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Effect of HIV infection on growth and bone density in peripubertal children in the era of antiretroviral therapy: a cross-sectional study in Zimbabwe

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Abstract:	Background Poor linear growth and pubertal delay, both common in children with HIV (CWH) in sub-Saharan Africa, may affect adolescent bone accrual and future fragility fracture risk. We investigated the association of HIV with size-adjusted bone density in peri-pubertal children in Zimbabwe. Methods CWH aged 8-16 years taking ART for ≥2 years from public-sector HIV clinics in Harare, and HIV-uninfected children from schools in the same suburbs, were recruited into a cross-sectional study. Sociodemographic, clinical and anthropometric data were collected. The prevalence of dual-energy X-ray absorptiometry (DXA) measured bone outcomes, total-body less-head bone mineral content-for-lean mass adjusted for height (TBLH-BMCLBM) and lumbar spine bone mineral apparent density (LS-BMAD), were determined. Linear regression models, using multiple imputation for missing data, assessed relationships between risk factors and TBLH-BMCLBM and LS-BMAD Z-scores. Findings We recruited 303 CWH and 306 without HIV, mean (SD) age 12.5 (2.5) years and 50% female. Median ART duration was 8.1 (IQR 6.2-9.5) years; for 102 (34%) ART included tenofovir disproxil fumarate (TDF). Compared to children without HIV, those with HIV

	had higher prevalence of TBLH-BMCLBM (10.4% vs. 6.2%, p=0.066) and LS-BMAD Z-Scores <-2 (14.3% vs. 5.8%, p=0.001). HIV and male sex were associated with earlier Tanner stage. The negative associations between HIV and both TBLH-BMCLBM and LS-BMAD were more pronounced with pubertal maturation, particularly in females. Among CWH, years of TDF exposure and orphanhood were associated with lower TBLH-BMCLBM Z-Score. Current TDF use (v.s., non-TDF containing ART) was associated with a reduction in TBLH-BMCLBM and LS-BMAD Z-Scores of 0.41 (95%CI 0.08, 0.74; p=0.015) and 0.31 (0.08, 0.69; p=0.116) respectively. Interpretation Despite ART, HIV is associated with substantial skeletal deficits towards the end of puberty. The size of bone deficits associated with TDF and its widespread use in children in sub-Saharan Africa raise concern for future adult fracture risk.

Effect of HIV infection on growth and bone density in peripubertal children in the era of antiretroviral therapy: a cross-sectional study in Zimbabwe

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Abstract

Background: Poor linear growth and pubertal delay, both common in children with HIV (CWH) in sub-Saharan Africa, may affect adolescent bone accrual and future fragility fracture risk. We investigated the association of HIV with size-adjusted bone density in peri-pubertal children in Zimbabwe.

Methods: CWH aged 8-16 years taking ART for ≥2 years from public-sector HIV clinics in Harare, and HIV-uninfected children from schools in the same suburbs, were recruited into a cross-sectional study. Sociodemographic, clinical and anthropometric data were collected. The prevalence of dual-energy X-ray absorptiometry (DXA) measured bone outcomes, total-body less-head bone mineral content-for-lean mass adjusted for height (TBLH-BMC^{LBM}) and lumbar spine bone mineral apparent density (LS-BMAD), were determined. Linear regression models, using multiple imputation for missing data, assessed relationships between risk factors and TBLH-BMC^{LBM} and LS-BMAD Z-scores.

Findings: We recruited 303 CWH and 306 without HIV, mean (SD) age 12.5 (2.5) years and 50% female. Median ART duration was 8.1 (IQR 6.2~~,~~ ~~–~~9.5) years; for 102 (34%) ART included tenofovir disproxil fumarate (TDF). Compared to children without HIV, those with HIV had higher prevalence of TBLH-BMC^{LBM} (10.4% *vs.* 6.2%, p=0.066) and LS-BMAD Z-Scores <-2 (14.3% *vs.* 5.8%, p=0.001). HIV and male sex were associated with earlier Tanner stage. The negative ~~effects~~ associations between~~of~~ HIV ~~on~~ and both TBLH-BMC^{LBM} and LS-BMAD were more pronounced with pubertal maturation, particularly in females. Among CWH, years of TDF exposure and orphanhood were associated with lower TBLH-BMC^{LBM} Z-Score. Current TDF use (*v.s.*, non-TDF containing ART) was associated with a reduction in TBLH-BMC^{LBM} and LS-BMAD Z-Scores of 0.41 (95%CI 0.08, 0.74; p=0.015) and 0.31 (0.08, 0.69; p=0.116) respectively.

Interpretation: Despite ART, HIV is associated with substantial skeletal deficits towards the end of puberty. The size of bone deficits associated with TDF and its widespread use in children in sub-Saharan Africa raise concern for future adult fracture risk.

Funding: Wellcome Trust

Introduction

Stunting and delayed puberty are common manifestations of perinatally-acquired HIV infection; up to 50% of children with HIV (CWH) experience poor linear growth.[1] In sub-Saharan Africa, where 90% of the world's children with HIV live, higher background rates of malnutrition and intercurrent infections further impair linear growth, so that the prevalence and extent of stunting is much higher than in high-income settings.[1,2] Puberty is a critical period for bone mass accrual; after cessation of linear growth, consolidation of mineral continues until peak bone mass (PBM) (the reservoir of bone for later life) is achieved in early adulthood. Disturbances in growth and/or pubertal delay due to HIV infection, therefore, have implications for bone mass accrual and achievement of PBM. Low PBM is a principal determinant of subsequent adult osteoporotic fracture risk; a 10% reduction in PBM doubles fracture risk in adulthood.[3]

The scale-up of antiretroviral therapy (ART) programmes globally has dramatically improved survival of people living with HIV, so that increasing numbers of children with HIV, who would otherwise have died in early childhood, are surviving to adolescence and adulthood.[4] Catch-up linear growth occurs once ART is initiated, but those who start ART in older childhood may not realise their full growth potential and not attain population age-norms.[5] In sub-Saharan Africa, children start ART much later than in high-income settings, at 7.9 (6.0–9.3) years compared to 0.9 (0.4–2.6) years in North America. [6] Furthermore, the prevalence of stunting is higher, so that HIV may have a more detrimental effect on skeletal growth than in high-income settings.[2] Notably, small cross-sectional studies from South Africa have shown reduced bone mass,[7] and strength,[8] in mostly pre-pubertal children living with HIV.

Most studies investigating the effect of HIV infection on skeletal growth have been conducted in high-income settings and have not taken into account the impact of poor growth on bone density.[9] Dual energy X-ray absorptiometry (DXA) is commonly used to measure bone density; when skeletons are small (in the case of HIV, due to stunting) DXA underestimates bone density.[10] Our aim was to understand the relationship between HIV infection and skeletal health in peripubertal children taking

ART in Zimbabwe, a country with a generalised sustained severe HIV epidemic. Specifically, we sought to determine the prevalence of low *size-adjusted* bone density in children with and without HIV ~~compared to their uninfected peers~~, and to investigate risk factors associated with lower size-adjusted bone density.

Methods

Study design and participants

A cross-sectional study was conducted using baseline DXA bone measurements in the IMVASK study (The IMpact of Vertical HIV infection on child and Adolescent SKeletal development in Harare, Zimbabwe), as per published protocol (ISRCTN12266984).[11] CWH aged 8-16 years were recruited from outpatient clinics at the two large public-sector general hospitals in Harare (Parirenyatwa and Harare Central Hospital). Studies in children suggest that ART initiation is followed by an initial decline in bone mass which stabilizes after two years,[12] hence we enrolled CWH who had been taking ART for at least two years. Systematic quota-based sampling, stratified by age and sex, was used to recruit 50 male and 50 female CWH in each of three age-groups (8-10, 11-13 and 14-16 years). Exclusion criteria were being acutely unwell (defined as requiring immediate hospitalisation), not residing in Harare and being unaware of one's HIV status (to avoid inadvertent disclosure through study participation). A maximum of five CWH were recruited each day for logistical reasons.

A comparison group of children without HIV was recruited from six government primary and secondary schools randomly selected from the 109 primary and 44 secondary schools within the same suburbs in Harare where the hospitals provide HIV care. Younger children (8-12 years) were sampled from primary schools and older children (14-16 years) from secondary schools, with thirteen-year-olds sampled from both schools. The number of children selected from each school was proportional to school size, thereby giving each child equal probability of being sampled. A random number sequence applied to school registers was used to select participants using the same quota-based sampling approach of 50 males and 50 females in each of the three age strata (8-10, 11-13 and 14-16

years). Children underwent HIV testing prior to recruitment; those testing positive and not in care were referred to HIV services.

Ethical and governance approvals were granted by the London School of Hygiene and Tropical Medicine Ethics Committee (Ref: 15333), the Institutional Review Board of the Biomedical Research and Training Institute in Harare (Ref: AP145/2018), the Joint Research Ethics Committee for University of Zimbabwe College of Health Sciences and the Parirenyatwa Group of Hospitals (Ref: 11/18), Harare Central Hospital Ethics Committee (Ref: 170118/04), the Medical Research Council of Zimbabwe (Ref: MRCZ/A/2297) and the Ministry of Primary and Secondary Education Zimbabwe (Ref: C/426/Harare). Parents/ guardians provided written informed consent for study participation and HIV testing, and children provided written assent.

Procedures

An interviewer-administered questionnaire was used to collect socio-demographic and clinical data including smoking, alcohol and steroid use. The International Physical Activity Questionnaire (IPAQ), validated in multiple countries including South Africa, but not in Zimbabwe, was used to assess physical activity as multiples of the resting metabolic rate (MET) in MET-minutes. Diet and nutrition were assessed using a tool based on a validated dietary diversity and food frequency tool from India and Malawi, and adapted to the Zimbabwean context using international guidelines applicable to SSA. This tool quantified dietary calcium and vitamin D intake plus sunlight exposure; adaptations reflected the local context where fortification of oils and margarine with vitamin D is mandated and specific vitamin D rich foods, *e.g.* kapenta fish, are commonly eaten.

All anthropometric measurements were carried out by trained research nurses and research assistants. Standing and sitting height, measured to the nearest 0.1 cm (using a Seca 213 stadiometer), and weight (using Seca 875 weight scales), measured to the nearest 0.1 kg, were taken by two separate readers. If height measurements differed by more than 0.5 cm, or weight measurements by more than 0.5 kg, a

third reading was taken, and final height and weight values were taken as means of the two or three measurements. The same ~~readers~~ researchers measured both the children with and without HIV. All equipment was calibrated annually. Tanner pubertal staging was performed by the study nurse and doctor, with an orchidometer used to assess testicular volume in males. Pubertal delay was defined as Tanner stage 2 in girls aged 13 years or older, and in boys aged 14 years or older.

Details about HIV including age at HIV diagnosis, ART regimen and duration, and current CD4 count and HIV viral load were collected for participants with HIV. CD4 cell count was measured using an Alere PIMA CD4 machine (Waltham, Massachusetts, USA) and HIV viral load using the GeneXpert HIV-1 viral load platform (Cepheid Inc, Sunnyvale, California, USA), with viral suppression defined as <1,000 copies/ml (as per WHO guidelines).

