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Research Article

Changes in peripheral quantitative computed tomography measured bone density, size, and strength in Zimbabwean children with and without HIV over one year: a cohort study

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Abstract

Understanding bone accrual in adolescents may inform approaches to improve skeletal health and reduce adult fracture risk. We investigated the effect of HIV on bone mineral accrual assessed by peripheral quantitative computed tomography (pQCT). Children with HIV (CWH) on ART for ≥2 years, and children without HIV (CWOH), aged 8-16 years ($n = 609$), had tibial pQCT scans at 0 and 12 months. Linear regression estimated sex-stratified differences in change (Δ) and mean pQCT bone density (trabecular and cortical), size (total cross-sectional area [CSA]), and strength (SSI) between CWH and CWOH, adjusting for socio-economic status (SES) and orphanhood and incorporating an interaction term for baseline pubertal status (Tanner 1-2[pre/early] vs 3-5[mid/late]). Structural equation modeling tested whether baseline height-for-age-Z-scores (HAZ) mediate the effect of HIV on Δbone outcomes. CWH were more likely than CWOH to be orphans (44% vs 7%), of lower SES (43% vs 27%), and be stunted (30% vs 8%); but similar in age. At baseline and follow-up, CWH had lower trabecular density, CSA, and SSI than CWOH. After adjustment, bone density and strength increased similarly in CWH and CWOH. CWH in mid/late puberty at baseline had greater 12 months increases in CSA than CWOH, particularly males (mean difference [31.3(95%CI:–3.1, 65.6) mm^2 in mid/late puberty vs –2.04(–23.8, 19.7) mm^2 in pre/early puberty; interaction p-value = 0.013]). HAZ mediated the effect of HIV on Δbone outcomes only in females. as follows: indirect pathways from HIV to Δtrabecular density [–2.47 (–4.4, –0.5) mg/cm^3], Δcortical density [–3.26 (–5.5, –0.9) mg/cm^3], and ΔSSI [–15.76 (–27.3, –4.2) mm^3]. In conclusion, CWH show bone deficits at follow-up. Investigations of bone mineral accrual earlier in life and post-puberty to peak bone mass are needed.

Keywords: pQCT, HIV, children, bone density, bone size, bone strength

Lay Summary

We measured bone density, bone size, and bone strength at 0 and 12 months in 609, 8-16 year old children living with HIV (CWH) and children living without HIV (CWOH). CWH were more likely to be orphans, to be of a lower socio-economic status, to be shorter, and to have lower bone density, size, and strength than CWOH who are of the same age. After 12 months, there were persistent bone deficits in CWH, despite that CWH who were in their mid/late puberty (especially males) showed greater increases in bone size than CWOH. Investigations of bone accrual in early life and beyond puberty are necessary.

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Introduction

People living with HIV are at increased risk of fragility fractures, particularly hip fracture.[1] Bone mineral density (BMD), size, and strength are important determinants of fracture risk.[2] Bone health in later life reflects both bone accrual in childhood and adolescence and bone loss in adult years. Understanding factors that influence bone accrual may inform approaches to improve skeletal health, for example through maximizing acquisition of peak bone mass (PBM) to reduce future adult fracture risk. Most studies investigating BMD by dual energy x-ray absorptiometry (DXA) have reported lower BMD in children living with HIV (CWH) than in children without HIV (CWOH).[3–5] In a cross-sectional analysis, we previously demonstrated lower bone size and predicted bone strength in CWH compared with CWOH; deficits were larger in the later stages of puberty, suggesting lasting effects into early adulthood.[5] Underlying etiological mechanisms remain unclear, and furthermore, it is uncertain whether CWH "catch-up" with their uninfected counterparts as the skeleton matures.[6] Treatment of HIV is associated with poor bone outcomes.[7] In addition, traditional factors that contribute to poor growth and bone accrual such as low dietary calcium, low physical activity, and social deprivation are common in CWH.[6] Although data on bone development in CWH have recently begun to emerge from sub-Saharan Africa where 90% of CWH live,[8] available studies to date have either largely used DXA, which only measures 2-dimensional areal bone density,[3,4,6,9] or have been cross-sectional in study design.[10]

Peripheral quantitative computed tomography (pQCT) uses very low-dose radiation to measure volumetric BMD and bone geometry of cortical and trabecular bone compartments. The skeleton constitutes 80% cortical bone and 20% trabecular bone.[11] Use of pQCT offers an opportunity to increase our understanding of how HIV affects trabecular and cortical bone development during puberty.

Longitudinal data are necessary to understand bone development, particularly in the context of HIV-associated growth impairment.[3,12] Longitudinal studies assessing the effect of HIV on bone mineral accrual from sub-Saharan Africa are lacking despite that 90% of CWH are living in this region. We have previously demonstrated that stunting disproportionately affects CWH compared with CWOH in Zimbabwe, where 32% CWH, age 8-16 years, are stunted.[6] It is unclear how much of the effect of HIV on bone accrual is explained by, and therefore proportionate to, impaired longitudinal growth. Hence, this study aimed to use pQCT to study a pediatric population in sub-Saharan Africa and determine the effect of HIV on the change in pQCT measured bone density, size, and strength over a 12-month period. We also determined to what extent impairment in longitudinal growth explains any detrimental effects of HIV on pQCT-assessed bone outcomes.

Materials and methods

Study setting

A cohort study, the IMpact of Vertical HIV infection on child and Adolescent Skeletal development (IMVASK), with 2 visits 12 months apart, was conducted as per published protocol (ISRCTN12266984).[5,6,13] Perinatally infected CWH attending either of the 2 main public hospitals in Harare, Zimbabwe, were quota sampled, stratified by sex and 3-year age band (8-10, 11-13, 14-16 years). Perinatal infection was defined as HIV acquired since birth and not via blood transfusion or sex. Inclusion criteria were being established on antiretroviral therapy (ART) for at least 2 years and being aware of their HIV status. Stratified random sampling was used to recruit CWOH from 3 primary and 3 secondary schools randomly selected from the 109 primary schools and 44 secondary schools serving the population residing within the same catchment area as the hospitals. The number of children selected from each school was proportional to school size. A random number sequence was generated, and school registers were used to select participants of similar age and sex as the CWH using the same quota- based approach of 50 males and 50 females in each of the 3 age strata. CWOH were tested for HIV to confirm their status and excluded if positive. Those with acute illness requiring hospitalization, and who had no parental consent were excluded.

