CLINICAL TRIAL

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Immediate Initiation of Antiretroviral Therapy for HIV Infection Accelerates Bone Loss Relative to Deferring Therapy: Findings from the START Bone Mineral Density Substudy, a Randomized Trial

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ABSTRACT

Both HIV infection and antiretroviral therapy (ART) are associated with lower bone mineral density (BMD) and increased fracture risk. Because the relative contributions of ART and untreated HIV to BMD loss are unclear, it is important to quantify the effect of ART on bone. We compared the effect of early ART initiation (CD4 >500 cells/μL) with deferred ART on change in BMD in the START Bone Mineral Density substudy, a randomized trial evaluating the effect of immediate ART initiation versus deferring ART (to CD4 <350 cells/μL). BMD was measured annually at the lumbar spine and hip by dual-energy X-ray absorptiometry (DXA). Percent change in BMD by treatment assignment (intent-to-treat analysis) was estimated using longitudinal mixed models and linear regression. Baseline and follow-up DXA scans were available for 399 (195 immediate, 204 deferred) participants (median age 32 years, 80% non-white, 26% women, median CD4 count 642 cells/μL). ART (most commonly including tenofovir and efavirenz) was used for 95% and 18% of follow-up in the immediate and deferred ART groups, respectively. Through 2.2 years mean follow-up, immediate ART resulted in greater BMD declines than deferred ART at the hip (–2.5% versus –1.0%; difference –1.5%, 95% confidence interval [CI] –2.2 to –0.8, $p < 0.001$) and spine (–1.9% versus –0.4%; difference –1.6%, 95% CI –2.2 to –1.0, $p < 0.001$). BMD declines were greatest in the first year of ART. In the immediate ART group, spine BMD stabilized after year 1, whereas hip BMD declined progressively over 2 years. After year 1, BMD changes were similar in the immediate and deferred groups. No clinical, HIV-related, or ART characteristic predicted greater BMD loss in either group. All HIV treatment guidelines now recommend ART initiation at HIV diagnosis because of the reduced risk of serious clinical outcomes. Better understanding of the longer-term consequences of the observed reductions in BMD is needed. Clinical Trials Registration: NCT00867048. © 2017 American Society for Bone and Mineral Research.

KEY WORDS: BONE MINERAL DENSITY; HIV; ANTIRETROVIRAL THERAPY; CLINICAL TRIALS; DXA

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Introduction

Low bone mineral density (BMD), osteoporosis, and fractures are more common in HIV-infected adults than in HIV-negative controls.[1,2] Uncontrolled studies have found that initiation of antiretroviral therapy (ART) is followed by a 2% to 6% reduction in BMD, mainly over the first 1 to 2 years. Most studies report stabilization thereafter, although follow-up was generally less than 3 years.[1,3] Antiretroviral guidelines now recommend immediate ART initiation regardless of CD4 count, in large part because of the results of the INSIGHT Strategic Timing of Antiretroviral Therapy (START) trial, which reported a 57% reduction in serious AIDS and non-AIDS–related morbidity and mortality.[4,5]

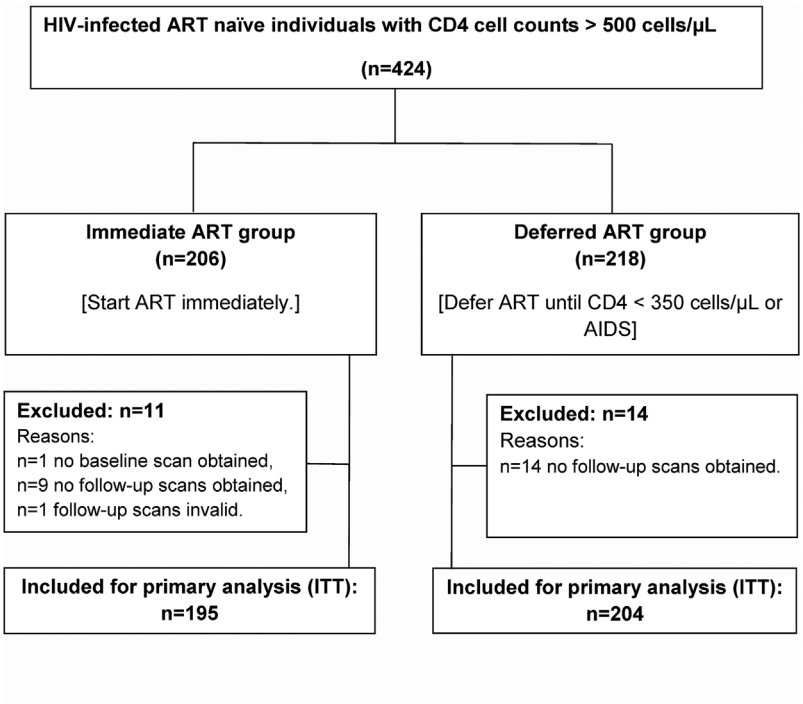
Bone loss in HIV-infected adults is multifactorial. Greater bone loss has been reported with use of tenofovir disoproxil fumarate (TDF) and ritonavir-boosted protease inhibitors (PIs) in the ART regimen.[3,6] However, some bone loss occurs with all ART,[7] probably because of increased bone catabolism after suppression of HIV viral load and immune reconstitution.[8,9] Untreated HIV is also associated with lower BMD, possibly because of greater prevalence of conventional risk factors or because of HIV infection of osteoblasts or increased bone metabolism.[10,11] Cross-sectional studies, however, have not reported consistent relationships between BMD and duration of HIV infection, viral load, or CD4 count.[12,13]

The relative contributions of ART and untreated HIV infection to BMD loss are unclear, and it is important to quantify the bone effects of ART to fully determine its risk-benefit profile. There are no prospective, randomized trial data describing the effect of ART versus no ART on bone. We report the results of the Bone Mineral Density substudy of START, a randomized trial comparing the effects of immediate versus deferred ART on hip and spine BMD. We hypothesized that immediate ART would result in greater BMD loss than deferred ART.

Materials and Methods

Study design and participants

The START study randomized 4685 HIV-positive, ART-naïve adults with high CD4 counts (>500 cells/μL) to one of two strategies of



Participant Disposition at Years 1-3

	Numbers of Participants					
	Immediate ART Group (N=195)			Deferred ART Group (N=204)		
Disposition	Year 1	Year 2	Year 3	Year 1	Year 2	Year 3
Included	194	169	51	197	180	59
Not included because follow-up was censored on May 26, 2015	0	16	142	0	12	142
Not included for other reasons:						
Missed visit	1	8	0	7	11	2
Scan was invalid	0	0	0	0	0	0
Consent withdrawn	0	2	2	0	0	0
Death	0	0	0	0	1	1

Fig. 1. Study design, CONSORT diagram, and participant disposition at each follow-up visit. Of the 399 participants included in the primary analysis, 349 (87.5%) had year 2 BMD data and 110 (27.6%) had year 3 data. For 284 of the 289 participants without year 3 data, the year 3 visit was due after the common censoring date, May 26, 2015.

