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Short term bone loss in HIV infected premenopausal women

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Abstract

Background—Low bone mineral density (BMD) has been reported in HIV + women, but less is known about the longitudinal evolution of BMD and fracture incidence.

Methods—In 100 HIV+ and 68 HIV− premenopausal women in the Women's Interagency HIV Study (WIHS), BMD was measured by dual energy x-ray absorptiometry at the femoral neck (FN) and lumbar spine (LS) at index visit and after a median of 2.5 years.

Results—In HIV+ women, BMD at index visit was normal but 5% lower at the LS and FN than in HIV− women. Annual percent decrease in BMD did not differ between HIV+ and HIV− women at the LS ($-0.8 \pm 0.2\%$ vs $-0.4 \pm 0.2\%$, $p=0.20$) or FN ($-0.8 \pm 0.3\%$ vs $-0.6 \pm 0.3\%$, $p=0.56$), and remained similar after adjustment for age, weight, and BMD at index visit. Among HIV+ women, bone loss was associated with vitamin D deficiency and opiate use but not with use or class of antiretrovirals. Incidence of self-reported fracture was 0.74/100 person-years in HIV+ women, and similar in HIV− women.

Conclusions—In premenopausal HIV+ women, index BMD was lower than comparable HIV− women; however, rates of bone loss at the LS and FN were similar over 2.5 years of observation, irrespective of ART.

Introduction

Low bone mineral density (BMD) has been reported in several cross-sectional studies of HIV + women¹⁻⁵. Lower BMD measurements are strongly related to the generally lower weight of HIV+ individuals^{2, 6, 7} as well as other established risk factors such as calciotropic or gonadal hormone deficiency,^{2, 8} and lifestyle factors such as smoking, alcohol and opiate use which may be more common among HIV+ individuals. In addition, recent longitudinal studies suggest that bone loss accelerates during ART initiation, with BMD declining by 2-6% within the first two years of initiating various ART regimens⁹⁻¹¹. Whether this translates to increased fractures is still uncertain. Although a recent study suggests that fractures occur more commonly in HIV+ individuals¹², data is lacking from large cohorts with suitably matched HIV− controls.

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In contrast to initiation of ART, BMD appears to be relatively stable in patients on established ART 13, 14. Several factors may explain these discrepant findings. HIV infection results in upregulation of cytokines, such as Tumor Necrosis Factor (TNF)- α , Interleukin (IL)-6 15-17, and Receptor Activator of Nuclear Factor κ B Ligand (RANKL), the final mediator of osteoclastogenesis 18, which may induce osteoclast activation and bone resorption. Accelerated bone loss associated with the initiation of ART may result from the addition of antiretrovirals with direct effects on bone cells in a microenvironment that is already primed by pro-resorptive cytokines. Stabilization of bone loss over time may result from modulation of these cytokines and improvement of hormonal and nutritional factors with effective ART. In this study, we assessed the rate and determinants of bone loss and fracture incidence in a well-characterized cohort of predominantly ART-experienced HIV+ women and compared them to HIV- controls.

Methods

Study Population

WIHS is a multicenter prospective study of HIV infection in women. A total of 3,766 women (2,791 HIV-infected and 975 HIV-uninfected) were enrolled in 1994-95 (n=2,623) or 2001-02 (n=1,143) from six sites (Bronx/Manhattan, Brooklyn, Chicago, Los Angeles, San Francisco and Washington DC). WIHS methods and baseline cohort characteristics have been described previously 19, 20. At each semiannual visit, participants completed a physical examination and provided biological specimens and information on demographics, disease characteristics, and ART use. Informed consent was obtained in accordance with procedures approved by the committees on human research at each of the collaborating institutions.

From April 2001 through October 2005, 581 women from the Bronx, San Francisco and Chicago sites underwent DXA scanning. Eligible subjects weighed ≤ 264 lbs (weight restriction of densitometer), did not have type I diabetes and did not use corticosteroids, drugs used to treat osteoporosis, or exogenous gonadal hormones including oral contraceptives. The cross sectional results have been published 21 and preliminary longitudinal results were presented as an abstract 22. A reanalysis of the cross sectional cohort including bone turnover markers and cytokine levels are presented in this paper along with the longitudinal analysis.

