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Interruption or Deferral of Antiretroviral Therapy Reduces Markers of Bone Turnover Compared With Continuous Therapy: The SMART Body Composition Substudy

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ABSTRACT

Bone mineral density (BMD) declines significantly in HIV patients on antiretroviral therapy (ART). We compared the effects of intermittent versus continuous ART on markers of bone turnover in the Body Composition substudy of the Strategies for Management of AntiRetroviral Therapy (SMART) trial and determined whether early changes in markers predicted subsequent change in BMD. For 202 participants (median age 44 years, 17% female, 74% on ART) randomized to continuous or intermittent ART, plasma markers of inflammation and bone turnover were evaluated at baseline and months 4 and 12; BMD at the spine (dual-energy X-ray absorptiometry [DXA] and computed tomography) and hip (DXA) was evaluated annually. Compared with the continuous ART group, mean bone-specific alkaline phosphatase (bALP), osteocalcin, procollagen type 1 N-terminal propeptide (P1NP), N-terminal cross-linking telopeptide of type 1 collagen (NTX), and C-terminal cross-linking telopeptide of type 1 collagen (BCTX) decreased significantly in the intermittent ART group, whereas RANKL and the RANKL:osteoprotegerin (OPG) ratio increased (all $p \le 0.002$ at month 4 and month 12). Increases in bALP, osteocalcin, P1NP, NTX, and β CTX at month 4 predicted decrease in hip BMD at month 12, whereas increases in RANKL and the RANKL:OPG ratio at month 4 predicted increase in hip and spine BMD at month 12. This study has shown that compared with continuous ART, interruption of ART results in a reduction in markers of bone turnover and increase in BMD at hip and spine, and that early changes in markers of bone turnover predict BMD changes at 12 months. © 2013 American Society for Bone and Mineral Research.

KEY WORDS: HIV; BONE MINERAL DENSITY; ANTIRETROVIRAL THERAPY; BONE TURNOVER MARKER

Introduction

A lthough adults infected with the human immunodeficiency virus (HIV) have increased fracture risk⁽¹⁾ and lower bone mineral density (BMD) compared with the general population,⁽²⁻⁴⁾ the relative contributions of HIV infection versus

the use of antiretroviral therapy (ART) are unknown. The immune system also plays a key role in bone homeostasis, as both B and T lymphocytes regulate osteoclast activity,⁽⁵⁾ and higher circulating levels of interleukin 6 (IL-6) are independently associated with greater bone loss.⁽⁶⁾ In untreated HIV infection, osteocalcin is lower, and beta C-terminal cross-linking telopeptide of type 1

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collagen (β CTX) is higher, respectively, with more advanced HIV immunodeficiency.^(7–9) HIV may induce bone resorption by several mechanisms including increased receptor activator for nuclear factor- κ B ligand (RANKL) expression through chronic immune activation and increased synthesis of pro-inflammatory cytokines.⁽¹⁰⁾ Initiation of combination ART is associated with increases in markers of bone turnover, identified by increases in levels of both bone formation and resorption markers.^(7,11,12) For ART-naïve patients initiating abacavir-lamivudine or tenofovir-emtricitabine with efavirenz, bone turnover markers increased to 24 weeks after ART initiation and remained relatively stable thereafter, and the increase in markers of bone turnover correlated with reduction in BMD, with *r* values ranging from –0.19 to –0.40.⁽¹¹⁾

No prospective study has investigated the effects of cessation of ART on markers of turnover and inflammation, nor correlated changes in markers of bone turnover and inflammatory markers with subsequent changes in BMD on cessation of ART.

The INSIGHT Strategies for Management of AntiRetroviral Therapy (SMART) study was an international, randomized strategy treatment trial comparing intermittent, CD4+ T lymphocyte count-guided ART with continuous ART. BMD was measured at baseline and annually in a substudy (n = 275).^(13,14) In the intermittent ART group, participants stopped or deferred ART at baseline, and BMD increased or remained stable during the first year after study entry. In contrast, BMD decreased in the continuous ART group, by 0.8% per year at the hip and 0.4% per year at the spine by DXA, over 2.4 years mean follow-up.⁽¹⁴⁾

To investigate why stopping/deferring ART resulted in higher BMD compared with continuous ART, we evaluated markers of bone turnover and inflammation at baseline, month 4, and month 12. The aims of the analyses were: 1) to evaluate the effect of intermittent compared with continuous ART on bone turnover markers, regulators of bone turnover, and inflammatory markers by comparing the randomized treatment groups in a subset of the SMART study; and 2) to determine whether early changes in biomarkers (baseline to month 4) predict change in BMD at the lumbar spine and hip at 12 months.