Table 1. Baseline Characteristics

	Total ($n = 399$) Median [IQR] or n (%)	Immediate ART group ($n = 195$) Median or %	Deferred ART group ($n = 204$) Median or %
Demographics			
Age (years)	32 [26, 41]	32	33
Sex			
Male	295 (73.9)	73.3	74.5
Premenopausal female	91 (22.8)	24.1	21.6
Postmenopausal female	13 (3.3)	2.6	3.9
Race			
Asian	126 (31.6)	30.8	32.4
Black	74 (18.5)	16.9	20.1
Latino/Hispanic	97 (24.3)	25.6	23.0
White	80 (20.1)	19.5	20.6
Other	22 (5.5)	7.2	3.9
Clinical factors			
Previous fracture (any)[a]	31 (7.8)	8.7	6.9
Previous fragility fracture[a]	17 (4.3)	4.1	4.4
BMI (kg/m^2)	23.9 [21.4, 27.3]	24.1	23.8
Current smoker	77 (19.3)	16.9	21.6
Alcohol use[b]	16 (4.0)	4.6	3.4
Current medication use			
Corticosteroids	1 (0.3)	0.0	0.5
Vitamin D	22 (5.5)	4.1	6.9
Calcium supplements	19 (4.8)	4.1	5.4
Hormone-replacement therapy	1 (0.3)	0.0	0.5
HIV history			
Known HIV duration (years)	0.7 [0.3, 2.8]	0.6	0.9
ART, prespecified before randomization			
Tenofovir DF	334 (83.7)	83.1	84.3
Efavirenz	333 (83.5)	82.6	84.3
Protease inhibitor	42 (10.5)	11.8	9.3
Laboratory results			
CD4 count (cells/μL)	642 [579, 738]	644	640
HIV viral load (copies/mL)	14,940 [3399, 53,293]	20,257	12,332
eGFR (mL/min/1.73 m^2)[c]	114.0 [98.9, 122.4]	114.2	113.4
Bone mineral density			
Spine			
BMD (g/cm^2)	1.01 [0.94, 1.11]	1.02	1.01
T-score[d]	−0.31 [−1.00, 0.59]	−0.24	−0.33
Z-score[e]	−0.66 [−1.33, 0.25]	−0.63	−0.69
Total hip			
BMD (g/cm^2)	0.96 [0.87, 1.04]	0.94	0.97
T-score[d]	0.14 [−0.61, 0.84]	0.02	0.24
Z-score[e]	−0.37 [−0.94, 0.18]	−0.47	−0.31
Femoral neck			
BMD (g/cm^2)	0.84 [0.76, 0.93]	0.83	0.85
T-score[d]	−0.06 [−0.84, 0.77]	−0.16	0.04
Z-score[e]	−0.44 [−1.05, 0.19]	−0.53	−0.39
Low BMD relative to age group[f]	45 (11.3)	13.3	9.3
T-score $\leq$ −2.5 at the spine, hip or femoral neck	8 (2.0)	3.1	1.0

Tenofovir DF = tenofovir disoproxil fumarate; NSAID = nonsteroidal anti-inflammatory drug; eGFR = estimated glomerular filtration rate.

[a]Fractures after age 18 years. Fragility fracture defined as fracture occurring after fall from standing height or equivalent.

[b]Consumed alcohol 4 to 7 days a week with at least 2 drinks per day.

[c]eGFR was calculated using the CKD-EPI formula.

[d]BMD *T*-scores were standardized relative to young adult white women.

[e]BMD *Z*-scores were standardized relative to age, sex, and race/ethnicity (black/white/hispanic) matched reference populations. White reference populations are used for all other races/ethnicities.

[f]BMD *Z*-score $\leq$ −2 at the lumbar spine, hip, or femoral neck.

ART initiation, either immediate ART initiation or deferred ART (initiation when CD4 count fell below 350 cells/μL or HIV disease progression).[14] ART regimens were not protocol-specified, but the initial regimen was selected by investigators pre-randomization. At 33 clinical sites in 11 countries, all eligible START participants were offered BMD substudy co-enrollment. Eligibility criteria were broad and excluded only those receiving treatment for low BMD (calcium, vitamin D, and hormone-replacement therapy were permitted) or for whom valid BMD scans could not be obtained. The substudy was approved by the institutional review board at each participating clinical site and was performed in compliance with the principles of the Declaration of Helsinki and local regulatory requirements. All participants provided written, informed consent before enrollment.

BMD at the hip and lumbar spine (L_1 to L_4) was measured at baseline (within 120 days before randomization) and annually by dual-energy X-ray absorptiometry (DXA). Each of the 16 radiology centers used either a Hologic (Hologic Inc., Bedford, MA, USA; $n = 290$ participants) or GE Lunar (GE Healthcare, Madison, WI, USA; $n = 134$) scanner. All DXA images were obtained using a standardized protocol and read centrally at the study's DXA quality assurance (QA) center (University of California, San Francisco, CA, USA). Procedures to ensure quality and standardization of BMD measurements have been described.[15] In brief, equipment and radiology technicians at each radiology center were certified by the DXA QA center; the quality of each scan submitted was evaluated immediately at the QA center, and unacceptable scans were repeated. All scans were standardized for longitudinal and cross-sectional consistency by the DXA QA center, using two types of phantom scans: 1) phantoms provided by the DXA equipment manufacturers were scanned before each participant scan and at least 3 times a week; 2) a set of three cross-calibration phantoms were scanned at each radiology center. BMD measures obtained on GE Lunar equipment were standardized to Hologic measures using validated linear transformation equations.[16,17] *T*-scores and *Z*-scores were calculated from longitudinally and cross-sectionally adjusted BMD readings. *T*-scores were calculated relative to peak bone mass in young white women.[18] *Z*-scores were calculated relative to US reference populations matched by age, sex, and race/ethnicity.[19] Low BMD (below the expected range for age) was defined by a BMD *Z*-score (spine, hip, femoral neck) ≤ -2, consistent with recommendations for young populations by the US National Osteoporosis Foundation and the International Society for Clinical Densitometry.[20,21] WHO classifies BMD *T*-scores ≤ -2.5 as osteoporosis.[22]

