant changes within the placebo group. Between-group percent changes were also significant from baseline to 24 weeks ($P = .04$). The absolute decrease in P1NP in the rosiglitazone group was also significant from baseline to the 48-week time point ($P = .04$), but the percent decrease was not significant. Neither the between-group changes nor within-group changes for the placebo group from baseline to 48 weeks were statistically significant.

Only the absolute value for the bone formation marker, OC, decreased significantly from baseline to 24 weeks in the rosiglitazone group ($P = .04$), but the percent and absolute change from baseline to 24 weeks and week 48 changes were not significant. Changes within the placebo group and between-group differences were not significant for OC for either time point. There were no significant changes for either group for the bone resorption marker, CTX.

Groups were similar at baseline for OPG/sRANKL, and there were no within-group or between-group changes throughout the study period (data not shown). Inflammatory marker data are published elsewhere.³⁹ Briefly, hsCRP, sTNFR-I, and sTNFR-II increased significantly within both groups, without between-group differences; thus, rosiglitazone did not independently affect inflammatory cytokines.

Univariate Analyses

Baseline or changes in BMD were not correlated with baseline or changes in bone turnover markers, OPG/sRANKL, inflammatory markers, or limb fat for the rosiglitazone group. In the placebo group, the only significant correlation was between changes in BMD and changes in sTNFR-I at 24 weeks ($R = 0.42$, $P = .01$).

In the rosiglitazone group, significant correlations among baseline inflammation markers and change in bone turnover markers included baseline hsCRP with the change in CTX ($R = 0.39$, $P = .04$) and the change in OPG ($R = -0.38$, $P = 0.04$) at 48 weeks. Among the change in inflammation markers and the change in bone turnover markers, significant correlations at 24 weeks included the change in hsCRP and the change in sRANKL ($R = -0.38$, $P = .03$). At 48 weeks, the change in hsCRP was correlated with the change in CTX ($R = -0.41$, $P = .03$) and OPG ($R = .41$, $P = .02$), and the change in sTNFR-1 was correlated with the change in CTX ($R = .39$, $P = .04$).

For the placebo group, significant correlations among baseline inflammation markers and change in bone turnover markers included baseline hsCRP ($R = 0.37$, $P = .01$) and baseline

sTNFR-II ($R = 0.42$, $P = 0.03$) with change in sRANKL at week 24. Among the change in inflammation markers and the change in bone turnover markers, the change in sTNFR-1 was correlated with the change in P1NP ($P[M1] = 0.36$; $P = .03$).

None of the bone markers were correlated with HOMA-IR for either group.

Multivariable Regression

To investigate predictors of percent changes in P1NP over 48 weeks in the rosiglitazone group, an exploratory multivariable regression analysis model was constructed. The final model included the combination of variables that produced the best R^2 (Table 2). Variables that remained in the final model included percent change in limb fat over 48 weeks, percent change in IL-6 over 48 weeks, sex, smoking, nadir CD4 cell count, ART duration, current EFV use, and current TDF use. Percent change in limb fat and EFV and TDF use were associated with percent changes in P1NP.

DISCUSSION

Thiazolidinediones improve peripheral tissue insulin sensitivity in diabetics by activating the nuclear receptor PPAR- γ .²² Thiazolidinediones also affect the differentiation of mesenchymal stem cells, whereby an increase in adipogenesis occurs at the expense of osteoblast formation.²⁴⁻²⁶ Thus, it has been suggested for use in HIV-infected individuals with lipodystrophy, a complication of tNRTI agents (stavudine and zidovudine). We have previously shown that rosiglitazone improves limb fat in HIV-infected individuals stable on thymidine-sparing ART regimens who still suffer from lipodystrophy.²²

However, because the skeleton undergoes constant remodeling with osteoclasts resorbing older bone and osteoblasts laying down new bone in a normally tightly coupled manner, anything that disrupts this balance, such as the use of TZDs, may affect BMD. Thus, in the general population, this side effect of increasing adipogenesis at the expense of osteoblast production, while osteoclast production remains constant, leads to a decrease in BMD and ultimately increases the risk of fractures.^{29,40,41}

The action of osteoclasts and osteoblasts can be assessed in vivo using markers of bone turnover that can be quantified in the serum, urine, or plasma. CTX is a breakdown product of type 1 collagen whose circulating concentrations represent osteoclast activity⁴² and increase during conditions marked by high bone resorption, such as menopause and hyperparathyroidism.^{43,44} and decrease by 50% or more during antiresorptive therapy with bisphosphonates.^{45,46} Osteocalcin is a polypeptide of unknown function, secreted primarily by osteoblasts into the bone matrix. Circulating OC represents the 10% to 40% of secreted OC that is not adsorbed to hydroxyapatite.⁴⁷ Owing to its osteoblast origin, OC is considered a marker of bone formation. Procollagen type 1 contains N- and C-terminal extensions, which are removed by specific proteases during conversion of procollagen to collagen. The extensions are the C- and N-terminal propeptides of procollagen type 1 (P1CP and P1NP). The serum concentration of P1NP is directly proportional to the amount of new collagen produced by osteoblasts⁴⁸ and thus is a sensitive marker of bone formation.