Study procedures

Baseline data were collected from May 2018 to January 2020, and follow-up from May 2019 to January 2021. Study procedures at the baseline and follow-up visits were the same, as previously described.[6,13] Questionnaires, completed by the parents/guardians, were used to collect demographic and clinical data including age, sex, orphanhood, socio-economic status (SES), medical history, physical activity patterns, and dietary data concerning calcium and vitamin D intake. SES was derived using the first principal component combining a list of factors (number in household, head of household age, highest maternal and paternal education levels, household ownership, monthly household income, access to electricity, water supply, household sanitation, and ownership of a fridge, bicycle, car, television, and/or radio) and was split into tertiles for analysis. Physical activity, assessed using the International Physical Activity Questionnaire (IPAQ) short version,[14] classified participants into low (<600 metabolic rate (MET) minutes per week), moderate (600 to 3000 MET minutes per week), and vigorous intensity (3000 MET minutes per week). A diet and nutrition assessment food frequency tool, validated in India and Malawi[15] and then adapted to the Zimbabwean context with international guidelines applicable to sub-Saharan Africa, was used to quantify dietary calcium and vitamin D intake.[16] The mandatory fortification of oils and margarine with vitamin D in Zimbabwe was included in the adaptation. Daily dietary calcium intake was classified as very low (<150 mg/day), low (150–299 mg/day), and moderate (300–450 mg/day). Daily dietary vitamin D intake was classified as very low (<4.0mcg/day), low (4.0–5.9mcg/day), and moderate (6.0–8.0mcg/day).[16]

Puberty was assessed by a trained study nurse or doctor using Tanner staging. For males, testicular volume, penile size (length and circumference), and pubic hair growth (quality, distribution, and length) were assessed. For females, breast growth (size and contour) as well as pubic hair growth and age of menarche were assessed. Testicular, breast, and penile growth were graded from 1 to 5 based on Tanner descriptions.[17,18] Where there was a discordance between the indicators, testicular and breast development stage, respectively, for males and females were used to assign Tanner stage. Participants were grouped into Tanner stages 1 and 2 (pre/early puberty) and Tanner stages 3 to 5 (mid/late puberty). A mean of 3 standing height measurements, to the nearest 0.1 cm, using a Seca 213 stadiometer (Hamburg, Germany) and a mean of 3 weight measurements, to the nearest 0.1 kg, using a Seca 875 weight scale (Hamburg, Germany) were obtained.

As World Health Organization data for WAZ are not available beyond 10 years of age,[19] weight and BMI Z-scores were generated using external growth standards (from the United Kingdom in 1990).[20] Height Z-scores were generated in the same way[20]; as a validation step Height Z-scores were generated using height measurements from the HIV negative children recruited in this study, as reference data. Height-for-age Z-score < –2, weight-for-age Z-score < –2, and low weight-for-height BMI Z-score < –2 were used to define stunting, underweight, and wasting, respectively.[20,21] CD4 cell count was measured using a PIMA CD4 (Waltham, Massachusetts, United States) machine and HIV viral load using a GeneXpert HIV-1 viral load platform (Cepheid, Sunnyvale, California, United States).

pQCT scan acquisition at baseline and follow-up

Non-dominant tibial pQCT scans were performed using a single XCT 2000™ (Stratec Medizintecknik, Pforzheim, Germany), with voxel size 0.5 × 0.5 mm and slice thickness 2 mm (CT scan speed 30 mm/s; scout view scan speed 40 mm/s). At each visit, tibia length (in millimeters) was measured once, as the distance from the distal medial malleolus to the tibial plateau. As previously described,[5] scan sites were determined as a percentage of tibia length. The exact scan site position was determined by scout view placement of a reference line on the growth plate or on the end plate (for those with fused growth plates).[22] As long bones grow in length, the growth plate moves upward and the wider metaphysis is reshaped into a diaphysis by continuous resorption by osteoclasts beneath the periosteum.[23] To allow for consistency of a scan site the reference line was placed at the growth plate in those children whose end plate and growth plate were not yet fused.[5] If the growth plates were not fused, the reference line was placed on a line dissecting the growth plate along the medial line. If a growth plate had fused during the follow-up interval, the reference line was placed on the end plate. Scan sites were at 4% (trabecular vBMD) and 38% (cortical vBMD) of the measured tibial length. Other measurements included 4% and 38% total cross-sectional area (CSA), 38% cortical thickness, and 38% stress strain index (SSI). The same software (version 6.20 Stratec Medizintechnik) was used for image processing and analysis at both baseline and follow-up.

A phantom was scanned daily for quality assurance. To assess reproducibility, 30 participants were scanned twice after repositioning. Short-term precision (root mean square % CV) was 1.26% for trabecular vBMD, 0.37% for cortical vBMD, 2.08% for 4% total CSA, and 0.93% for 38% CSA. One radiographer qualitatively assessed and graded all pQCT images for movement artifacts from 0 to 3: (0) none, (1) slight streaking, (2) moderate streaking, and (3) scan unusable. Scans graded as a 3 were excluded from analysis. Baseline and follow-up scans were assessed to check that scan positioning at baseline and follow-up were consistent. In addition to movement artifacts, other scan errors such as disagreement in the side scanned (left or right tibia) or in the positioning of the reference line placement (growth plate or end plate) between baseline and follow-up scans were reasons to exclude participant scans from analyses.

Ethical considerations

This study was approved by the Parirenyatwa Hospital and College of Health Sciences joint research ethics committee (JREC/123/19), the Biomedical Research and Training Institute Institutional Review Board (AP150/2019), the Medical Research Council of Zimbabwe (MRCZ/A2494), and the London School of Hygiene and Tropical Medicine (17154) Ethics Committee. Written informed consent and/or age-appropriate assent was obtained from parents/guardians and participants.