Assessment of bone density

Dual energy X-ray absorptiometry (DXA) scans of the lumbar spine and total body were performed by one of two trained radiographers using standard procedures on a Hologic QDR Wi densitometer (Hologic Inc., Bedford, MA, USA) with Apex Version 4.5 software for scan analysis. Daily calibration was conducted using the manufacturer provided spine phantom. DXA scans were repeated in a subgroup (n=30) to determine reproducibility. The precision error was a root mean square-standard deviation (RMS-SD) of 0.011 g/cm^2 (lumbar spine) and 0.010 g/cm^2 (total body) with an RMS-coefficient of variation (CV) of 1.35% (lumbar spine) and 1.22% (total body). An important limitation of DXA in paediatric populations with chronic disease, is that the two-dimensional (areal) bone density values are highly dependent upon body, and therefore bone size; hence DXA underestimates bone density in small children.[10] The two main size adjustment techniques recommended by the International Society for Clinical Densitometry (ISCD) to overcome the problem of size dependence of DXA measurement,[13] were employed, namely to measure (i) total-body less-head (TBLH) Bone Mineral Content (BMC) for lean mass adjusted for height (TBLH-BMC^{LBM}) and (ii) lumbar spine bone mineral apparent density (LS-BMAD). LS-BMAD was calculated from DXA-measured lumbar spine data using the Carter method.[14] TBLH-BMC^{LBM} was calculated from the whole body scan using published derived

equations, for Hologic DXAs, which adjust for log-transformed total body lean mass, total body fat mass and height.[15] Sex and age-matched Z-scores were generated using Hologic UK population reference data as recommended by ISCD guidelines, as there were no local reference data available.[13] Low TBLH-BMC^{LBM} and LS-BMAD were defined as Z-score < -2.0.[15]

Statistical analysis

A sample size of 300 in each group permitted detection of a difference between children with and without HIV in DXA-measured size-adjusted bone density Z-scores of 0.23 with 80% power and a significance level of 0.05 assuming a standard deviation of 1.3.[16] The study had 80% power to detect a 4.8% difference in prevalence of low TBLH-BMC^{LBM} between those with and without HIV, assuming a prevalence in those without HIV of 1%.[17]

~~*Definition and derivation of variables*~~

Height-for-age and weight-for-age Z-scores were calculated using 1990 UK reference data,[18] with Z-scores < -2.0 defining stunting and underweight respectively. Socio-economic status (SES) was derived using the first component from a principal component analysis combining an asset list (detailing: number in household, head of household age, highest maternal and paternal education levels, household ownership, monthly household income, access to electricity, water, a flush toilet and/or pit latrine and ownership of a fridge, bicycle, car, television, and/or radio) and was split into tertiles for analysis.

Analyses were conducted using Stata 16.1 (StatCorp, Texas, USA). The primary exposure was HIV and primary outcomes were TBLH-BMC^{LBM} and LS-BMAD Z-Scores (as described above). The characteristics of participants with HIV were compared with those without HIV, using independent sample t-tests for means, with unequal variance as required, Wilcoxon signed-rank tests for non-parametric variables, and chi-squared or Fisher's exact tests for proportions. To first understand the role of sex, puberty and HIV on bone outcomes, mean differences in TBLH-BMC^{LBM} and LS-BMAD Z-scores between those with and without HIV were examined using linear regression with robust standard errors, overall and stratified by sex and Tanner stage (stages 1 and 2 *vs.* 3 to 5), similarly, mean risk

differences for low TBLH-BMC^{LBM} and LS-BMAD Z-scores (< ~~-~~2 compared to ≥ -2~~-~~) were examined using generalised linear models, with Poisson distributions and log links with robust standard errors. Three-way interactions between sex, pubertal stage and HIV were assessed using Wald tests in linear regression models. In secondary analyses of absolute measures of TBLH-BMC^{LBM} and LS-BMAD generalised linear models with log link and gamma distribution were used, and marginal means estimated.

Associations between potential risk factors and TBLH-BMC^{LBM} and LS-BMAD Z-score measures were investigated using linear regression separately for participants with and without HIV. Adjustment was made for (i) *a priori* confounders (age, sex, pubertal stage[19]), (ii) potential risk factors (socio-economic status,[20] physical activity,[21] calcium and vitamin D intake[22] and, in those with HIV, CD4 count, viral load, tenofovir exposure and age at ART initiation),[23] (iii) variables associated in complete case analysis with any outcome (at $p<0.2$) and (iv) variables associated with missingness.

To account for missing data, including DXA-measured outcomes, we used multiple imputation by chained equations with seven imputed datasets, which allowed for imputation of categorical and continuous data jointly. Our imputation models included all outcomes, auxiliary variables associated with missingness, with group membership (with or without HIV), and variables determined in complete case analysis to be associated with either outcome ($p<0.200$).

~~***Ethical considerations***~~

~~Ethical and governance approvals were granted by the London School of Hygiene and Tropical Medicine Ethics Committee (Ref: 15333), the Institutional Review Board of the Biomedical Research and Training Institute in Harare (Ref: AP145/2018), the Joint Research Ethics Committee for University of Zimbabwe College of Health Sciences and the Parirenyatwa Group of Hospitals (Ref: 11/18), Harare Central Hospital Ethics Committee (Ref: 170118/04), the Medical Research Council of Zimbabwe (Ref: MRCZ/A/2297) and the Ministry of Primary and Secondary Education Zimbabwe (Ref: C/426/Harare).~~

~~Parents/ guardians provided written informed consent for study participation and HIV testing, and children provided written assent.~~

Role of funding source

The funders had no role in study design, data collection, data analysis, data interpretation, report writing or in the decision to submit this paper for publication.

Results

Study population

In total, 303 participants with HIV were recruited, representing 62% of the 486 eligible CWH attending HIV clinics, with 4 additional participants, identified from screening in schools, who met inclusion criteria (Figure 1). Participants with HIV were a mean (SE) 1.2 (0.26) years older, more commonly female (151 [50%] *vs.* 75 [41%]) and more likely to attend school (295 [97] *vs.* 168 [92%]) than CWH who were eligible but not enrolled. Of 536 children randomly selected from schools, 500 (93%) were eligible, of whom 307 (61%) consented to participate and one was excluded after testing HIV-positive, giving a total of 306 participants without HIV. Participants without HIV were a mean (SE) 0.6 (0.24) years older but with no difference by sex (155 [51%] -*vs.* 95 [49%] female) compared to children who were eligible but not enrolled.

A higher proportion of participants with HIV were of low socio-economic status, were orphaned, had past or current tuberculosis, were less physically active, and reported greater outdoor time than those without HIV (Table 1). Across the whole study population consumption of vitamin D and calcium was low, with 79% (479/609) consuming <6.0 mcg/day vitamin D (Recommended Daily Allowance [RDA] ≥15 mcg/day), and 66% (399/609) consuming <300 mg/day of calcium. No participant reported smoking, drinking alcohol or use of oral steroids. The 24 CWH and 14 children without HIV who were missing DXA data (Figure 1) were thinner and shorter than those with DXA data and spent more time outdoors (Supplementary Table 1). Only orphanhood status and earlier pubertal stage were otherwise associated with missingness of data (Supplementary Table 2).

All but five of the 303 participants with HIV were perinatally-infected. The median age of HIV diagnosis was 3.0 (IQR 1.2-5.8) years, with ART initiated at median age 3.7 (IQR 1.8-6.9) years. At enrolment, 102/303 (34%) were taking a tenofovir disproxil fumarate (TDF)-containing regime, with a median duration of TDF use of 3.0 (IQR 1.4-5.5) years. Overall, 211 (70%) were taking a non-nucleoside reverse-transcriptase inhibitor (NNRTI) based ART regime, and 89 (29%) a protease

inhibitor (PI) based ART regime. The median CD4 count was 766 (IQR 537–1019) cells/µl and 212 (79%) had a suppressed HIV viral load (Table 1).

Growth and bone density by HIV status

A significantly greater proportion of participants with HIV compared to those without HIV were stunted (97 [32%] *vs.* 24 [8%]) and were underweight (79 [26%] *vs.* 26 [9%]) (Table 1). Interestingly, differences between those with and without HIV were greater for standing than sitting height (mean Z-Score differences 1.05 [95% CI 0.87, 1.24] and 0.73 [95% CI 0.55, 0.92] respectively). At all ages participants with HIV were more likely to be earlier in Tanner stage, and to have pubertal delay compared to participants without HIV (8 [6%] *vs.* 0 [0%], p=0.003) (Table 1; Supplementary Table 3).

Arithmetic mean TBLH-BMC^{LBM} and LS-BMAD absolute and Z-score were all lower among participants with HIV than those without HIV. The prevalence of low TBLH-BMC^{LBM} and LS-BMAD Z-scores in those with HIV were 10.4% (n=29) and 14.3% (n=40) respectively, significantly higher than the prevalence in those without HIV (6.2% [n=18] and 5.8% [n=17] respectively) (Table 1). Overall CWH had 0.20 (95%CI 0.03, 0.37) lower TBLH-BMC^{LBM} and 0.26 (0.04, 0.48) lower LS-BMAD Z-Scores than children without HIV (Table 2); differences in LS-BMAD Z-Scores were similar in females and males, whilst differences in TBLH-BMC^{LBM} were more apparent in females. Correspondingly, both male and female CWH had an increased the risk of low LS-BMAD (Table 2). In CWH, low LS-BMAD was strongly associated with stunting (p<0.001). This association was not observed among participants without HIV (Supplementary Table 4).

~~Modification of the effect of~~Interactions by sex and puberty on the association between HIV ~~on~~ and size-adjusted bone density ~~by sex and puberty~~

In each age stratum, both male and female CWH were on average at a lower Tanner stage than their HIV-negative peers of the same sex (Supplementary Table 3). Mean absolute measures of TBLH-BMC^{LBM} and LS-BMAD differed by pubertal stage and sex (3-way interactions: TBLH-BMC^{LBM} p=0.041, LS-BMAD p=0.88), such that differences in TBLH-BMC^{LBM} and LS-BMAD absolute and

Z-score values between participants with and without HIV were more marked in later pubertal stages (Figure 2). Differences in size-adjusted bone density between participants with and without HIV were generally small in the early stages of puberty (Table 2). Whereas, in the later stages of puberty, differences in bone outcomes between those with and without HIV, were more apparent for both sexes, but with females particularly showing evidence of differences in both TBLH-BMC^{LBM} and LS-BMAD (Z-Scores and absolute values) (Figure 2; Table 2). Stratification widened confidence intervals as fewer older participants with HIV (14-16 years) had entered the later stages of puberty, *i.e.,* Tanner stages 4 and 5, compared to those without HIV (Supplementary Table 3). When models assessing differences in TBLH-BMC^{LBM} and LS-BMAD Z-scores between children with and without HIV were adjusted for age, sex and pubertal stage, confidence intervals widened. However, even after adjustment for age, male CWH in the early stages of puberty had a greater risk of low LS-BMAD than did males without HIV (risk difference 0.14 [95%CI 0.04, 0.24]) (Table 2).

