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The association of fat and lean tissue with whole body and spine bone mineral density is modified by HIV status and sex in children and youth

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Abstract

Background—HIV-infected (HIV-pos) male children/youth showed lower bone mineral density at sexual maturity than HIV-uninfected (HIV-neg) **females.** It is not known whether complications of HIV disease, including abnormal body fat distribution, contribute to lower bone accrual in male HIV-pos adolescents.

Methods—In a cross-sectional study, we evaluated the relationship between body composition (fat and lean mass) and bone mass in HIV-pos and HIV-neg children/youth and determined if it is modified by HIV status and sex. We used generalized estimating equations to simultaneously model the effect of fat/lean mass on multiple bone outcomes, including total body bone mineral density (BMD) and bone mineral content (BMC), and spine BMD. We evaluated effect modification by HIV and sex.

Results—The analysis cohort consisted of 143 HIV-neg and 236 HIV-pos, of whom 55% were black Non-Hispanic and 53% were male. Ages ranged from 7 to <25 years. Half of the children/

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Author's contributions:

Denise Jacobson – Project conception and development of overall research plan, provided input on analysis, interpreted analysis, wrote paper, and approved final paper

Jane Lindsey – Project conception and development of overall research plan, analyzed data, interpreted analysis, contributed to writing of paper, and approved final paper

Brent Coull – Development of overall research plan, advised on statistical analysis and interpretation, provided input to paper and approved final paper

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youth were at Tanner stage 1 and 20% at Tanner 5. Fat mass was more strongly positively correlated with bone mass in HIV-neg than HIV-pos children/youth and these relationships were more evident for total body bone than spine outcomes. Within HIV strata, fat mass and bone were more correlated in female than male children/youth. The relationship between lean mass and bone varied by sex, but not by HIV status.

Conclusions—HIV disease diminishes the positive relationship of greater fat mass on bone mass in children/youth. Disruptions in body fat distribution, which are common in HIV disease, may have an impact on bone accretion during pubertal development.

Keywords

Perinatal HIV-infection; children/youth; DXA; bone-fat interactions

Introduction

Life expectancy of perinatally HIV-infected (HIV-pos) children has increased dramatically since the introduction of combination antiretroviral (ARV) therapy. However, cumulative effects of chronic HIV infection and prolonged exposure to ARVs during childhood may impact adult health. Inadequate bone accrual during adolescence may decrease bone strength and increase risk of fracture later in life. [1] Numerous studies report lower bone mineral density (BMD) in HIV-pos relative to HIV-uninfected (HIV-neg) children/youth. [2–4] We previously showed that male HIV-pos children/youth were more affected than females. [5] In two other studies, BMD was lower in HIV-pos than HIV-neg males, no differences were seen in females[6] [7], and BMD was lower in HIV-pos males than HIV-pos females.[7]

Bone, muscle and adipose tissue have complex functional interactions. Skeletal muscle is a major source of anabolic stimulus for bone tissue, [8] and leptin, secreted by adipocytes, modulates bone formation and bone resorption, and may be influenced by sex hormones. Bone mass generally increases with body weight, although obese children/adolescents have lower BMD than expected given their weight. [9] Fat and lean mass have differential effects on bone in males and females [10–13], and varies by bone site (appendicular versus axial). [11, 13] In perinatally HIV-infected children, percentage total body fat and trunk fat are lower in males than females, but percentage extremity fat and trunk-to-extremity fat ratio do not differ by sex.[14] Given lower bone mass in HIV-pos male children/youth [5] and effects of HIV and ARVs on body composition, [14–17], we explored how fat and lean mass correlated with total body and spinal bone mass and whether these relationships were modified by HIV status and sex. We hypothesized that body fat would be more correlated with bone in HIV-pos female than male children/youth.