Included in this analysis are 240 women from the Bronx and San Francisco sites who completed at least two DXA scans on the same Lunar Prodigy bone densitometer (Madison, WI) separated by a median of 2.5 years. The visit at which the first DXA scan was completed will be referred to as the index visit. After exclusion of three women with a history of bilateral oophorectomy, current antiepileptic drug use, or active thyroid disease at the index visit, there were 181 premenopausal (107 HIV+, 74 HIV-) and 56 self-identified postmenopausal (54 HIV+, 2 HIV-). Because menopausal status is a strong predictor of BMD and the imbalance in proportion postmenopausal women between HIV+ and HIV- groups might bias our results, we limited the analysis to premenopausal women. Additionally, since hormone levels were not available, in order to avoid misclassification, we further restricted the analyses to premenopausal women who were less than 48 yrs of age at the index visit (100 HIV+, 68 HIV-).

Covariates of interest included: 1) HIV-related factors: history of AIDS defining conditions, ART use, CD4 cell count (nadir and at index visit), and hepatitis C seropositivity and 2) menstrual data: self report of irregular periods (defined as missing at least one period in last 6 months), number of lifetime pregnancies, and menopause. Other covariates of interest included self-reported cigarette smoking (at index and ever), opiate (including methadone) and cocaine use (at index and duration of use), alcohol use (at index and amount of use), and vitamin D and calcium supplementation. Race and ethnicity was based upon self-categorization: African-American (Hispanic and non-Hispanic), white (Hispanic and non-Hispanic), and Latina

(women identifying as Hispanic but neither black nor white). Diabetes was defined as self-report of diabetes or anti-diabetic medication, or a fasting glucose of ≥ 126 mg/dl. Body mass index (BMI) was calculated at time of the DXA evaluation.

Bone mineral density and Fracture assessment—BMD of the lumbar spine (LS) and the femoral neck (FN) was measured by DXA using the same Lunar Prodigy densitometer (Madison, WI) at index and each subsequent visit. An established program of instrument calibration and ongoing quality control was used to allow for accurate comparisons of BMD data between patients measured at different time points. For follow up exams, all instruments were calibrated prior to beginning the study with ‘gold standard’ reference spine and hip phantoms with anatomically correct contours to read BMD within 1%. Subsequent calibration strategy included re-scanning of the reference phantoms at regular intervals throughout the study. T scores were calculated as a standard deviation score compared to BMD at peak bone mass (age 30) using data from NHANES, III and categorized according to the World Health Organization criteria for postmenopausal white women: T scores ≤ -2.5 defined as osteoporosis and T scores ≤ -1.0 as “low BMD”²³ Z scores, which compare a young individual’s BMD to the mean of an age-, gender-, and ethnicity-matched reference population, were also calculated as recommended by the International Society for Clinical Densitometry (ISCD) for premenopausal women; Z scores > -2.0 are within the normal range for age 24.

Personal history of fracture was obtained at the first WIHS metabolic study visit and incident fracture events were obtained by self-report at each subsequent visit. Information was obtained about site of fracture and whether the event was atraumatic, as defined by a fall from standing height or less.

Biochemical measures—Serum calciotropic hormones, bone turnover markers and pro-inflammatory cytokine levels were measured in 82 HIV+ and 58 HIV– women. Parathyroid hormone (PTH) was measured by immunoradiometric assay (IRMA, Intact PTH, Scantibodies, Santee, CA); osteocalcin by ELISA (N-mid Osteocalcin, IDS Ltd, Fountain Hills, AZ); bone alkaline phosphatase (BAP) by enzyme immunoassay (Quidel, San Diego, CA); serum N-Telopeptide (NTX) by a competitive-inhibition ELISA (Inverness Medical, Princeton, NJ); IL-6 and TNF- α by highly sensitive ELISA (R&D Systems, Inc. Minneapolis, MN); serum total soluble RANKL by a two-site sandwich ELISA (Immundiagnostik AG, Bensheim, Germany)²⁵; and serum 25-hydroxyvitamin D (25-OHD) by High Performance Liquid Chromatography²⁶.