Risk factors associated with size-adjusted bone density outcomes

Among children without HIV, male sex and earlier pubertal stage were associated with lower TBLH-BMC^{LBM} and LS-BMAD Z-scores in crude but not adjusted analyses (Supplementary Table 5). Among children with HIV, orphanhood and TDF exposure were associated with lower TBLH-BMC^{LBM} Z-score in both unadjusted analyses and after adjustment for age, sex, pubertal stage, socioeconomic status, physical activity, calcium and vitamin D intake, CD4 count, HIV viral load and age at ART initiation (Table 3). After adjustment, TDF exposure for four or more years was associated with a 0.52 SD deficit in TBLH-BMC^{LBM} Z-Score.

Older age, male sex, orphanhood, as well as older age at ART initiation and TDF use, were all associated with lower LS-BMAD Z-Score in unadjusted analyses (Table 4). A weak association was detected between low CD4 count and low LS-BMAD Z-Score. Older age, male sex and earlier Tanner stage remained associated with low LS-BMAD Z-Score after adjustment for orphanhood, socioeconomic status, physical activity, calcium and vitamin D intake, ART initiation, TDF exposure, CD4 count and viral load.

Current TDF use was associated with 0.41 (95%CI 0.08, 0.74; p=0.015) lower TBLH-BMC^{LBM} Z-Score and 0.31 (95%CI 0.08, 0.69; p=0.116) lower LS-BMAD Z-Score, compared to those with HIV on non-TDF containing ART, after adjustment for age, sex, pubertal stage, orphanhood, socioeconomic status, physical activity, calcium and vitamin D intake, CD4 count, HIV viral load and age at ART initiation.

Discussion

The main finding of this study was that despite ART, marked deficits in size-adjusted bone density are common among children with HIV, who have a substantially higher prevalence of low bone density (a deficit of 2 SDs or more) than do HIV-uninfected peers. The negative ~~effects~~ associations between~~of~~ HIV and~~on~~ size-adjusted bone density were more pronounced with pubertal maturation. Orphanhood and use of tenofovir are associated with bone deficits in peripubertal children with HIV.

Across all age groups, CWH were behind HIV-uninfected peers in terms of pubertal stage. Despite low numbers, the association between HIV and size-adjusted bone density is most marked towards the end of puberty. This suggests cumulatively lower bone accrual through puberty in those with HIV relative to children without HIV. Importantly, if bone accrual remains compromised, those with HIV will achieve substantially lower PBM. Female CWH had substantially lower TBLH-BMC^{LBM} Z-Scores than females without HIV, this difference was more apparent in later puberty; this deficit was not seen in boys. Both male and female CWH had lower LS-BMAD Z-Scores and a greater risk of low LS-BMAD (*i.e.*, a Z-Score <-2), compared to those without HIV. Male CWH in early puberty were at particular risk of low LS-BMAD. These results highlight the need for interventions to optimise bone density before skeletal growth is completed. In addition to minimising exposure to TDF (discussed further below), a number of interventions may be beneficial. In Zimbabwe and Zambia, a trial is currently underway to determine whether supplementation of vitamin D_3 and calcium ameliorates bone mineralisation deficits in adolescents growing up with HIV (trial registration PACTR202009897660297). Furthermore, our study identified lower levels of physical activity in

CWH, than their uninfected peers. High impact physical activity is thought to be osteogenic, and has been associated with improved bone density at the hip in healthy adolescents.[21] Recently a small, randomised trial in young adults living with HIV, has suggested the combination of resistance and aerobic exercise may improve bone mass,[24] whether the same holds for CWH remains to be determined. Recently the first, small randomised controlled trial of the oral bisphosphonate, alendronate, given to CWH showed some evidence of improved bone density over 48 weeks of treatment; however, longer term effects on PBM, fracture risk, and rare side effects are unknown.

The deficits we have identified in TBLH-BMC^{LBM} in females at the end of puberty is a concern. Bone density is compromised during pregnancy and lactation when skeletal calcium reserves are mobilised,[25] and even healthy adolescent mothers may achieve a compromised PBM.[26] Given in 2019 24% of young women living in Zimbabwe had given birth before the age of 18,[27] our findings have implications for the recovery of skeletal mineralisation post-partum and subsequent adult fracture risk.[28]

Not only did CWH have a higher prevalence of stunting compared to uninfected children, but a higher proportion of CWH who were stunted had lower lumbar spine bone density than stunted children without HIV. While stunting increases risk of poor skeletal growth, we have shown that stunting is not a sufficient clinical proxy for low size-adjusted bone density. Interestingly, deficits in standing height were greater than sitting height, suggesting that HIV infection may have a greater effect on appendicular (limb length) than axial (spinal length) skeletal growth. Appendicular growth occurs more rapidly than axial growth before puberty. It is thought that exposures shortly before puberty preferentially affect appendicular development, whilst exposures during puberty may have a greater impact on axial development.[29] Thus, these findings suggest that HIV and its treatment pre-pubertally may be particularly important in influencing linear growth.

Notably, ART drugs themselves may cause accelerated bone loss. There was a strong and consistent association between TDF exposure and bone deficits, particularly affecting TBLH-BMC^{LBM}. Those exposed to TDF for four or more years had on average a 0.5 SD lower TBLH-BMC^{LBM} Z-Score

compared with CWH who had not received TDF. This is a clinically important effect size, as a 0.5 SD reduction in bone density increases by 50% both childhood and, if sustained, future adult fracture risk.[30] Whilst bone loss following TDF initiation in adults is well recognised, hitherto studies of TDF on bone outcomes in children have been inconsistent, for example among 74 Brazilian adolescents, mean age 17 years, length of TDF use was associated with lower (albeit non size-adjusted) lumbar spine and total body bone density,[31] whereas a larger study of 394 Thai adolescents of a similar age, found no such association with both (non-size adjusted) total body bone density and (size-adjusted) LS-BMAD.[32]

Our study supports the hypothesis that TDF use has a detrimental effect on bone health in children; notably we saw a dose response with longer TDF exposure associated with more pronounced bone deficits. TBLH-BMC^{LBM} largely represents cortical bone (whereas LS-BMAD reflects predominantly trabecular bone), thus our findings suggest TDF may particularly affect the mineralisation of cortical bone to reduce density; consistent with TDF induced renal tubulopathy with phosphaturia leading to skeletal hypomineralization.[33] TDF is a well-tolerated drug and remains one of the recommended component drugs of a first line ART regimen, and is thus extensively used in sub-Saharan Africa, in both children and adults. In Zimbabwe, TDF is mostly available at an adult dose as part of a combination ART regime, and use is recommended only in children weighing over 25kg or age above 10 years. However, due to limitation in the availability of ART options, TDF is often prescribed in younger children, and/or those with lower weight, and therefore those children will effectively receive a higher than recommended dose. Our findings add to a growing body of evidence to support the replacement of TDF with tenofovir alafenamide, a prodrug of TDF that is associated with significantly fewer effects on bone and kidneys.[34] This is of particular importance in adolescence, a period of rapid bone accrual, as achievement of PBM may otherwise be compromised by TDF use.

The mechanism by which HIV compromises musculoskeletal development is likely multifactorial. HIV promotes dysregulated systemic immune activation, which is not completely reversed by ART.[35] During childhood, formation predominates over resorption, but the pro-inflammatory milieu leads to an imbalance in osteoblastic/osteoclastic activity, promoting increased bone resorption relative to

formation. Other factors that can compromise skeletal development include inadequate dietary calcium, vitamin D deficiency[22] and low levels of physical activity.[21] Whilst in this study CWH reported lower levels of physical activity than HIV-uninfected children, low intake of both calcium and vitamin D were common regardless of HIV status. Orphanhood, more common in CWH, was independently associated with low TBLH-BMC^{LBM}. Orphanhood may be a proxy for social and emotional deprivation, which along with biological and nutritional factors, ~~can~~ may disrupt growth.

The strengths of the study were being well-powered, the inclusion of an HIV-uninfected comparison group from broadly the same socioeconomic background, and collection of detailed data on risk factors including physical activity as well as vitamin D and calcium intake using a standardised tool adapted for the local context. 'Gold standard' size-adjustment methods were used for DXA data, which were particularly crucial given height differences (levels of stunting) in the population studied.[13,15] In addition, sex and age-matched Z-scores were used for size-adjusted bone density measures which have greater clinical interpretability than absolute measures.

We acknowledge several limitations: data are cross-sectional and therefore causality cannot be inferred. In the absence of local or African reference population data, the reference data used to generate the Z-scores for TBLH-BMC^{LBM} and LS-BMAD were obtained from a paediatric reference population in the UK using the same manufacturer and software version to derive Z-scores (as recommended by the ISCD),[13] who may not be comparable to the children in this study. Whilst the study population Z-Score SDs for TBLH-BMC^{LBM} were close to 1, those for LS-BMAD were higher, suggesting the UK reference population may be a better fit for total body than lumbar spine data, and/or that the size-adjustment should be further validated in African populations. DXA is unable to distinguish between bone density deficits due to hypomineralization versus other architectural properties of bone. Only current CD4 count and HIV viral load were available as markers of HIV disease severity; annual trends in these measures would be more appropriate as growth occurs over a long period. Only five (1.6%) CWH reported non-perinatal infection and their inclusion is highly unlikely to have biased results. Aside from examining TDF *v.s.*, non-TDF containing ART regimes, we lacked sufficient power to further categorise the many

ART regimes to understand associations with other individual treatments ~~which have been reported~~. Further, although we examined three-way interactions between age, puberty and HIV on size-adjusted bone density, the study was not powered to detect the true differences between these strata. While we aimed to collect comprehensive data on factors associated with size-adjusted bone density and growth, there may be residual confounding by other past or current biological or environmental factors. Due to delays in engineering maintenance, DXA measurements were missing for a minority of children, in addition to a small amount of missing covariate data. We used multiple imputation with chained equations to impute these data, which are subject to a missing at random (MAR) assumption. Our findings are therefore valid under this MAR assumption and the specified imputation model. Finally, achievement of PBM is estimated to occur in the early twenties and therefore bone accrual may continue to consolidate beyond the age-range examined.

To our knowledge this is the largest study investigating the ~~potential~~ relationship between~~effect of~~ HIV infection ~~on~~ and skeletal health in children in sub-Saharan Africa in the ART era. While ART results in immune reconstitution and has dramatically improved survival, this study underscores the importance of addressing the long-term adverse impacts of HIV infection on musculoskeletal health in the current cohort of children, particularly in sub-Saharan Africa. There is an urgent need for HIV programmes to focus beyond delivery of ART, to develop strategies for prevention and management of the long-term effects of HIV infection and its treatment on musculoskeletal health to ensure optimum health in children as they enter adulthood. Longitudinal studies with follow-up of children through and after puberty are required to understand the extent of catch-up growth and bone accrual, and hence the population reported here are now in follow-up.

Contributors: RR, RAF, CLG conceived the study. SF, KAW, RAF, CLG and RR and AMR designed the study protocol. RR, CK, FK, GM, HM collected the data. JC, CK and RR were responsible for data management. AMR, CLG, TM, VS and RR conducted data analyses. RR and CLG wrote the first draft. ~~All authors contributed to the report and approved the final draft for submission~~. RR and AR had full access to all the study data and had final responsibility for the decision to submit for publication. All authors contributed to the report and approved the final submitted text. Authors thank Dr Nicola Crabtree for her advice regarding calculation of TBLH-BMC^{LBM} and LS-BMAD Z-scores.