Materials and Methods

Overall study design

Participants in SMART (n = 5472) were randomized to two groups: 1) intermittent, CD4+ T-cell count-guided ART, where ART was stopped or deferred at study entry, and restarted when the CD4+ T-cell count declined below 250 cells/µL, and stopped again at CD4+ T-cell counts above 350 cells/µL; or 2) continuous ART.⁽¹³⁾ ART drugs were not protocol specified. The SMART study was approved by the institutional review board (IRB) or ethics committee at each clinical site. All participants provided written, informed consent. The study was registered at ClinicalTrials.gov: NCT00027352.

Study population

The DSMB closed the parent SMART study and its substudies early because of safety concerns in the intermittent ART arm of the parent study. This analysis includes the 202 of 275 participants co-enrolled in the SMART Body Composition substudy who had BMD measurements as well as stored samples for biomarker analysis available at baseline and month 12 (25 and 33 participants in the intermittent and continuous ART study arms had no month 12 follow-up visits) (Fig. 1). Design and primary results of the Body Composition substudy were described elsewhere.^(14,15) Participants were enrolled at 32 sites in the United States, Australia, and Spain.

Measurement of BMD

BMD was assessed at baseline and month 12 by dual-energy X-ray absorptiometry (DXA) of the hip and lumbar spine (L_1 to L_4) and quantitative computed tomography (qCT) of the spine (L_2 to L_4). Each patient had all scans performed by the same imaging. Scans were obtained at eight radiology centers and were centrally analyzed (Bio-Imaging Technologies, Inc., Newtown, PA, USA). All sites used a standardized scanning protocol, and the Central Reading Center corrected BMD measurements for cross-sectional and longitudinal consistency based on phantom scans.¹⁴ BMD assessments were blinded to treatment group.

Measurement of markers of bone turnover and inflammation

At baseline and months 4 and 12, plasma was collected into EDTA tubes, after participant fasting for at least 8 hours, processed within 4 hours of collection, and stored at -70° C. All assays were performed blinded to treatment group and BMD measurements.



The 73 excluded participants had similar baseline characteristics to those who were included, except that they had shorter prior duration of HIV infection (median 6 versus 9 years), shorter duration of prior ART use (5 versus 6 years), lower BMI (24.5 versus 26.3), slightly lower total hip BMD (median 0.98 versus 1.00 g/cm²), and lower use of protease inhibitors in the intermittent ART group.

Fig. 1. Study design and CONSORT flow diagram.

Markers of bone formation assayed were bone-specific alkaline phosphatase (bALP) (Ostase BAP, ImmunoDiagnostic Systems, Fountain Hills, AZ, USA) (reference range: men 3.7 to 20.9 μ g/L, premenopausal women 2.9 to 14.5 μ g/L, and postmenopausal women 3.8 to 22.6 µg/L, interassay coefficient of variation [CV] <5.3%), osteocalcin (N-MID Osteocalcin, Roche, Indianapolis, IN, USA) (reference range: men 10.2 to 36.7 ng/mL, premenopausal women 9.7 to 35.1 ng/mL, postmenopausal women 7.3 to 37.8 ng/mL, interassay CV <4.3%), and procollagen type 1 N terminal propeptide (P1NP) (Intact N-terminal propeptide of type 1 procollagen, ImmunoDiagnostic Systems) (Laboratory for Clinical Biochemistry Research [LCBR] reference range: 25.91 to 132.5 µg/L, interassay CV <12.4%). Markers of bone resorptions assayed were N-telopeptide (NTX) (Osteomark N-Tx, Wampole Laboratories, Princeton, NJ, USA) (reference range: men 5.4 to 24.2 nM BCE, premenopausal women 6.2 to 19.0 nM BCE, interassay CV <12.8%), C-terminal cross-linking telopeptide of type 1 collagen (βCTX) (B-Crosslaps, Roche) (reference range: men 0.158 to 0.584 ng/mL, premenopausal women 0.162 to 0.573 ng/mL, postmenopausal women 0.330 to 1.008 ng/mL, interassay CV <8.9%). The bone remodeling markers assayed were osteoprotegerin (OPG) (Alpco Diagnostics, Salem, NH, USA) (LCBR reference range: 2.58 to 7.16 pmol/L, interassay CV <12.5%), RANKL (Millipore, Billerica, MA, USA) (LCBR reference range: 0.160 pg/mL, interassay CV <10.2%). Additionally, the inflammatory markers interleukin (IL)-1 (IL-1b, Panel B, Millipore) (LCBR reference range: 0 to 1.35 pg/mL, interassay CV <13%), IL-6 (R&D Systems, Minneapolis, MN, USA) (LCBR reference range: 0.68 to 6.36 pg/mL, interassay CV <12.2%), and TNF- α (Panel B, Millipore) (LCBR reference range: 0.79-9.41 pg/mL, interassay CV <8.1%) were assayed.