Statistical Methods

LS and FN BMD and biochemical indices in HIV+ and HIV– were compared in univariate analyses. Demographic and clinical predictors were assessed, including age, race/ethnicity, BMI, smoking, alcohol use, illicit drug use, HCV serostatus, vitamin D deficiency. Among HIV+ participants, LS and FN BMD were also analyzed according to use and class of ART, CD4 cell counts and plasma HIV RNA levels. Categorical characteristics in HIV+ versus HIV– were expressed as number and percentages and compared by Fisher exact tests. Continuous variables were presented as mean and standard deviation and compared by two-sample t-test.

Annual percent change in BMD was calculated as change in BMD between index and last visit divided by years between index and last visit. Analysis of covariance models evaluated associations between annual percent change in BMD and covariates overall and within the HIV+ group. Variables with $p < 0.10$ in the univariate analyses were fit simultaneously in multivariate models.

Linear regression models were used to evaluate associations between change in BMD and baseline covariates and biochemical indices. Backward stepwise linear regression was used to determine the final model. R-square, estimate coefficient and p-value were reported.

All analyses were performed using SAS software (Version 9.1.3).

Results

Participant characteristics at the index visit

Table 1 presents the characteristics of the 168 women at the index visit stratified by HIV status. HIV+ women were older and there was a trend towards lower weight and BMI than HIV- controls. The distribution by race/ethnicity was similar as were reported opiate, cocaine, cigarette and alcohol use. Reported use of calcium supplements was low in both groups, but use of vitamin D supplements was common. A greater proportion of HIV- women reported irregular periods, but the groups were similar with regard to numbers of pregnancies and live births. HCV seropositivity was more common in HIV+ women.

Among the HIV-infected women, 59% reported ART use at index visit (22% PI-ART, 25% NNRTI-ART, 6% on NRTI only, 5% on NNRTI and PI, and 1% on NNRTI only), 15% were ART-naïve, and the remaining 26% had previously been on ART but had not taken ART for a median duration of 427 days (IQR 157-1281). The majority of women remained on the same class of ART throughout the duration of study.

Bone mineral density at the index visit

Absolute BMD was approximately 5% lower in HIV+ women at the LS (1.25 ± 0.17 vs 1.31 ± 0.16 g/cm², $p=0.05$) and FN (1.05 ± 0.13 vs 1.10 ± 0.15 g/cm², $p=0.03$). Mean T scores were in the normal range (>-1.0) in both groups, but were significantly lower in HIV+ women (Figure 1). After adjustment for BMI, T scores remained lower in HIV+ women at FN (0.27 ± 0.11 vs 0.64 ± 0.13 , $p=0.03$) and trended towards being lower at the LS (0.46 ± 0.14 vs 0.87 ± 0.17 , $p=0.06$). Mean Z scores were also in the normal range for age (>-2.0) in both groups, and trended towards being lower in the HIV+ group (Figure 2).

Prevalence of low BMD (T score <-1.0) was higher in HIV+ women at the LS (14% vs 6%, $p=0.11$) but the difference did not reach significance, and was similar at the FN (9% vs 7%, $p=0.71$). Among HIV+ women, osteoporosis at the LS and FN was present in 9% and 0% of subjects, respectively. None of the HIV- women had osteoporosis.

Calcitropic hormone, bone turnover marker and pro-inflammatory cytokine levels at the index visit

Mean PTH and 25-OHD levels were similar between HIV+ and HIV-. However, mean 25-OHD levels were well below the level currently considered optimal for bone health (32 ng/ml) in both groups (Table 2): 91% of HIV+ and 91% of HIV- had 25-OHD levels <32 ng/ml ($p=0.97$) and 69% of HIV+ and 60% of HIV- had 25-OHD levels <20 ng/ml ($p=0.24$). Thirty percent of HIV+ and 24% of HIV- women ($p=0.36$) had severe vitamin D deficiency (25-OHD <10 ng/ml). There was a trend towards an association between older age and serum 25-OHD ($r=-0.17$, $p=0.06$). Serum 25-OHD levels were significantly higher in whites than blacks, although both groups had levels in the insufficient range (25 ± 8 vs 20 ± 8 ng/ml, $p=0.004$). There was no significant correlation between 25-OHD and PTH or bone turnover markers. Within HIV+ women, 25-OHD levels did not differ between ART groups.