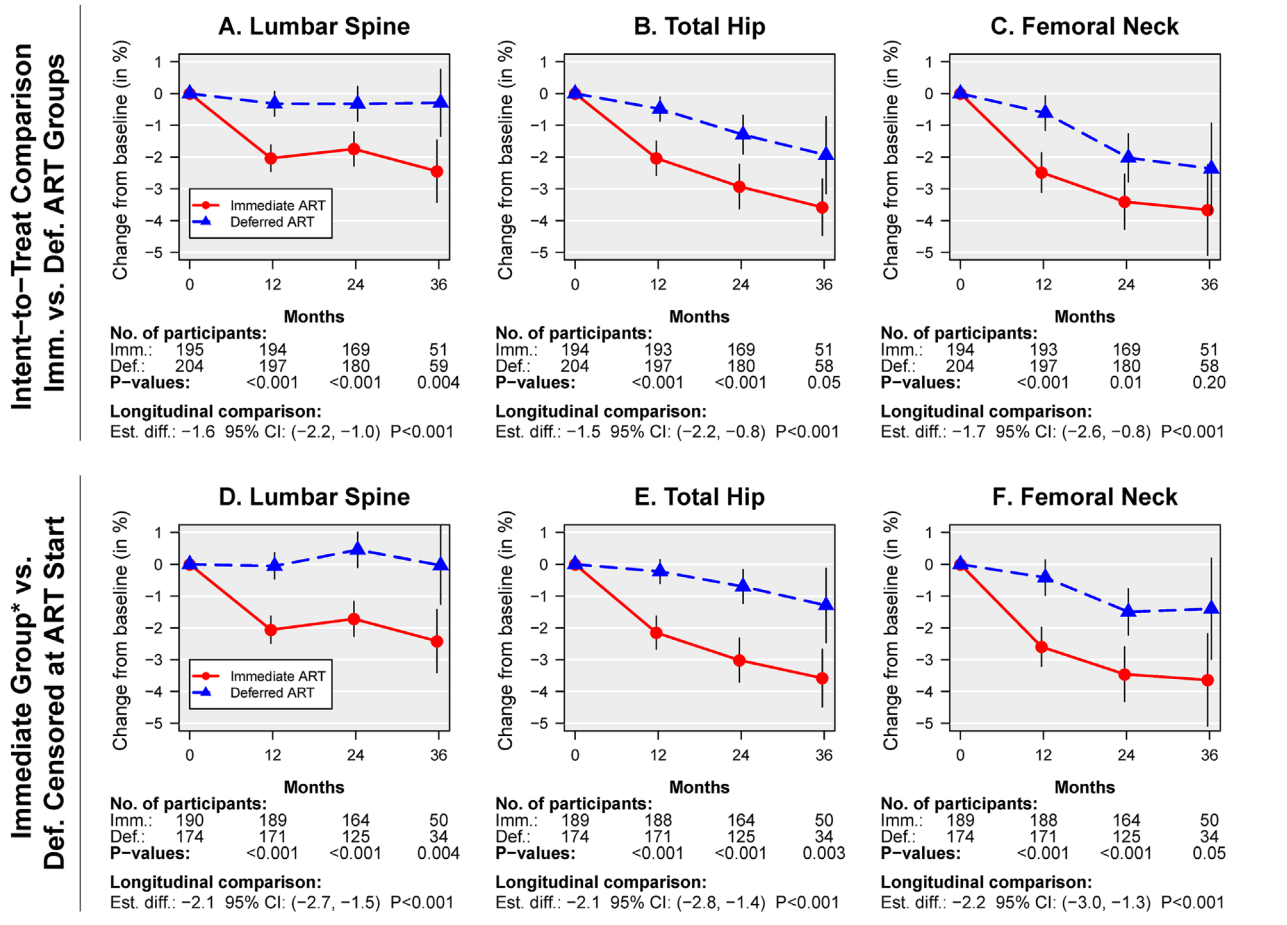


Fig. 2. Mean percent change (95% CIs) in BMD by treatment group. (*A–C*) Intent-to-treat comparisons. In *D–F*, follow-up in the deferred group is censored at ART start, and participants in the immediate group who did not start ART within the first year are excluded. (*A*, *D*) Lumbar spine (L_1 to L_4) BMD, (*B*, *E*) total hip BMD, (*C*, *F*) femoral neck BMD.

Fractures and osteoporosis treatment were recorded at baseline and annually for all START participants. Use of vitamin D and calcium supplements was recorded at baseline and annually in the BMD substudy participants.

Study outcomes

The co-primary outcomes were changes from baseline in total hip BMD and lumbar spine BMD. The primary objective was to compare changes in hip and spine BMD through follow-up between the immediate and deferred ART groups by intent-to-treat. Prespecified secondary objectives included change in femoral neck BMD, incidence of osteoporosis or low BMD, rates of BMD loss upon ART initiation in the immediate ART group and among participants in the deferred group before ART start (untreated HIV), and evaluation of clinical parameters associated with rates of BMD change.

Statistical analyses

The sample size of 400 participants was estimated to detect between-group differences of 1.0% and 1.2% in mean percent change in total hip and spine BMD, respectively, from baseline through follow-up, with 80% power at a 5% significance level, assuming standard deviations of 3.8% and 4.8%, respectively.[3,23]

All changes in BMD from baseline were expressed as percent of baseline BMD. Follow-up was censored at each participant's last DXA scan before May 27, 2015 (when the Data and Safety Monitoring Board recommended all START participants be offered ART).[14] Unless noted otherwise, all treatment group comparisons were by intent-to-treat. The primary analysis was the intent-to-treat comparison between the immediate and deferred ART groups for percent change in BMD using longitudinal mixed models, adjusted for baseline BMD and visit. Groups were compared for changes in BMD to each year of follow-up using ANCOVA models adjusted for baseline BMD. Rates of BMD change were estimated within each group using unadjusted, longitudinal mixed models with the subject-specific annual percent change in BMD as response variable; to compare the groups, models were adjusted for baseline BMD and visit. The groups were compared for incidence of low BMD and *T*-scores ≤ -2.5 using unadjusted Cox regression models. To assess the effect of ART versus strictly untreated HIV, we compared the immediate group (excluding participants who did not start ART within the first year) versus the deferred group censored at ART start. Subgroup analyses for the co-primary outcomes were performed to determine whether the treatment effect differed across baseline characteristics; we considered only subgroups that included at least 20 participants pooled across the two

Table 2. Percent Change in BMD at the Spine (L_1 to L_4), Hip, and Femoral Neck Compared Between the Immediate and Deferred ART Groups by Intent-to-Treat