Most, but not all studies, have shown that differences in bone turnover markers predict fracture risk in the non-HIV-infected population.⁴⁹ However, there are many limitations to using them as surrogate markers for BMD and fracture risk. Studies differ among patient populations, the markers examined, the statistical methods, and which markers appeared predictive of fracture. Bone resorption markers tend to be more predictive than bone formation markers. At this time, there are no cut-off values for a particular bone marker that place a person at risk for fracture. Nevertheless, comparison of bone turnover markers

between treatment groups and changes over time can lend insight into the bone microenvironment and the changes that may affect fracture risk.

The effects of TZDs on bone turnover markers have not been evaluated in HIV-infected individuals. This is critical as HIV-infected individuals already have an increased risk of osteoporosis and fractures.¹⁻³ In addition, because bone disease in HIV infection is multifactorial, the effect of TZDs may be different than in the general population. In this current analysis, we investigated the changes in the plasma levels of OC, P1NP, and CTX after 24 and 48 weeks of rosiglitazone use in HIV-infected subjects on ART. We observed a significant decrease in P1NP throughout the study period and a decrease in OC in the first 24 weeks of the study in subjects who received rosiglitazone compared to placebo. There were no accompanying changes in CTX or BMD. This observed decrease in bone formation without an accompanying change in bone resorption is consistent with the use of TZDs in the general population.⁵⁰

In contrast to studies in non-HIV populations, however, rosiglitazone did not significantly change BMD over the 48-week study period. There are a number of reasons as to why we did not observe any significant BMD changes. First, several studies that found decreases in BMD with study lengths similar to or shorter than this current study measured changes in BMD specifically at the hip,^{40,41} as compared to total body BMD. Changes in BMD at the hip are most predictive of subsequent fractures⁵¹ and appear to be a sensitive location to estimate BMD changes,⁵² so it is possible that had we measured site-specific BMD, we may have detected a change. It is also possible that had we continued the study drug for a longer time, the observed decrease in bone formation would have eventually led to a decrease in BMD, especially in light of the fact we observed no changes in the bone resorption marker, CTX. The relatively small sample size may have also limited our ability to detect small changes in BMD. Finally, the young age of our study population may also have been a factor.

To investigate variables associated with the decrease in P1NP, we conducted an exploratory multivariable regression analysis. The decrease in P1NP was associated with an increase in limb fat. This finding is consistent with the known effects of rosiglitazone on adipogenesis and bone metabolism, where adipocyte production increases as bone formation falls. This may have implications for using rosiglitazone (and other TZDs) in the clinical setting for HIV-associated lipodystrophy, as those individuals who may experience the most improvement in their lipodystrophy would also be the ones who have the most decrease in bone formation and, thus, may be at the most risk for osteoporosis with long-term use of TZDs. In addition, TDF use was associated with a decrease in P1NP, and EFV use was associated with an increase. As most HIV-infected patients who are on EFV are not also on a PI, and vice versa, we repeated the regression using PI use instead of EFV use and found the opposite effect (ie, PI use was associated with a decrease in P1NP; data not shown). This is consistent with previous studies that show that TDF and PIs appear to be associated with greater bone losses than other antiretrovirals,⁵³ although the etiology behind this is not well-established. These findings may suggest that bone loss would be greater with rosiglitazone among individuals who are concomitantly using TDF or a PI.

Bone metabolism is also mediated in part by inflammatory cytokines (eg, IL-6 and TNF- α), which have been shown to stimulate osteoclast activity and/or inhibit bone formation.¹¹ These cytokine effects appear to contribute to bone loss in other inflammatory conditions.^{54,55} We know that HIV-infected individuals have increased levels of proinflammatory cytokines compared to healthy individuals^{56,57}; therefore, it was important in this study to evaluate the contribution of inflammation to the bone effects seen with rosiglitazone. In addition, TZDs have been shown to possess anti-inflammatory

properties.^{31,32} In our study, changes in BMD and bone markers did not appear to be related to either baseline or changes in inflammatory cytokine levels. Likewise, percent change in IL-6 was not predictive of PINP changes in the regression analysis. One explanation for this may be due to our study population, who were all suppressed on ART for at least 24 weeks prior to study entry. Although ART-treated HIV-infected individuals have increased inflammation compared to healthy individuals, ART initiation and virological suppression is accompanied by a significant decrease in cytokine levels.^{13,56} This effect may have attenuated any association we may have otherwise seen if some or all of our subjects had not been on ART during the study. Likewise, given the increased inflammatory state in the HIV population regardless of ART status and its multifactorial nature, the anti-inflammatory effects of rosiglitazone may be too weak to affect inflammation in any significant way. Moreover, there may have been unmeasured confounders that increased some of the inflammatory markers over the course of the study.