Mean levels of bone turnover markers (osteocalcin, BAP, NTX) and cytokines (TNF- α , RANKL, IL-6) were similar between HIV+ and HIV- groups (Table 2).

Among HIV+, women on ART at the index visit had higher BAP than women not on ART, but did not differ with regard to other bone turnover markers or cytokine levels (Table 2). Results were similar when ART experienced (N=68) and ART naïve (N=14) groups were compared. RANKL levels were higher in women with CD4 $\geq$ 200 cells/ml than in women with CD4<200 cells/ml (1.5 ± 1.7 vs 0.6 ± 0.8 nmol/L, $p=0.01$), but did not differ between women by ART use or by virologic suppression, (HIV-1 RNA $\leq$ 80 copies/ml). There were no significant differences in levels of TNF- α and IL6 between CD4, ART, and virologic suppression groups.

Factors associated with BMD at index visit

A stepwise regression analysis with fit model testing for index LS and FN BMD was performed in HIV+ and HIV- women (Table 3). HIV status, serum osteocalcin and TNF- α were all independently associated with lower LS BMD, and serum PTH and alcohol use with higher LS BMD. Weight, alcohol use, and RANKL were positively associated with FN BMD.

In regression analysis restricted to the HIV+ women, traditional osteoporosis risks such as weight ($\beta=0.0022$ g/cm² per kg increase, $p=0.003$) and alcohol use ($\beta=0.0758$, $p=0.005$) at index visit were associated with greater FN BMD. There were no significant associations between HIV specific factors including CD4 nadir, AIDS-defining events, duration of ART, and class of ART and BMD (data not shown).

Bone Loss: Rate and Determinants

The median duration between index and follow-up BMD was 2.5 years. Among HIV+, a small but significant decrease in LS ($-1.9\pm5.6\%$, $p=0.001$) and FN BMD ($-1.9\pm7.6\%$, $p=0.02$) was observed. There was an annual percent decrease of $-0.8\pm2.1\%$ ($p=0.0004$) at the LS and $-0.8\pm3.4\%$ ($p=0.002$) at FN. Among HIV- women, total decreases were seen in LS ($-1.0\pm5.0\%$, $p=0.11$) and FN BMD ($-1.4\pm4.6\%$, $p=0.01$), with an annual rate of $-0.4\pm1.9\%$ ($p=0.1$) at the LS and $-0.6\pm1.6\%$ ($p=0.02$) at the FN.

When compared to HIV- women, the annual percent decrease in BMD appeared greater in HIV+, but differences were not significant (Figure 2), and were further attenuated after adjusting for age, weight and index BMD: LS ($-0.7\pm0.3\%$ vs $-0.5\pm0.3\%$ $p=0.54$) and FN ($-0.6\pm0.2\%$ vs $-0.4\pm0.3\%$, $p=0.50$).

Rapid bone loss is defined as having more than a 3% decrease in BMD per year. Similar proportions of HIV+ and HIV- women had rapid bone loss at the LS (14% vs 10%, $p=0.54$) and FN (10% vs 6%, $p=0.34$).

Among HIV+ women, differences in rates of LS bone loss were observed in women not receiving ART (N=41), on NNRTI-ART (N=22), or on PI-ART (N=25) at index visit, ($-0.92\pm0.3\%$ vs. $-1.55\pm0.4\%$ vs $-0.78\pm0.4\%$, respectively, $p=0.39$); however, given the small sample size of this subset, these differences could not be determined statistically. Similarly, non-statistical differences in rates of FN bone loss were observed in women not receiving ART, on NNRTI-ART, or on PI-ART ($-0.65\pm0.6\%$ vs $-0.48\pm0.7\%$ vs $-1.91\pm0.8\%$, respectively, $p=0.33$). Cumulative exposure to tenofovir was not correlated with bone loss at either the LS ($r=0.09$, $p=0.74$) or FN ($r=-0.12$, $p=0.70$).