	Immediate ART group		Deferred ART group		Immediate – Deferred ART groups	
	n	Mean (95% CI)	*n*	Mean (95% CI)	Estimated difference (95% CI)	*p*
Spine (L_1 to L_4)						
Baseline to year 1	194	−2.04 (−2.46, −1.62)	197	−0.32 (−0.71, 0.07)	−1.72 (−2.29, −1.14)	<0.001
Baseline to year 2	169	−1.74 (−2.28, −1.21)	180	−0.33 (−0.87, 0.22)	−1.41 (−2.18, −0.65)	<0.001
Baseline to year 3	51	−2.45 (−3.41, −1.48)	59	−0.29 (−1.33, 0.75)	−2.16 (−3.60, −0.71)	<0.01
Overall	195	−1.92 (−2.34, −1.50)	204	−0.35 (−0.76, 0.06)	−1.57 (−2.16, −0.98)	<0.001
Change from year 1 to year 2 (%)	168	0.29 (−0.16, 0.74)	173	−0.10 (−0.54, 0.35)	0.39 (−0.25, 1.03)	0.23
Change from year 2 to year 3 (%)	50	0.25 (−0.54, 1.03)	59	−0.49 (−1.21, 0.23)	0.78 (−0.31, 1.86)	0.16
Rate of change after year 1 (% per year)		0.24 (−0.11, 0.59)		−0.17 (−0.51, 0.16)	0.42 (−0.07, 0.90)	0.10
Total hip						
Baseline to year 1	193	−2.03 (−2.57, −1.50)	197	−0.49 (−0.87, −0.11)	−1.54 (−2.21, −0.88)	<0.001
Baseline to year 2	169	−2.93 (−3.62, −2.23)	180	−1.30 (−1.90, −0.69)	−1.58 (−2.50, −0.65)	<0.001
Baseline to year 3	51	−3.58 (−4.46, −2.70)	58	−1.94 (−3.14, −0.73)	−1.57 (−3.09, −0.04)	0.05
Overall	194	−2.53 (−3.03, −2.02)	204	−0.99 (−1.48, −0.49)	−1.53 (−2.24, −0.82)	<0.001
Change from year 1 to year 2 (%)	168	−0.97 (−1.45, −0.49)	173	−0.81 (−1.28, −0.34)	−0.11 (−0.79, 0.57)	0.75
Change from year 2 to year 3 (%)	50	0.03 (−0.64, 0.69)	58	−0.96 (−1.58, −0.34)	1.03 (0.11, 1.95)	0.03
Rate of change after year 1 (% per year)		−0.74 (−1.12, −0.36)		−0.84 (−1.20, −0.47)	0.15 (−0.38, 0.68)	0.58
Femoral neck						
Baseline to year 1	193	−2.49 (−3.11, −1.86)	197	−0.62 (−1.15, −0.08)	−1.96 (−2.78, −1.14)	<0.001
Baseline to year 2	169	−3.41 (−4.27, −2.54)	180	−2.02 (−2.78, −1.27)	−1.46 (−2.61, −0.32)	0.01
Baseline to year 3	51	−3.66 (−5.08, −2.25)	58	−2.36 (−3.78, −0.95)	−1.32 (−3.34, 0.69)	0.20
Overall	194	−3.02 (−3.64, −2.40)	204	−1.44 (−2.05, −0.83)	−1.70 (−2.57, −0.83)	<0.001
Change from year 1 to year 2 (%)	168	−1.00 (−1.63, −0.37)	173	−1.46 (−2.08, −0.84)	0.46 (−0.42, 1.35)	0.30
Change from year 2 to year 3 (%)	50	−0.32 (−1.37, 0.74)	58	−0.58 (−1.56, 0.40)	0.25 (−1.21, 1.71)	0.74
Rate of change after year 1 (% per year)		−0.87 (−1.37, −0.38)		−1.23 (−1.70, −0.75)	0.36 (−0.33, 1.06)	0.30

Follow-up was censored at the start of osteoporosis therapy for 2 participants (both in the deferred group, month 24). Mean changes in BMD from baseline to each year were calculated without adjustments, differences at each year were estimated in regression models adjusted for baseline BMD, and overall changes in BMD were estimated and compared using longitudinal mixed models adjusted for both baseline BMD and visit. Rates of changes in spine BMD after year 1 were estimated in longitudinal mixed models using annual BMD changes as response.

treatment arms. Homogeneity of treatment effect was assessed by testing for interaction between the subgroup variable and treatment group indicator in longitudinal mixed models adjusted for baseline BMD and visit. Associations of baseline factors with changes in BMD after ART initiation in the immediate ART group were estimated in longitudinal mixed models. To evaluate the effect of time-updated ART use, the annual percent change in BMD was used as response in longitudinal mixed models, and the subject-specific proportion of follow-up time that specific drugs (or any ART) were used during each year were included as time-updated predictors in the models, along with baseline predictors.

Fracture incidence rates were estimated in the parent START population, and groups were compared using a Cox proportional hazards model. Analyses were performed with SAS version 9.3 (SAS Institute, Cary, NC, USA) and R version 3.[24]

Results

Participant characteristics

The BMD substudy co-enrolled 424 START participants between June 2011 and June 2013. Baseline characteristics of the substudy population have been reported;[15] 25 participants (5.9%) did not have analyzable baseline or follow-up scans and were excluded from analysis (Fig. 1). Table 1 shows the baseline characteristics of the 399 analyzed participants (195 in the immediate group, 204 in the deferred ART groups). The racially diverse population had a median age of 32 years; 26.1% were women; median time since HIV diagnosis was 0.7 years. Forty-five participants (11.3%) had low BMD relative to their age group (Z-score ≤ -2) at the spine, total hip, or femoral neck.

Participants were followed for a mean of 2.2 years. In the immediate group, 95% of participants started ART within 8 weeks of randomization. In the deferred group, 14.7%, 27.9%, and 44.6% had started ART by months 12, 24, and 36, respectively. ART was used for 95% and 18% of cumulative follow-up in the immediate and deferred ART groups, respectively. In the immediate ART group, initial ART contained TDF for 82.8% of participants, efavirenz (EFV) for 78.1%, and a PI for 13.0%. No tenofovir alafenamide was used. Almost all participants on ART had plasma HIV viral load $\leq$200 copies/mL.[14] Two deferred arm participants commenced treatment for osteoporosis after month 12.