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Data sharing: Anonymised research data will be made available for sharing through the LSHTM open access data repository (LSHTM Data Compass, https://datacompass.lshtm.ac.uk/).

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Research in context

Evidence before this study

We and others have previously shown that poor linear growth resulting in stunting is common in children growing up with HIV in sub-Saharan Africa. Successful roll-out of antiretroviral therapy (ART) programmes has enabled many more children to survive adolescence to reach adulthood. Puberty is a critical period of skeletal development during which bone mass accrues to achieve, by early adulthood, peak bone mass (PBM), which has been shown to be a key determinant of lifetime fracture risk. We searched EMBASE, Ovid MEDLINE(R) (including Epub and Daily databases), Cochrane databases and conference proceedings (through Web of Science) from inception until~~in~~ March 10th 2021, adapting search strategies for each database to include combinations of medical subject headings (MeSH terms) relevant to bone, growth, development, HIV and ART in children, and without language restriction. Few studies had investigated the effect of HIV on adolescent skeletal growth in low-income settings and even fewer had adequately considered the effect of skeletal size on bone density measurement techniques; this is crucial as we know the most commonly used measure, dual energy X-ray absorptiometry (DXA), underestimates bone density when skeletons are small (as is the case with stunting). The International Society for Clinical Densitometry (ISCD) recommended specific size-adjustment techniques to overcome this problem. These techniques had not been used to understand the effect of HIV on bone mass accrual in populations in sub-Saharan Africa.

Added value of this study

To our knowledge, ~~T~~this is the largest study to investigate the effect of HIV infection on skeletal health in children in sub-Saharan Africa. Findings are strengthened using 'gold standard' size-adjustment methods for DXA data, particularly important given observed height differences (levels of stunting). The study found marked deficits in bone density were common in children with HIV, who had a substantially higher prevalence of low bone density (a deficit of 2 SDs or more) compared with their HIV-uninfected peers. The effect of HIV on bone density was most marked in the last stage of puberty, especially at the spine in females.

Use of tenofovir disproxil fumarate (TDF) was strongly associated with bone deficits, particularly affecting the total body (predominantly reflecting cortical bone). TDF exposure for four or more years was associated with a 0.5 SD lower total body Z-Score (which translates clinically to an approximately 50% increase in both childhood and, if sustained, adult fracture risk).

Implications of all the available evidence

This study underscores the importance of addressing the long-term adverse impacts of HIV infection on musculoskeletal health in children living in sub-Saharan Africa. If the bone deficits here identified persist into adulthood, this African region risks increasing fracture incidence within the current birth cohort. The finding that bone mineral deficits appear greatest at the end of puberty in females is of concern, as these women may soon draw upon these potentially inadequate skeletal calcium reserves to support pregnancy and lactation, which may increase their vertebral fracture risk. TDF is currently one of the recommended component drugs of a first line ART regimen and is thus extensively used in sub-Saharan Africa. The detrimental effects associated with TDF use in this study, support re-evaluation of first-line use during skeletal growth.

Figure Legends

Figure 1. Flow diagram illustrating recruitment of children living with HIV from HIV clinic, and children from schools residing in the same suburbs.

Figure 2. Arithmetic means and 95% confidence intervals for A) TBLH-BMC^{LBM} Z-score B) LS-BMAD Z-score C) absolute TBLH-BMC^{LBM} D) absolute LS-BMAD, stratified by sex, age, pubertal stage and HIV status (with no adjustment). Raw data presented as open circles (HIV) and open triangles (non-HIV)

Effect of HIV infection on growth and bone density in peripubertal children in the era of antiretroviral therapy: a cross-sectional study in Zimbabwe

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Abstract

Background: Poor linear growth and pubertal delay, both common in children with HIV (CWH) in sub-Saharan Africa, may affect adolescent bone accrual and future fragility fracture risk. We investigated the association of HIV with size-adjusted bone density in peri-pubertal children in Zimbabwe.

Methods: CWH aged 8-16 years taking ART for ≥2 years from public-sector HIV clinics in Harare, and HIV-uninfected children from schools in the same suburbs, were recruited into a cross-sectional study. Sociodemographic, clinical and anthropometric data were collected. The prevalence of dual-energy X-ray absorptiometry (DXA) measured bone outcomes, total-body less-head bone mineral content-for-lean mass adjusted for height (TBLH-BMC^{LBM}) and lumbar spine bone mineral apparent density (LS-BMAD), were determined. Linear regression models, using multiple imputation for missing data, assessed relationships between risk factors and TBLH-BMC^{LBM} and LS-BMAD Z-scores.

Findings: We recruited 303 CWH and 306 without HIV, mean (SD) age 12.5 (2.5) years and 50% female. Median ART duration was 8.1 (IQR 6.2-9.5) years; for 102 (34%) ART included tenofovir disproxil fumarate (TDF). Compared to children without HIV, those with HIV had higher prevalence of TBLH-BMC^{LBM} (10.4% *vs.* 6.2%, p=0.066) and LS-BMAD Z-Scores <-2 (14.3% *vs.* 5.8%, p=0.001). HIV and male sex were associated with earlier Tanner stage. The negative associations between HIV and both TBLH-BMC^{LBM} and LS-BMAD were more pronounced with pubertal maturation, particularly in females. Among CWH, years of TDF exposure and orphanhood were associated with lower TBLH-BMC^{LBM} Z-Score. Current TDF use (*v.s.*, non-TDF containing ART) was associated with a reduction in TBLH-BMC^{LBM} and LS-BMAD Z-Scores of 0.41 (95%CI 0.08, 0.74; p=0.015) and 0.31 (0.08, 0.69; p=0.116) respectively.

Interpretation: Despite ART, HIV is associated with substantial skeletal deficits towards the end of puberty. The size of bone deficits associated with TDF and its widespread use in children in sub-Saharan Africa raise concern for future adult fracture risk.

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Introduction

Stunting and delayed puberty are common manifestations of perinatally-acquired HIV infection; up to 50% of children with HIV (CWH) experience poor linear growth.[1] In sub-Saharan Africa, where 90% of the world's children with HIV live, higher background rates of malnutrition and intercurrent infections further impair linear growth, so that the prevalence and extent of stunting is much higher than in high-income settings.[1,2] Puberty is a critical period for bone mass accrual; after cessation of linear growth, consolidation of mineral continues until peak bone mass (PBM) (the reservoir of bone for later life) is achieved in early adulthood. Disturbances in growth and/or pubertal delay due to HIV infection, therefore, have implications for bone mass accrual and achievement of PBM. Low PBM is a principal determinant of subsequent adult osteoporotic fracture risk; a 10% reduction in PBM doubles fracture risk in adulthood.[3]

The scale-up of antiretroviral therapy (ART) programmes globally has dramatically improved survival of people living with HIV, so that increasing numbers of children with HIV, who would otherwise have died in early childhood, are surviving to adolescence and adulthood.[4] Catch-up linear growth occurs once ART is initiated, but those who start ART in older childhood may not realise their full growth potential and not attain population age-norms.[5] In sub-Saharan Africa, children start ART much later than in high-income settings, at 7.9 (6.0–9.3) years compared to 0.9 (0.4–2.6) years in North America. [6] Furthermore, the prevalence of stunting is higher, so that HIV may have a more detrimental effect on skeletal growth than in high-income settings.[2] Notably, small cross-sectional studies from South Africa have shown reduced bone mass,[7] and strength,[8] in mostly pre-pubertal children living with HIV.

Most studies investigating the effect of HIV infection on skeletal growth have been conducted in high-income settings and have not taken into account the impact of poor growth on bone density.[9] Dual energy X-ray absorptiometry (DXA) is commonly used to measure bone density; when skeletons are small (in the case of HIV, due to stunting) DXA underestimates bone density.[10] Our aim was to understand the relationship between HIV infection and skeletal health in peripubertal children taking

ART in Zimbabwe, a country with a generalised sustained severe HIV epidemic. Specifically, we sought to determine the prevalence of low *size-adjusted* bone density in children with and without HIV, and to investigate risk factors associated with lower size-adjusted bone density.

Methods

Study design and participants

A cross-sectional study was conducted using baseline DXA bone measurements in the IMVASK study (The IMpact of Vertical HIV infection on child and Adolescent SKeletal development in Harare, Zimbabwe), as per published protocol (ISRCTN12266984).[11] CWH aged 8-16 years were recruited from outpatient clinics at the two large public-sector general hospitals in Harare (Parirenyatwa and Harare Central Hospital). Studies in children suggest that ART initiation is followed by an initial decline in bone mass which stabilizes after two years,[12] hence we enrolled CWH who had been taking ART for at least two years. Systematic quota-based sampling, stratified by age and sex, was used to recruit 50 male and 50 female CWH in each of three age-groups (8-10, 11-13 and 14-16 years). Exclusion criteria were being acutely unwell (defined as requiring immediate hospitalisation), not residing in Harare and being unaware of one's HIV status (to avoid inadvertent disclosure through study participation). A maximum of five CWH were recruited each day for logistical reasons.

A comparison group of children without HIV was recruited from six government primary and secondary schools randomly selected from the 109 primary and 44 secondary schools within the same suburbs in Harare where the hospitals provide HIV care. Younger children (8-12 years) were sampled from primary schools and older children (14-16 years) from secondary schools, with thirteen-year-olds sampled from both schools. The number of children selected from each school was proportional to school size, thereby giving each child equal probability of being sampled. A random number sequence applied to school registers was used to select participants using the same quota-based sampling approach of 50 males and 50 females in each of the three age strata (8-10, 11-13 and 14-16

years). Children underwent HIV testing prior to recruitment; those testing positive and not in care were referred to HIV services.

Ethical and governance approvals were granted by the London School of Hygiene and Tropical Medicine Ethics Committee (Ref: 15333), the Institutional Review Board of the Biomedical Research and Training Institute in Harare (Ref: AP145/2018), the Joint Research Ethics Committee for University of Zimbabwe College of Health Sciences and the Parirenyatwa Group of Hospitals (Ref: 11/18), Harare Central Hospital Ethics Committee (Ref: 170118/04), the Medical Research Council of Zimbabwe (Ref: MRCZ/A/2297) and the Ministry of Primary and Secondary Education Zimbabwe (Ref: C/426/Harare). Parents/ guardians provided written informed consent for study participation and HIV testing, and children provided written assent.

Procedures

An interviewer-administered questionnaire was used to collect socio-demographic and clinical data including smoking, alcohol and steroid use. The International Physical Activity Questionnaire (IPAQ), validated in multiple countries including South Africa, but not in Zimbabwe, was used to assess physical activity as multiples of the resting metabolic rate (MET) in MET-minutes. Diet and nutrition were assessed using a tool based on a validated dietary diversity and food frequency tool from India and Malawi, and adapted to the Zimbabwean context using international guidelines applicable to SSA. This tool quantified dietary calcium and vitamin D intake plus sunlight exposure; adaptations reflected the local context where fortification of oils and margarine with vitamin D is mandated and specific vitamin D rich foods, *e.g.* kapenta fish, are commonly eaten.