Among HIV+ women, higher BMI at index visit was associated with less FN bone loss ($r=-0.22$, $p=0.03$), while opiate use ($p=0.003$) and vitamin D deficiency (25-OHD<20ng/ml) ($p=0.0014$) were associated with more FN bone loss. Higher baseline BAP ($r=-0.30$, $p=0.01$) was associated with LS bone loss. Among HIV+ women, there was a -1.3 ± 0.9 kg loss in weight from index to follow-up DXA visit; however, the correlation between change in weight/BMI and change in BMD was modest and did not reach statistical significance. Associations of bone loss with AIDS diagnosis, CD4, use or class of ART, and cumulative exposure to PI- or NNRTI-

ART did not reach significance at either site. In multivariate analysis, opiate use ($p=0.04$) and vitamin D deficiency ($p=0.011$) remained predictive of FN bone loss, and BAP level ($p=0.009$) remained predictive of LS bone loss.

Fracture incidence

At the index visit, there were 3 self-reported fractures in HIV+ and 2 fractures in HIV- women. There were 2 new fractures in the HIV+ and 2 new fractures in the HIV- group during follow up, with one atraumatic and one traumatic fracture in each group. The overall fracture incidence was 0.74/100 person-years in the HIV+ and 1.03/100 person-years ($p=1.0$) in the HIV- group.

Discussion

We found that premenopausal HIV+ women had lower LS and FN BMD, lower T-scores and lower Z-scores than HIV- women, although BMD was within the normal range on average. After adjustment for traditional osteoporosis risk factors, levels of calciotropic hormones, bone turnover markers and bone-resorbing cytokines, HIV infection remained associated with lower LS BMD. Over two years of observation, we observed similar decreases in LS and FN BMD in HIV+ and HIV- groups, with a mean decrease of approximately 0.4-0.8% per year.

In the only other longitudinal study of BMD in HIV+ women that included matched HIV- controls, Dolan et al. found no significant difference by HIV serostatus in rates of bone loss over 2 years, despite higher baseline NTX and osteocalcin in the HIV+ group²⁷. Other longitudinal BMD studies in younger men on established ART also support our findings of stable BMD or relatively small amounts of bone loss with short term follow up^{14, 28-30}, despite elevations in bone turnover marker¹⁴. Associations between ART and change in BMD were found in only the two smaller of these studies^{29, 30}.

We found no difference by HIV serostatus in serum concentrations of calciotropic hormones, bone-resorbing cytokines, and bone turnover markers. In this regard, our results differ from those of Dolan et al., who found higher bone turnover markers in HIV+ women². However, most other studies comparing normal weight HIV+ individuals mostly on ART with HIV- controls reported no significant differences^{2, 31-33}. Among untreated HIV+ individuals, increased serum TNF- α and RANKL concentrations have been associated with higher bone resorption markers and lower BMD^{34, 35}. However, few studies have examined the association of serum cytokine levels and BMD in ART-experienced individuals. Since TNF- α levels decrease with ART³⁶, one might theorize that other cytokine levels would be lower in ART experienced individuals and thus the association with BMD less significant. In our study, TNF- α , IL-6, and total RANKL levels did not differ by HIV serostatus, correlate with bone turnover markers or predict BMD change, in contrast to smaller studies of RANKL^{18, 37}. These inconsistencies may be related to differences in study populations, antiretroviral regimens, or choice of assays, especially in the case of soluble RANKL³⁸. We were not able to detect free RANKL in the majority of the WIHS specimens utilizing the same commercial assay as others^{18, 37}, and therefore used an ELISA that specifically measures total soluble RANKL levels in serum. Another limitation is that measured serum levels of RANKL or other cytokines may not reflect levels within the bone microenvironment³⁸, making it difficult to interpret the lack of correlation of serum cytokine levels with bone turnover or BMD.

Our data suggest that in contrast to advanced untreated HIV infection or initiation of ART, cytokine levels and bone turnover markers are not elevated in ART-experienced premenopausal women, and rates of bone loss are modest in such women. Longitudinal studies that measure cytokines, bone turnover, and rates of BMD change before and after initiation of ART are necessary to confirm these findings.