Statistical analysis

Statistical analyses were performed using Stata version 17 (Stata Corporation Inc., College Station, TX, United States). Data were cleaned and checked for consistency and outliers. Primary outcomes were annualized mean 4% Δ trabecular and 38% Δ cortical vBMD (mg/cm^3), 4% and 38% Δ CSA (mm^2), 38% Δ cortical thickness (mm), and 38% Δ stress–strain index (SSI) (mm^4). Annualized changes of pQCT bone outcomes were defined as the pQCT bone outcomes at follow-up minus the pQCT bone outcome at baseline, divided by number of days between the baseline and follow-up visits and multiplied by 365.25. All analyses were stratified by sex as bone accrual rates differ between males and females.[24] Independent t-tests were used to compare group means for continuous data and chi-squared tests were used for categorical percentages. Linear regression (with robust standard errors) was used to estimate mean differences (presented with 95% CI) between CWH and CWOH. Adjustments were made for SES in tertiles,[25] and orphanhood.[6] Potential differences in the effect of HIV on Δbone outcomes by pubertal status were tested by incorporating an interaction term for baseline binary pubertal status (Tanners 1 and 2 vs Tanners 3, 4, and 5). Structural equation modeling (SEM) for mediation analysis was used to evaluate whether the effect of HIV infection on change in bone outcomes was mediated first by internally generated baseline height Z-scores and secondly by internally generated baseline tibia length Z-scores at baseline. SEM was used, irrespective of the overall association between HIV and the pQCT bone outcomes since it is possible for an exposure to exert an effect on an outcome indirectly through a mediator even if one cannot establish evidence of an association through a hypothesis test, for a total effect of an exposure on an outcome.[26] The structural equation models used linear regression and were adjusted for SES and orphanhood.

Results

Study population

At baseline 609 participants, 303 CWH (151 [49.8%] male and 152 [50.2%] female), and 306 CWOH (151 [49.8%] male and 152 [50.2%] female) were recruited, and 492 (80.7%) participants, 244 CWH (125 [51.2%] male and 119 [48.8%] female), and 248 CWOH (122 [49.2%] male and 126 [50.8%] female) had a follow-up visit (Supplementary Figure 2). In the complete case analysis, 419 (68.8%) participants had usable pQCT scans at both baseline and follow-up and were not missing covariate data. The 190 (31.2%) participants (108 CWH and 82 CWOH) not included in analyses were similar to those included in terms of age, sex, height, weight, pubertal stage, SES, physical activity levels, calcium, and vitamin D intake (Supplementary Table 1). Eleven of the 419 children (3%) underwent complete fusion of epiphyses and had a change in reference line placement from the growth plate to end plate.

At baseline, males were aged mean 12.3 (standard deviation [SD] 2.5) years and females 12.4 (SD 2.5) years with no difference by HIV status (Table 1). Follow-up was challenged in 2020-2021 by national travel restrictions

due to COVID-19 lockdown. Between-visit duration was on average 66 days longer among CWH compared with CWOH; therefore, at follow-up CWH were older than CWOH (Supplementary Table 1). Most males ($n = 132$ [62%]) were in Tanner stages 1 or 2 at baseline, while fewer than half were so at follow-up ($n = 94$ [44%]); this did not differ by HIV status. More female CWH were in Tanner stages 1 and 2 compared with CWOH (at both baseline and follow-up) (Table 1, Supplementary Table 1). Having one or both parents deceased was more common among CWH, compared with those without HIV (42% vs 7% in males; 46% vs 6% in females, Table 1). CWH were more likely to have a lower SES than CWOH (43% vs 25% in males; 42% vs 29% in females). Dietary calcium intake and levels of physical activity were generally low, with no difference by HIV status (Table 1).

Both height and weight were lower in CWH than CWOH, but during follow-up CWH experienced greater absolute gains in height compared with CWOH (Table 1). At baseline, male and female CWH were 6 and 8 cm shorter than CWOH, respectively, and more likely to be stunted (33% vs 7% [males]; 26% vs 9% [females]). Stunting prevalence increased slightly at follow-up when CWH were more likely to be stunted than CWOH (43% vs 9% [males]; 33% vs 11% [females]) (Supplementary Table 2). Although overall the study population gained weight during follow-up, the proportion who were classified as having low weight-for-age increased; at follow-up this was more likely in CWH than CWOH (35% vs 14% in males; 27% vs 7% in females) (Supplementary Table 2). During follow-up a greater proportion of CWH than CWOH advanced in Tanner stage, 62% vs 49% in males and 66% vs 59% in females (Table 1).

The effect of HIV on pQCT bone outcomes

At baseline, all CWH had lower distal trabecular density, lower distal and diaphyseal tibial CSA and lower predicted bone strength (38% SSI) than CWOH, while diaphyseal cortical density and thickness were comparable by HIV status, in both males and females (Table 2); these findings persisted at follow-up and remained robust to adjustment for SES and orphanhood (Table 2). In analyses of annualized change in bone parameters, adjusted for SES and orphanhood, the annual change in pQCT measures was similar by HIV status (Table 2). Baseline pubertal stage appeared to modify the effect of HIV on growth in bone size, specifically distal tibial CSA in males, such that in late puberty male CWH had a greater increase in tibial CSA than CWOH, with a mean adjusted difference of 31.3 mm^2 [95%CI –3.1, 65.6; p=.074; interaction p=.013] (Figure 1, Table 3). In females the same pattern was seen at the diaphyseal site for tibial CSA (rather than the distal site in males), such that female CWH in late puberty at baseline had greater increases in bone size than CWOH. No evidence was detected for an interaction between pubertal stage and HIV for bone density or strength measures.

The effect of HIV on change in pQCT bone outcomes mediated by height for age z-scores.

In females, there was some evidence that the effect of HIV on annualized change in pQCT bone outcomes was mediated via HAZ (Table 4). In both males and females, there was strong evidence of an association between HIV and lower baseline HAZ (the mediator). The effect of HIV on HAZ was β –0.88 (95%CI –1.19, –0.57); p<.001 in males, and –0.90 (–1.24, –0.57); p<.001 in females. In males, there was no evidence of an indirect effect of HIV on change in pQCT outcomes via HAZ and hence no evidence for mediation by linear growth, except for Δcortical density only (–3.19 (–5.55, –0.82); p=.008), which was completely mediated by HAZ. In females, there appeared to be an indirect effect of HIV via HAZ on Δtrabecular density, Δcortical density, and Δpredicted bone strength (SSI), while no evidence for a direct effect of HIV on these bone outcomes was seen.

In sensitivity analyses (1) modeling change in bone outcomes adjusted for duration of follow-up, findings were unchanged, (2) excluding 11 children who underwent epiphyseal fusion, findings were unchanged, and (3) assessing whether tibial length Z-Score mediated the effect of HIV in bone outcomes differently from height Z-Score, we identified broadly consistent patterns, although indirect effect estimates were smaller (Supplementary Table 3).