All anthropometric measurements were carried out by trained research nurses and research assistants. Standing and sitting height, measured to the nearest 0.1 cm (using a Seca 213 stadiometer), and weight (using Seca 875 weight scales), measured to the nearest 0.1 kg, were taken by two separate readers. If height measurements differed by more than 0.5 cm, or weight measurements by more than 0.5 kg, a third reading was taken, and final height and weight values were taken as means of the two or three

measurements. The same researchers measured both the children with and without HIV. All equipment was calibrated annually. Tanner pubertal staging was performed by the study nurse and doctor, with an orchidometer used to assess testicular volume in males. Pubertal delay was defined as Tanner stage 2 in girls aged 13 years or older, and in boys aged 14 years or older.

Details about HIV including age at HIV diagnosis, ART regimen and duration, and current CD4 count and HIV viral load were collected for participants with HIV. CD4 cell count was measured using an Alere PIMA CD4 machine (Waltham, Massachusetts, USA) and HIV viral load using the GeneXpert HIV-1 viral load platform (Cepheid Inc, Sunnyvale, California, USA), with viral suppression defined as <1,000 copies/ml (as per WHO guidelines).

Assessment of bone density

Dual energy X-ray absorptiometry (DXA) scans of the lumbar spine and total body were performed by one of two trained radiographers using standard procedures on a Hologic QDR Wi densitometer (Hologic Inc., Bedford, MA, USA) with Apex Version 4.5 software for scan analysis. Daily calibration was conducted using the manufacturer provided spine phantom. DXA scans were repeated in a subgroup (n=30) to determine reproducibility. The precision error was a root mean square-standard deviation (RMS-SD) of 0.011 g/cm^2 (lumbar spine) and 0.010 g/cm^2 (total body) with an RMS-coefficient of variation (CV) of 1.35% (lumbar spine) and 1.22% (total body). An important limitation of DXA in paediatric populations with chronic disease, is that the two-dimensional (areal) bone density values are highly dependent upon body, and therefore bone size; hence DXA underestimates bone density in small children.[10] The two main size adjustment techniques recommended by the International Society for Clinical Densitometry (ISCD) to overcome the problem of size dependence of DXA measurement,[13] were employed, namely to measure (i) total-body less-head (TBLH) Bone Mineral Content (BMC) for lean mass adjusted for height (TBLH-BMC^{LBM}) and (ii) lumbar spine bone mineral apparent density (LS-BMAD). LS-BMAD was calculated from DXA-measured lumbar spine data using the Carter method.[14] TBLH-BMC^{LBM} was calculated from the whole body scan using published derived equations, for Hologic DXAs, which adjust for log-transformed total body lean mass, total body fat

mass and height.[15] Sex and age-matched Z-scores were generated using Hologic UK population reference data as recommended by ISCD guidelines, as there were no local reference data available.[13] Low TBLH-BMC^{LBM} and LS-BMAD were defined as Z-score < -2.0.[15]

Statistical analysis

A sample size of 300 in each group permitted detection of a difference between children with and without HIV in DXA-measured size-adjusted bone density Z-scores of 0.23 with 80% power and a significance level of 0.05 assuming a standard deviation of 1.3.[16] The study had 80% power to detect a 4.8% difference in prevalence of low TBLH-BMC^{LBM} between those with and without HIV, assuming a prevalence in those without HIV of 1%.[17]

Height-for-age and weight-for-age Z-scores were calculated using 1990 UK reference data,[18] with Z-scores < -2.0 defining stunting and underweight respectively. Socio-economic status (SES) was derived using the first component from a principal component analysis combining an asset list (detailing: number in household, head of household age, highest maternal and paternal education levels, household ownership, monthly household income, access to electricity, water, a flush toilet and/or pit latrine and ownership of a fridge, bicycle, car, television, and/or radio) and was split into tertiles for analysis.

Analyses were conducted using Stata 16.1 (StatCorp, Texas, USA). The primary exposure was HIV and primary outcomes were TBLH-BMC^{LBM} and LS-BMAD Z-Scores (as described above). The characteristics of participants with HIV were compared with those without HIV, using independent sample t-tests for means, with unequal variance as required, Wilcoxon signed-rank tests for non-parametric variables, and chi-squared or Fisher's exact tests for proportions. To first understand the role of sex, puberty and HIV on bone outcomes, mean differences in TBLH-BMC^{LBM} and LS-BMAD Z-scores between those with and without HIV were examined using linear regression with robust standard errors, overall and stratified by sex and Tanner stage (stages 1 and 2 *vs.* 3 to 5), similarly, mean risk differences for low TBLH-BMC^{LBM} and LS-BMAD Z-scores (< -2 compared to ≥ -2) were examined using generalised linear models, with Poisson distributions and log links with robust standard errors.

Three-way interactions between sex, pubertal stage and HIV were assessed using Wald tests in linear regression models. In secondary analyses of absolute measures of TBLH-BMC^{LBM} and LS-BMAD generalised linear models with log link and gamma distribution were used, and marginal means estimated.

Associations between potential risk factors and TBLH-BMC^{LBM} and LS-BMAD Z-score measures were investigated using linear regression separately for participants with and without HIV. Adjustment was made for (i) *a priori* confounders (age, sex, pubertal stage[19]), (ii) potential risk factors (socio-economic status,[20] physical activity,[21] calcium and vitamin D intake[22] and, in those with HIV, CD4 count, viral load, tenofovir exposure and age at ART initiation),[23] (iii) variables associated in complete case analysis with any outcome (at $p<0.2$) and (iv) variables associated with missingness.

To account for missing data, including DXA-measured outcomes, we used multiple imputation by chained equations with seven imputed datasets, which allowed for imputation of categorical and continuous data jointly. Our imputation models included all outcomes, auxiliary variables associated with missingness, with group membership (with or without HIV), and variables determined in complete case analysis to be associated with either outcome ($p<0.200$).

Role of funding source

The funders had no role in study design, data collection, data analysis, data interpretation, report writing or in the decision to submit this paper for publication.

Results

Study population

In total, 303 participants with HIV were recruited, representing 62% of the 486 eligible CWH attending HIV clinics, with 4 additional participants, identified from screening in schools, who met inclusion criteria (Figure 1). Participants with HIV were a mean (SE) 1.2 (0.26) years older, more commonly female (151 [50%] *vs.* 75 [41%]) and more likely to attend school (295 [97] *vs*. 168 [92%]) than CWH who were eligible but not enrolled. Of 536 children randomly selected from schools, 500 (93%) were eligible, of whom 307 (61%) consented to participate and one was excluded after testing HIV-positive, giving a total of 306 participants without HIV. Participants without HIV were a mean (SE) 0.6 (0.24) years older but with no difference by sex (155 [51%] *vs.* 95 [49%] female) compared to children who were eligible but not enrolled.

A higher proportion of participants with HIV were of low socio-economic status, were orphaned, had past or current tuberculosis, were less physically active, and reported greater outdoor time than those without HIV (Table 1). Across the whole study population consumption of vitamin D and calcium was low, with 79% (479/609) consuming <6.0 mcg/day vitamin D (Recommended Daily Allowance [RDA] ≥15 mcg/day), and 66% (399/609) consuming <300 mg/day of calcium. No participant reported smoking, drinking alcohol or use of oral steroids. The 24 CWH and 14 children without HIV who were missing DXA data (Figure 1) were thinner and shorter than those with DXA data and spent more time outdoors (Supplementary Table 1). Only orphanhood status and earlier pubertal stage were otherwise associated with missingness of data (Supplementary Table 2).

All but five of the 303 participants with HIV were perinatally-infected. The median age of HIV diagnosis was 3.0 (IQR 1.2-5.8) years, with ART initiated at median age 3.7 (IQR 1.8-6.9) years. At enrolment, 102/303 (34%) were taking a tenofovir disproxil fumarate (TDF)-containing regime, with a median duration of TDF use of 3.0 (IQR 1.4-5.5) years. Overall, 211 (70%) were taking a non-nucleoside reverse-transcriptase inhibitor (NNRTI) based ART regime, and 89 (29%) a protease

inhibitor (PI) based ART regime. The median CD4 count was 766 (IQR 537–1019) cells/μl and 212 (79%) had a suppressed HIV viral load (Table 1).

Growth and bone density by HIV status

A significantly greater proportion of participants with HIV compared to those without HIV were stunted (97 [32%] *vs.* 24 [8%]) and were underweight (79 [26%] *vs.* 26 [9%]) (Table 1). Interestingly, differences between those with and without HIV were greater for standing than sitting height (mean Z-Score differences 1.05 [95% CI 0.87, 1.24] and 0.73 [95% CI 0.55, 0.92] respectively). At all ages participants with HIV were more likely to be earlier in Tanner stage, and to have pubertal delay compared to participants without HIV (8 [6%] *vs.* 0 [0%], p=0.003) (Table 1; Supplementary Table 3).

Arithmetic mean TBLH-BMC^{LBM} and LS-BMAD absolute and Z-score were all lower among participants with HIV than those without HIV. The prevalence of low TBLH-BMC^{LBM} and LS-BMAD Z-scores in those with HIV were 10.4% (n=29) and 14.3% (n=40) respectively, significantly higher than the prevalence in those without HIV (6.2% [n=18] and 5.8% [n=17] respectively) (Table 1). Overall CWH had 0.20 (95%CI 0.03, 0.37) lower TBLH-BMC^{LBM} and 0.26 (0.04, 0.48) lower LS-BMAD Z-Scores than children without HIV (Table 2); differences in LS-BMAD Z-Scores were similar in females and males, whilst differences in TBLH-BMC^{LBM} were more apparent in females. Correspondingly, both male and female CWH had an increased the risk of low LS-BMAD (Table 2). In CWH, low LS-BMAD was strongly associated with stunting (p<0.001). This association was not observed among participants without HIV (Supplementary Table 4).

Interactions by sex and puberty on the association between HIV and size-adjusted bone density

In each age stratum, both male and female CWH were on average at a lower Tanner stage than their HIV-negative peers of the same sex (Supplementary Table 3). Mean absolute measures of TBLH-BMC^{LBM} and LS-BMAD differed by pubertal stage and sex (3-way interactions: TBLH-BMC^{LBM} p=0.041, LS-BMAD p=0.88), such that differences in TBLH-BMC^{LBM} and LS-BMAD absolute and Z-score values between participants with and without HIV were more marked in later pubertal stages

(Figure 2). Differences in size-adjusted bone density between participants with and without HIV were generally small in the early stages of puberty (Table 2). Whereas, in the later stages of puberty, differences in bone outcomes between those with and without HIV, were more apparent for both sexes, but with females particularly showing evidence of differences in both TBLH-BMC^{LBM} and LS-BMAD (Z-Scores and absolute values) (Figure 2; Table 2). Stratification widened confidence intervals as fewer older participants with HIV (14-16 years) had entered the later stages of puberty, *i.e.*, Tanner stages 4 and 5, compared to those without HIV (Supplementary Table 3). When models assessing differences in TBLH-BMC^{LBM} and LS-BMAD Z-scores between children with and without HIV were adjusted for age, sex and pubertal stage, confidence intervals widened. However, even after adjustment for age, male CWH in the early stages of puberty had a greater risk of low LS-BMAD than did males without HIV (risk difference 0.14 [95%CI 0.04, 0.24]) (Table 2).

