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Original Article

Hylonome

Bone geometry and strength are adapted to muscle force in children and adolescents perinatally infected with HIV

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Abstract

Objectives: To determine if bone health is compromised in perinatally HIV-infected youth. **Methods:** We assessed BMC at the proximal femur, lumbar spine and total body using DXA in perinatally HIV-infected youth (n=31; 9-18y). Using pQCT, we assessed muscle CSA, total and cortical bone area, cortical BMD and thickness and strength strain index at the tibial shaft. Thirty and 18 participants returned at 12- and 24-months, respectively. We calculated age- and sex-specific z-scores for the HIV-infected youth using data from a healthy cohort (n=883; 9-18y). **Results:** At baseline, height and MCSA were reduced in HIV-infected youth (-0.79 to -0.23, $p<0.05$). BMC z-scores adjusted for height and lean mass were lower than controls at all sites except the lumbar spine (-0.57 to -0.27, $p<0.05$). Bone area and strength z-scores were not different from zero after adjusting for tibial length and MCSA. In contrast, cortical BMD z-scores were greater in HIV-infected youth (0.46, $p=0.011$). Z-scores for all bone outcomes showed positive trends over time in HIV-infected youth. **Conclusion:** Although HIV infection may be associated with bone mass deficits during growth, bone geometry and strength appear adapted to muscle force. Further, deficits in bone mass may dissipate over time in this population.

Keywords: HIV, Bone Strength, peripheral QCT, Children

Introduction

Highly active antiretroviral therapy (HAART) has dramatically improved the life expectancy of human immunodeficiency virus (HIV)-infected individuals; perinatally-infected children are now growing well into adolescence and adulthood[1-5]. However, as these youth survive into adulthood with chronic HIV infection, lifelong HAART and persistent inflammation may be associated with the development of new complications such as central nervous system and metabolic abnormalities, increased risk of cardiovascular disease, renal disease and disturbances in bone metabolism[6].

Low bone mineral content (BMC, grams) and/or areal bone mineral density (aBMD, g/cm^2), as measured with dual energy X-ray absorptiometry (DXA), has been reported in several studies of children and adolescents infected with HIV[7-12]. These bone mass deficits are likely associated with a number of disease-related factors including increased levels of proinflammatory cytokines, delayed growth and puberty, low muscle mass, endocrine disruptions, vitamin D deficiency and decreased levels of physical activity[8,12,13]. In addition, treatment with antiretrovirals, particularly tenofovir and protease inhibitors, was associated with lower bone mass in previous studies of HIV-infected children and youth[14,15]. Compromised bone mass is of particular concern when present during the period of rapid adolescent growth and peak bone mineral accrual; failure to achieve optimal bone mass and strength during this critical window may further increase the risk for osteoporosis and related fracture later in life[16,17].

The authors have no conflict of interest.

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Currently, we know little about the potential impact of HIV infection and disease-related factors on bone cross-sectional geometry and cortical and trabecular BMD, which are also determinants of whole bone strength. Planar DXA measures of BMC and aBMD do not capture such 3-dimensional (3D) characteristics, and are unable to separate cortical and trabecular bone compartments. Further, DXA's inability to account for bone size is of concern when measuring the growing skeleton, particularly when there may be impaired growth or delayed maturation in a clinical population[18,19]. Thus, conclusions regarding deficits in BMC or aBMD in previous studies of HIV-infected youth may be confounded by patients' shorter stature and smaller bone size. This is highlighted by results from a computed tomography (CT) study that found reduced lumbar spine aBMD (by DXA) in HIV-infected patients aged 5-19 yrs compared with healthy controls, but no difference in 3D measures of vertebral BMD (by CT) between HIV-infected youth and healthy controls[20].

Thus, imaging tools that capture a more comprehensive picture of bone health in HIV-infected youth are needed. Peripheral quantitative CT (pQCT) is a safe and accurate tool for measuring BMD (cortical and trabecular), bone cross-sectional geometry and estimates of bone strength of the pediatric peripheral skeleton (e.g., distal and shaft sites of the radius and tibia) with a lower radiation dose than clinical CT. To date, pQCT has not been used to evaluate bone health in perinatally HIV-infected youth in either a cross-sectional or longitudinal study.

Therefore, in this exploratory analysis we first aimed to compare BMD, bone cross-sectional geometry and estimates of bone strength (by pQCT) in addition to traditional measures of bone mass (BMC by DXA) between perinatally HIV-infected children and adolescents and a group of healthy, uninfected youth. Second, we aimed to describe changes in bone outcomes over two years in HIV-infected youth. Finally, we aimed to investigate associations between disease-related factors such as HAART use, calcium and vitamin D status and pQCT-derived bone outcomes in HIV-infected children and adolescents.

Materials and Methods

Participants

We offered participation in this two-year longitudinal study to perinatally HIV-infected children, adolescents and young adults between the ages of 8 and 25 years who were receiving regular medical care (clinic visits every 3 months) at the Oak Tree Clinic, a specialized pediatric and adult HIV interdisciplinary clinic located at the British Columbia Women's Hospital and Health Centre in Vancouver, Canada, during 2009-2011. Exclusion criteria were history of corticosteroid use (>3 months), severe concomitant illness, congenital diseases affecting bone health or current pregnancy. Of 45 eligible patients, 36 (80%) volunteered to participate. Among the 9 patients who did not participate, one was on prolonged steroid therapy for disseminated lupus, 2 were not attending the clinic regularly, 3 were not interested in the study and 3 declined for practical reasons (work schedule, distance from the clinic). In addition, due to the age range of our healthy controls (9-18 yrs), we limited the age range of participants for the present analysis to 9-18 yrs. Of the 36 who volunteered we excluded 5 from this analysis because they were older than 18 years of age at their first study visit. Thus, the present analysis includes 31 HIV-infected individuals (19 males, 12 females; 13.7±2.6 yrs; range: 9.0-18.6 yrs) with at least one study visit (n=18 with 3 study visits, n=30 with 2 study visits, n=1 with one study visit). Twenty-four of these patients were enrolled in 2009, 6 in 2010 and 1 in 2011.

We compared the HIV-infected group to healthy controls who were participants in the University of British Columbia Healthy Bones III follow-up study (HBSIII; n=883; 469 females, 414 males, ages 9-18 yrs). HBSIII participants were recruited from elementary schools in Vancouver and Richmond, BC between 1999 and 2009[21-24]. For the purpose of this analysis, we included data from annual measurements conducted between 2001 (first year of pQCT measurements) and 2011. Across the 883 HBSIII participants, the average number of annual measurements was three (range: 1 to 9). As some participants were involved in a school-based exercise intervention[24], we excluded 410 observations from the 2004 spring data collection. We included all additional follow-up measurements (2005-2012) regardless of group assignment, as we showed previously that participation in an exercise intervention was not associated with sustained benefits at the tibial shaft[25].