Subjects and methods

Participants

In this US-based cross-sectional study, we selected a representative sample of HIV-pos children/youth 7 to <25 years across six strata defined by protease inhibitor use/nonuse and Tanner stage (Tanner 1, 2–3, 4–5) [5, 16, 18]. For comparison, we enrolled 50 HIV-neg 7-<25 year-olds into the three Tanner strata with sex and race/ethnicity distributions similar to the

HIV-pos. Participants >18 years old provided informed consent. Those < 18 provided assent and their parent/guardian provided informed consent. We received approval from the institutional review board at each site.

Measurements

Clinical—Tanner stage was determined by inspection by certified pediatric medical practitioners who regularly assess growth and development in HIV-pos children/youth. For females it was the most advanced stage for breasts and pubic hair, and for males, the most advanced of genitalia and pubic hair. [5, 16] Growth velocity is greatest at Tanner 3 and 4, so Tanner stage was categorized as Tanner 1–2, 3–4 and 5.

Dual energy X-ray Absorptiometry (DXA)—DXA scans were performed on the whole body to assess total body with head BMD (TB-BMD) and bone mineral content (TB-BMC) and on the lumbar spine to assess spinal BMD (SP-BMD) of L1-L4, using Hologic QDR 4500, Delphi or Discovery (Hologic, Waltham, MA) or Lunar DPX, DPXL or Prodigy (GE Medical Systems, Madison, WI) machines. Fat and lean mass (body composition) measures included total body fat mass (TBF), trunk fat (TrF), extremity (arms and legs) fat (ExF) and extremity lean mass (ExL). The Body Composition Analysis Center at Tufts University School of Medicine analyzed all scans to standardize across machines and clinical sites. [5, 16] Results were available on 379 of 386 enrollees (four were not ambulatory, two were too heavy for the machine and one had no results).

Age/sex-adjusted Z-scores—Z-scores were calculated for fat, lean and bone measures using the HIV-neg cohort as the reference group [16]. We used our HIV-neg cohort because there were no published norms in HIV-uninfected children/youth that included BMD, fat and lean mass for both Hologic and Lunar machines. [19]. Spine bone mineral apparent density (SP-BMAD) was calculated [20] to estimate volumetric BMD.

Outcomes/Predictors—Our goal was to evaluate the relationship between body composition and bone outcomes and determine if these relationships were modified by sex and HIV status. The *four bone outcomes* were TB-BMD, TB-BMC, SP-BMD and SP-BMAD. The main *predictors* were the four body composition measures (TBF, TrF, ExF, ExL). Extremity lean mass was an index of skeletal muscle mass [21]. The effect modifiers were sex and HIV status. Four groups of sex by HIV status were established (color and line pattern for graphs): HIV-neg females (blue solid line), HIV-neg males (blue dotted line), HIV-pos females (red solid line) and HIV-pos males (red dotted line).

Statistical analysis

Mean z-scores—Mean z-scores for each body composition/bone outcome were plotted for each group of sex by HIV status across Tanner stages 1–2, 3–4 and 5.

Generalized estimating equations (GEEs) for multiple outcomes—To increase statistical power and test whether the relationship between body composition and bone was the same across bone outcomes, we fit GEEs [22–25] jointly modeling the effects of body composition, HIV status and sex on the four bone outcomes. Models were adjusted for race

(Black non-Hispanic, Hispanic and White/Other) and Tanner stage (1–2, 3–4, 5). Age, height and type of DXA machine (Lunar versus Hologic) were not included because conclusions were similar without them.

Separate models were fit for each body composition measure. For each model, there was one record for each bone type for each participant. Backwards stepwise regression was used to find the most parsimonious model, starting with all main effects (race/ethnicity, Tanner stage, sex, HIV status, body composition, bone) and higher order interactions between sex, HIV status, body composition and bone type (See Text, Supplemental Digital Content 1). With four bone outcomes and four combinations of HIV and sex, there were 16 possible slopes in a saturated model (including 2, 3 and 4-way interactions). Higher order interactions were dropped sequentially until only interactions significant at the $P<0.10$ level remained. All lower order interactions and main effects contained within the highest interaction were kept in the model.