Discussion

To our knowledge, this is the first study to investigate longitudinal changes in detailed pQCT measured bone parameters in CWH in sub-Saharan Africa. The study showed that CWH have consistently lower trabecular bone density, bone size and bone strength than CWOH over 1 year of follow-up. Our findings suggest that in children aged 8-16 years, puberty modifies the effect of HIV on bone mineral accrual, such that in the later stages of puberty both male and female CWH increase their bone size more than CWOH, although at the end of follow-up, and despite more CWH transitioning pubertal stage, bone size remains smaller in CWH. Furthermore, we have demonstrated there is a mediation effect of height on pQCT bone outcomes in females only, although total effect sizes are similar in males and females.

There were greater increases in height over the 1-year of this study in CWH than CWOH. In addition, over the same period, a greater proportion of CWH than CWOH advanced in Tanner stage. We have previously reported pubertal delay in the same cohort[6] and other studies have shown height is strongly determined by pubertal growth.[27] Pubertal transition is classically characterized by a "growth spurt" (ie, peak height velocity). The differences in height change in our cohort could be explained by more CWH than CWOH still undergoing pubertal transition from early to late puberty. Despite that, male CWH who were in the later stages of puberty gained more bone size at the 4% site than CWOH; however, male CWH still had lower mean bone size than CWOH at the follow-up visit. These results suggest that, although CWH demonstrate a degree of catch-up growth in bone size, as they transition through puberty, this catch-up growth may be insufficient to reach full skeletal potential. These findings are concerning since deficits in bone accrual at the end of puberty are likely to persist into adulthood.

We have shown that CWH still had lower bone outcomes at follow-up despite the greater increase in height over the study period. Moreover, we have demonstrated that the effect of HIV on Δbone size, Δbone strength, and Δcortical density in females is due to the indirect effect of HIV on height rather than the effect of HIV on changing bone turnover and mineral accrual. Childhood HIV infection can manifest as stunting (poor linear growth). Our study reports a higher prevalence of stunting in CWH than in CWOH for both males (33% [CWH] vs 7% [CWOH]) and females (26% [CWH] vs 10% [CWOH]). This is similar to several studies that have assessed growth patterns in African countries which have consistently

Table 1. Clinical characteristics and anthropometry of children living with and without HIV stratified by sex.

	Males ($n=213$)			Females ($n=206$)		
	CWOH ($n=113$)	CWH ($n=100$)	*p*-value	CWOH ($n=111$)	CWH ($n=95$)	*p*-value
Baseline clinical characteristics						
Age, years, mean (SD)	12.2 (2.4)	12.5 (2.5)	0.29	12.6 (2.5)	12.1 (2.4)	0.14
Age group, %			0.96			0.23
8-10 years	40 (35)	34 (34)		35 (32)	36 (38)	
11-13 years	39 (35)	34 (34)		33 (30)	33 (35)	
14-16 years	34 (30)	32 (32)		43 (39)	26 (27)	
Tanner stage, %			0.59			<0.001
Tanner 1	35 (31)	38 (38)		19 (17)	43 (45)	
Tanner 2	31 (27)	28 (28)		22 (20)	15 (16)	
Tanner 3	18 (16)	15 (15)		22 (20)	20 (21)	
Tanner 4	27 (24)	16 (16)		35 (32)	14 (15)	
Tanner 5	2 (2)	3 (3)		13 (12)	3 (3)	
Pubertal status, %			0.26			<0.001
Early puberty	66 (58)	66 (66)		41 (37)	58 (61)	
Late puberty	47 (42)	34 (34)		70 (63)	37 (39)	
Socio-economic status, %			0.009			0.077
Low, tertile 1	28 (25)	43 (43)		32 (29)	40 (42)	
Middle, tertile 2	36 (32)	30 (30)		37 (33)	31 (33)	
High, tertile 3	49 (43)	27 (27)		42 (38)	24 (25)	
Orphanhood, %			<0.001			<0.001
Not an orphan	105 (93)	58 (58)		105 (95)	51 (54)	
One parent alive	5 (4)	36 (36)		4 (4)	36 (38)	
Orphan	3 (3)	6 (6)		2 (2)	8 (8)	
Physical activity, %			0.061			0.16
Low, <600	37 (33)	43 (43)		43 (39)	49 (52)	
Moderate, 600-3000	40 (35)	21 (21)		32 (29)	24 (25)	
High, >3000	36 (32)	36 (36)		36 (32)	22 (23)	
Calcium intake, %			0.98			0.81
Low, <150 mg	49 (43)	42 (42)		48 (43)	38 (40)	
Moderate, 150-299 mg	24 (21)	22 (22)		24 (22)	24 (25)	
High, 300-449 mg	40 (35)	36 (36)		39 (35)	33 (35)	
Change in clinical characteristics during 1 year follow-up						
Tanner stage	55 (49)	61 (62)	0.072	65 (59)	63 (66)	0.32
Baseline anthropometry						
Height, cm, mean(SD)	146.2 (14.5)	140.6 (12.1)	0.003	147.7 (11.6)	139.4 (13.0)	<0.001
Height for age Z-score, mean (SD)[a]	–0.7 (1.0)	–1.7 (1.1)	<0.001	–0.5 (1.1)	–1.5 (1.0)	<0.001
Height for age Z-score, mean (SD)[b]	0 (1.5)	–1.6 (1.6)	<0.001	0 (1.4)	–1.4 (1.5)	<0.001
Stunting, %	8 (7)	33 (33)	<0.001	10 (9)	25 (26)	<0.001
Weight, kgs, mean (SD)	37.1 (11.0)	33.2 (7.7)	0.004	42.8 (13.1)	34.3 (10.3)	<0.001
Weight for age Z-score, mean (SD)	–0.8 (1.0)	–1.6 (1.2)	<0.001	–0.2 (1.1)	–1.3 (1.1)	<0.001
Underweight, %	12 (11)	29 (29)	<0.001	7 (6)	19 (20)	0.003
Body mass index, kg/cm^2	16.9 (2.2)	16.5 (1.4)	0.17	19.1 (3.8)	17.2 (2.5)	<0.001
Body mass index Z-scores, mean (SD)	–0.6 (1.0)	–0.8 (0.8)	0.086	0.0 (1.2)	–0.6 (0.9)	<0.001
Wasting, %	9 (8)	7 (7)	0.79	3 (3)	7 (7)	0.12
Tibia length, mm, mean (SD)	341.7 (47.3)	327.6 (36.1)	0.017	344.7 (37.6)	323.5 (39.2)	<0.001
Change in anthropometry						
Height, cm, mean (SD)	5.0 (2.4)	6.8 (4.7)	<0.001	3.7 (3.3)	4.9 (3.0)	0.005
Weight, kgs, mean (SD)	4.0 (2.4)	4.3 (3.1)	0.53	3.9 (3.5)	4.1 (3.3)	0.6
Body mass index, kg/cm^2, mean (SD)	0.7 (0.8)	0.4 (0.8)	0.029	0.9 (1.2)	0.8 (1.3)	0.83
Tibia length, mm, mean (SD)	20.3 (21.6)	20.3 (13.2)	0.980	18.3 (18.6)	16.9 (17.6)	0.59