All HBSIII participants were healthy and normally active, and none were taking medications known to influence bone metabolism. They were of diverse ethnic backgrounds in accordance with the demographics of the Vancouver and Richmond regions[26]. Based on parental report of ethnicity in a health history questionnaire, 40% (n=350) of the healthy controls were Caucasian (both parents or all 4 grandparents born in North America or Europe), 49% (n=428) were Asian (both parents or all 4 grandparents born in Hong Kong, China, India, Philippines, Vietnam, Korea or Taiwan) and 12% (n=105) were of mixed ethnicity or other ethnic origins.

We obtained written informed consent from the parents or legal guardians, written assent from participants younger than 18 years of age and informed consent only from participants aged 18 years of age and older. The University of British Columbia's Clinical Research Ethics Board approved this study (#H08-01846).

Measurements

Data collection for the HIV-infected participants took place in February and March 2009, 2010 and 2011. With the exception of clinical and laboratory measures which were collected at the Oak Tree Clinic, all data were collected at the Centre for Hip Health and Mobility, Vancouver Coastal Health Research Institute. We used the same procedures (except clinical and laboratory measures) to assess the healthy controls during annual spring data collection sessions between 2001 and 2011[21-23].

Clinical history and laboratory measures

We obtained demographic data, maturity status (physician-reported Tanner stage and age at menarche), smoking status, antiretroviral treatment history, disease stage (based on the Revised HIV Pediatric Classification System from the Center for Disease Control and Prevention[27], CD4 count (nadir, absolute and %) and plasma HIV RNA viral load (TaqMan HIV-1 assay, Roche Diagnostics, Indianapolis, IN) from the patients' medical charts within 3 months of the study visit. We used the log10 of HIV plasma viral load for all analyses. In addition, at the clinic visit closest to the study visit we collected venous blood samples and analyzed for serum calcium (colorimetric assay, VITROS 5600, Ortho-Clinical Diagnostics, Inc., Rochester, NY) and 25 OH-vitamin D (ELISA assay, Immunodiagnostic Systems, Scottsdale, AZ). Trained technicians at the British Columbia Children's Hospital laboratory performed the blood analyses according to standard procedures.

Anthropometry

We measured standing height (stretch stature) to the nearest 0.1 cm with a wall-mounted digital stadiometer (Seca Model 242, Hanover, MD, USA), body weight to the nearest 0.1kg with an electronic scale (Seca Model 840, Hanover, MD, USA) and tibial length (distance from the distal edge of the medial malleolus to the tibial joint line) to the nearest 0.1 cm using an anthropometric tape. For each variable we used the mean of two measures for analyses.

Questionnaires

We used a validated food frequency questionnaire[28] to estimate daily dietary calcium intake (mg/day). A trained research assistant administered the questionnaire and provided visual and physical cues to help participants with food recall and specific serving sizes. We used the validated Physical Activity Questionnaire for Children (PAQ-C) and Adolescents (PAQ-A) to estimate leisure-time physical activity[29,30]. A trained research assistant administered the PAQ-C/A, which are 7-day recall questionnaires that assess habitual moderate to vigorous physical activity. We report two outcomes from the PAQ-C/A: 1) a general physical activity score (1-low active and 5-highly active) and 2) hours/week of moderate to vigorous physical activity, which provides an estimate of the time spent in common sports and activities[21].

Dual energy X-ray absorptiometry

We used a Hologic QDR 4500W bone densitometer (DXA, Hologic Inc, Waltham, MA) to assess bone mineral content (BMC, grams) of the total body, total proximal femur and femoral neck sub-region (left femur) and lumbar spine (L1-L4). We also obtained measures of total body lean mass (kg) and percent body fat (%) from the total body DXA scan. Two experienced technicians acquired and analyzed all DXA scans according to standard procedures[31] and performed daily quality assurance scans. Coefficients of variation (%CV) for repeated BMC measurements in our laboratory ranged from 0.6% to 2.2% in 15 healthy adult volunteers[32].

Peripheral quantitative computed tomography (pQCT)

We used pQCT (Norland/Stratec XCT 3000; Stratec Medizintechnic GmbH, Pforzheim, Germany) to assess cortical bone at the 50% site of the left tibia, measured proximally from the tibial plafond. To begin, we acquired a 10-20 mm planar scout scan over the joint line (the minimum scan region required to obtain an image of the tibial plafond for placement of the reference line), and located a standard anatomical reference (the tibial plafond). We then acquired a single 2.3 +/- 0.2 mm slice with a scan speed of 30 mm/sec and a 0.4 mm voxel size. Two trained technicians acquired all scans according to standard procedures. A cone phantom was scanned daily to maintain quality assurance. In our laboratory, short-term precision (reproducibility, %CV) for 14 participants (mean age, 12-27 years) was less than 1% for all outcomes.

All pQCT scans were analyzed using Stratec software, Version 6.0 by the same trained technicians who acquired the scans. As in previous analyses[24,33], to obtain values for total bone cross-sectional area (Tt.Ar, mm^2) and total bone mineral density (Tt.BMD, mg/cm^3) we used Contour mode 1 (200 mg/cm^3) and Peel mode 2. To obtain values for cortical BMD (Ct.BMD, mg/cm^3) and cortical thickness (Ct.Th, mm) we used Cort mode 1 (711 mg/cm^3) and for cortical area (Ct.Ar, mm^2) and the polar strength strain index (SSI_p, mm^3) we used Cort mode 1 (480 mg/cm^3).