Statistical analyses

Treatment groups were compared for baseline characteristics using Kruskal-Wallis tests to compare medians and chi-square tests for proportions. For all analyses involving change in biomarkers, the biomarkers were analyzed on the log₂ scale, to satisfy distribution assumptions for the statistical tests. An increase in 1 log₂ biomarker unit corresponds to a twofold increase (doubling) on the original biomarker scale.⁽¹⁶⁾ As the primary analysis for the first aim (see Introduction), the intermittent ART and continuous ART groups were compared for mean changes in bone biomarkers and in BMD by intent-to-treat, using unadjusted t tests. Significant differences between the randomized treatment groups, where present, provide evidence for a causal relationship between ART use and biomarker changes. As sensitivity analyses, we repeated the comparisons with adjustment for sex, ART status at study entry, and baseline CD4 cell counts. Within treatment groups, statistical significance of changes in BMD and markers of bone turnover was assessed using two-sided t tests. As secondary analyses, we compared participants who stopped ART and stayed off ART through 12 months in the intermittent ART group with participants in the continuous ART group who were on ART at baseline and continued ART for 12 months (Fig. 1).

were randomized to the continuous ART arm and the race distribution varied by sex. To describe associations of each biomarker with BMD change (11 biomarkers, each with 4 BMD outcomes), estimated coefficients for the biomarker change were presented with 95% confidence intervals (CI) and p values. These regressions were not adjusted for treatment group because the treatment intervention was thought to cause changes in biomarkers, and such could mask the investigated relationship. For multiple regression, for each BMD outcome, we started with a linear regression model that included all 11 biomarkers and the adjustment factors, and performed stepwise backward selection with the Akaike Information Criterion (AIC).⁽¹⁷⁾ For each of the BMD outcomes, we reported which markers remained in the model and whether the association with BMD change was significant. Analyses used SAS version 9.2 (SAS Institute, Cary, NC, USA) and R version 2.15.⁽¹⁸⁾ A p value < 0.05 was considered statistically significant. All p values are two-sided. Results **Baseline characteristics** Baseline characteristics of the study population are shown in

For the second aim, associations of changes in biomarkers

from baseline to month 4 with changes in BMD from baseline to

month 12 were estimated using linear regression; all models

were adjusted for age, sex, race, BMI, HIV duration, ART use at

study entry, and baseline CD4 cell counts. We also included

sex-by-race interaction terms because by chance more women

Table 1. Median age was 44 years, 17% were female, 27% were black, and 56% were white. The treatment groups were well balanced, except that 62% of the intermittent ART group had HIV RNA < 400 copies/mL at baseline compared with 47% in the continuous ART group (p = 0.03 for difference), more participants in the intermittent ART group had used ART at baseline (80% versus 68%; p = 0.05), and more participants in the intermittent ART group had used protease inhibitors (p = 0.04). Among those using ART at study entry, however, protease inhibitor use was balanced across treatment groups (p = 0.15). The intermittent ART group also had a lower proportion of women (13% versus 23%); the difference was not statistically significant. Few participants had osteoporosis and none was receiving osteoporosis therapy. Baseline values for the bone turnover and regulatory markers and inflammatory cytokines are described in Table 2; differences between treatment groups were not significant.

Antiretroviral therapy

Of the participants assigned to continuous ART, 68% were using ART at baseline, and 32% started ART; 97% were on ART at month 4 and 91% at month 12. Of those assigned to intermittent ART, 80% were using ART at baseline and ceased ART, whereas the others continued to defer ART according to the CD4-guided intermittent ART strategy. Through follow-up, 18% had recommenced ART at month 4 and 46% at month 12 because of CD4 cell decline or clinical events.