[a]Height for age Z-score based on British reference data. [b]Height for age Z-score based on internally generated reference data. Abbreviations: CWH, Children living with HIV; CWOH, Children without HIV; SD, Standard Deviation; BMI, body mass index.

reported high prevalence of stunting as measured by height for age Z-scores in CWH.[12,28–34] In Zimbabwe CWH have higher odds of stunting; eight times greater in those who have acquired HIV in utero, and 4 times greater in those who have acquired HIV around the time of birth.[34] Improvements in growth have been reported in CWH who have an early HIV diagnosis and less severe HIV symptoms or who start ART early.[35] However, in Africa, CWH start ART later in life than other parts of the world, often when the disease is already in a more advanced stage, potentially affecting growth.[36]

To date, only one other study conducted in Canada has assessed longitudinal changes in pQCT bone outcomes in 31 CWH and reported no change in pQCT bone outcomes over 2 years,[36] probably because the study was underpowered. Our findings demonstrate lower bone outcomes in CWH than in CWOH at both baseline and follow-up, despite evidence of

Table 2. pQCT bone outcomes of children living with and without HIV, stratified by sex.

	Males ($n = 213$)				Females ($n = 206$)			
	Mean (SD) CWOH ($n = 113$)[a]	Mean (SD) CWH ($n = 100$)[a]	Adjusted MD (95% CI)[b]	*p*-value	Mean (SD) CWOH ($n = 111$)[a]	Mean (SD) CWH ($n = 95$)[a]	Adjusted MD (95% CI)[b]	*p*-value
Baseline								
4% trabecular density, mg/cm^3	208.0 (42.1)	197.3 (37.7)	−10.52 (−22.8, 1.7)	0.092	210.7 (31.5)	198.5 (33.1)	−12.63 (−22.9, −2.4)	0.016
38% cortical density, mg/cm^3	1070.3 (33.5)	1067.8 (37.1)	−3.56 (−14.4, 7.3)	0.518	1098.6 (45.7)	1095.7 (40.2)	−8.21 (−21.9, 5.5)	0.239
4% total cross-sectional area, mm^2	752.7 (230.2)	679.2 (177.9)	−99.79 (−163.0, −36.6)	0.002	731.7 (171.1)	661.1 (166.9)	−104.89 (−157.8, −51.9)	<0.001
38% total cross-sectional area, mm^2	341.79 (78.01)	313.00 (64.35)	−39.04 (−60.9, −17.2)	0.001	330.25 (60.4)	286.52 (57.6)	−51.18 (−69.8, −32.5)	<0.001
38% cortical thickness, mm	3.8 (0.6)	3.8 (0.6)	−0.13 (−0.3, 0)	0.153	3.8 (0.6)	3.7 (0.6)	−0.18 (−0.4, 0)	0.062
38% stress strain index, mm^3	1162.0 (386.9)	1021.3 (311.1)	−197.54 (−304.9, −90.2)	<0.001	1124.0 (326.4)	923.1 (284.2)	−241.28 (−338.3, −144.3)	<0.001
Follow-up								
4% trabecular density, mg/cm^3	203.8 (34.5)	192.1 (36.1)	−10.87 (−21.6, −0.1)	0.048	210.9 (33.1)	196.2 (32.6)	−14.52 (−25.0, −4.1)	0.007
38% cortical density, mg/cm^3	1077.1 (38.8)	1074.0 (38.8)	−4.80 (−16.8, 7.2)	0.429	1115.5 (45.6)	1109.0 (39.2)	−11.17 (−24.7, 2.4)	0.106
4% total cross-sectional area, mm^2	783.0 (221.8)	728.9 (187.3)	−81.91 (−144.8, −19.0)	0.011	748.6 (152.7)	685.0 (151.1)	−94.32 (−142.0, −46.6)	<0.001
38% total cross-sectional area, mm^2	368.1 (79.1)	342.1 (70.0)	−36.31 (−59.2, −13.5)	0.002	346.6 (56.5)	306.6 (57.5)	−48.16 (−66.1, −30.2)	<0.001
38% cortical thickness, mm	4.0 (0.6)	3.9 (0.7)	−0.17 (−0.4, 0)	0.074	4.0 (0.6)	3.8 (0.6)	−0.21 (−0.4, 0)	0.033
38% stress strain index, mm^3	1306.8 (410.2)	1175.6 (358.3)	−190.72 (−308.4, −73.0)	0.002	1229.4 (314.6)	1034.3 (295.1)	−240.50 (−336.9, −144.1)	<0.001
Change over 12 months								
4% trabecular density, mg/cm^3	−3.8 (19.5)	−4.4 (17.2)	−0.35 (−6.0, 5.3)	0.903	0.1 (13.4)	−2.1 (13.9)	−1.61 (−5.9, 2.7)	0.463
38% cortical density, mg/cm^3	6.2 (18.3)	5.1 (18.4)	−1.90 (−7.6, 3.8)	0.508	14.8 (16.7)	10.6 (14.5)	−3.61 (−8.6, 1.4)	0.157
4% total cross-sectional area, mm^2	27.5 (65.9)	41.3 (53.2)	13.23 (−5.3, 31.8)	0.161	15.7 (57.6)	18.5 (61.8)	6.42 (−12.4, 25.3)	0.503
38% total cross-sectional area, mm^2	24.7 (15.7)	24.7 (18.6)	0.10 (−5.2, 5.4)	0.970	14.6 (16.4)	16.4 (17.0)	1.44 (−3.8, 6.7)	0.590
38% cortical thickness, mm	0.2 (0.2)	0.1 (0.2)	−0.05 (−0.1, 0)	0.101	0.2 (0.2)	0.1 (0.2)	−0.04 (−0.1, 0)	0.252
38% stress strain index, mm^3	136.5 (77.9)	130.8 (95.6)	−7.12 (−33.7, 19.5)	0.599	94.2 (73.5)	90.4 (89.8)	−6.52 (−32.2, 19.2)	0.617

[a]Unadjusted. [b]Adjusted for socioeconomic status and orphanhood. MD (95% CI); Mean Difference (95 % Confidence Interval) with CWOH as the reference group, such that negative values mean that those with HIV have lower values than those without HIV. Abbreviations: CWH, Children living with HIV; CWOH, Children without HIV; SD, Standard deviation.