Final models focused on interpreting differences in slopes (correlations between body composition measures and bone outcomes) across sex and HIV status and across bone outcomes, using the highest order interaction term(s) containing body composition. For example, for TBF*sex*bone, the interpretation would be that the difference in slopes of TBF on bone between males and females varied across bone outcomes. Results were presented graphically using predicted lines (for a Tanner 1–2 Black non-Hispanic child/youth) for each sex by HIV group. We did not adjust for multiple comparisons and analyses were exploratory and hypothesis-generating. Analyses were done in SAS 9.2 (SAS Institute, Cary, North Carolina, USA).

Results

Population characteristics

Approximately half the HIV-pos and HIV-neg children/youth were male and Black Non-Hispanic, with 20% at Tanner stage 5 and 60% participating in some regular exercise. Median age of the cohort was approximately 12.0 years with the HIV-pos slightly older than the HIV-neg children/youth (Table 1). HIV-neg children had higher weight and height z-scores than the HIV-pos (Table 1). Few HIV-pos children were severely immunosuppressed and approximately half had undetectable viral load. Almost all HIV-pos children were receiving NRTIs (98%), and 37% and 67% were receiving NNRTIs and PIs, respectively. The percent receiving specific antiretrovirals is shown in Table 1.

Mean z-scores for body composition and bone

Figure 1 shows mean z-scores for each sex by HIV group (colored lines) across Tanner stage. The first row contains each fat and lean measure and the second row each of the four bone measures. For all three fat measures (row 1 columns 1–3), mean z-scores were similar between males and females within each HIV group. Fat z-scores were lower for HIV-pos compared to HIV-neg at Tanner stages 1–4, but converged at Tanner 5. For extremity lean mass (row 1 column 4), HIV-neg males and females tracked together across Tanner stages. HIV-pos males were lower at earlier Tanner stages than HIV-neg, but there were no

differences at Tanner 5 by HIV or sex. For all bone outcomes (row 2), HIV-neg and HIV-pos had similar mean levels at Tanner 1–2, but HIV-pos tended to be lower at subsequent Tanner stages, with the lowest levels in HIV-pos males.

GEE models

Raw data are illustrated in in the Supplemental Digital Content (Figure 1). Final GEE models are summarized in Table 2. All models had at least one three-way interaction that included fat or lean. All models with fat as the predictor included a three-way interaction term for fat*HIV*bone, and TBF and ExF included a two way interaction for fat*sex (at $P < 0.10$). The model with ExL included a term for lean*sex*bone.

The models in Table 2 are illustrated in Figure 2. Each column represents one body composition predictor with a row for each bone outcome. Each cell shows the predicted lines (slope) of the relationship between each bone z-score (Y-axis) and body composition z-score (X-axis) for each sex by HIV group. Interpretation of the higher order interactions is helped by mentally stacking the four graphs for each bone outcome on top of one another (collapsing across rows). If there were no differences in how sex, HIV, and body composition were related to the four bone outcomes, the stacked graphs could be reduced to one common line. If HIV, but not sex, modified the relationship of a body composition measure on a bone outcome, the predicted lines for males (dotted lines) and females (solid lines) would be parallel within HIV status. If a body composition variable was not correlated with a bone outcome, the four lines would be flat (slope=0).

Fat and bone

The impression from columns 1–3 of Figure 2 was that across all three fat measures, the pattern of the four slopes was similar for TB-BMD and TB-BMC, but differed from the relatively similar patterns for SP-BMD and SP-BMAD. However interactions with bone remained statistically (if not clinically) significant in models including only TB-BMD and TB-BMC. The same was true with the spine outcomes.