Statistical analysis

To determine if bone outcomes in the HIV-infected youth were different from the healthy controls, we calculated age- and sex-specific z-scores. First, we calculated age- (whole year) and sex-specific means (SD) for the healthy control group for each bone outcome (as well as for the anthropometric, body composition, physical activity and calcium outcomes). As we conducted annual measurements on our healthy cohort, we included all available data when calculating the age- and sex-specific means and standard deviations. Thus, for the 883 healthy children we included between 3177 and 3227 observations, depending on the outcome variable. We visually inspected histograms of each variable in the healthy cohort to ensure the outcomes were normally distributed at each age (9-18 yrs) and in each sex. Using the age- and sex-specific means and standard deviations for the healthy youth we then calculated z-scores for each variable (anthropometric and bone outcomes) in the HIV-infected youth. Due to the small number of HIV-infected youth, we used the nonparametric Wilcoxon signed rank test to determine if z-scores in the HIV-infected youth were significantly different from zero (expected average of z scores in the healthy cohort) at baseline. To account for the important influence of body size on bone mass, structure and strength, we then fit multivariable regression models to: 1) adjust BMC z-scores for height and weight z-scores and 2) adjust bone area, BMD, cortical thickness and SSI_p z-scores for tibial length and weight z-scores. We also fit a second model for DXA and pQCT z-scores in which we replaced weight z-score by lean mass z-score (DXA outcomes) and

	Baseline (n=31)
Demographics	
Sex (males/females)	19/12
Age (yrs)	13.6 (11.6, 16.0)
Ethnicity (Asian/Caucasian/Aboriginal/Black/Mixed)	2/4/7/8/10
Disease severity and treatment hx	
CDC stage (N1/N2/A1/A2/B1/B2)	15/7/4/1/2/2
Current use of HAART (Yes/No)	22/9
Ever use of HAART (Yes/No)	27/4
Lifetime months on HAART	111 (59, 146)
Use of NNRTI (Yes/No)	25/6
Lifetime months on NNRTI	26 (12, 58)
Use of PIs (Yes/No)	22/9
Lifetime months on PI	79 (46, 109)
Use of Tenofovir (yes/no)	11/20
Age Tenofovir initiated (yrs)	12.1 (9.9, 13.0)
Lifetime months on Tenofovir	33 (19, 41)
$CD4^+$ cells nadir (x 10^6 cells/ mm^3)	320 (190, 470)
$CD4^+$ cells nadir (%)	22 (10, 29)
$CD4^+$ cells (x 10^6 cells/mm^3)	580 (480, 740)
$CD4^+$ cells (%)	30 (24, 38)
HIV $\log_{10}$ copies/mL in current HAART users (n=22)	1.3 (1.3, 1.3)
HIV $\log_{10}$ copies/mL in current non-HAART users (n=9)	4.3 (3.6, 4.5)
Anthropometrics	
Height (cm)	158.7 (143.3, 165.0)
Weight (kg)	48.9 (38.7, 61.3)
BMI (kg/m^2)	20.4 (17.4, 22.5)
Tibial length (mm)	390 (350, 412)
Body composition	
Lean mass (kg)	38.2 (28.7, 46.9)
Percent body fat	19.3 (13.7, 30.0)
Muscle CSA (cm^2)	33.7 (26.2, 40.7)
Maturity	
Females' Tanner stage (1/2/3/4/5)	4/0/2/4/2
Females' menarche status (# pre/post)	6/6
Age at menarche (yrs)	12.3 (11.0, 12.7)
Males' Tanner stage (1/2/3/4/5)	1/4/3/10/1
Lifestyle factors	
PAQ score (/5)	2.2 (1.7, 2.4)
MVPA (hrs/week)	6.4 (2.7, 9.6)
Dietary calcium intake (mg/day)	950 (640, 1702)
Ever smoked (yes/no)	5/26
Current smoker (yes/no)	4/27
Serum calcium (n=29)	2.4 (2.3, 2.4)
Serum vitamin D (n=28)	55.5 (40, 79)

CDC=Centers for Disease Control, HAART=highly active anti-retroviral therapy, NNRTI=non-nucleoside reverse transcriptase inhibitor, PI=protease inhibitor, BMI=body mass index, CSA=cross-sectional area, PAQ=physical activity questionnaire, MVPA=moderate to vigorous physical activity

Table 1. Anthropometric and descriptive characteristics and disease status of HIV-infected youth at baseline. Values are presented as median (p25, p75) unless otherwise indicated.

MCSA z-score (pQCT outcomes) to account for the well-established relationship between these surrogates of muscle force and bone mass, cross-sectional geometry and estimates of bone strength[33,34]. To determine if z-scores for the anthropometric and bone outcomes changed during follow-up in the HIV-infected youth, we first determined a slope from a linear regression model for each individual; the slope represents the annual change. We then used a one-sample t-test to determine whether the average slope across individuals was different from zero.

Finally, to identify potential predictors of bone mass, struc-

ture and strength in HIV-infected youth, we fit univariable regression models between DXA and pQCT outcomes at baseline and disease-related outcomes including disease stage, CD4%, $\log_{10}$ HIV plasma viral load and ever use of HIV therapies. We used Stata, Version 10 (Stata Corp, TX) for all analyses and we considered $p<0.05$ statistically significant.

Results

Baseline Characteristics

The median age of the HIV-infected youth (n=31) was 13.6 yrs and 61% were male (Table 1). The majority of the HIV-infected patients were of mixed ethnicity (32%), Black (26%) or Aboriginal (23%) whereas the remainder were Caucasian (13%) or Asian (6%). At baseline most of the HIV-infected youth were non-symptomatic (category N, n=22, 71%) or had mild symptoms of lymphadenopathy, chronic parotitis, dermatitis or recurrent upper respiratory infections (category A, n=5, 16%); a minority had moderate symptoms such as diarrhea and poor weight gain, recurrent herpes zoster infection (shingles) or HIV-related hepatitis (category B, n=4, 13%). None had severe HIV-related disease (category C) at the time of the study but 5 (16%) previously experienced AIDS-defining illnesses such as an opportunistic infection or HIV encephalopathy. Similarly, at baseline, the majority of the HIV-infected youth (n=21, 68%) showed no or minimal immune suppression (absolute CD4 >500 cells x 10^6 cells/mm^3 and CD4 % >25%) while 10 (32%) had moderate suppression (absolute CD4 200-500 cells x 10^6 cells/mm^3 or CD4 % of 15-25%) and none had severe immune suppression (absolute CD4 <200 cells x 10^6 cells/mm^3 or CD4 % <15%). The majority (22/31, 71%) of the HIV-infected youth were receiving HAART at the time of the study. Three participants initiated HAART between visit one and two and one participant initiated HAART between visit two and three. HAART included nucleoside reverse transcriptase inhibitors (NRTI, 100%) with non-nucleoside reverse transcriptase inhibitors (NNRTI, 81%) and/or protease inhibitors (PI, 71%). Just over one third of the HIV-infected youth (35%) started taking tenofovir at a median age of 11.5 years (range 8.5 to 16) and had taken it for a median of 30 months (range 3 to 60). Among those treated with HAART, 20/26 (77%) reported being optimally adherent (taking >95% of prescribed ARV doses) to their regimen and had an undetectable HIV plasma viral load (<40 copies/ml) across the entire study period. Lipodystrophy, defined as limb or facial atrophy with or without accumulation of abdominal fat, was clinically observed in seven of the 22 youth who had received at least three years of HAART in their lifetime.