Table 3. pQCT bone outcomes of children living with and without HIV, stratified by sex and pubertal status.

	Unadjusted ($n=419$)					Adjusted ($n=419$)[a]				
	Tanner Stages 1 and 2		Tanner Stages 3, 4, and 5		Interaction	Tanner Stages 1 and 2		Tanner Stages 3, 4, and 5		Interaction
Characteristic	MD (95% CI)	*p*-value	MD (95% CI)	*p*-value	*p*-value	MD (95% CI)	*p*-value	MD (95% CI)	*p*-value	*p*-value
Males ($n=213$)										
Δ 4% trabecular density, mg/cm^3	−3.10 (−9.0, 2.8)	0.298	3.59 (−5.6, 12.8)	0.441	**0.208**	−2.91 (−9.6, 3.7)	0.389	3.52 (−7.6, 14.7)	0.531	**0.174**
Δ 38% cortical density, mg/cm^3	3.49 (−2.1, 9.1)	0.218	−7.13 (−16.1, 1.9)	0.118	**0.051**	3.05 (−3.2, 9.3)	0.337	−6.77 (−17.5, 3.9)	0.212	**0.042**
Δ 4% total CSA, mm^2	−4.17 (−23.4, 15.1)	0.669	40.81 (12.0, 69.6)	0.006	**0.011**	−2.04 (−23.8, 19.7)	0.853	31.27 (−3.1, 65.6)	0.074	**0.013**
Δ 38% total CSA, mm^2	−0.78 (−6.7, 5.1)	0.792	0.89 (−6.9, 8.7)	0.820	**0.728**	−0.72 (−7.4, 5.9)	0.831	1.47 (−7.9, 10.8)	0.754	**0.728**
Δ 38% cortical thickness, mm	−0.05 (−0.1, 0)	0.185	−0.03 (−0.1, 0.0)	0.372	**0.653**	−0.05 (−0.1, 0)	0.275	−0.03 (−0.1, 0.1)	0.480	**0.713**
Δ 38% stress strain index, mm^3	−3.16 (−33.6, 27.3)	0.838	−4.41 (−41.0, 32.2)	0.811	**0.958**	−3.05 (−37.5, 31.3)	0.861	4.42 (−39.3, 48.1)	0.841	**0.966**
Females ($n=206$)										
Δ 4% trabecular density, mg/cm^3	0.98 (−4.5, 6.5)	0.723	−4.53 (−10.0, 1.0)	0.106	**0.163**	0.25 (−6.1, 6.6)	0.938	−2.97 (−9.6, 3.6)	0.374	**0.144**
Δ 38% cortical density, mg/cm^3	−1.98 (−8.6, 4.7)	0.556	−3.26 (−9.2, 2.7)	0.278	**0.781**	−1.65 (−9.3, 6.0)	0.669	−0.49 (−7.6, 6.6)	0.892	**0.845**
Δ 4% total CSA, mm^2	−14.76 (−40.5, 11.0)	0.259	−2.10 (−20.7, 16.5)	0.824	**0.444**	−17.88 (−47.2, 11.4)	0.229	1.72 (−20.9, 24.3)	0.880	**0.546**
Δ 38% total CSA, mm^2	−6.51 (−13.4, 0.3)	0.063	4.76 (−1.1, 10.6)	0.110	**0.013**	−7.04 (−14.9, 0.9)	0.080	3.32 (−3.7, 10.3)	0.349	**0.022**
Δ 38% cortical thickness, mm	−0.09 (−0.2, 0.0)	0.072	0.02 (−0.0, 0.1)	0.501	**0.064**	−0.11 (−0.2, 0)	0.061	0.02 (−0.1, 0.1)	0.639	**0.096**
Δ 38% stress strain index, mm^3	−31.62 (−66.2, 3.0)	0.073	9.68 (−20.5, 39.9)	0.526	**0.072**	−37.27 (−76.9, 2.4)	0.065	9.57 (−26.7, 45.9)	0.602	**0.097**

[a]Adjusted for socioeconomic status and orphanhood. MD (95% CI); Mean Difference (95 % Confidence Interval) with CWOH as the reference group, such that negative values mean that those with HIV have lower values than those without HIV. Abbreviations: CSA, Cross-sectional area; Δ, Linear regression estimated sex-stratified differences in change.

Table 4. pQCT bone outcomes of children living with and without HIV after mediation analysis with HIV as exposure, internally generated height for age Z-scores as the mediator and adjusted for SES and orphanhood status.