Fat z-scores influenced bone more in females than males as shown by the steeper positive slopes in females (solid lines) compared to males (dotted lines) for all bone outcomes. There was a term for fat*sex, but no term for fat*sex*bone in each fat model (Table 2).

The relationship between fat and bone also differed by HIV status (fat*HIV*bone included in all fat models, Table 2). This is visualized by averaging across blue lines (HIV-neg) and then red lines (HIV-pos) and comparing the red-blue differences across bone outcomes. For all fat measures predicting TB-BMD and SP-BMD (row 1 and row 3), and for TrF predicting SP-BMAD (row 4 column 2), slopes for the HIV-neg were steeper than for the HIV-pos, with larger differences in slopes for TB-BMD compared to spine. This suggests that HIV infection diminishes the benefit of higher fat levels on bone health, particularly for these bone outcomes. For TBF and ExF predicting TB-BMC and SP-BMAD, the HIV-pos slopes were steeper than HIV-neg, although the size of the differences in slopes was small.

Lean and bone

The relationship between ExL and bone was different from that observed for fat when comparing patterns in the fourth column of Figure 2 to the first three columns. For extremity lean, the four sex by HIV lines were closer to being parallel for all bone outcomes than was observed for the fat measures. As with fat, patterns for lean were more similar for BMD and BMC and distinct from patterns for the two spine outcomes. The differences in slopes between males and females differed significantly across bone types (ExL*sex*bone, Table 2), visualized by comparing lines formed by averaging across solid lines (females) and dotted lines (males). For TB-BMD and the two spine outcomes, females had steeper slopes than males. However for TB-BMC, this pattern was reversed, with the slope for males being slightly steeper, although differences were small. HIV infection was not a moderator of the relationship of lean on any bone outcome (no ExL*HIV*bone or ExL*HIV in the final model, Table 2).

Discussion

In a previous analysis, we observed lower BMD at sexual maturity in HIV-pos compared to HIV-neg males.[5] A similar trend in girls was non-significant and less pronounced. We wished to explore possible explanations related to body composition. During puberty, females accumulate fat at the hips and thighs while males experience reductions in extremity fat and rapid gains in lean body mass. [8, 26]

HIV-pos children/youth have lower total and extremity fat and higher trunk-to-extremity fat ratios than healthy children/youth. [14, 16] [27] We hypothesized that the relationship between body composition and bone might vary by HIV status and sex. Both fat and lean mass had a larger positive correlation with whole body bone than with spine. Fat mass was also more positively correlated with bone in females than male children/youth and HIV diminished the positive effect of fat on bone in both sexes. It is possible that the diminished relationship between fat and bone in HIVpos children/youth reflects the differential effects of certain ARVs on fat and bone mass. For example, thymidine analogue NRTIs are specifically associated with subcutaneous fat loss.[28] On the other hand, tenofovir disoproxil fumarate is associated with greater bone loss than NRTIs but there is little evidence of effects on fat.[29] The direct or indirect involvement with other classes of ARVs in fat and bone changes are less clear. The effect of lean mass on bone was modified by sex, but not by HIV infection. Greater lean mass was associated with higher bone mass in both sexes, but was somewhat more related in females than males and the relationship was stronger for whole body bone than spine.

Our finding that total body fat and extremity fat mass were more correlated with bone outcomes in female compared to male children/youth has precedent in healthy children/youth. Lean mass has a greater effect than fat mass on bone, and the effect of fat on bone may be greater in girls than boys. [10, 11] Regional lean mass and weight are associated with regional BMC, but obesity status is not correlated with BMC for any region. Furthermore, fat and extremity lean are positively associated with both TB-BMD and SP-BMD in healthy girls, but only extremity lean is associated with BMD in boys. [13]