Among the HIV-infected youth, z-scores for height and muscle cross-sectional area were negative and significantly different from zero (Table 2, Figure 1). However, upon visual inspection we noted that the height z-scores for three participants were noticeably lower than the other HIV-infected youth. These individuals came from endemic countries and did not receive HAART until after age 11. When we removed these individuals from the analysis the height z-score remained negative (median=-0.12) in the HIV-infected youth, but was no longer significantly different from zero. We included these participants in subsequent analyses, which were adjusted for height z-scores. Z-scores for weight, BMI, lean mass and percent body fat were also negative, but were not significantly different from zero. Maturity status ranged from pre- to post-pubertal in both HIV-infected females and males; however, the majority of the HIV-infected males were Tanner stage 4 or 5 (58%). Half of the HIV-infected females were postmenarcheal with a median age at menarche of 12.3 yrs, and this was not significantly different from the healthy controls. Regarding lifestyle factors, the HIV-infected youth were less active than their healthy peers as indicated by the physical activity z-score whereas dietary calcium intake was similar in the HIV-infected youth and healthy controls. Serum levels of calcium were also within the normal range (n=28) and of the 28 HIV-infected youth for whom we had valid data, none were vitamin D deficient according to recent guidelines[35]. Four of the 31 HIV-infected youth (13%) were current smokers at baseline.

At baseline, unadjusted BMC z-scores were negative and significantly different from zero at all sites, with the exception of the lumbar spine (Table 3, Figure 2). Similarly, z-scores for Tt.Ar and Ct.Ar were negative and significantly different from zero (Table 3, Figure 3). The z-score for SSI_p was also negative; however, it was not significantly different from zero. In contrast, the z-score for Ct.BMD was positive and significantly different from zero.

After adjusting for height and weight z-scores, WB and PF BMC z-scores were no longer significantly different from zero (Table 3) whereas adjusted FN BMC z-score remained significantly lower than zero. When weight z-score was replaced by lean mass z-score, WB, PF and FN BMC z-scores were negative and significantly different from zero whereas LS BMC z-score was not.

At the tibial midshaft, Tt.Ar and Ct.Ar z-scores remained significantly different from zero after adjusting for tibial length and weight; however, when MCSA replaced body weight, the z-scores were not significantly different from zero (Table 3). In both models, the z-score for Ct.BMD remained positive and significantly different from zero while the z-score for SSI_p was not significantly different from zero.

Change in DXA and pQCT z-scores in HIV-infected youth

During the follow-up period, 30 out of 31 HIV-infected youth returned for measurement at 12 months and 18 of 31 returned at 24 months. The clinical health of most subjects remained stable and the one participant who initiated HAART went from moderately symptomatic to non-symptomatic.

In the HIV-infected youth, the slopes for height and weight z-scores over time were positive as were the slopes for LS and FN BMC (Table 4). The slopes for WB and PF BMC were also positive, but not statistically different from zero. Similarly, the slopes for Tt.Ar, Ct.BMD and SSI_p at the tibial midshaft were positive and the slopes for Ct.Ar and Ct.Th were positive, but not significantly different from zero.

	Baseline (n=31)	p-value
Anthropometrics		
Height z-score	-0.23 (-0.88, 0.20)	0.044
Weight z-score	-0.25 (-0.74, 0.17)	0.063
BMI z-score	-0.01 (-0.77, 0.32)	0.40
Tibial length z-score	0.45 (-0.22, 0.82)	0.10
Body composition		
Lean mass z-score	-0.05 (-0.70, 0.69)	0.95
Percent body fat z-score	-0.33 (-0.85, 0.66)	0.38
MCSA z-score	-0.79 (-1.36, -0.46)	<0.001
Maturity		
Age at menarche z-score*	0.05 (-1.1, 0.7)	0.92
Lifestyle factors		
PAQ score z-score	-0.73 (-1.29, -0.06)	<0.001
MVPA z-score	-0.64 (-1.08, -0.09)	0.002
Dietary calcium intake z-score	0.14 (-0.49, 1.16)	0.22

BMI=body mass index, MCSA=muscle cross-sectional area, PAQ=physical activity questionnaire, MVPA=moderate-to-vigorous physical activity.
** n=6 postmenarcheal HIV-infected females.*

Table 2. Age- and sex-specific z-scores for anthropometric, body composition, maturity and lifestyle outcomes in the HIV-infected youth at baseline. Baseline values are median (p25, p75) and p-values indicate whether z-scores are significantly different from zero (Wilcoxon sign rank test).

	Unadjusted	p-value	Adjusted: Model 1[a]	p-value	Adjusted: Model 2[b]	p-value
DXA						
WB BMC (g)	1643.2 (1175.7, 1936.9)					
WB BMC z-score	-0.32 (-0.88, 0.24)	0.034	0.06 (-0.14, 0.26)	0.54	-0.20 (-0.37, -0.03)	0.024
LS BMC (g)	39.8 (25.7, 48.8)					
LS BMC z-score	-0.43 (-1.11, 0.27)	0.10	0.03 (-0.22, 0.29)	0.81	-0.18 (-0.41, 0.06)	0.15
PF BMC (g)	25.6 (16.7, 31.0)					
PF BMC z-score	-0.45 (-0.97, 0.11)	0.030	-0.03 (-0.27, 0.21)	0.81	-0.27 (-0.49, 0.06)	0.013
FN BMC (g)	3.29 (2.22, 3.98)					
FN BMC z-score	-0.80 (-1.23, -0.16)	0.005	-0.32 (-0.57, -0.06)	0.015	-0.57 (-0.80, -0.34)	<0.001
pQCT – Tibia 50% site						
Tt.Ar (mm^2)	376.8 (298.6, 468.0)					
Tt.Ar z-score	-0.28 (-1.09, 0.14)	0.022	-0.26 (-0.49, -0.04)	0.022	-0.11 (-0.35, 0.12)	0.34
Ct.Ar (mm^2)	280.8 (220.2, 329.6)					
Ct.Ar z-score	-0.46 (-1.03, 0.05)	0.011	-0.31 (-0.54, -0.08)	0.008	-0.15 (-0.39, 0.09)	0.23
Ct.Th (mm)	4.9 (4.0, 5.2)					
Ct.Th z-score	-0.32 (-0.98, 0.52)	0.14	-0.19 (-0.50, 0.11)	0.21	0.06 (-0.37, 0.25)	0.70
Ct.BMD (mg/cm^3)	1092.0 (1065.3, 1121.2)					
Ct.BMD z-score	0.48 (-0.19, 1.06)	0.020	0.44 (0.08, 0.79)	0.014	0.46 (0.10, 0.81)	0.011
SSI_p (mm^3)	1455.1 (1045.8, 1917.8)					
SSI_p z-score	-0.37 (-0.98, 0.37)	0.063	-0.16 (-0.41, 0.10)	0.23	0.003 (-0.26, 0.26)	0.98