	Adjusted for SES and orphanhood status			
	Direct effect of HIV on pQCT outcome, β (95% CI); p value	Effect of Height Z-score on pQCT bone outcome, β (95% CI); p value	Indirect effect of HIV on pQCT outcome, β (95% CI); p value	Total effect of HIV on pQCT outcome, β (95% CI); p value
Males ($n=213$)				
Δ 4% trabecular density	1.02 (–0.57, 2.60); 0.210	1.02 (–4.95, 6.98); 0.738	–1.37 (–3.56, 0.82); 0.221	–0.35 (–5.94, 5.24); 0.902
Δ 38% cortical density	1.29 (–4.55, 7.12); 0.666	2.36 (0.81, 3.91); 0.003	–3.19 (–5.55, –0.82); 0.008	–1.90 (–7.46, 3.65); 0.502
Δ 4% total CSA	7.56 (–11.83, 26.95); 0.445	–4.20 (–9.36, 0.95); 0.110	5.67 (–1.56, 12.90); 0.124	13.2 (–4.98, 31.44); 0.154
Δ 38% total CSA	0.48 (–6.02, 6.215.06); 0.864	–0.43 (–1.91, 1.04); 0.565	–0.58 (–1.41, 2.58); 0.567	0.10 (–5.07, 5.28); 0.969
Δ 38% cortical thickness	–0.07 (–0.13, 0.01); 0.047	–0.01 (–0.03, 0.01); 0.241	0.01 (–0.01, 0.04); 0.251	–0.05 (–0.11, 0.01); 0.095
Δ 38% SSI	–4.09 (–32.10, 23.91); 0.774	2.23 (–5.21, 9.68); 0.560	–3.02 (–13.12, 7.08); 0.558	–7.12 (–33.28, 19.05); 0.594
Females ($n=206$)				
Δ 4% trabecular density	0.86 (–3.66, 5.38); 0.709	1.74 (0.51, 2.96); 0.005	–2.47 (–4.40, –0.55); 0.012	–1.61 (–5.86, 2.64); 0.457
Δ 38% cortical density	–0.35 (–5.56, 4.86); 0.896	2.28 (0.87, 3.69); 0.002	–3.26 (–5.54, –0.98); 0.005	–3.61 (–8.53, 1.31); 0.151
Δ 4% total CSA	6.71 (–13.31, 26.83); 0.509	0.24 (–5.18, 5.66); 0.931	–0.34 (–8.08, 7.39); 0.931	6.41 (–12.10, 24.94); 0.417
Δ 38% total CSA	2.62 (–2.95, 8.17); 0.357	0.83 (–0.68, 2.33); 0.282	–1.18 (–3.37, 1.01); 0.289	1.44 (–3.72, 6.59); 0.585
Δ 38% cortical thickness	–0.03 (–0.09, 0.04); 0.364	0.01 (–0.01, 0.02); 0.667	–0.01 (–0.03, 0.02); 0.667	–0.04 (–0.10, 0.03); 0.244
Δ 38% SSI	9.24 (–-17.54, 36.03); 0.499	11.02 (3.78, 18.27); 0.003	–15.76 (–27.34, –4.19); 0.008	–6.52 (–31.75, 18.71); 0.612

The effect of HIV on Zim height z-score at baseline, (β (95% CI); p value) was '–0.88 (–1.19, –0.57); <0.001 (males) and '–0.90(–1.24, –0.57); <0.001 (females). Direct effect is the pathway from HIV to the pQCT outcome. The indirect effect is the pathway from HIV via Zim height for age Z-score at baseline to the pQCT outcome. The total effect is the sum of the direct and indirect effects. MD (95% CI); Mean Difference (95 % Confidence Interval) with CWOH as the reference group, such that negative values mean that those with HIV have lower values than those with HIV. Abbreviations: CSA, Cross-sectional area; SSI, Stress strain index; Δ, Linear regression estimated sex-stratified differences in change.

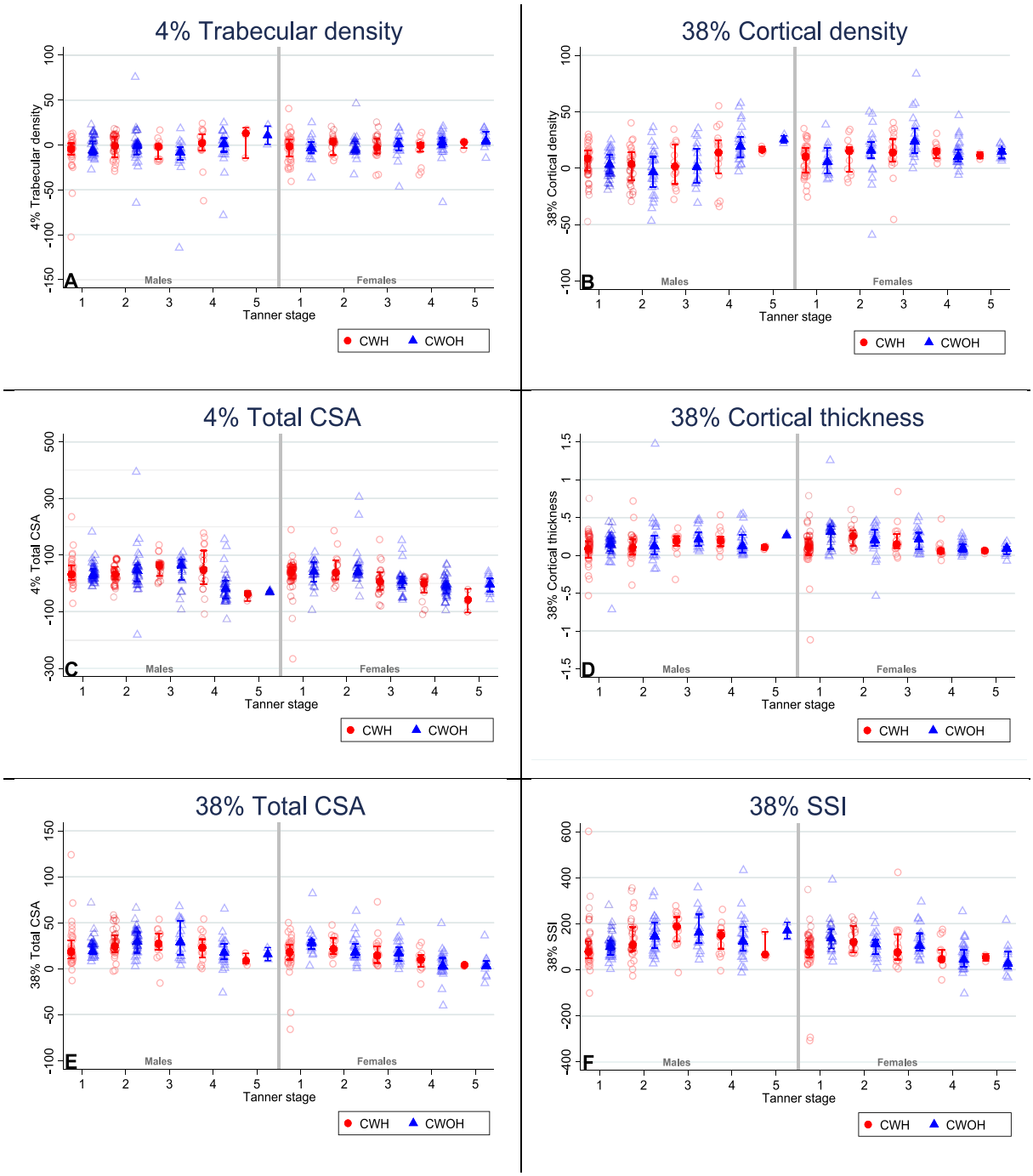


Figure 1. Unadjusted comparison of change in pQCT measured bone outcomes, over 12 months, between children living with and without HIV infection by sex and pubertal status; Figure 1 shows unadjusted comparison of change in pQCT measured bone outcomes, over 12 months, between children living with and without HIV infection by sex and pubertal status. Abbreviations: CWH, Children living with HIV; CWOH, Children without HIV; CSA, Cross-sectional area; SSI, Stress strain index.

some catch up growth as CWH transition through puberty. Bone formation occurs at a faster rate before the age of 4 years, and during puberty than in any other time of one's life. Our cohort were enrolled into the study at minimum age of 8 years. It is unclear whether or not the lower bone density, bone size and one strength we observed in CWH is a result of factors influencing bone in the early years of life and whether or not CWH will eventually catch-up to their uninfected counterparts.