In our study, strength of the association between fat and bone was stronger in HIV-neg compared to HIV-pos children/youth in both males and females, perhaps explained by the lower weight in HIV-pos children/youth. It was weakest in HIV-pos males. In healthy children, earlier age at pubertal onset is associated with higher BMC and BMD at skeletal maturity, independent of height and bone mineralization at pubertal onset and length of puberty. [30] Peak increases in lean mass [31] and height [32] precede rapid acceleration of bone mineral accretion or at least coincide during the pubertal growth spurt. [33] The velocity of fat increase is greater in girls than boys during this period. [33] In HIV-pos children, abnormal fat patterns [14] [16], later pubertal onset [16, 34], especially in boys, and reduced linear growth [14] and accrual of fat and lean mass before sexual maturation may have had a negative impact on bone mineralization. Alterations in fat and bone metabolism may share a common etiology, such as inflammation [35, 36], differentiation of mesenchymal stem cells into osteocytes and adipocytes,[37] or hormonal dysregulation, [38] each of which may be disrupted by HIV and/or ARVs.[39]

While lean mass accrual occurred later in HIV-pos children/youth in our study, the relationship between lean mass and bone did not differ by HIV status, only by sex. For a given lean mass z-score, the bone z-score did not differ from HIV-neg children/youth. In healthy adolescent girls, bone size, mass and density may accrue at different times across different body sites [32], perhaps explaining differences we observed between total body and spine outcomes. Pollock et al showed that fat mass is negatively correlated with cortical BMD, but not associated with trabecular BMD.[40]

BMD is lower in HIV-uninfected obese children who have greater amounts of visceral fat, despite positive associations between weight and BMD. [9, 41] While we found similar relationships for all three fat measures across bone outcomes, DXA cannot distinguish between visceral and subcutaneous abdominal fat, possibly obscuring a true relationship between visceral fat and bone.

We used multiple outcome GEE models that pooled evidence across outcomes. This increased power to detect associations but still allowed estimation of outcome-specific slopes. Although robust to miss-specification of the correlation structure between outcomes and to distributional assumptions, there are no established goodness-of-fit tests for GEE models, and nested models cannot be directly compared using likelihood-based methods. We used Wald tests to find the most parsimonious models and retained interactions with significance levels of $P < 0.10$ to avoid missing potentially meaningful associations. Rather than focusing on parameter estimates, we used graphical methods to look for patterns in associations. This approach was admittedly subjective, but this was an exploratory analysis on a relatively small dataset with a lot of covariates, and the graphs ‘told the story’ better than tables of numbers.

Our study was cross-sectional. While this limited our ability to establish temporal relationships between fat and lean mass on bone accrual, a study in healthy children shows that BMD level relative to peers tends to track over time. [42] Many of our HIV-pos participants were born prior to use of ARVs in children and are survivors, limiting generalizability to current adolescents with HIV. Finally, while Tanner stage is critical to

understanding bone accumulation, our data were too sparse to stratify by sex, HIV and Tanner stage.