Table 1. Baseline Characteristics

	Intermittent ART group	Continuous ART group	Total
	(<i>n</i> = 109)	(n = 93)	(N = 202)
Characteristic	% or median	% or median	% or median (IQR)
Demographics			
Age (years)	45.0	43.0	44.0 (40.0, 50.0)
Female participants (%)	12.8	22.6	17.3
Race/ethnicity (%)			
Black	25.7	28.0	26.7
Latino or Hispanic	16.5	14.0	15.3
White	56.0	57.0	56.4
Other or unknown	1.8	1.1	1.5
HIV transmission modes (%)			
MSM	70.6	58.1	64.9
Heterosexual	30.3	34.4	32.2
Intravenous drug use	13.8	14.0	13.9
Other or unknown	4.6	10.8	7.4
Clinical characteristics			
BMI	26.3	26.3	26.3 (23.6, 29.3)
Current smoker (%)	41.3	49.5	45.0
Corticosteroid use (%)	0.9	3.2	2.0
Duration of HIV infection (years)	9.0	9.0	9.0 (5.0, 14.0)
Prior AIDS diagnosis (%)	24.8	22.6	23.8
Hepatitis B (%)	3.7	5.5	4.5
Hepatitis C (%)	15.6	17.2	16.3
CD4 cell count (cells/µL)	580	525	558 (441, 766)
Nadir CD4 cell count (cells/µL)	225	268	250.0 (136, 371)
HIV-RNA \leq 400 copies/mL plasma (%)	62.4	47.3	55.4 [*]
Antiretroviral therapy (ART)			
Naive (%)	5.5	6.5	5.9
Duration of prior ART (years)	6	7	6 (3, 8)
Current ART (%)	79.8	67.7	74.3*
Current PI use (%)	43.1	29.0	36.6*
Current tenofovir use (%)	11.9	15.1	13.4
Current TA-NRTI use (%)	58.7	49.5	54.5
Current NRTIs ^a other than	11.9	9.7	10.9
tenofovir or TA-NRTIs (%)			
Current NNRTI use (%)	36.7	35.5	36.1
Bone mineral density			
Spine, qCT (mg/cm ³)	153	150	152 (128, 173)
Z-score	-0.10	-0.11	-0.11 (-0.88, 0.56)
T-score	-0.89	-0.97	-0.91 (-1.79, -0.02)
7-score < −2.5 (%)	7.3	12.9	9.9
Spine, DXA (g/cm²)	1.11	1.09	1.10 (1.01, 1.21)
Z-score	-0.40	-0.70	-0.60 (-1.33, 0.30)
<i>T</i> -score	-0.64	-0.70	-0.70 (-1.40, 0.30)
7-score < -2.5 (%)	4.6	4.3	4.5
Total hip, DXA (g/cm ²)	1.00	1.00	1.00 (0.94, 1.12)
Z-score	-0.23	-0.10	-0.20 (-0.80, 0.49)
T-score	-0.50	-0.41	-0.46 (-1.00, 0.30)
<i>T</i> -score < -2.5 (%)	2.8	1.1	2.0
			(Continued)

Intermittent ART group (n = 109)	Continuous ART group (n = 93)	Total (N = 202)
% or median	% or median	% or median (IQR)
0.92	0.91	0.91 (0.83, 1.05)
-0.26	-0.30	-0.30 (-0.88, 0.42)
-0.80	-0.70	-0.79 (-1.40, 0.08)
0.9	3.3	2.0
42.2	38.7	40.6
5.5	4.3	5.0
	Intermittent ART group (n = 109) % or median 0.92 -0.26 -0.80 0.9 42.2 5.5	IntermittentContinuousART groupART group $(n = 109)$ $(n = 93)$ $\%$ or median $\%$ or median0.920.91 -0.26 -0.30 -0.80 -0.70 0.93.342.238.75.54.3

ART = antiretroviral therapy; IQR = interquartile range; MSM = men who have sex with men; NNRTI = non-nucleoside reverse transcriptase inhibitor; QCT = quantitative computed tomography; TA-NRTI = thymidine analogue-reverse transcriptase inhibitor.

p value \leq 0.05 for the comparison between the treatment groups; chi-square tests were used to compare percentages and Kruskal-Wallis tests to compare medians.

^aIncludes abacavir (73%), didanosine (36%), emtricitabine (5%), and lamivudine (73%).

^bLow BMD = at least one of the *T*-scores for DXA spine and hip was below -1; osteoporosis, at least one of the *T*-scores for DXA spine and hip was below -2.5.

Changes in BMD and markers of bone turnover and inflammation by treatment group

Changes in BMD and estimated between-group differences are shown in Fig. 2. By year 1, each BMD measure was significantly higher in the intermittent ART group (increase of 0.33% at the hip and 1.64% at the spine by DXA) compared with the continuous ART group (decrease of -1.04% at the hip and -0.13% at the spine by DXA) at month 12. Within the continuous ART group, mean BMD declined by all measures at hip and spine, although the decline was not statistically significant for spine BMD by DXA.

In the intermittent ART group, BMD increased, although only the increase in spine BMD by DXA was significant.

Mean changes from baseline for (log transformed) markers of bone turnover, bone regulation, and chronic inflammation at 4 and 12 months, and the between-group differences (change in the intermittent ART minus the continuous ART group) are presented in Fig. 3. In the intermittent ART group, bALP, osteocalcin, P1NP, NTX, and β CTX decreased compared with an increase in the same markers in the continuous ART group. RANKL, the RANKL:OPG ratio, and TNF- α increased in the intermittent ART group and decreased in the continuous ART