Risk factors associated with size-adjusted bone density outcomes

Among children without HIV, male sex and earlier pubertal stage were associated with lower TBLH-BMC^{LBM} and LS-BMAD Z-scores in crude but not adjusted analyses (Supplementary Table 5). Among children with HIV, orphanhood and TDF exposure were associated with lower TBLH-BMC^{LBM} Z-score in both unadjusted analyses and after adjustment for age, sex, pubertal stage, socioeconomic status, physical activity, calcium and vitamin D intake, CD4 count, HIV viral load and age at ART initiation (Table 3). After adjustment, TDF exposure for four or more years was associated with a 0.52 SD deficit in TBLH-BMC^{LBM} Z-Score.

Older age, male sex, orphanhood, as well as older age at ART initiation and TDF use, were all associated with lower LS-BMAD Z-Score in unadjusted analyses (Table 4). A weak association was detected between low CD4 count and low LS-BMAD Z-Score. Older age, male sex and earlier Tanner stage remained associated with low LS-BMAD Z-Score after adjustment for orphanhood, socioeconomic status, physical activity, calcium and vitamin D intake, ART initiation, TDF exposure, CD4 count and viral load.

Current TDF use was associated with 0.41 (95%CI 0.08, 0.74; p=0.015) lower TBLH-BMC^{LBM} Z-Score and 0.31 (95%CI 0.08, 0.69; p=0.116) lower LS-BMAD Z-Score, compared to those with HIV on non-TDF containing ART, after adjustment for age, sex, pubertal stage, orphanhood, socioeconomic status, physical activity, calcium and vitamin D intake, CD4 count, HIV viral load and age at ART initiation.

Discussion

The main finding of this study was that despite ART, marked deficits in size-adjusted bone density are common among children with HIV, who have a substantially higher prevalence of low bone density (a deficit of 2 SDs or more) than do HIV-uninfected peers. The negative associations between HIV and size-adjusted bone density were more pronounced with pubertal maturation. Orphanhood and use of tenofovir are associated with bone deficits in peripubertal children with HIV.

Across all age groups, CWH were behind HIV-uninfected peers in terms of pubertal stage. Despite low numbers, the association between HIV and size-adjusted bone density is most marked towards the end of puberty. This suggests cumulatively lower bone accrual through puberty in those with HIV relative to children without HIV. Importantly, if bone accrual remains compromised, those with HIV will achieve substantially lower PBM. Female CWH had substantially lower TBLH-BMC^{LBM} Z-Scores than females without HIV, this difference was more apparent in later puberty; this deficit was not seen in boys. Both male and female CWH had lower LS-BMAD Z-Scores and a greater risk of low LS-BMAD (*i.e.*, a Z-Score <-2), compared to those without HIV. Male CWH in early puberty were at particular risk of low LS-BMAD. These results highlight the need for interventions to optimise bone density before skeletal growth is completed. In addition to minimising exposure to TDF (discussed further below), a number of interventions may be beneficial. In Zimbabwe and Zambia, a trial is currently underway to determine whether supplementation of vitamin D_3 and calcium ameliorates bone mineralisation deficits in adolescents growing up with HIV (trial registration PACTR202009897660297). Furthermore, our study identified lower levels of physical activity in CWH, than their uninfected peers. High impact physical activity is thought to be osteogenic, and has

been associated with improved bone density at the hip in healthy adolescents.[21] Recently a small, randomised trial in young adults living with HIV, has suggested the combination of resistance and aerobic exercise may improve bone mass,[24] whether the same holds for CWH remains to be determined. Recently the first, small randomised controlled trial of the oral bisphosphonate, alendronate, given to CWH showed some evidence of improved bone density over 48 weeks of treatment; however, longer term effects on PBM, fracture risk, and rare side effects are unknown.

The deficits we have identified in TBLH-BMC^{LBM} in females at the end of puberty is a concern. Bone density is compromised during pregnancy and lactation when skeletal calcium reserves are mobilised,[25] and even healthy adolescent mothers may achieve a compromised PBM.[26] Given in 2019 24% of young women living in Zimbabwe had given birth before the age of 18,[27] our findings have implications for the recovery of skeletal mineralisation post-partum and subsequent adult fracture risk.[28]

Not only did CWH have a higher prevalence of stunting compared to uninfected children, but a higher proportion of CWH who were stunted had lower lumbar spine bone density than stunted children without HIV. While stunting increases risk of poor skeletal growth, we have shown that stunting is not a sufficient clinical proxy for low size-adjusted bone density. Interestingly, deficits in standing height were greater than sitting height, suggesting that HIV infection may have a greater effect on appendicular (limb length) than axial (spinal length) skeletal growth. Appendicular growth occurs more rapidly than axial growth before puberty. It is thought that exposures shortly before puberty preferentially affect appendicular development, whilst exposures during puberty may have a greater impact on axial development.[29] Thus, these findings suggest that HIV and its treatment pre-pubertally may be particularly important in influencing linear growth.

Notably, ART drugs themselves may cause accelerated bone loss. There was a strong and consistent association between TDF exposure and bone deficits, particularly affecting TBLH-BMC^{LBM}. Those exposed to TDF for four or more years had on average a 0.5 SD lower TBLH-BMC^{LBM} Z-Score compared with CWH who had not received TDF. This is a clinically important effect size, as a 0.5 SD

reduction in bone density increases by 50% both childhood and, if sustained, future adult fracture risk.[30] Whilst bone loss following TDF initiation in adults is well recognised, hitherto studies of TDF on bone outcomes in children have been inconsistent, for example among 74 Brazilian adolescents, mean age 17 years, length of TDF use was associated with lower (albeit non size-adjusted) lumbar spine and total body bone density,[31] whereas a larger study of 394 Thai adolescents of a similar age, found no such association with both (non-size adjusted) total body bone density and (size-adjusted) LS-BMAD.[32]

Our study supports the hypothesis that TDF use has a detrimental effect on bone health in children; notably we saw a dose response with longer TDF exposure associated with more pronounced bone deficits. TBLH-BMC^{LBM} largely represents cortical bone (whereas LS-BMAD reflects predominantly trabecular bone), thus our findings suggest TDF may particularly affect the mineralisation of cortical bone to reduce density; consistent with TDF induced renal tubulopathy with phosphaturia leading to skeletal hypomineralization.[33] TDF is a well-tolerated drug and remains one of the recommended component drugs of a first line ART regimen, and is thus extensively used in sub-Saharan Africa, in both children and adults. In Zimbabwe, TDF is mostly available at an adult dose as part of a combination ART regime, and use is recommended only in children weighing over 25kg or age above 10 years. However, due to limitation in the availability of ART options, TDF is often prescribed in younger children, and/or those with lower weight, and therefore those children will effectively receive a higher than recommended dose. Our findings add to a growing body of evidence to support the replacement of TDF with tenofovir alafenamide, a prodrug of TDF that is associated with significantly fewer effects on bone and kidneys.[34] This is of particular importance in adolescence, a period of rapid bone accrual, as achievement of PBM may otherwise be compromised by TDF use.

The mechanism by which HIV compromises musculoskeletal development is likely multifactorial. HIV promotes dysregulated systemic immune activation, which is not completely reversed by ART.[35] During childhood, formation predominates over resorption, but the pro-inflammatory milieu leads to an imbalance in osteoblastic/osteoclastic activity, promoting increased bone resorption relative to formation. Other factors that can compromise skeletal development include inadequate dietary calcium,

vitamin D deficiency[22] and low levels of physical activity.[21] Whilst in this study CWH reported lower levels of physical activity than HIV-uninfected children, low intake of both calcium and vitamin D were common regardless of HIV status. Orphanhood, more common in CWH, was independently associated with low TBLH-BMC^{LBM}. Orphanhood may be a proxy for social and emotional deprivation, which along with biological and nutritional factors, may disrupt growth.

The strengths of the study were being well-powered, the inclusion of an HIV-uninfected comparison group from broadly the same socioeconomic background, and collection of detailed data on risk factors including physical activity as well as vitamin D and calcium intake using a standardised tool adapted for the local context. 'Gold standard' size-adjustment methods were used for DXA data, which were particularly crucial given height differences (levels of stunting) in the population studied.[13,15] In addition, sex and age-matched Z-scores were used for size-adjusted bone density measures which have greater clinical interpretability than absolute measures.

We acknowledge several limitations: data are cross-sectional and therefore causality cannot be inferred. In the absence of local or African reference population data, the reference data used to generate the Z-scores for TBLH-BMC^{LBM} and LS-BMAD were obtained from a paediatric reference population in the UK using the same manufacturer and software version to derive Z-scores (as recommended by the ISCD),[13] who may not be comparable to the children in this study. Whilst the study population Z-Score SDs for TBLH-BMC^{LBM} were close to 1, those for LS-BMAD were higher, suggesting the UK reference population may be a better fit for total body than lumbar spine data, and/or that the size-adjustment should be further validated in African populations. DXA is unable to distinguish between bone density deficits due to hypomineralization versus other architectural properties of bone. Only current CD4 count and HIV viral load were available as markers of HIV disease severity; annual trends in these measures would be more appropriate as growth occurs over a long period. Only five (1.6%) CWH reported non-perinatal infection and their inclusion is highly unlikely to have biased results. Aside from examining TDF *v.s.*, non-TDF containing ART regimes, we lacked sufficient power to further categorise the many ART regimes to understand associations with other individual treatments. Further, although we

examined three-way interactions between age, puberty and HIV on size-adjusted bone density, the study was not powered to detect the true differences between these strata. While we aimed to collect comprehensive data on factors associated with size-adjusted bone density and growth, there may be residual confounding by other past or current biological or environmental factors. Due to delays in engineering maintenance, DXA measurements were missing for a minority of children, in addition to a small amount of missing covariate data. We used multiple imputation with chained equations to impute these data, which are subject to a missing at random (MAR) assumption. Our findings are therefore valid under this MAR assumption and the specified imputation model. Finally, achievement of PBM is estimated to occur in the early twenties and therefore bone accrual may continue to consolidate beyond the age-range examined.

To our knowledge this is the largest study investigating the relationship between HIV infection and skeletal health in children in sub-Saharan Africa in the ART era. While ART results in immune reconstitution and has dramatically improved survival, this study underscores the importance of addressing the long-term adverse impacts of HIV infection on musculoskeletal health in the current cohort of children, particularly in sub-Saharan Africa. There is an urgent need for HIV programmes to focus beyond delivery of ART, to develop strategies for prevention and management of the long-term effects of HIV infection and its treatment on musculoskeletal health to ensure optimum health in children as they enter adulthood. Longitudinal studies with follow-up of children through and after puberty are required to understand the extent of catch-up growth and bone accrual, and hence the population reported here are now in follow-up.

Contributors: RR, RAF, CLG conceived the study. SF, KAW, RAF, CLG and RR and AMR designed the study protocol. RR, CK, FK, GM, HM collected the data. JC, CK and RR were responsible for data management. AMR, CLG, TM, VS and RR conducted data analyses. RR and CLG wrote the first draft.. RR and AR had full access to all the study data and had final responsibility for the decision to submit for publication. All authors contributed to the report and approved the final submitted text. Authors thank Dr Nicola Crabtree for her advice regarding calculation of TBLH-BMC^{LBM} and LS-BMAD Z-scores.