We found that traditional risk factors such as opiate use, vitamin D deficiency and higher bone turnover, as assessed by BAP levels, were associated with increased bone loss. In contrast, CD4 and class of ART did not predict decreases in BMD. Only two prior longitudinal studies were large enough to assess predictors of change in BMD^{14, 27}. Dolan et al. reported that CD4 count, weight, serum FSH and baseline BMD were independent predictors of change in BMD in multivariate modeling²⁷. Mondy et al. reported correlations of change in CD4 count on ART and HIV viral suppression at enrollment with greater gains in spine BMD in HIV+ men, but did not specifically report multivariate modeling¹⁴. Differences in study population likely account for most of the observed discrepancies. In contrast to our study, Dolan et al. included postmenopausal women, excluded substance abusers, and had women with BMIs ~2-3kg/m² lower²⁷. Additionally, their risk factor analysis may have been limited by a 75% dropout rate during the study.

Our study has several strengths, including a large, well-characterized, racially diverse cohort of women, with HIV- women who are similar with regard to lifestyle factors that could predispose to bone loss (opiate and alcohol use), and measurements of 25-OHD, PTH, bone turnover markers, and pro-inflammatory cytokines. Limitations include lack of FSH and gonadal hormone measurements, data on body composition and nutritional assessment. Therefore, we could not assess potential relationships between gonadal hormones and BMD change or evaluate whether the association between opiate use and increased bone loss was mediated by central hypogonadism. We also did not measure serum levels of osteoprotegerin which limits our ability to fully interpret associations with RANKL and relied solely on self-report for fracture analysis. Because of the unique demographic feature of the WIHS cohort, generalization of our findings to women of other race/ethnicities and weight status may not be valid.

Lastly, the smaller sample size of the longitudinal analysis did not permit us to draw inferences regarding the effects of antiretrovirals on BMD as was possible in the cross sectional study [2]. Although we detected no significant difference in rates of bone loss between women not receiving ART, on PI-ART or NNRTI-ART, there was tremendous variability within class and differential effects by class at the LS and FN. This suggests that an understanding of the impact of ART on bone loss can be obtained only through careful analyses of the effects of specific combinations of antiretrovirals and possibly their interactions with factors such cytokine and hormone levels and weight change. This may be possible with longer follow-up and longitudinal data from the full BMD WIHS substudy.

In conclusion, premenopausal HIV+ women with ART exposure had slightly lower BMD than comparable HIV- women, but on average experienced similar modest rates of short-term bone loss. Traditional osteoporosis risk factors such as vitamin D deficiency and opiate use and increased bone turnover as measured by BAP were associated with bone loss, while ART was not. Our results provide some reassurance that short-term bone loss is modest in the majority of premenopausal, weight stable HIV+ women. However, a cumulative annual 1% decrease in young women, given the expected acceleration during the menopausal transition, may predispose these women to premature fragility fractures as they age. In this regard, a large population-based study demonstrated a higher prevalence of vertebral and wrist fractures in HIV+ women as compared to age and race matched controls, especially over the age of 50¹². Long-term studies addressing BMD evolution across the menopausal transition and fracture incidence are required to address overall clinical significance.

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Figure 1. Bone Mineral Density at index visit in HIV+ and HIV- women. Comparison of Mean T scores (1A) and Z scores (1B)

P values for comparison between HIV+ and HIV- groups at the lumbar spine and femoral neck.

Figure 2. Annual percent decrease in Bone Mineral Density in HIV+ and HIV- women
P values for comparison between HIV+ and HIV- groups at the lumbar spine and femoral neck.

Table 1

Demographic, menstrual, medical and HIV data (mean±SD)