			Conti	nuous ART		
	Intermitte	ent ART group		group		Total
Marker	n	Median	n	Median	N	Median (IQR)
Bone formation						
bALP (μg/L)	109	20.3	93	18.7	202	19.6 (16.1, 26.1)
Osteocalcin (ng/mL)	107	18.9	92	17.0	199	17.6 (13.9, 22.6)
P1NP (µg/L)	109	49.4	93	45.9	202	47.6 (36.2, 62.1)
Bone resorption						
NTX (nM BCE)	109	11.9	93	10.8	202	11.3 (9.7, 14.1)
βCTX (ng/mL)	106	0.3	92	0.3	198	0.3 (0.2, 0.4)
Bone regulators						
OPG (pmol/L)	107	4.0	90	3.8	197	3.9 (3.3, 4.6)
RANKL (pg/mL)	109	7.9	91	8.6	200	8.3 (2.0, 23.2)
RANKL:OPG ratio	107	2.0	89	3.2	196	2.2 (0.6, 6.1)
Inflammation						
IL-6 (pg/mL)	109	2.0	93	1.7	202	1.9 (1.2, 3.0)
IL-1 (pg/mL)	104	0.1	86	0.1	190	0.1 (0.1, 0.1)
TNFα (pg/mL)	104	9.7	86	9.2	190	9.2 (6.7, 12.2)

Table 2. Markers of Bone Turnover at Baseline

bALP = bone-specific alkaline phosphatase; β CTX = C-terminal cross-linking telopeptide of type 1 collagen; IL-1 = interleukin 1beta; IL-6 = interleukin 6; NTX = N-telopeptide; OPG = osteoprotegerin; P1NP = procollagen type 1 N terminal propeptide; RANKL = receptor of nuclear factor- κ B ligand; TNF α = tumor necrosis factor α .

Chang	e from Bas	seline to	o Month	12 (in % o	f baseline)	Diff. IntermConti	n. (in %)
BMD	Group	Ν	Mean	P−val.	Mean and SE	Mean and 95% CI	P-val.
Total Hip, DX/	A Interm. Contin.	103 90	0.33 -1.04	0.20 0.002]+ +■	⊢ ⊷i	0.001
Femur, DXA	Interm. Contin.	103 90	0.49 -1.06	0.19 0.05]+ ⊢ ≣	⊢•-1	0.02
Spine, DXA	Interm. Contin.	106 91	1.64 -0.13	<0.001 0.78	H H	 -1	0.003
Spine, qCT	Interm. Contin.	107 93	0.48 -3.78	0.51 <0.001	₽-1 	F	<0.001
					$\xrightarrow{-4 -2 0} \xrightarrow{2}$	$\stackrel{-2}{\leftarrow} \stackrel{0}{\longrightarrow} \stackrel{2}{\longrightarrow} \stackrel{4}{\longrightarrow} \stackrel{6}{\longrightarrow}$	

Fig. 2. Change in BMD from baseline to year 1, and estimated difference between the intermittent and continuous ART groups. CI = confidence interval; SE = standard error.

group. The changes were apparent by month 4 and remained at about the same level at month 12. Between-group differences in mean change were significant at both months 4 and 12 for each of these biomarkers (all *p* values \leq 0.002). IL-6 followed a similar pattern to TNF- α , but the treatment difference was significant only at month 12 (*p* = 0.05). There was no significant between-group difference in mean change in OPG or IL-1. Adjustment for sex, ART status at study entry, and baseline CD4 cell counts had minimal impact on the between-group comparison (data not shown).

Changes in markers of bone turnover and inflammation by first-year ART use

When restricting the analyses to participants who were on ART at baseline and either remained on ART for 12 months (n = 53) or stopped and remained off ART for 12 months (n = 44) (Fig. 1), differences between the ART groups at month 4 were similar to the intent-to-treat comparisons in all 202 participants. Differences tended to increase further by month 12, although most of the treatment difference was attained by month 4 (data not shown). There was no evidence for a difference between the treatment groups in OPG or IL-1.

Effect of early change in markers (baseline to month 4) on change in BMD (baseline to month 12)

Associations between early changes in bone biomarkers (baseline to month 4) and changes in BMD between baseline and month 12 are summarized in Fig. 4. For example, an increase of $1 \log_2 \text{ unit in bALP}$ (twofold increase from baseline to month 4) was associated with a 2.3% per year steeper decrease in hip BMD. Pooled across treatment groups, increases in bone formation

markers (bALP, osteocalcin) as well as increases in bone resorption markers (NTX and β CTX) at month 4 were significantly correlated with loss of total hip BMD, whereas increased RANKL and RANKL:OPG ratios were associated with increases of hip BMD. The association of increased P1NP with a decrease in hip BMD was borderline significant (p = 0.06). The direction of the associations of these seven biomarkers with change in BMD were similar across all four BMD outcomes (total hip, femoral neck, spine by DXA, and spine by qCT), although not all associations were significant. An increase in TNF- α by month 4 was associated with increased spine BMD by qCT, but there was no evidence for an association of TNF- α , IL-1, or IL-6 with any of the other BMD outcomes. The analyses were adjusted for age, sex, race, BMI, HIV duration, ART use at study entry, and baseline CD4 cell counts.