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Data sharing: Anonymised research data will be made available for sharing through the LSHTM open access data repository (LSHTM Data Compass, https://datacompass.lshtm.ac.uk/).

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Research in context

Evidence before this study

We and others have previously shown that poor linear growth resulting in stunting is common in children growing up with HIV in sub-Saharan Africa. Successful roll-out of antiretroviral therapy (ART) programmes has enabled many more children to survive adolescence to reach adulthood. Puberty is a critical period of skeletal development during which bone mass accrues to achieve, by early adulthood, peak bone mass (PBM), which has been shown to be a key determinant of lifetime fracture risk. We searched EMBASE, Ovid MEDLINE(R) (including Epub and Daily databases), Cochrane databases and conference proceedings (through Web of Science) from inception until March 10th 2021, adapting search strategies for each database to include combinations of medical subject headings (MeSH terms) relevant to bone, growth, development, HIV and ART in children, and without language restriction. Few studies had investigated the effect of HIV on adolescent skeletal growth in low-income settings and even fewer had adequately considered the effect of skeletal size on bone density measurement techniques; this is crucial as we know the most commonly used measure, dual energy X-ray absorptiometry (DXA), underestimates bone density when skeletons are small (as is the case with stunting). The International Society for Clinical Densitometry (ISCD) recommended specific size-adjustment techniques to overcome this problem. These techniques had not been used to understand the effect of HIV on bone mass accrual in populations in sub-Saharan Africa.

Added value of this study

To our knowledge, this is the largest study to investigate the effect of HIV infection on skeletal health in children in sub-Saharan Africa. Findings are strengthened using 'gold standard' size-adjustment methods for DXA data, particularly important given observed height differences (levels of stunting). The study found marked deficits in bone density were common in children with HIV, who had a substantially higher prevalence of low bone density (a deficit of 2 SDs or more) compared with their HIV-uninfected peers. The effect of HIV on bone density was most marked in the last stage of puberty, especially at the spine in females.

Use of tenofovir disproxil fumarate (TDF) was strongly associated with bone deficits, particularly affecting the total body (predominantly reflecting cortical bone). TDF exposure for four or more years was associated with a 0.5 SD lower total body Z-Score (which translates clinically to an approximately 50% increase in both childhood and, if sustained, adult fracture risk).

Implications of all the available evidence

This study underscores the importance of addressing the long-term adverse impacts of HIV infection on musculoskeletal health in children living in sub-Saharan Africa. If the bone deficits here identified persist into adulthood, this African region risks increasing fracture incidence within the current birth cohort. The finding that bone mineral deficits appear greatest at the end of puberty in females is of concern, as these women may soon draw upon these potentially inadequate skeletal calcium reserves to support pregnancy and lactation, which may increase their vertebral fracture risk. TDF is currently one of the recommended component drugs of a first line ART regimen and is thus extensively used in sub-Saharan Africa. The detrimental effects associated with TDF use in this study, support re-evaluation of first-line use during skeletal growth.

Figure Legends

Figure 1. Flow diagram illustrating recruitment of children living with HIV from HIV clinic, and children from schools residing in the same suburbs.

Figure 2. Arithmetic means and 95% confidence intervals for A) TBLH-BMC^{LBM} Z-score B) LS-BMAD Z-score C) absolute TBLH-BMC^{LBM} D) absolute LS-BMAD, stratified by sex, age, pubertal stage and HIV status (with no adjustment). Raw data presented as open circles (HIV) and open triangles (non-HIV)

Table

Table 1. Socio-demographic, lifestyle, anthropometric and DXA-measured characteristics of study participants

		HIV positive		HIV negative		p value
		N	n (%)[a]	N	n (%)[a]	
Socio-demographic factors	Age years, mean (SD)	303	12.4 (2.5)	306	12.5 (2.5)	0.688
	Female sex	303	151 (49.8)	306	155 (50.7)	0.840
	Socioeconomic status (SES)	303		306		0.005
	Tertile 1 (low)		115 (38.0)		88 (28.8)	
	Tertile 2 (middle)		105 (34.7)		98 (32.0)	
	Tertile 3 (high)		83 (27.4)		120 (39.2)	
	Orphanhood (one or both parents dead)[b]	290	123 (42.4)	303	20 (6.6)	<0.001
Lifestyle factors	Outdoor time >2 hours/day	303	224 (73.9)	306	171 (55.9)	<0.001
	Physical activity level	303		306		0.012
	Low, <600 MET mins/week		148 (48.8)		114 (37.3)	
	Moderate, 600-3000 MET mins/week		77 (25.4)		88 (28.8)	
	High, >3000 MET mins/week		78 (25.7)		104 (34.0)	
	Daily dietary calcium intake	303		306		0.936
	Very low, <150 mg/day		135 (44.6)		136 (44.4)	
	Low, 150-299 mg/day		62 (20.5)		66 (21.6)	
	Moderate, 300–450 mg/day		106 (35.0)		104 (34.0)	
	Daily dietary vitamin D	303		306		0.413
	Very low, <4.0 mcg/day		40 (13.2)		37 (12.1)	
	Low, 4.0-5.9 mcg/day		205 (67.7)		197 (64.4)	
	Moderate, 6.0-8.0 mcg/day		58 (19.1)		72 (23.5)	
Pubertal stage	Tanner I	287	117 (40.8)	303	69 (22.8)	<0.001
	Tanner II		59 (20.6)		69 (22.8)	
	Tanner III		55 (19.2)		53 (17.5)	
	Tanner IV		44 (15.3)		92 (30.4)	
	Tanner V		12 (4.2)		20 (6.6)	
	Pubertal delay[c]	127	8 (6.3)	132	0 (0.0)	0.003
HIV characteristics	Age at HIV diagnosis years, median (IQR)	303	3.0 (1.2-5.8)			
	Age at ART initiation years, median (IQR)	303	3.7 (1.8-6.9)			
	ART duration years, median (IQR)	303	8.1 (6.2-9.5)			
	% life on ART, mean (SD)	303	65.4 (22.1)			
	Current TDF use	303	102 (33.7)			
	Viral load <1,000 copies/ml	268	212 (79.1)			
	CD4 count <500 cells/µL	288	58 (20.1)			
Anthropometry	Standing height Z-score, mean (SD)	302	-1.68 (1.23)	306	-0.63 (1.08)	<0.001
	Height-for-age Z-score <-2	302	97 (32.1)	306	24 (7.8)	<0.001
	Sitting height for age Z-score, mean (SD)	303	-2.11 (1.12)	303	-1.38 (1.21)	<0.001
	Sitting height-for-age Z-score <-2	303	152 (50.2)	303	76 (25.1)	<0.001
	Weight-for-age Z-score, mean (SD)	303	-1.46 (1.20)	304	-0.55 (1.24)	<0.001
	Weight-for-age Z-score <-2	303	79 (26.1)	304	26 (8.6)	<0.001
	BMI Z-score, mean (SD)	302	-0.63 (1.01)	304	-0.28 (1.17)	<0.001
	BMI Z-score <-2	302	28 (9.3)	304	18 (5.9)	0.119
Bone density measures	TBLH-BMC^{LBM} (g), mean (SD)	279	950.9 (272.8)	292[l]	1090.3 (336.1)	<0.001
	TBLH-BMC^{LBM} Z-score, mean (SD)	279	-0.61 (1.08)	292[l]	-0.41 (1.00)	0.018
	TBLH-BMC^{LBM} Z-score <-2	279	29 (10.4)	292[l]	18 (6.2)	0.066
	LS-BMAD (g/cm^3), mean (SD)	279	0.202 (0.035)	293	0.210 (0.037)	0.013
	LS-BMAD Z-score, mean (SD)	279	-0.51 (1.40)	293	-0.24 (1.21)	0.014
	LS-BMAD Z-score <-2	279	40 (14.3)	293	17 (5.8)	0.001

[a] n(%) unless stated otherwise; [b] one or both parents dead; [c] definition included girls >13 years and boys >14 years

Table 2. Mean differences (95% CI) in Z-scores and risk differences (95% CI) of Z-scores <-2, between participants with and without HIV

Outcome & Stratification	Strata	N[1]	Crude Mean Difference[2]	Adjusted MD[2,3]
TBLH-BMC^{LBM} Z-score	**Overall**[4]	609	-0.20 (-0.37, -0.03)	-0.13 (-0.31, 0.05)
Sex[5]	Males	303	-0.12 (-0.36, 0.11)	-0.06 (-0.30, 0.18)
	Females	306	-0.27 (-0.52, -0.02)	-0.20 (-0.46, 0.06)
Sex & Tanner stage[6]	**Males**			
	Stage 1-2	175	-0.18 (-0.49, 0.12)	-0.17 (-0.47, 0.14)
	Stage 3-5	117	-0.12 (-0.50, 0.26)	-0.11 (-0.50, 0.27)
	Females			
	Stage 1-2	139	-0.14 (-0.50, 0.22)	-0.12 (-0.48, 0.23)
	Stage 3-5	159	-0.35 (-0.71, 0.01)	-0.34 (-0.70, 0.03)
LS-BMAD Z-score	**Overall**[4]	609	-0.26 (-0.48, -0.04)	-0.11 (-0.32, 0.11)
Sex[5]	Males	303	-0.27 (-0.58, 0.04)	-0.17 (-0.46, 0.13)
	Females	306	-0.23 (-0.51, 0.04)	-0.04 (-0.32, 0.24)
Sex & Tanner stage[6]	**Males**			
	Stage 1-2	175	-0.29 (-0.68, 0.10)	-0.16 (-0.54, 0.22)
	Stage 3-5	117	-0.44 (-0.95, 0.07)	-0.46 (-0.95, 0.03)
	Females			
	Stage 1-2	139	-0.09 (-0.53, 0.34)	0.004 (-0.42, 0.42)
	Stage 3-5	159	-0.30 (-0.66, 0.07)	-0.24 (-0.61, 0.13)
		N	**Crude Risk Difference**[7]	**Adjusted RD**[3,7]
TBLH-BMC^{LBM} Z-score <-2	**Overall**[4]	609	0.04 (-0.005, 0.08)	0.01 (-0.03, 0.05)
Sex[5]	Males	303	0.05 (-0.008, 0.11)	0.02 (-0.03, 0.07)
	Females	306	0.02 (-0.04, 0.08)	0.004 (-0.04, 0.05)
Sex & Tanner stage[6]	**Males**			
	Stage 1-2	175	0.04 (-0.03, 0.12)	0.04 (-0.05, 0.13)
	Stage 3-5	117	0.05 (-0.05, 0.16)	0.04 (-0.04, 0.12)
	Females			
	Stage 1-2	139	0.003 (-0.09, 0.10)	-0.006 (-0.13, 0.12)
	Stage 3-5	159	0.03 (-0.04, 0.11)	0.02 (-0.04, 0.09)
LS-BMAD Z-score <-2	**Overall**[4]	609	0.08 (0.03, 0.12)	0.02 (-0.02, 0.06)
Sex[5]	Males	303	0.09 (0.009, 0.17)	0.03 (-0.03, 0.09)
	Females	306	0.06 (0.008, 0.11)	0.02 (-0.01, 0.05)
Sex & Tanner stage[6]	**Males**			
	Stage 1-2	175	0.13 (0.05, 0.22)	0.14 (0.04, 0.24)
	Stage 3-5	117	0.07 (-0.09, 0.23)	0.04 (-0.06, 0.13)
	Females			
	Stage 1-2	139	0.08 (-0.003, 0.17)	0.09 (-0.03, 0.21)
	Stage 3-5	159	0.03 (-0.03, 0.08)	0.01 (-0.02, 0.04)

MD: Mean Difference, negative MD values indicate lower mean in CWH

RD: Risk Difference, positive values indicate an increased risk of low Z-Score (<-2.0) in CWH.