Changes in BMD

The mean percent changes in BMD in the immediate and deferred ART groups and the estimated overall mean treatment

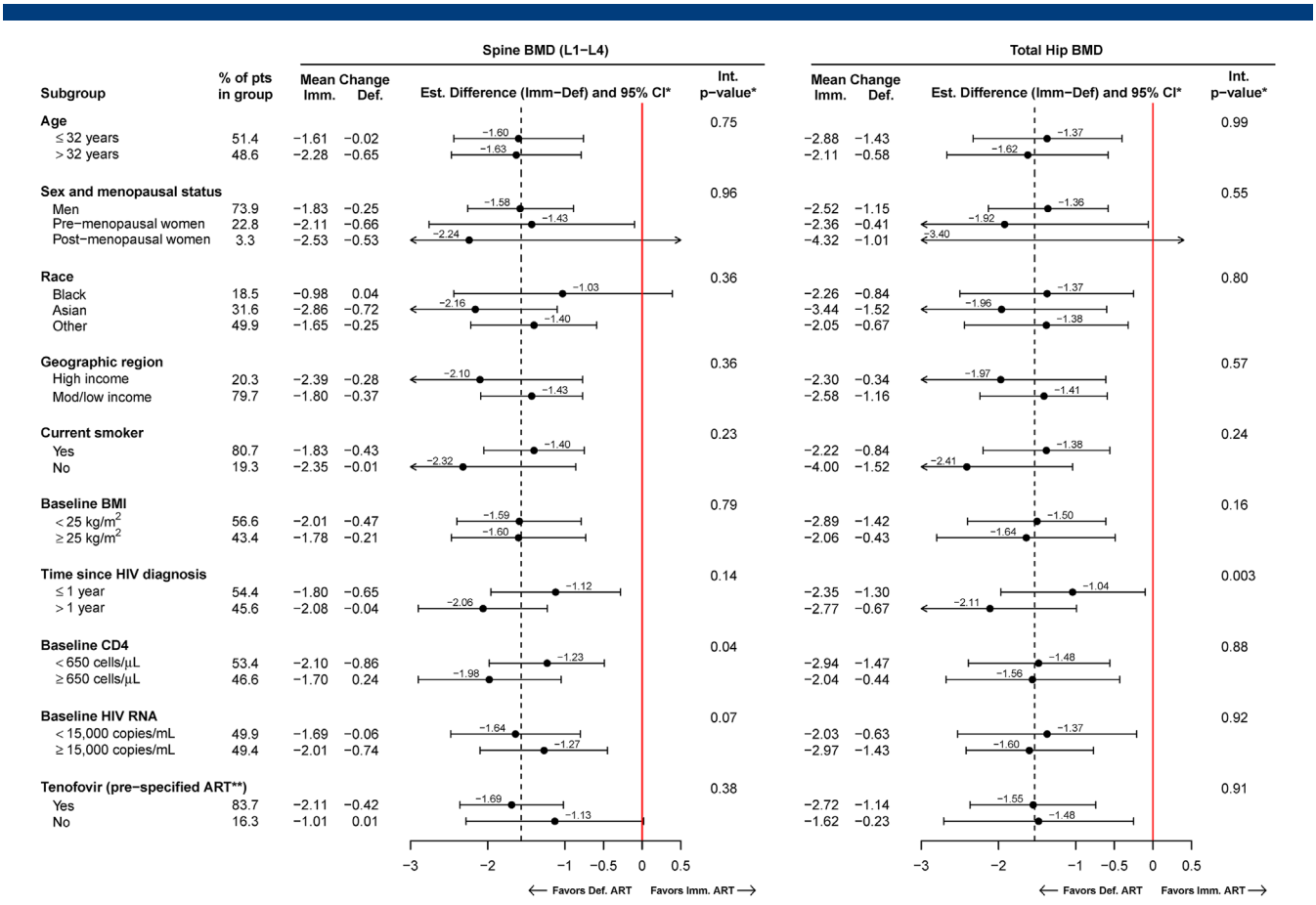


Fig. 3. Subgroup analyses: Mean percent change from baseline and treatment differences (immediate minus deferred ART groups) in spine (L_1 to L_4) and total hip BMD are estimated within subgroups, with 95% confidence intervals. The p values are for tests of heterogeneity of the treatment difference across subgroups. *Estimated in a longitudinal mixed model, adjusted for visit and baseline BMD. The interaction p value for heterogeneity across subgroups was calculated using continuous variables for age, BMI, time since HIV diagnosis, CD4 count, and $\log_{10}$ HIV RNA levels. **In the immediate ART group, a tenofovir-containing ART regimen was selected before randomization ("prespecified") for 162 participants. Of those who were prespecified tenofovir and had the corresponding follow-up scans, 155 (96.3%), 138 (97.2%), and 45 (97.8%) used tenofovir at years 1, 2, and 3, respectively.

differences were: −1.9% versus −0.4% (difference −1.6% [95% CI −2.2 to −1.0]) at the lumbar spine; −2.5% versus −1.0% (difference −1.5% [95% CI −2.2 to −0.8]) at the total hip; and −3.0% versus −1.4% (difference −1.7% [95% CI −2.6 to −0.8]) at the femoral neck (Fig. 2*A*–*C*, Table 2; all $p < 0.001$). Treatment groups differed significantly in BMD decline from baseline to months 12, 24, and 36 for spine and total hip BMD. In the deferred ART group, when censoring follow-up at ART initiation, BMD changed by 0.1%, −0.3%, and −0.6% per year at the spine, hip, and femoral neck, respectively (Fig. 2*D*–*F*, Supplemental Table S1). The differences between the immediate versus deferred groups were evident at year 1 and remained about constant afterwards. Rates of BMD decline were steeper in the immediate ART group at all 3 skeletal sites. In the immediate ART group, spine BMD declined sharply during the first year (by 2.0%) and remained stable afterward. At the total hip and femoral neck, BMD declined during year 1 by 2.0% and 2.5%, respectively, and from year 1 to year 2 by 1.0% and 1.0%, respectively (Table 2, Fig. 2*B*, *C*, Supplemental Table S1). The apparent continued decline in hip BMD after year 2 in Fig. 2 is due to a cohort effect; hip BMD remains about constant after year 2 when restricting the analysis to participants with 3 years of follow-up (Table 2, Supplemental Fig. S1*B*, *C*). After year 1, rates of BMD decline were similar in the immediate and deferred ART groups (Table 2).