Changes in markers of bone turnover and inflammation were correlated (data not shown). When including all 11 bone biomarkers as predictors for change in BMD in multiple regression models, a backwards selection procedure retained early declines in β CTX (p < 0.001) and the RANKL:OPG ratio (p = 0.03) as independent predictors for increase in total hip BMD. Results for the other BMD outcomes are summarized in Fig. 4; superscripts to the p values denote which bone markers were selected for the final model (s, *) and whether they were independently associated with the BMD outcome at year 1 (* for p < 0.05).

Changes in CD4+ T-cell counts

In the intermittent ART group, mean CD4+ T-cell counts declined by 198 and 232 cells/ μ L from baseline to months 4 and 12, respectively. In the continuous ART group, mean CD4+ T-cell

		Cha	nge from l	Baseline (in	log ₂ scale)	Diff. IntermContin. (ir	log ₂ scale)
Marker	Month	Group	Mean	P−val.	Mean and SE	Mean and 95% CI	P-val.
Bone Formation bALP	4	Interm. Contin.	-0.12 0.08	0.002 0.04	-	184	<0.001
	12	Interm. Contin.	-0.12 0.14	0.01 0.002		181	<0.001
Osteocalcin	4	Interm. Contin.	-0.25 0.10	<0.001 0.07	H===	H B -1	<0.001
	12	Interm. Contin.	-0.27 0.13	<0.001 0.02		HEH	<0.001
P1NP	4	Interm. Contin.	-0.16 0.12	0.001 0.05		HEH	<0.001
	12	Interm. Contin.	-0.19 0.15	0.003 0.01		+8-1	<0.001
Bone Resorption							
NTX	4	Interm. Contin.	-0.19 0.01	<0.001 0.77		4 14	<0.001
	12	Interm. Contin.	-0.13 0.07	0.002 0.16	e e		0.002
βCTX	4	Interm. Contin.	-0.48 0.16	<0.001 0.08	+===		<0.001
	12	Interm. Contin.	-0.38 0.26	<0.001 0.004		⊢ ∎-1	<0.001
Bone Regulators							
OPG	4	Interm. Contin.	0.01 -0.05	0.77 0.30	4	H B -1	0.30
	12	Interm. Contin.	0.10 0.01	0.004 0.71	D ⁺ ⊨		0.10
RANKL	4	Interm. Contin.	0.81 -0.40	<0.001 0.02			<0.001
	12	Interm. Contin.	0.62 -0.45	0.001 0.03			<0.001
RANKL:OPG ratio	4	Interm. Contin.	0.83 -0.39	<0.001 0.04			<0.001
	12	Interm. Contin.	0.62 -0.46	0.002 0.04			<0.001
Inflammation							
IL-6	4	Interm. Contin.	0.12 -0.07	0.34 0.60		⊢ ∎→	0.30
	12	Interm. Contin.	0.24 -0.07	0.05 0.45			0.05
IL-1	4	Interm. Contin.	0.05 0.09	0.62 0.55	₽	F-81	0.79
	12	Interm. Contin.	0.27 0.09	0.06 0.42			0.33
ΤΝΓα	4	Interm. Contin.	0.34 -0.14	0.002 0.15			0.001
	12	Interm. Contin.	0.36 -0.14	<0.001 0.01			<0.001
					1.0 -0.5 0.0 0.5 1.0		
				•	Decrease Increase		

Fig. 3. Change in biomarkers from baseline to months 4 and 12 within the intermittent ART and continuous ART groups, and estimated treatment difference. Bars denote the mean change from baseline. All biomarkers were analyzed on the log_2 scale; an increase of $1 log_2$ biomarker unit corresponds to a twofold increase (doubling) in the original measurement scale. bALP = bone-specific alkaline phosphatase; $\beta CTX = C$ -terminal cross-linking telopeptide of type 1 collagen; IL-1 = interleukin 1beta; IL-6 = interleukin 6; NTX = N-telopeptide; OPG = osteoprotegerin; P1NP = procollagen type 1 N terminal propeptide; RANKL = receptor of nuclear factor- κB ligand; TNF α , tumor necrosis factor α ; CI = confidence interval; SE = standard error.

counts increased by 25 cells/ μ L to month 4, and 33 cells/ μ L from baseline to month 12. A month 4 increase in CD4+ T cells by 100 cells/ μ L was associated with a decrease in spine DXA BMD by -0.40% (95% CI -0.71 to -0.08%, p = 0.01), and a decrease in spine qCT BMD by -0.89% (95% CI -1.44 to -0.35%, p = 0.002) in

univariate regression pooled across treatment groups. After adjustment for treatment group, these associations were not significant. There was no evidence for an association between changes in CD4+ T-cell count at month 4 and changes in hip BMD at month 12.