[a] DXA outcomes adjusted for height and weight z scores, pQCT outcomes adjusted for tibial length and weight z-scores;
[b] DXA outcomes adjusted for height and lean mass z-scores, pQCT outcomes adjusted for tibial length and muscle CSA z scores;
WB=whole body, LS=lumbar spine, PF=proximal femur, FN=femoral neck, Tt.Ar=total bone cross-sectional area, Ct.Ar=cortical cross-sectional area, Ct.Th=cortical thickness, Ct.BMD=cortical bone mineral density, SSI_p=polar strength strain index.

Table 3. Unadjusted and adjusted DXA and pQCT outcomes and z-scores in HIV-infected youth at baseline. Values are median (p25, p75) for unadjusted outcomes and z-scores and mean (95% CI) for adjusted z-scores.

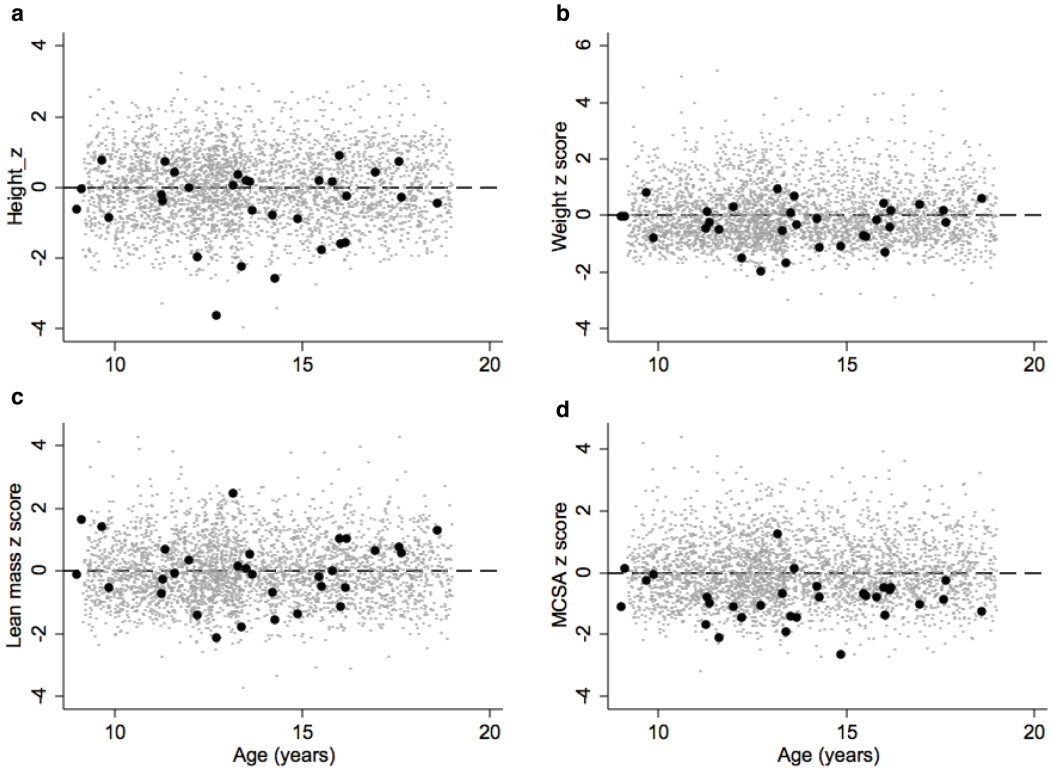

Figure 1. Z-scores for (**a**) height, (**b**) weight, (**c**) total body lean mass and (**d**) muscle cross-sectional area (MCSA) in the HIV-infected (solid circles) and healthy (grey points) youth.

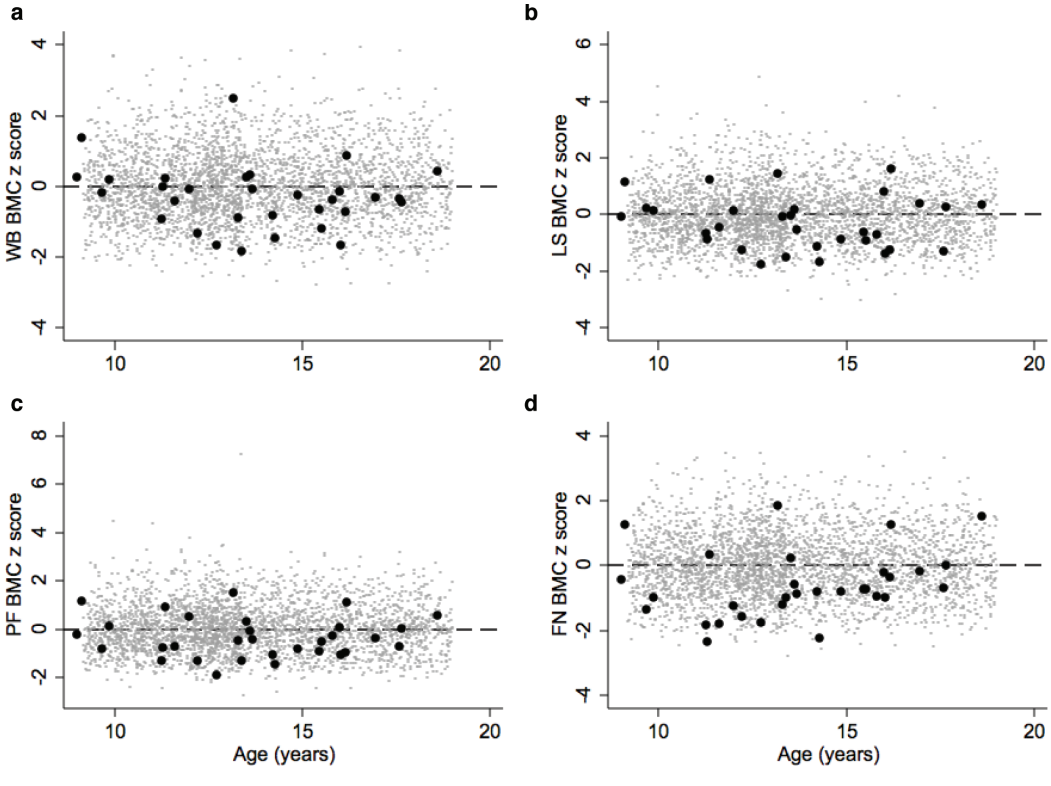

Figure 2. Bone mineral content Z-scores for the (**a**) total body, (**b**) lumbar spine, (**c**) proximal femur and (**d**) femoral neck in the HIV-infected (solid circles) and healthy (grey points) youth.