In all subgroups, spine and hip BMD declined more in the immediate ART group (Fig. 3). In addition to the subgroups shown in Fig. 3, subgroups were analyzed by baseline BMD, season of enrollment, mode of HIV infection, CD8 count, CD4:CD8 ratio, calcium or vitamin D supplements, recreational drug use, eGFR, and prespecified PI use. Treatment differences were homogenous across all subgroups, except for 2 cases. For spine BMD, the treatment effect was stronger among those with higher baseline CD4 count ($p = 0.04$). For hip BMD, the treatment effect was stronger in those diagnosed with HIV more than 1 year earlier ($p = 0.003$). Notably, there was no evidence for larger differences between the immediate and deferred ART groups in the TDF or PI subgroups; however, power to detect heterogeneity across TDF and PI subgroups was low because of the low sample size in the non-TDF and the PI subgroup categories. Results were similar when restricting follow-up to the first 2 years, except that there was no evidence for a differential treatment effect by baseline CD4 counts (Supplemental Fig. S2).

Predictors of BMD decline

No clinical, demographic, or HIV-related factor was consistently associated with BMD change across both spine and hip in either

Table 3. Factors Associated With Change in BMD in the Immediate ART Group ($n = 190$) (Participants Who Did Not Start ART Within the First Year Were Excluded)

		Spine (L_1 to L_4)		Total hip		Femoral neck	
Factor	*n* (%) in subgroup	Est. coefficient (95% CI)	p^+	Est. coefficient (95% CI)	p^+	Est. coefficient (95% CI)	p^+
Age (per 10 years)		−0.10 (−0.69, 0.50)	0.75	0.56 (−0.19, 1.31)	0.14	0.88 (−0.08, 1.85)	0.07
Sex and menopausal status			0.43		0.49		0.41
Male	138 (72.6%)	Ref.	—	Ref.	—	Ref.	—
Premenopausal female	47 (24.7%)	−0.85 (−2.55, 0.84)	0.32	1.12 (−1.05, 3.29)	0.31	−0.51 (−3.10, 2.07)	0.69
Postmenopausal female	5 (2.6%)	−2.01 (−5.35, 1.34)	0.24	−0.36 (−4.65, 3.94)	0.87	−3.46 (−8.58, 1.66)	0.18
Race			0.03		0.81		0.27
Black	33 (17.4%)	0.60 (−0.97, 2.18)	0.45	0.35 (−1.65, 2.34)	0.73	1.80 (−0.65, 4.25)	0.15
Asian	58 (30.5%)	−1.31 (−2.47, −0.15)	0.03	−0.33 (−1.79, 1.14)	0.66	−0.32 (−2.10, 1.45)	0.72
White/other	99 (52.1%)	Ref.	—	Ref.	—	Ref.	—
Location of enrollment							
Low or medium income (versus high income)	153 (80.5%)	0.84 (−0.43, 2.11)	0.19	0.22 (−1.38, 1.82)	0.78	0.32 (−1.62, 2.27)	0.74
Season of enrollment							
Summer/autumn (versus spring/winter)	92 (48.4%)	0.21 (−0.66, 1.07)	0.59	−0.04 (−1.12, 1.04)	0.94	0.06 (−1.25, 1.37)	0.93
Current smoker	33 (17.4%)	−0.22 (−1.36, 0.92)	0.70	−1.38 (−2.82, 0.07)	0.06	−0.82 (−2.58, 0.94)	0.36
Recreational drug use	21 (11.1%)	−0.68 (−2.09, 0.72)	0.34	2.18 (0.39, 3.97)	0.02	0.62 (−1.54, 2.79)	0.57
BMI (per kg/m^2)		−0.02 (−0.13, 0.09)	0.70	0.01 (−0.13, 0.15)	0.89	0.04 (−0.13, 0.21)	0.62
eGFR (per 1 mL/min/1.73m^2)		0.02 (−0.01, 0.06)	0.21	0.02 (−0.03, 0.06)	0.47	0.02 (−0.03, 0.07)	0.46
Mode of HIV infection			0.83		0.25		0.22
Opposite sex contact	57 (30.0%)	Ref.	—	Ref.	—	Ref.	—
Same sex contact	118 (62.1%)	−0.04 (−1.66, 1.59)	0.96	1.48 (−0.56, 3.53)	0.15	0.98 (−1.48, 3.45)	0.43
Other	15 (7.9%)	0.54 (−1.36, 2.44)	0.57	−0.40 (−2.79, 1.99)	0.74	−1.79 (−4.70, 1.12)	0.23
Time since HIV diagnosis (per year)		0.07 (−0.10, 0.25)	0.39	−0.19 (−0.41, 0.02)	0.08	−0.15 (−0.41, 0.11)	0.27
CD4 count (per 100 cells/μL)		0.07 (−0.22, 0.36)	0.62	0.22 (−0.15, 0.58)	0.25	0.12 (−0.32, 0.57)	0.58
CD8 count (per 100 cells/μL)		−0.06 (−0.15, 0.03)	0.18	0.03 (−0.08, 0.14)	0.61	−0.03 (−0.16, 0.10)	0.64
HIV viral load (per $\log_{10}$)		0.14 (−0.39, 0.68)	0.60	−0.57 (−1.25, 0.11)	0.10	−0.25 (−1.07, 0.57)	0.55
Calcium or vitamin D use	9 (4.7%)	−0.03 (−2.07, 2.01)	0.98	−0.44 (−3.03, 2.15)	0.74	−1.83 (−4.97, 1.30)	0.25
Baseline BMD		1.52 (−1.98, 5.01)	0.39	0.83 (−3.94, 5.60)	0.73	−4.92 (−10.8, 0.91)	0.10

eGFR = estimated glomerular filtration rate.

^+p value for association of the factor with percent change in BMD, estimated in longitudinal mixed models, adjusted for all factors listed and visit.

treatment group (Table 3). In the immediate ART group, being Asian (Table 3), and using PIs in the initial regimen (Table 4) was associated with steeper BMD decline at the spine. At the hip, TDF use was associated with steeper BMD decline (Table 4) and recreational drug use with less decline (Table 3). In a time-updated analysis, no single drug nor drug class was associated with steeper BMD declines (Supplemental Table S2).

In the deferred ART group, only low baseline CD4 cell counts were independently associated with steeper spine BMD decline, by –0.34% per 100 cells/μL lower (Table 5). At the femoral neck, lower baseline body mass index (BMI), calcium and vitamin D use, and higher baseline BMD were independently associated with steeper BMD loss (Table 5).

Incidence of low BMD and fractures

Among participants without low BMD at baseline (Z-score > -2), 22 participants in the immediate ART group developed low BMD at the spine, hip, or femoral neck (Z-score ≤ -2) (rate 6.2 per 100 person-years) compared with 6 (rate 1.4 per 100 person-years) in the deferred ART group, hazard ratio (HR) = 4.7 (95% CI 1.9 to 11.7, $p < 0.001$). For 4 participants (rate 1.0 per 100 person-years) in the immediate ART group, the T-score at the spine, hip, or femoral neck newly declined to ≤ -2.5 compared with 6 (rate 1.3 per 100 person-years) in the deferred group, HR = 0.78 (95% CI 0.22 to 2.76, $p = 0.70$).