Peak bone mass is influenced by genetic potential, clinical and environmental factors. We showed that the relationship between fat mass and bone varied by HIV status and sex, with the weakest relationship in HIV-pos males. Prolonged exposure to HIV, ARVs, and HIV-related illnesses can disrupt metabolic pathways and may have affected bone accrual in these HIV-pos children, at least partially through disruptions in body composition. To further understand the underlying mechanisms behind these complex interactions, we should focus our efforts on studying factors that modulate both fat and bone metabolism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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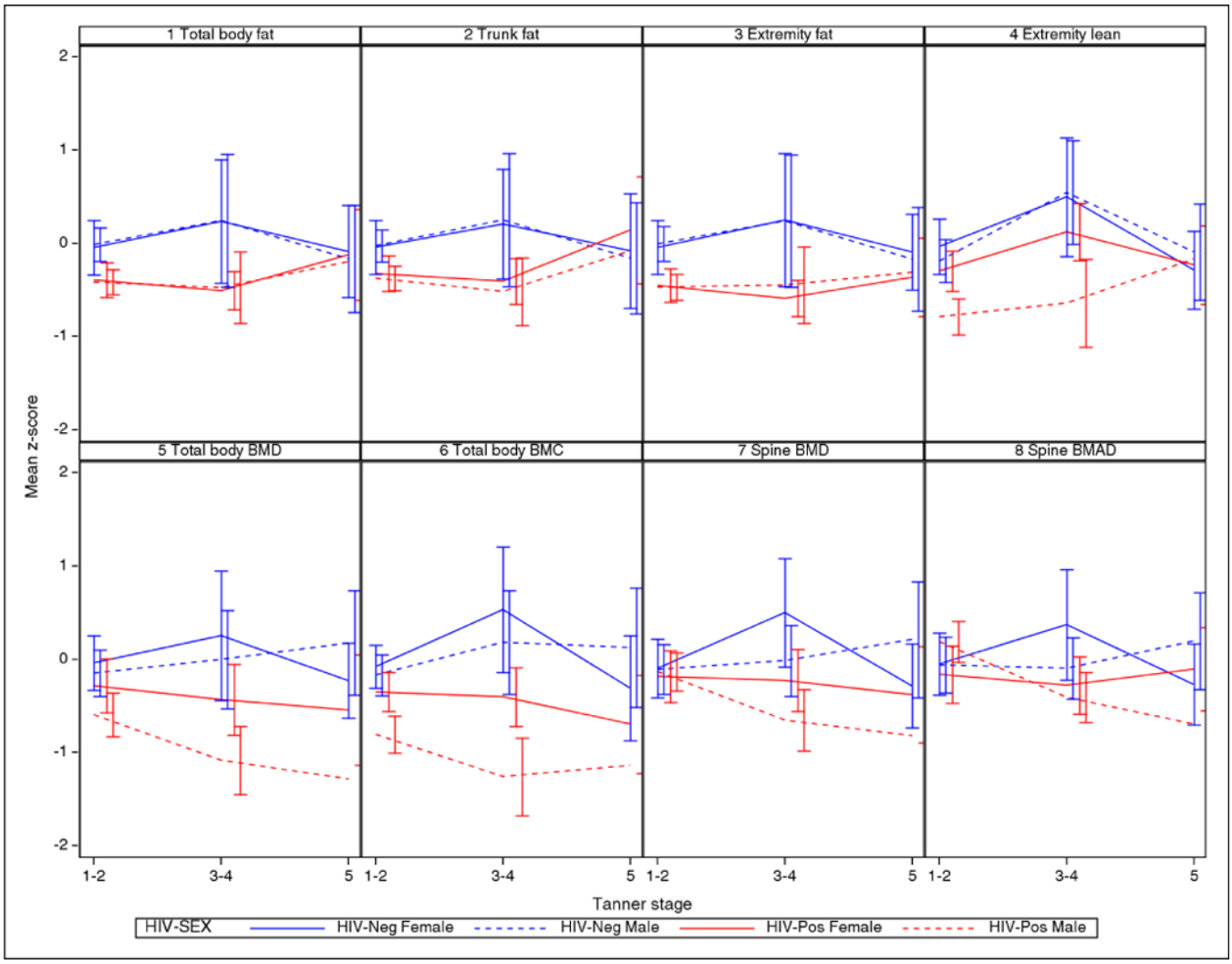

Figure 1.