	Slope of z-score over time[a]	p-value
Anthropometry		
Height z-score	0.22 (0.07, 0.37)	0.006
Weight z-score	0.13 (0.01, 0.24)	0.034
BMI z-score	0.07 (-0.05, 0.12)	0.24
Lean mass z-score	0.20 (0.03, 0.36)	0.020
Tibial length z-score	0.03 (-0.07, 0.13)	0.58
MCSA z-score	0.11 (0.007, 0.21)	0.037
DXA		
WB BMC z-score	0.07 (-0.03, 0.17)	0.15
LS BMC z-score	0.17 (0.05, 0.28)	0.007
PF BMC z-score	0.10 (-0.01, 0.21)	0.081
FN BMC z-score	0.20 (0.10, 0.30)	<0.001
pQCT – Tibia 50% site		
Tt.Ar z-score	0.11 (0.01, 0.20)	0.025
Ct.Ar z-score	0.10 (-0.009, 0.21)	0.071
Ct.Th z-score	0.09 (-0.02, 0.20)	0.12
Ct.BMD z-score	0.16 (0.02, 0.29)	0.024
SSI_p z-score	0.14 (0.05, 0.24)	0.006

[a] *N=30 participants with 0-12 month slopes and 18 participants with 0-12-24 month slopes.*
BMI=body mass index, MCSA=muscle cross-sectional area, WB=whole body, BMC=bone mineral content, LS=lumbar spine, PF=proximal femur, FN=femoral neck, Tt.Ar=total bone cross-sectional area, Ct.Ar=cortical cross-sectional area, Ct.Th=cortical thickness, Ct.BMD=cortical bone mineral density, SSI_p=polar strength strain index.

Table 4. Mean (95% CI) of the slope of the z-scores over time.

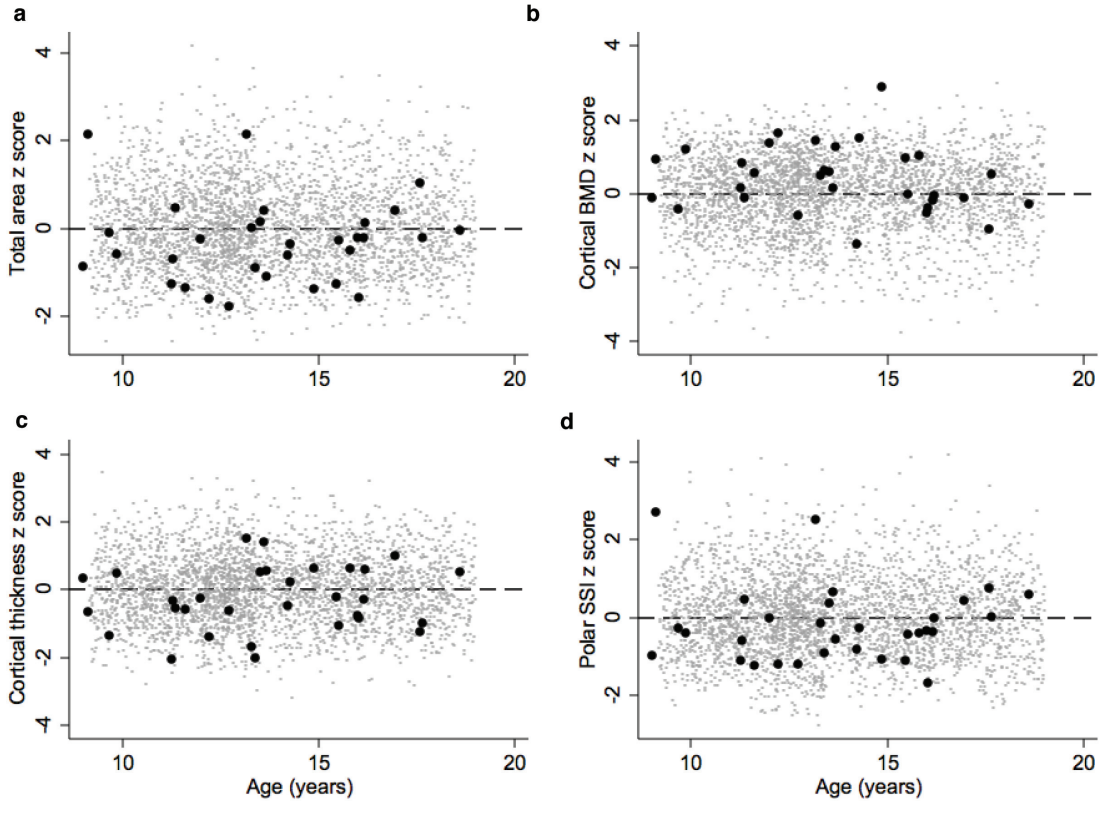

Figure 3. Z-scores for (**a**) total bone area, (**b**) cortical bone mineral density (BMD), (**c**) cortical thickness and (**d**) polar strength strain index (SSI_p) at the tibial midshaft in the HIV-infected (solid circles) and healthy (grey points) youth.