	HIV+(N=100)	HIV- (N=68)	P value
Demographics			
Age	40 ± 5	36 ± 7	0.0001
Race			0.55
White	18%	25%	
Black	61%	56%	
Latina	21%	19%	
Weight (kg)	75.0±17.8	79.2±17.1	0.13
BMI (kg/m ²)	28.8±6.6	30.2±6.5	0.16
Lowest BMI (kg/m ²)	25.7±5.7	27.2±6.2	0.12
Smoking at index visit	59%	65%	0.46
Smoking ever	79%	78%	0.77
Intravenous drug use	28%	23%	0.28
Opiate use at index visit	4%	9%	0.19
Ever opiate use	18%	29%	0.08
Duration of opiates (years)	0.7±1.9	1.1 ± 2.3	0.26
Cocaine use at index visit	14%	18%	0.52
Ever cocaine use	44%	46%	0.84
Duration of cocaine (years)	1.0±1.5	1.2 ± 1.8	0.54
Alcohol use at index visit ¹	45%	56%	0.16
Ever alcohol consumption	83%	82%	0.91
Alcohol consumption (g/day)	45±110	58 ± 113	0.48
Menstrual history			
Number of pregnancies	4.4±3.1	5.2 ± 3.4	0.13
Irregular menses	11%	22%	0.05
Medical history			
Calcium supplementation	3%	2%	0.52
Vitamin D supplementation	39%	26%	0.09
Type II diabetes	5%	7%	0.53
HCV seropositive	31%	12%	0.004
HIV			
AIDS defining criteria	44%		
CD4 at index visit (cells/ul)	438±273		
CD4 nadir (cells/ul)	242±163		
ART duration (years)	2.6±2.4		
ART naïve	15%		
ART at index	59%		
NRTIs only at index visit	6%		

	HIV+(N=100)	HIV- (N=68)	P value
PI-ART at index visit	22%		
NNRTI-ART at index visit	25%		

Abbreviations: BMI, body mass index; ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor.

¹ Alcohol consumption determined by averaging number of drinks per day over a one week period multiplied by 112 gm of alcohol per drink

Table 2

Baseline biochemical indices in premenopausal women (mean±SD)

	HIV+ (N=82)	HIV- (N=58)	P value	HIV+ On ART (N=46)	HIV+ Not on ART (N=36)	P value
PTH (pg/ml)	29.7±10.3	26.0±9.0	0.11	27.8±10.7	29.9±9.6	0.37
25-OHD (ng/ml)	20.1±8.6	20.1±7.8	0.62	19.5±8.7	20.8±8.9	0.51
Osteocalcin (ng/ml)	11.8±7.0	12.0±5.8	0.81	12.6±7.4	10.7±6.4	0.22
BAP (U/L)	29.3±12.2	27.4±10.0	0.31	32.7±13.5	25.2±8.8	0.004
NTX (nmol/BCE/L)	11.3±4.3	10.4±3.2	0.17	11.8±4.8	10.6±3.6	0.21
IL-6 (pg/ml)	2.9±2.5	3.0±2.2	0.77	3.2±3.0	2.6±1.8	0.27
TNF-α (pg/ml)	3.7±6.5	2.8±4.0	0.36	4.0±8.2	3.4±3.2	0.69
RANKL (nmol/L)	1.3±1.6	1.5±1.6	0.55	1.3±1.6	1.2 ± 1.6	0.82

Normal premenopausal ranges: parathyroid hormone (PTH) 14-66 pg/ml; 25-hydroxyvitamin D (25-OHD) 32-100 ng/ml; osteocalcin 4.9-30.9 ng/ml; bone alkaline phosphatase (BAP) 11.6-29.6 U/L; N-telopeptide (NTX) 6.2-19.0 nmol BCE/L; Interleukin-6 (IL-6) 0.4-10.0 pg/mL; Tumor necrosis factor-α (TNF-α) 0.6-2.8 pg/mL; R Receptor activator of nuclear factor kb ligand (RANKL) 0.8-4.4nmol/L;

Table 3

Linear regression of variables associated with Bone Mineral Density at index visit

Variables	Lumbar spine ($r^2=0.288$)		Femoral neck ($r^2=0.310$)	
	Beta Estimate	P value	Estimate	P value
Weight			0.0021	0.0012
Alcohol use	0.0868	0.007	0.0918	0.0002
Osteocalcin	-0.011	0.0001	-0.0032	0.06
RANKL			0.0158	0.02
TNF- α	-0.0049	0.05		
Bone alkaline phosphatase	-0.0022	0.09		
Parathyroid hormone	0.0029	0.05		
HIV status	-0.058	0.04	-0.029	0.17

Abbreviations: RANKL, Receptor activator of nuclear factor kb ligand