Other cellular mechanisms that are important mediators of bone loss and bone turnover changes include the OPG/RANKL system. Mouse and in vitro studies suggest some of the bone effects seen with TZDs may occur through OPG/RANKL.³³⁻³⁶ This study investigated whether this system played a role in bone metabolism in HIV-infected individuals on rosiglitazone. Serum levels of RANKL and OPG did not change in either group throughout the 48-week study period, and neither was associated with bone turnover markers or BMD. This may be due to the fact that the plasma concentrations of RANKL and OPG did not reflect their concentrations in the bone micro-environment or that in vivo the RANKL/OPG system plays only a minor role in the bone effects of TZDs.

The main limitations to this study included the relatively short study duration and small sample size and, as previously discussed, the BMD evaluation was limited to total body and soluble levels of RANKL/OPG may not accurately measure cellular levels. The small sample size with the number of variables we investigated increases the probability of type 2 errors. Likewise, since the study was limited to HIV-infected individuals with lipoatrophy, this may limit the generalizability of the results. Finally, rosiglitazone may not be available everywhere, since it has been withdrawn from some markets. However, these results are likely generalizable to other TZDs, such as pioglitazone, as the bone data from studies in the general population have been similar for these 2 drugs.^{28,58}

Despite the limitations, our study provides insight into the balance of osteoclast and osteoblast activity with the addition of rosiglitazone in HIV-infected, ART-suppressed individuals with lipoatrophy. In this study, we demonstrated that bone formation decreases with rosiglitazone, similar to what is seen in the general population. This may have negative implications for osteoporosis and fracture risk over the long run, despite the fact that we did not see any significant changes in total BMD. Additional studies are needed to determine the long-term effect of TZDs on bone health in HIV-infected individuals. Until then, caution and monitoring of bone status may be warranted with TZD use, especially for those already on TDF or a PI-based regimen.

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Figure 1. Percent changes for the bone turnover markers. CTX = C-terminal telopeptide of type I collagen; OC = osteocalcin; P1NP = procollagen type 1 amino-terminal propeptide; Rosi = rosiglitazone.

Table 1

Baseline characteristics

	Rosiglitazone group (n = 34)	Placebo group (n = 37)
Age, years	47 (44-52)	52 (44-54)
Men, n (%)	29 (85)	30 (81)
White, n (%)	19 (56)	17 (45)
CD4+ cell count, cells/mm ³	596 (437-706)	691 (423-861)
HIV-1 RNA <50 copies/mL, n (%)	28 (82)	28 (78)
Duration of HIV diagnosis, months	146 (122-194)	170 (122-231)
Duration on ART, months	114 (96-127)	105 (74-123)
Duration on tNRTI, months	65 (48-59)	69 (24-83)
Duration off tNRTI, months	47 (14-59)	42 (11-64)
NNRTI at study entry, n (%)	15 (44)	14 (37)
PI at study entry, n (%)	19 (56)	23 (62)
Limb fat, g	4,696 (3,644-7,758)	5,967 (3,514-8,435)
BMD, g/cm ²	1.08 (1.01-1.16)	1.11 (1.04-1.17)
P1NP, ng/mL	54.2 (38.7-70.9)	56.0 (43.0-88.1)
OC, ng/mL	6.5 (5.1-9.3)	8.8 (5.5-10.5)
CTX, ng/mL	0.64 (0.5-0.9)	0.7 (0.5-0.8)

Note: All values are median (IQR), unless otherwise indicated. There were no significant differences between groups at baseline for any of the above variables. ART = antiretroviral; BMD = bone mineral density; CTX = C-terminal telopeptide of type 1 collagen; IQR = interquartile range; NNRTI = non-nucleoside reverse transcriptase inhibitor; OC = osteocalcin; P1NP = procollagen type 1 amino-terminal propeptide; PI = protease inhibitor; tNRTI = thymidine nucleoside reverse transcriptase inhibitor.

Table 2

Multivariable regression model for predictors of percent changes in P1NP over 48 weeks in rosiglitazone group

Variable ^a	β	SE (β)	P
Intercept	1.317	0.526	.02
% change in limb fat over 48 weeks	-0.014	0.005	<.01
% change in IL-6 over 48 weeks	-3.411×10^{-5}	2.098×10^{-5}	.12
Sex	0.211	0.265	.44
Smoking	-0.377	0.200	.08
Nadir CD4 cell count	-0.001	6.315×10^{-5}	.10
ART duration	-0.004	0.003	.15
On EFV	0.587	0.183	<.01
On TDF	-0.989	0.347	.01
Adjusted R^2		0.516	

Note: % change in P1NP was log-transformed prior to analysis. TDF use = 25 subjects (74%); EFV use = 14 subjects (41%). ART = antiretroviral; EFV = efavirenz; IL-6 = interleukin-6; TDF = tenofovir; P1NP = procollagen type 1 amino-terminal propeptide.

^aVariables were selected based on clinical significance and the combination of variables that led to the best adjusted R^2 value.