	CDC stage (N + A vs. B)	CD4 %	Log_{10} viral load	HAART ever (yes vs. no)	NNRTI ever (yes vs. no)	PI ever (yes vs. no)	TDF ever (yes vs. no)
DXA z-scores							
WB BMC	-0.52	**0.040**	-0.094	0.35	0.38	-0.06	0.31
	(-1.52, 0.47)	**(0.0005, 0.080)**	(-0.34, 0.15)	(-0.65, 1.36)	(-0.46, 1.24)	(-0.81, 0.69)	(-0.39, 1.01)
LS BMC	-0.43	0.032	-0.062	0.44	0.51	0.33	0.29
	(-1.47, 0.60)	(-0.010, 0.074)	(-0.32, 0.19)	(-0.60, 1.47)	(-0.35, 1.38)	(-0.43, 1.09)	(-0.44, 1.01)
PF BMC	-0.48	0.029	-0.064	0.58	0.63	0.31	0.31
	(-1.40, 0.44)	(-0.008, 0.067)	(-0.29, 0.16)	(-0.33, 1.49)	(-0.13, 1.39)	(-0.38, 0.99)	(-0.34, 0.96)
FN BMC	-0.70	-0.0031	0.034	0.063	0.33	0.020	0.38
	(-1.83, 0.43)	(-0.052, 0.045)	(-0.25, 0.32)	(-1.10, 1.23)	(-0.65, 1.30)	(-0.84, 0.88)	(-0.42, 1.18)
pQCT z-scores							
Tt.Ar	-0.57	0.006	-0.047	0.33	0.51	0.046	0.45
	(-1.61, 0.48)	(-0.039, 0.050)	(-0.31, 0.21)	(-0.73, 1.39)	(-0.38, 1.39)	(-0.74, 0.83)	(-0.28, 1.17)
Ct.Ar	-0.48	0.012	-0.012	0.002	0.16	-0.38	0.16
	(-1.42, 0.46)	(-0.028, 0.052)	(-0.25, 0.22)	(-0.96, 0.96)	(-0.65, 0.97)	(1.07, 0.32)	(-0.51, 0.84)
Ct.Th	-0.07	0.019	0.009	-0.45	-0.22	**-0.84**	-0.11
	(-1.12, 0.99)	(-0.024, 0.063)	(-0.25, 0.27)	(-1.48, 0.59)	(-1.11, 0.66)	**(-1.55, -0.13)**	(-0.85, 0.62)
Ct.BMD	0.64	**0.047**	-0.15	0.77	**0.90**	-0.13	0.062
	(-0.32, 1.61)	**(0.010, 0.085)**	(-0.38, 0.090)	(-0.18, 1.72)	**(0.13, 1.67)**	(-0.87, 0.60)	(-0.63, 0.76)
SSI_p	-0.43	0.013	-0.062	0.37	0.60	0.046	0.47
	(-1.52, 0.66)	(-0.032, 0.059)	(-0.33, 0.21)	(-0.73, 1.46)	(-0.30, 1.51)	(-0.77, 0.86)	(-0.28, 1.22)

CDC=Centers for Disease Control, N=non-symptomatic (n=22), A=mild symptoms (n=5), B=moderate symptoms (4), WB=whole body, LS=lumbar spine, PF=proximal femur, FN=femoral neck, Tt.Ar=total bone area, Ct.Ar=cortical bone area, Ct.Th=cortical thickness, Ct.BMD=cortical bone mineral density, SSI_p=polar strength strain index, HAART=highly active antiretroviral therapy, NNRTI=non-nucleoside reverse transcriptase inhibitors, PI=protease inhibitor, TDF=tenofovir.

Table 5. Univariate relationships between baseline DXA and pQCT z-scores and disease-related factors in HIV-infected youth (n=31). Values are unstandardized regression coefficients (95% CI).

Predictors of bone mass, geometry and strength in HIV-infected youth

Results of the univariable regression analyses are presented in Table 5. Few of the HIV disease-related variables were significantly associated with DXA or pQCT z-scores. For DXA outcomes, WB BMC z-score was positively associated with CD4 percentage. For pQCT outcomes, cortical BMD z-score was positively associated with use of NNRTIs and CD4 percentage and cortical thickness z-score was negatively associated with use of PIs.

Discussion

This two-year longitudinal study extends and complements the findings of earlier studies of HIV-infected youth by using pQCT to examine tibial bone cross-sectional geometry, cortical BMD and estimated bone strength in addition to standard measures of bone mass (BMC) as measured with DXA. Despite lower muscle cross-sectional area (MCSA), total and cortical bone area z-scores in the HIV-infected youth were not significantly different from the healthy controls and cortical BMD z-scores were, in fact, higher in the HIV-infected youth. In contrast, whole body and hip (total proximal femur and femoral neck) BMC z-scores were lower in HIV-infected youth even after adjusting for lean mass and body size. Finally, our longitudinal analysis suggests that deficits in bone mass do not worsen over two years in HIV-infected youth.

Before discussing our findings in detail, we acknowledge that due to our small sample of HIV-infected youth across a wide range of ages, ethnicities and maturational stages we must consider the role of sampling error in our analysis. Our sample included the majority of perinatally HIV-infected youth in British Columbia; however, it may not represent the population of youth living with HIV in other regions. Further, sampling error may have increased the likelihood of Type I errors, particularly given the number of bone outcomes and associated statistical tests. Thus, although we used more conservative non-parametric tests and report interquartile ranges and confidence intervals, our results should be interpreted with caution and considered hypothesis generating.

Baseline comparisons

The HIV-infected youth in our study tended to be shorter and weigh less than their healthy peers. However, as we noted previously, three participants who emigrated from endemic countries, and who had not received HAART until after age 11, were substantially shorter than the other HIV-infected youth at baseline (height z-scores -3.6 to -2.2). Their short stature is consis-

tent with stunting related to ongoing HIV replication since birth, in addition to a genetic predisposition for short stature in one participant. Although HIV-infected children are known to experience delays in both linear growth and weight gain[36,37] the incidence of stunting and malnutrition has been attenuated due to use of HAART in developed countries[38]. Previous cross-sectional studies reported that heights and weights for age in HIV-infected youth were not significantly different from the population norm z-score of zero[13]. Further, in a preliminary analysis of peak height velocity in our population of HIV-infected youth[39] we found that both males and females achieved peak height velocity at approximately the same age as their same-sex peers as reported in the University of Saskatchewan Bone Mineral Accrual Study[40]. Thus, it appears that with the advent of HAART in recent decades, and with current guidelines recommending earlier start of HAART[41], children and adolescents living with HIV can be expected to grow at a similar rate to their non-infected peers.

In contrast to previous studies[37], we did not observe any deficits in bone mineral free lean mass as measured with DXA in our HIV-infected youth. However, z-scores for MCSA, a common surrogate for muscle force[33,42], were significantly lower in HIV-infected youth compared with controls. We cannot determine the mechanism underpinning the reduced MCSA in this study; however, the lower levels of physical activity among the HIV-infected youth may play a role. A small proportion (13%) of participants in our study had subjectively reduced physical abilities secondary to HIV encephalopathy in infancy and/or to *in utero* exposure to alcohol or substances of addiction. In addition, antiretroviral (ARV)-related lipodystrophy, including limb muscle atrophy is a common finding in patients treated with ARVs for many years, and was clinically observed in one third of the HIV-infected youth in our study population[43]. Finally, HIV-related muscle wasting is also associated with malnutrition, abnormal cytokine production and endocrine dysfunction[44]. Thus, further investigation is warranted to elucidate the causes of lower muscle CSA in this cohort, and whether deficits in functional measures of muscle force are also apparent.