Rates of fracture and of fragility fracture were similar between groups in the parent START population. In the immediate ART group, 66 participants (rate of 0.92 per 100 person-years) experienced a new fracture compared with 61 (rate 0.83) in the deferred group, HR = 1.09 (95% CI 0.77 to 1.55, $p = 0.62$). Of these, 17 and 27 fractures occurred with minimal trauma in the immediate and deferred groups, respectively, at rates of 0.23 and 0.37 per 100 person-years, HR = 0.63 (95% CI 0.35 to 1.16, $p = 0.14$).

Discussion

In this diverse population of adults with HIV infection and near-normal CD4 counts, immediate initiation of ART resulted in significantly greater reductions in BMD at the spine and total hip compared with deferred ART. During the first year, participants in the immediate ART group lost 2.0% of BMD at the lumbar spine and total hip. This 2.0% BMD decline is less than that reported in earlier studies of ART initiation (2% to 6% over 2 years)[3,25] but similar to recent studies (0.9% to 3.7% declines in BMD over 1 to 2 years).[26–29] The 2% loss of BMD is similar to the BMD loss found with administration of oral glucocorticoids (0.8% to 3.0%);[30] a BMD loss of 0.5% to 0.7% is associated with a fracture risk 1.5- to 2.4-fold greater than in non-glucocorticoid users.[31] Whether similar BMD declines in ART-treated HIV-infected patients will translate into increased risk of fractures is unknown.

After the first 12 months of ART, BMD remained stable at the spine but continued to decline through the second year at the hip, albeit at a lower rate (by 0.9%). In the deferred ART group, spine BMD remained stable, whereas hip BMD declined at a rate of 0.3% and 0.6% at the femoral neck before initiation of ART. In the general population, the annual decline in BMD at the hip and the lumbar spine for premenopausal women and men less than 50 years is 0.15% to 0.4%.[19,32] Our study population had a median age of 32 years, so stability of spine BMD is to be expected. With no ART, the annual 0.3% to 0.6% BMD decline at the hip suggests a role for untreated HIV. However, the magnitude of the overall steeper BMD decline in the immediate versus deferred ART group suggests that ART is a greater contributor to BMD loss than HIV itself.

The difference in percent change in BMD from baseline between those who started ART in the immediate group and the deferred group before ART start developed in year 1, and remained about constant thereafter, as participants in the deferred ART group gradually initiated ART. At the spine, the BMD loss was largely restricted to the first year of ART. At the hip, BMD continued to decline through the second year of ART use (see Fig. 2 and Supplemental Fig. S1, immediate ART group). Whether ART use causes ongoing BMD loss beyond the first year or two is uncertain, however. Stabilization of BMD after the first 2 years of ART has been demonstrated in clinical trials (usually less than 3 years' duration) and several cohort studies.[33,34] In other studies, BMD continues to decline beyond the first year of ART by approximately 1% per year.[23,25,35] We have previously observed ongoing BMD decline with ART in the SMART study, where hip BMD declined by 0.8% per year for up to 4 years of follow-up in participants who were ART-experienced at study entry, and continued using ART.[23,36] Continued decline in BMD has been confirmed by others, where the rate of BMD decline was steeper during the 2 years after ART initiation compared with HIV-negative people of similar age; although the rate of BMD loss slowed after 2 years, spine BMD loss remained significantly greater compared with the HIV-negative group through 7.5 years of follow-up.[37] Switching ART for virological

Table 4. Association of Specific Antiretroviral Drugs in the First ART Regimen With Percent Change in BMD, Estimated Within the Immediate ART Group ($n = 190$) (Participants Who Did Not Start ART Within the First Year Were Excluded)

		Spine (L_1 to L_4)		Total hip		Femoral neck	
Specific drug in first ART regimen	n (%)	Est. (95% CI)	p^+	Est. (95% CI)	p^+	Est. (95% CI)	p^+
EFV	148 (77.9%)	0.26 (–0.89, 1.42)	0.65	–0.08 (–1.57, 1.41)	0.92	0.13 (–1.67, 1.94)	0.88
TDF	157 (82.6%)	–0.75 (–2.09, 0.58)	0.27	–1.71 (–3.38, –0.03)	**0.05**	–1.88 (–3.90, 0.15)	0.07
PI	25 (13.2%)	–1.79 (–3.05, –0.53)	**<0.01**	–0.70 (–2.37, 0.97)	0.41	–1.13 (–3.14, 0.89)	0.27
NNRTI	156 (82.1%)	0.65 (–0.53, 1.83)	0.28	0.31 (–1.22, 1.83)	0.69	0.35 (–1.50, 2.20)	0.71

EFV = efavirenz; eGFR = estimated glomerular filtration rate; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; TDF = tenofovir disoproxil fumarate.

^+p value for association between use of the drug in the first ART regimen and percent change in BMD, estimated separately for each drug in longitudinal mixed models, which also included age, sex, race, location and season of enrollment, smoking status, alcohol use, drug use, diabetes, BMI, hepatitis B, hepatitis C, previous fracture, eGFR, mode of HIV infection, time since HIV diagnosis, baseline CD4 and CD8, CD4:CD8 ratio, HIV RNA, calcium or vitamin D supplements, baseline BMD and visit. Associations were estimated in separate longitudinal models for each drug. There was no adjustment for multiple comparisons.

failure has also been shown to be associated with another significant fall in BMD of 2% before stabilization again.[38] Given that ART use is lifelong in those with HIV, continued decline of BMD at rates greater than those observed in the general population, if that should be the case, may result in adverse outcomes in the aging HIV population.

There was no difference in fracture rates between immediate and deferred ART groups in the 4684 participants in the parent START study. Whether ART increases fracture risk is unclear, with contradictory results reported from multiple studies. The effect of ART is likely small in women under the age of 50 years and men under 60 years.[39] The young age of our study population may have contributed to the low rate of fractures and lack of treatment group difference.[14]

There was no independent association between baseline CD4 count and change in BMD in the immediate ART group. In contrast, lower CD4 count was a significant predictor of greater BMD loss at both the spine and the hip in the deferred ART group (before any ART). A lower CD4 count and higher HIV viral load have been associated with lower bone mass in cross-sectional studies, suggesting a role for HIV infection or the immunological response to HIV in bone loss.[7,40] Lower pre-ART CD4 counts were associated with greater bone loss after ART initiation in combined ACTG studies but only in those with CD4 counts <50 cells/μL.[8] The observed effect of ART was consistent across demographic, HIV-related, and traditional risk factors for BMD loss. Greater bone loss at the spine after ART initiation in Asians compared with white race participants has not been described previously, and this observation may be spurious. Most studies evaluating change in BMD after ART report greater bone loss with TDF and/or ritonavir-boosted PI-containing ART.[3,27–29] In START, ART type was not mandated, and most participants received TDF and EFV. Although we found that TDF use was associated with greater loss of BMD at the hip and PI use was associated with greater BMD decline at the spine, our study was underpowered to separate the effects of TDF from those of other ART drugs.