					Percent Cł	hange in BMI), baselin∉	e to Month 12				
Bone Biomarker, Change to Month 4		Total Hip, D)	XA	Ľ	emoral Neck,	DXA		Spine, DXA			Spine, qCT	
(in log ₂ scale)	Coef.	95% CI	P-value	Coef.	95% CI	P-value	Coef.	95% CI	P-value	Coef.	95% CI	P-value
Bone Formation												
bALP	-2.23	ŀ	<0.001 ^{\$}	-1.89	•	60.0	-1.93	ļ	0.04	-5.46	ŀ	<0.001 *
Osteocalcin	-1.19	ł	0.02	-2.34	ł	0.005 ^s	-1.54	ł	0.03 ^s	-2.03	ł	0.12 ^s
P1NP	-0.89	ł	0.06	-1.55	ł	0.05	-0.92	ł	0.17	-0.27	ł	0.82 *
Bone Resorption												
NTX	-2.47	ŀ	<0.001	-2.12	ł	0.05 ^s	-2.53	ļ	0.005	-4.04	ł	0.01
рстх	-1.37	Ŧ	<0.001 *	-1.84	ł	<0.001 *	-0.61	ł	0.14	-1.80	Ŧ	0.02 ^s
Bone Regulators												
Osteoprotegerin	-1.16	ł	0.10	-0.15	ļ	06.0	-0.10	ł	0.92	0.79	ł	0.62
RANKL	0.42	٠	s 200.0	0.47	÷	0.08	0.42	ě	0.05	0.90	٠	0.02 ^s
RANKL:OPG ratio	0.50	٠	0.001 *	0.50	ŧ	0.05	0.45	ě	0.03 ^s	1.05	٠	0.004
Inflammation												
IL-1	-0.23	Ť	0.35	0.01	Ŧ	0.99	0.21	ł	0.52	0.69	Ŧ	0.26
IL-6	-0.01	Ť	0.96	-0.04	Ŧ	0.89	0.18	Ŧ	0.55	0.18	Ť	0.72
$TNF\alpha$	0.21	Ŧ	0.45	-0.05	ł	0.91	0.61	ł	0.11	1.88	Ť	0.006 ^s
		-4 -2 0 2	L 4		-4 -2 0 2	L 4			۲ 4	L Ŧ I	0 -5 0 5	L 5

Fig. 4. Associations of early changes in biomarkers with change in BMD. Coefficients, 95% confidence intervals, and p values for association were calculated in linear regression models, using the containing all 11 bone biomarkers (log₂ scale) and adjustment factors. S = biomarker was selected but association was not significant; * = biomarker was independently associated with change in BMD in the final model ($p \le 0.05$). bALP = bone-specific alkaline phosphatase; BCTX = C-terminal cross-linking telopeptide of type 1 collagen; IL-1 = interleukin 1beta; IL-6 = interleukin 6; change in each biomarker (from baseline to month 4, log₂ scale) to estimate the change in BMD at year 1; models were adjusted for age, sex, race, BMI, HIV duration, ART use at study entry, and measurement scale. For each BMD outcome, superscripts for p values denote the final multiple regression model obtained through backwards variable selection with AIC, starting from a model baseline CD4 cell counts. The size of the coefficients is comparable across biomarkers because an increase of 1 log₂ biomarker unit corresponds to a twofold increase (doubling) in the original NTX = N-telopeptide; OPG = osteoprotegerin; P1NP = procollagen type 1N terminal propeptide; <math>RANKL = receptor of nuclear factor-kB ligand; $TNF\alpha = tumor$ necrosis factor α .

Discussion

To our knowledge, this is the first randomized study in HIV patients to compare changes in markers of bone turnover and inflammation between continuous ART (continue or start ART) and intermittent ART (stop or defer ART, and resume ART at CD4 counts <250 cells/ μ L). Relative to continuous ART, we found significant reductions in markers of both bone resorption and bone formation in the intermittent ART group. Markers of bone formation and resorption both decreased from baseline to month 4, and remained stable thereafter to month 12 in the intermittent ART group. Conversely, these markers tended to increase in the continuous ART group.

The RANKL:OPG ratio increased in the intermittent ART group by month 4 and remained elevated through month 12. In some studies of postmenopausal osteoporosis, increased RANKL:OPG was associated with net bone loss, whereas no association with BMD was demonstrated in others.⁽¹⁹⁾ In our study, the association was inverse; higher RANKL:OPG ratios were associated with increased BMD and reduction in other markers of bone resorption. The inverse association was also seen in a prospective study in 87 patients with HIV, which reported significant reductions in levels of RANKL and OPG as well as inflammatory markers, on initiation of ART, whereas markers of bone formation and resorption significantly increased. The OPG/sRANKL ratio remained unchanged over time, and there was no association with markers of bone turnover.⁽²⁰⁾