Fat and bone z-scores by HIV status sex and Tanner stage (Lines: Females-solid Males-dashed HIV status: Negative-blue Positive-red)

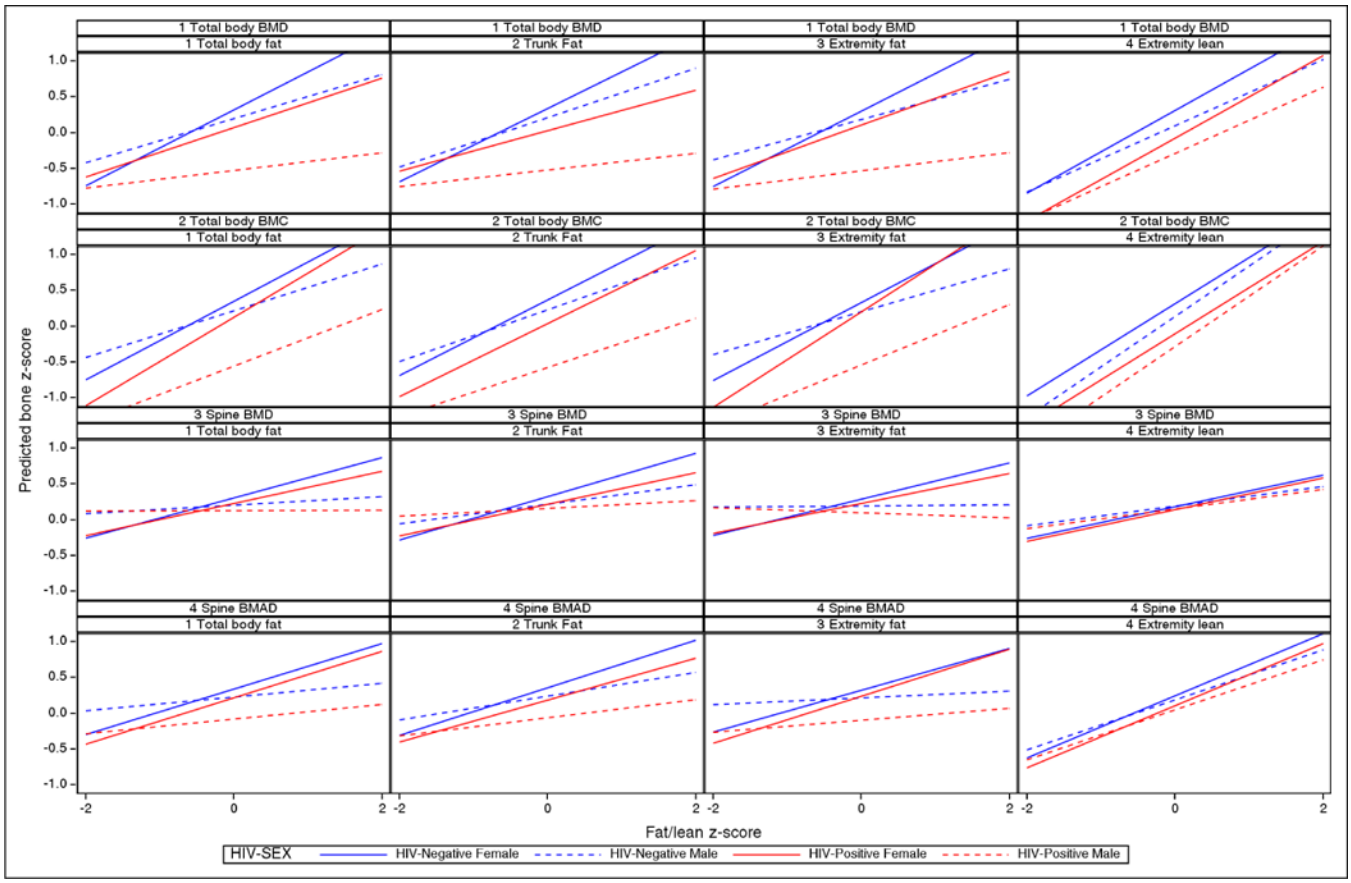

Figure 2.
Predicted bone outcomes by HIV status and sex

Table 1

Characteristics of participants by HIV status

Characteristic		HIV status	
		Negative (N=143)	Positive (N=236)
Sex	Male	83 (58%)	124 (53%)
	Female	60 (42%)	112 (47%)
Race/ethnicity	White Non-Hispanic/Other	20 (14%)	31 (13%)
	Black Non-Hispanic	78 (55%)	129 (55%)
	Hispanic	45 (31%)	76 (32%)
Tanner group	1–2	82 (57%)	117 (50%)
	3–4	33 (23%)	71 (30%)
	5	28 (20%)	48 (20%)
Age (years)	Median (Min, Max)	11.9 (7.1, 24.9)	12.6 (7.1, 22.8)
Weight z-score	Median (10^{th}, 90^{th}%le)	−0.2 (−1.0, 1.1)	−0.5 (−1.3, 0.5)
Height z-score	Median (10^{th}, 90^{th}%le)	0.1 (−1.3, 1.3)	−0.5 (−1.9, 0.8)
Exercise regularly	No	57 (42%)	98 (42%)
	1–6 hrs/week	41 (30%)	54 (23%)
	1–3 hrs/day	31 (23%)	46 (20%)
	>3 hrs/day	7 (5%)	36 (15%)
CD4 count (cells/mm^3)	<200		6 (3%)
	200 – < 500		61 (26%)
	≥ 500		164 (71%)
CD4%	<15%		14 (6%)
	15 – < 25%		49 (21%)
	≥ 25%		168 (73%)
HIV-1 RNA (copies/ml)	≤ 400		128 (56%)
Antiretroviral use	Any NRTI		232 (98%)
	Lamivudine (3TC)		122 (52%)
	Stavudine (d4T)		112 (48%)
	Zidovudine (ZDV)		78 (33%)
	Didanosine (ddI)		77 (33%)
	Tenofovir (TDF)		14 (6%)
	Other NRTI [a]		45 (19%)
	Any NNRTI		87 (37%)
	Efavirenz (EFZ)		53 (23%)
	Nevirapine (NVP)		34 (14%)