When we examined bone outcomes in the HIV-infected youth after adjusting for either body weight or a surrogate of muscle force (lean mass or MCSA), we noted divergent results for bone cross-sectional geometry and BMC. Whereas total and cortical area, cortical thickness and estimated bone strength (SSI_p) appeared to be appropriately adapted to the lower MCSA in HIV-infected youth, bone area z-scores remained lower after adjusting for body weight. In contrast, the negative z-scores for WB, PF and FN BMC after adjusting for lean mass suggest that perhaps BMC is not appropriately adapted to lean mass in this population. These findings highlight the importance of considering measures of bone mass, structure and strength in the context of muscle forces (or surrogates of muscle force) and the functional muscle-bone unit[34].

The greater cortical BMD at the tibial shaft in the HIV-infected youth is a somewhat surprising result. As this is the first pQCT study to measure cortical BMD in HIV-infected individuals, we are unable to compare our findings to previous studies. One cross-sectional QCT study of HIV-infected youth found no difference in vertebral BMD between HIV-infected youth and healthy controls[20]; however, the resolution of QCT was insufficient to differentiate between cortical and trabecular bone in the vertebral bodies. Thus, it is not known whether cortical bone is higher at other skeletal sites in HIV-infected youth when compared with healthy controls. Further, we can only speculate as to the causes of the higher cortical BMD in the HIV-infected youth. During normal growth, cortical BMD increases as rates of intracortical remodelling decrease[45]. Thus, it is possible that certain HIV therapies such as NNRTIs may negatively affect intracortical remodelling and lead to increases in cortical BMD. Similarly, lower levels of physical activity among our HIV-infected youth may also reduce intracortical remodelling, and result in accumulation of older, denser cortical bone. In contrast, our results suggest that lower levels of immune suppression (as evidenced by a higher CD4 count) are positively associated with cortical BMD. This association may reflect a reduced level of cytokine activity that in turn could lead to a decrease in bone resorption[8,46]. Future studies may benefit from assessing markers of inflammation and bone metabolism in addition to cortical BMD to clarify these relationships. In addition, longitudinal studies are needed to determine whether higher cortical BMD persists into adulthood in HIV-infected individuals and if so, what is the long-term impact of higher cortical BMD on bone strength and fracture risk in this population.

Longitudinal analysis: change in z-scores

We did not observe any declines in z-scores over the two-year follow-up for any of the bone outcomes. This finding suggests that BMC and pQCT measures of bone area, cortical thickness and bone strength are not increasingly compromised as children and youth age, as has been reported in previous cross-sectional studies[9,13]. On the contrary, our findings support that bone health may improve slightly over time. This positive result is similar to the increase across 12-months in lumbar spine and whole body aBMD, observed by Mora et al. in a study of 32 perinatally-infected youth aged 6.3 to 17.7 yrs[47]. The annual incremental increase in aBMD was similar to that observed in healthy children, but absolute values for aBMD remained lower in HIV-infected youth compared with controls. This suggests that despite a similar rate of change, the observed bone deficit may persist across maturity. However, our results and those of Mora et al.'s longitudinal analysis should be interpreted with caution due to the small sample sizes and the possibility of regression to the mean. Further, due to the small number of HIV-infected youth who returned for follow-up measurements in the present study we did not have sufficient power to fit a multivariable regression model to adjust for changes in body size, body composition, maturation, disease status and medication use that would likely influence change in bone outcomes.

Potential determinants of bone health in HIV-infected youth

In our small cohort, disease stage was not associated with any bone outcome; this may reflect the positive effect of

HAART on controlling highly inflammatory processes associated with HIV replication. The majority of youth in our cohort had well-controlled or slow-progressing disease. Similar to previous studies[9], we also did not find an association between plasma HIV viral load and either BMC by DXA or pQCT measures of BMD, bone geometry and bone strength. However, as noted by Arpadi et al.[9], a single measure of HIV viral load in chronically infected patients likely does not adequately capture the cumulative exposure to the virus. We also did not observe an association between tenofovir use and any bone outcome in our cohort of HIV-infected youth who initiated tenofovir therapy either later in childhood or in adolescence. This finding agrees with results from previous longitudinal studies that used DXA; tenofovir use did not have any deleterious effects on bone mass in HIV-infected children and youth aged 5-18 yrs over 12 months[48] or 60 months[49]. Importantly, we do not yet know the long-term effects of tenofovir use on bone health during adolescence and into adulthood.

Limitations

We note several limitations of our study. In addition to increased likelihood of Type I errors, the small sample size limited our statistical power and we were unable to fit multivariable regression models to identify additional predictors of bone outcomes in HIV-infected youth (other than disease-related variables). Second, our HIV-infected youth were from diverse ethnic backgrounds with the majority being either of mixed ethnic backgrounds, Aboriginal or Black. In contrast, our healthy comparison population was comprised mainly of Caucasian and Asian youth. Thus, we were not able to generate ethnic-specific z-scores for this study. Third, we measured the tibial midshaft with pQCT, which is predominantly a cortical bone site. We chose this site based on the availability of comparison data for our healthy cohort. Future pQCT studies that include a distal site (e.g., 8% site of the tibia) would permit us to examine trabecular bone while ensuring the scanning region does not include the growth plate[50]. Fourth, long-term reproducibility of pQCT measurements may be influenced by disproportionate longitudinal growth of the tibia (especially during adolescence) caused by a greater contribution of the proximal growth plate (60%) compared with the distal growth plate (40%)[51]. However, as in previous studies[24], we expect that despite small shifts in the measurement site associated with an increase in tibia length, the region of interest would still be within the 2.3-mm slice thickness. Further, we reduce this small effect by using a fixed anatomical landmark to locate the same relative region along the tibia length at each follow-up measurement.

In summary, our findings suggest that although HIV infection may be associated with deficits in bone mass during childhood and adolescence, bone geometry and estimates of bone strength appear appropriately adapted to estimates of muscle force. Further, our results suggest deficits in bone mass dissipate over time in these HIV-infected youth. Additional investigations are needed to determine the disease- or treatment-related mechanisms underpinning the greater cortical BMD in this population, and how this might influence bone strength and fracture risk as these youth move into adulthood. Further study is also warranted to determine whether deficits in muscle performance are associated with reduced muscle area in HIV-infected youth. This may serve as a foundation for development of intervention strategies to target muscle and bone health in HIV-infected youth.

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