RANKL, IL-6, and TNF- α significantly increased in the intermittent ART group and decreased in the continuous ART group. In contrast, osteoprotegerin levels and IL-1 remained relatively stable. Conditions associated with chronic immune activation and inflammation are well known to be associated with increased bone loss (eg, rheumatoid arthritis and inflammatory bowel disease).⁽²¹⁻²³⁾ Inflammation results in increased RANKL expression and production by activated T cells and B cells, increased numbers of osteoclast precursors, and reduction in production in osteoprotegerin, with resultant greater bone resorption and net bone loss.⁽²⁴⁻²⁶⁾ It is counterintuitive that the increases in RANKL and the RANKL:OPG ratio in the intermittent ART group occurred in association with reduction in markers of bone resorption and increased BMD. It is possible that ART increases bone turnover through a pathway that is not mediated by RANKL or osteoprotegerin and that the increase in RANKL at cessation of ART occurred as a compensatory response to the reduction in markers of bone turnover. Alternatively, the increase in RANKL may be a reflection of increased immune activation and subsequent expression and production of RANKL by activated T cells associated with untreated HIV infection.

Early increases (from baseline to month 4) in the markers of bone formation and bone resorption predicted decreases in BMD at the hip and spine at Month 12. The directions of associations between biomarkers and BMD were consistent across BMD at the hip (femoral neck and total hip) and spine (lumbar spine measured by DXA and qCT). However, only a few markers (β CTX and the RANKL:OPG ratio for hip BMD, bALP and P1NP for spine BMD by qCT) remained independently associated with changes in BMD in multiple regression and the strength of the associations varied by BMD site and measurement modality. This reflects the complex interrelationships between the bone biomarkers, with none clearly dominating with respect to predicting change in BMD.

The randomized comparison provides evidence that the observed decline in markers of bone turnover in the continuous ART group versus the increase in the intermittent ART group are likely due to the differences in ART use. However, the difference between the two treatment groups may underestimate the effect of stopping ART, because in the intermittent ART arm, 36% of participants had recommenced ART by month 12, and 20% had not used ART at study entry. When the analysis was restricted to those participants in the intermittent ART arm who stopped ART and stayed off ART through month 12, the levels of markers of bone formation and resorption continued to decline through month 12, although most of the reduction was evident by month 4. Conversely, in the continuous ART group, when restricting the analysis to those who were using ART at study entry and continued to use ART through month 12, markers of bone turnover within this subset of participants tended to increase, but the magnitude of the increase was less than among those randomized to the continuous ART group who were not taking ART at baseline and started ART.

In the setting of ART, increased markers of bone turnover, as observed in the continuous ART group, have been consistently associated with loss of BMD, particularly over the first year of treatment.^(11,12) Elucidating the mechanism of ART-induced bone loss is confounded by changes in HIV-induced immune activation and inflammation. Is the significant early bone loss observed over 24 to 48 weeks after initiation of ART driven by immune reconstitution, as proposed by some,^(27,28) or by the direct effects of ART? In our study, increased CD4 T-cell counts were associated with steeper decreases in spine BMD, pooled across treatment groups. It is not possible, however, to separate the effect of ART in our study from the effect of increased CD4 T cells. After adjustment for treatment group, there was no evidence for an association of CD4 T-cell increases with bone loss.

The main strength of our study is the randomized comparison of continuous versus intermittent ART. There were several limitations to our study. About half of the participants randomized to intermittent ART had restarted ART by month 12. The ART regimens used in SMART were heterogeneous and may not be entirely representative of modern ART regimens; moreover, this study was not designed to evaluate the effects of specific drugs. The first follow-up time point was at month 4, so we could not determine which biomarker or group of biomarkers changed first. The analysis excluded 73 of the 275 randomized participants in the SMART Body Composition substudy, mostly because month 12 data were not available because of study closure. Although the excluded participants were largely similar to those analyzed, they differed in some baseline characteristics. Finally, the proportion of women was higher and ART use at study entry was lower in one of the randomized treatment groups in the Body Composition substudy; however, after adjustment for sex and ART use at baseline in sensitivity analyses, results were similar to the unadjusted comparisons between treatment groups summarized in Fig. 3.

In conclusion, this study has shown that interruption of ART results in a reduction in markers of bone turnover and increase in BMD at hip and spine relative to continuous ART, and that early changes in markers of bone turnover predict BMD changes at 12 months. The stabilization of bone loss after ART interruption, despite increased inflammation, suggests a deleterious effect of ART on bone, either directly or indirectly. The clinical implication of these findings is unknown given the imperative need for ART to prevent major HIV-related outcomes; once started, ART needs to be used continuously. The trend to initiate ART earlier in the course of HIV infection means that low BMD may become more prevalent as more patients use ART for longer, highlighting the need for prospective studies with longer follow-up.

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