Combining our results, we hypothesize that the trajectory of skeletal growth for CWH has been preset at a lower trajectory than in CWOH before the age of 8 years or possibly even before the initiation of ART by age 4 years, in the critical window of the first 1000 days of life. Figure 2 illustrates this hypothesis. As the children transition through puberty, it appears that the development of bone density and bone strength runs in parallel to CWOH, meaning they are growing at the same rate but that the set point has been predetermined by something else earlier in life and by the end of our study's follow-up period, there are still deficits in all parameters of bone. There is evidence of catch-up growth as puberty proceeds with bone area increasing to accommodate height

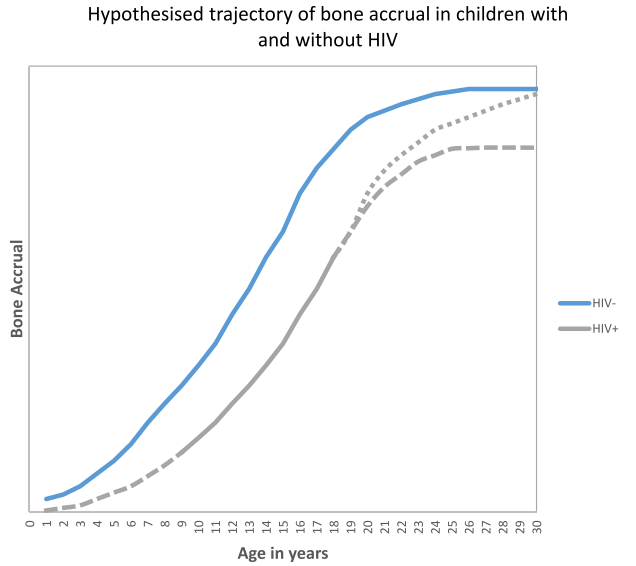


Figure 2. Conceptualized diagram showing bone accrual in children with and without HIV; Figure 2 is a hypothesized diagram, hypothesizing that CWH are pre-set at a lower trajectory than CWOH before the age of 8 years, accrue bone at the same rate as CWOH, and may or may not catch-up after pubertal years. Abbreviations: CWH, Children living with HIV; CWOH, Children without HIV; CSA, Cross-sectional area; SSI, Stress strain index.

growth. It is unknown whether the catch up growth in CWH is sufficient or not sufficient to enable them to get back on the trajectory they would have been had they not had HIV. It is also not known whether it is possible to reset that trajectory through interventions in the early life management of CWH. Moreover, when those children are reaching the end of puberty it is unclear whether CWH continue to accrue bone for a longer period of time to an older age than CWOH such that they do achieve the PBM at a later stage. Studies are needed to examine the pattern of growth in later adolescence and timing of the acquisition of PBM in CWH to assess whether deficits in bone and in height are likely to continue through adulthood and increase their risk of fracture.

Strengths and limitations

The strengths of this study include the novel use of pQCT's three-dimensional outcomes to evaluate bone measurements using a large sample. The inclusion of a comparison group of children without HIV, and longitudinal follow-up are also strengths. The participants are likely to be representative of children living in Harare due to our robust sampling methods. The precision results reported in our study are comparable with the expected precision results for pQCT bone outcomes from other authors, suggesting our results are likely not due to scan errors and this is another strength of this study. The use of pQCT may also be considered a potential limitation, as this is not a technique used in clinical practice, which limits the ability to relate our findings to routine clinical practice. There are currently no normative pQCT reference data for children living in sub-Saharan Africa, so it is not possible to determine how our measurements relate to a population norm. There is no validated reference growth database for children living in Zimbabwe or in sub Saharan Africa. In defining stunting, underweight and wasting in this study, the use of growth reference values from British children requires some caution in interpretation as changes in Z-scores may reflect changes relative to UK growth curves (for example timing of puberty), rather than changes in the children under study. This is a limitation of use of external references such as this and WHO standards (which we did not use because they do not cover the age range of our study population for weight) and highlights the importance of collecting independent country-specific references. Participants with missing data were excluded to allow SEM analysis, thereby limiting the number of participants and potentially reducing power in this study. Small numbers within Tanner stage categories limited our ability to test for interaction, as we needed to group participants in different pubertal stages based on numbers within each stage, rather than pubertal biological characteristics for each stage. We assessed reliability of pQCT bone outcomes but not of height and tibia length measurements and therefore we acknowledge this as another limitation of our study. The study was underpowered to assess the relationship between changes in height with changes in bone outcomes over a year, to determine whether changes were proportionate across compartments. Furthermore, the exact timing of peak height velocity could not be established, meaning it is not currently possible to determine whether the differences are delays in growth or persisting deficits in bone accrual. Follow-up of longer than 1 year may provide further insights into trajectories of bone mineral accrual.

Conclusion

Deficits were seen in bone size and strength associated with HIV infection over 1 year follow-up. CWH gained more height, bone size, and bone strength than CWOH over the follow-up period, showing some evidence to support catch-up growth in CWH, but not enough to rectify total deficits in bone density, size, and strength. These findings add to information on growth impairment in CWH and have potential implications for fracture risk in adulthood. If bone accrual does not catch up, CWH may be at higher risk of fracture in later life. To better understand trajectories of skeletal development, further research is needed to assess the effect of HIV on bone development in the early years of life as well as to follow-up CWH through to the end of young adult bone accrual.

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Author contributions

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Writing—review & editing), Rashida Ferrand (Project administration, Supervision, Writing—review & editing), Kate A Ward (Conceptualization, Methodology, Resources, Supervision, Writing—review & editing), and Andrea M. Rehman (Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing—original draft, Writing—review & editing)

Supplementary material

Supplementary material is available at *Journal of Bone and Mineral Research* online.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability

The data that support the findings of this study are available on request from the senior authors.

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