	Any PI		158 (67%)
	Nelfinavir (NFV)		63 (27%)
	Kaletra (KAL)		50 (21%)
	Ritonavir (RTV)		42 (18%)
	Other PI [b]		32 (14%)

[a] Includes abacavir (n=24), ddC (n=1) and others (n=9)

[b] Includes saquinavir (n=15), indinavir (n=5), amprenavir (n=7) and others (n=8)

Table 2

Final GEE models evaluating relationship of bone z-score with body composition z-scores by sex and HIV status and adjusted for Tanner stage and race/ethnicity

	Body composition predictor			
Main effects and interactions[1]	**Model 1: Total Body fat**	**Model 2: Trunk fat**	**Model 3: Extremity fat**	**Model 4: Extremity lean**
Main effects	P value	P value	P value	P value
Bone[2]	0.001	0.001	0.001	0.001
Tanner	0.061	0.043	0.089	0.001
Race/ethnicity	0.001	0.001	0.001	0.004
Fat/lean	0.001	0.001	0.001	0.001
HIV status	0.001	0.001	0.004	0.005
Sex	0.004	0.007	0.003	0.188
Two-way interactions				
Fat/lean*HIV	0.701	0.390	0.931	–
Fat/lean*Sex	0.039	0.110	0.028	0.526
Fat/lean*Bone	0.001	0.001	0.001	0.001
HIV*Bone	0.001	0.001	0.001	0.001
Sex*Bone	0.006	0.011	0.003	0.030
HIV*Sex	0.097	0.173	0.060	–
Three-way interactions				
Fat/lean*Sex*Bone	–	–	–	0.016
Fat/lean*HIV*Bone	0.068	0.089	0.066	–
HIV*Sex*Bone	0.017	0.026	0.010	–

[1] fat/lean – In models 1–3 the term is fat and in Model 4 the term is lean.

[2] bone – TB-BMD, TB-BMC, SP-MBD or SP-BMAD

Abbreviations: Bone, bone type; Tanner, Tanner group; fat/lean, fat/lean z-score; HIV, HIV status