The main strengths of our study are the randomized design, the racially diverse study population, and the standardized acquisition and reading of the DXA scans. There are several limitations. First, follow-up is only 2 years for most participants. Longer follow-up of our participants is needed to clarify whether the early BMD loss with ART is sustained over time. Second, only 26% of participants were women, resulting in low power for detecting sex differences. Third, the study was not designed to identify effects of specific drugs. Fourth, our study was not powered to detect a moderate increase of fracture risk because expected fracture rates would be very low in this young population. Finally, some of the factors identified as associated with change in BMD may be false positives; because of the high number of predictors, our results need to be interpreted with caution. If the 16 predictors were independent, the chance of

Table 5. Factors Associated With Change in BMD in the Deferred ART Group, With Follow-up Censored at ART Initiation ($n = 175$)

Factor	*n* (%)	Spine (L_1 to L_4)		Total hip		Femoral neck	
		Est. (95% CI)	p^+	Est. (95% CI)	p^+	Est. (95% CI)	p^+
Age (per 10 years)		−0.53 (−1.11, 0.04)	0.07	0.24 (−0.32, 0.80)	0.39	0.39 (−0.40, 1.19)	0.33
Sex and menopausal status			0.41		0.29		0.39
Male	127 (72.6%)	Ref.	—	Ref.	—	Ref.	—
Premenopausal female	40 (22.9%)	−0.94 (−2.36, 0.48)	0.19	0.64 (−0.75, 2.04)	0.36	−0.12 (−2.07, 1.84)	0.91
Postmenopausal female	8 (4.6%)	−0.88 (−3.19, 1.43)	0.45	−0.89 (−3.05, 1.27)	0.42	−2.05 (−5.16, 1.06)	0.19
Race			0.56		0.44		0.76
Black	34 (19.4%)	0.58 (−0.65, 1.80)	0.35	−0.53 (−1.71, 0.65)	0.38	0.66 (−1.09, 2.41)	0.46
Asian	59 (33.7%)	−0.07 (−1.17, 1.03)	0.90	0.27 (−0.77, 1.31)	0.61	0.26 (−1.22, 1.75)	0.73
White/other	82 (46.9%)	Ref.	—	Ref.	—	Ref.	—
Location of enrollment							
Low or medium income (versus high income)	139 (79.4%)	0.13 (−1.07, 1.32)	0.83	0.02 (−1.12, 1.16)	0.98	−0.07 (−1.67, 1.53)	0.93
Season of enrollment							
Summer/autumn (versus spring/winter)	80 (45.7%)	−0.04 (−0.86, 0.77)	0.91	−0.01 (−0.78, 0.76)	0.98	−0.20 (−1.30, 0.90)	0.72
Current smoker	39 (22.3%)	0.19 (−0.85, 1.23)	0.72	−0.63 (−1.62, 0.36)	0.21	−0.63 (−2.03, 0.78)	0.38
Recreational drug use	20 (11.4%)	0.04 (−1.24, 1.32)	0.95	0.63 (−0.58, 1.83)	0.31	−0.44 (−2.16, 1.28)	0.61
BMI (per kg/m^2)		−0.01 (−0.12, 0.10)	0.87	0.05 (−0.06, 0.16)	0.36	0.15 (0.00, 0.30)	**0.04**
eGFR (per 1 mL/min/1.73m^2)		0.00 (−0.02, 0.03)	0.84	0.01 (−0.02, 0.03)	0.45	−0.00 (−0.04, 0.03)	0.80
Mode of HIV infection			0.39		0.21		0.70
Opposite sex contact	66 (37.7%)	Ref.	—	Ref.	—	Ref.	—
Same sex contact	98 (56.0%)	−0.47 (−1.83, 0.89)	0.50	0.76 (−0.57, 2.08)	0.26	0.58 (−1.28, 2.44)	0.54
Other	11 (6.3%)	−1.26 (−3.11, 0.58)	0.18	1.50 (−0.26, 3.25)	0.09	−0.42 (−2.91, 2.07)	0.74
Time since HIV diagnosis (per year)		0.07 (−0.05, 0.19)	0.25	0.06 (−0.05, 0.18)	0.29	−0.09 (−0.26, 0.07)	0.25
CD4 count (per 100 cells/μL)		0.34 (0.04, 0.64)	**0.03**	0.19 (−0.09, 0.48)	0.18	0.39 (−0.01, 0.80)	0.06
CD8 count (per 100 cells/μL)		−0.08 (−0.16, 0.01)	0.09	−0.03 (−0.12, 0.05)	0.42	−0.05 (−0.17, 0.07)	0.41
HIV viral load (per $\log_{10}$)		−0.22 (−0.74, 0.30)	0.40	−0.09 (−0.59, 0.40)	0.71	0.25 (−0.44, 0.95)	0.47
Calcium or vitamin D use	13 (7.4%)	−0.20 (−1.84, 1.44)	0.81	−1.00 (−2.53, 0.53)	0.20	−2.62 (−4.80, −0.44)	**0.02**
Baseline BMD		1.33 (−2.18, 4.84)	0.45	2.03 (−1.66, 5.72)	0.28	−6.57 (−11.6, −1.53)	**0.01**

eGFR = estimated glomerular filtration rate.

^+p value for association of the factor with percent change in BMD, estimated in longitudinal mixed models, adjusted for all factors listed and visit.

observing one or more p values ≤ 0.05 would be at least 56% for each of the BMD outcomes.

In summary, immediate initiation of ART at high CD4 cell counts compared with deferring ART results in accelerated bone loss at the spine and hip, which may stabilize after a year or two. All key ART guidelines now recommend ART initiation at HIV diagnosis regardless of CD4 cell count because of the reduced risk of serious clinical outcomes relative to deferring ART. Although the START Bone Mineral Density substudy revealed an adverse effect of immediate ART, the overall benefits of ART for prevention of HIV transmission and adverse health outcomes prevail. It will be important to understand the longer-term consequences of the observed reductions in BMD and whether these reductions continue or stabilize with longer therapy.

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