[1] Missing data for TBLH-BMC^{LBM} Z-score (19 males and 19 females), LS-BMAD Z-score (18 males and 19 females), pubertal stage (11 males and 8 females), and orphanhood (9 males and 7 females) were estimated using multiple imputation models and numbers in each pubertal stage stratum varied by imputation dataset. Non-missing n are shown.

[2] Linear regression with robust standard errors

[3] Model adjusted for age, sex, pubertal stage

[4] Tanner stage included as a variable with 5 levels

[5] Estimated by fitting an interaction term for sex by HIV status; in models adjusted for Tanner stage (as 5 levels)

[6] Estimated by fitted a 3-way interaction term for sex by Tanner stage (2 categories) by HIV status

[7] Generalised linear model, Poisson distribution and log link with robust standard errors

Table 3. Characteristics associated with TBLH-BMC^{LBM} Z-Score in participants with HIV

	N (303)[1]	Mean outcome[2]	Crude beta coefficient (95%CI)[3]	p value	Adjusted beta coefficient (95% CI)[3,4]	p value
Age						
8-10 years	94	-0.579	Ref	0.202	Ref	0.315
11-13 years	94	-0.481	0.126 (-0.178, 0.430)		0.156 (-0.272, 0.584)	
14-16 years	91	-0.781	-0.162 (-0.476, 0.152)		-0.147 (-0.730, 0.436)	
Sex						
Male	142	-0.687	Ref	0.301	Ref	0.364
Female	137	-0.534	0.138 (-0.125, 0.401)		0.121 (-0.141, 0.382)	
Pubertal status						
Tanner I	107	-0.628	Ref	0.951	Ref	0.522
Tanner II	56	-0.742	-0.069 (-0.425, 0.288)		0.084 (-0.318, 0.487)	
Tanner III	49	-0.672	-0.021 (-0.374, 0.332)		0.024 (-0.457, 0.505)	
Tanner IV	40	-0.506	0.122 (-0.341, 0.584)		0.448 (-0.183, 1.080)	
Tanner V	12	-0.640	-0.054 (-0.703, 0.594)		0.204 (-0.602, 1.010)	
Socioeconomic status (SES)						
SES Tertile 3 (high)	78	-0.621	Ref	0.871	Ref	0.931
SES Tertile 2 (middle)	93	-0.637	-0.024 (-0.353, 0.304)		0.030 (-0.264, 0.388)	
SES Tertile 1 (low)	108	-0.584	0.054 (-0.268, 0.377)		0.061 (-0.264, 0.387)	
Orphanhood						
Not an orphan	154	-0.422	Ref	0.001	Ref	0.002
One or both parents dead	113	-0.843	-0.445 (-0.710, -0.181)		-0.461 (-0.750, -0.171)	
Physical activity level						
High, >3000 MET mins/week	76	-0.533	Ref	0.707	Ref	0.956
Mod, 600-3000 MET mins/week	69	-0.621	-0.087 (-0.434, 0.261)		0.016 (-0.341, 0.372)	
Low, <600 MET mins/week	134	-0.652	-0.129 (-0.438, 0.179)		-0.030 (-0.348, 0.287)	
Daily calcium (Ca) intake						
Moderate, 300–450 mg/day	95	-0.696	Ref	0.385	Ref	0.300
Low, 150-299 mg/day	60	-0.640	0.069 (-0.283, 0.420)		0.105 (-0.255, 0.465)	
Very low, <150 mg/day	124	-0.533	0.196 (-0.087, 0.478)		0.235 (-0.063, 0.533)	
Daily vitamin D intake						
Moderate, 6.0-7.9 mcg/day	50	-0.404	Ref	0.246	Ref	0.112
Low, 4.0-5.9 mcg/day	190	-0.624	-0.181 (-0.514, 0.152)		-0.247 (-0.581, 0.087)	
Very low, <4.0 mcg/day	39	-0.816	-0.383 (-0.826, 0.061)		-0.495 (-0.962, -0.027)	
CD4 count						
≥500 cells/µL	213	-0.593	Ref	0.638	Ref	0.711
<500 cells/µL	54	-0.696	-0.076 (-0.395, 0.242)		-0.066 (-0.416, 0.284)	
HIV viral load						
≥1,000 copies/ml	54	-0.530	Ref	0.303	Ref	0.186
<1,000 copies/ml	207	-0.653	-0.162 (-0.469, 0.146)		-0.241 (-0.600, 0.118)	
Age at ART initiation						
<4 years	148	-0.611	Ref	0.942	Ref	0.617
4-8 years	83	-0.577	0.041 (-0.265, 0.347)		0.108 (-0.229, 0.445)	
>8 years	48	-0.676	-0.021 (-0.372, 0.330)		0.209 (-0.210, 0.628)	
Tenofovir years of exposure						
None	184	-0.484	Ref	0.037	Ref	0.046
<4 years	59	-0.803	-0.298 (-0.613, 0.016)		-0.346 (-0.693, 0.0003)	
≥4 years	36	-0.950	-0.476 (-0.935, -0.017)		-0.520 (-1.036, -0.003)	

[1]Non-missing data

[2]Arithmetic mean of non-missing data

[3]Beta coefficient from linear regression indicates Z-Score difference from referent category, lower values indicate lower Z-Scores. Missing data for TBLH-BMC^{LBM} Z-score (n=38), LS-BMAD Z-score (n=37), pubertal stage (n=19), orphanhood (n=16), CD4 cell count (n=15), and HIV viral load (n=35) were estimated using multiple imputation models

[4]Adjusted model includes age, sex, pubertal stage, orphanhood, socioeconomic status, physical activity, calcium and vitamin D intake, age at ART initiation, years of tenofovir exposure, CD4 and viral load.

Table 4. Characteristics associated with LS-BMAD Z-Score in participants with HIV

	N (303)[1]	Mean outcome[2]	Crude beta coefficient (95%CI)[3]	p value	Adjusted beta coefficient (95% CI)[3,4]	p value
Age						
8-10 years	94	-0.110	Ref	<0.001	Ref	0.001
11-13 years	94	-0.313	-0.241 (-0.617, 0.134)		-0.357 (-0.864, 0.150)	
14-16 years	91	-1.133	-0.994 (-1.385, -0.604)		-1.255 (-1.961, -0.549)	
Sex						
Male	142	-0.889	Ref	<0.001	Ref	<0.001
Female	137	-0.122	0.755 (0.430, 1.081)		0.771 (0.451, 1.090)	
Pubertal status						
Tanner I	117	-0.368	Ref	0.669	Ref	0.029
Tanner II	59	-0.654	-0.297 (-0.747, 0.153)		0.380 (-0.122, 0.882)	
Tanner III	55	-0.505	-0.160 (-0.616, 0.297)		0.564 (-0.005, 1.134)	
Tanner IV	44	-0.698	-0.322 (-0.892, 0.249)		1.049 (0.316, 1.783)	
Tanner V	12	-0.564	-0.286 (-1.144, 0.573)		1.305 (0.363, 2.246)	
Socioeconomic status (SES)						
SES Tertile 3 (high)	78	-0.482	Ref	0.885	Ref	0.647
SES Tertile 2 (middle)	93	-0.488	-0.017 (-0.433, 0.399)		0.023 (-0.362, 0.409)	
SES Tertile 1 (low)	108	-0.555	-0.094 (-0.511, 0.324)		-0.143 (-0.550, 0.264)	
Orphanhood						
Not an orphan	154	-0.321	Ref	0.010	Ref	0.144
One or both parents dead	113	-0.772	-0.439 (-0.771, -0.107)		-0.252 (-0.590, 0.087)	
Physical activity level						
High, >3000 MET mins/week	76	-0.378	Ref	0.389	Ref	0.774
Mod, 600-3000 MET mins/week	69	-0.597	-0.232 (-0.682, 0.219)		-0.150 (-0.579, 0.278)	
Low, <600 MET mins/week	134	-0.545	-0.149 (-0.544, 0.246)		-0.109 (-0.498, 0.281)	
Daily calcium intake						
Moderate, 300–450 mg/day	95	-0.384	Ref	0.457	Ref	0.694
Low, 150-299 mg/day	60	-0.455	-0.069 (-0.528, 0.391)		-0.048 (-0.502, 0.405)	
Very low, <150 mg/day	124	-0.638	-0.228 (-0.591, 0.136)		-0.156 (-0.514, 0.201)	
Daily vitamin D intake						
Moderate, 6.0-7.9 mcg/day	50	-0.215	Ref	0.400	Ref	0.420
Low, 4.0-5.9 mcg/day	190	-0.564	-0.360 (-0.955, 0.234)		-0.281 (-0.738, 0.175)	
Very low, <4.0 mcg/day	39	-0.642	-0.273 (-0.735, 0.190)		-0.199 (-0.791, 0.393)	
CD4 count						
>500 cells/µL	213	-0.429	Ref	0.060	Ref	0.778
<500 cells/µL	54	-0.820	-0.392 (-0.801, 0.017)		-0.060 (-0.482, 0.361)	
HIV Viral load						
>1,000 copies/ml	54	-0.713	Ref	0.211	Ref	0.752
<1,000 copies/ml	207	-0.450	0.263 (-0.151, 0.678)		0.066 (-0.344, 0.476)	
Age at ART initiation, years						
<4 years	148	-0.181	Ref	0.0002	Ref	0.288
4-8 years	83	-0.788	-0.611 (-0.987, -0.235)		-0.280 (-0.688, 0.128)	
>8 years	48	-1.057	-0.826 (-1.270, -0.381)		-0.356 (-0.858, 0.146)	
Tenofovir years of exposure						
None	184	-0.262	Ref	0.0007	Ref	0.248
<4 years	59	-0.972	-0.700 (-1.108, -0.291)		-0.377 (-0.808, 0.054)	
≥4 years	36	-1.036	-0.721 (-1.249, -0.192)		-0.189 (-0.750, 0.375)	

[1]Non-missing data

[2]Arithmetic mean of non-missing data

[3]Beta coefficient from linear regression indicates Z-Score difference from referent category, lower values indicate lower Z-Scores. Missing data for TBLH-BMC^{LBM} Z-score (n=38), LS-BMAD Z-score (n=37), pubertal stage (n=19), orphanhood (n=16), CD4 cell count (n=15), and HIV viral load (n=35) were estimated using multiple imputation models

[4]Adjusted model includes age, sex, pubertal stage, orphanhood, socioeconomic status, physical activity, calcium and vitamin D intake, age at ART initiation, years of tenofovir exposure, CD4 and viral load.

Figure 1

Figure 1. Flow diagram illustrating recruitment of study participants

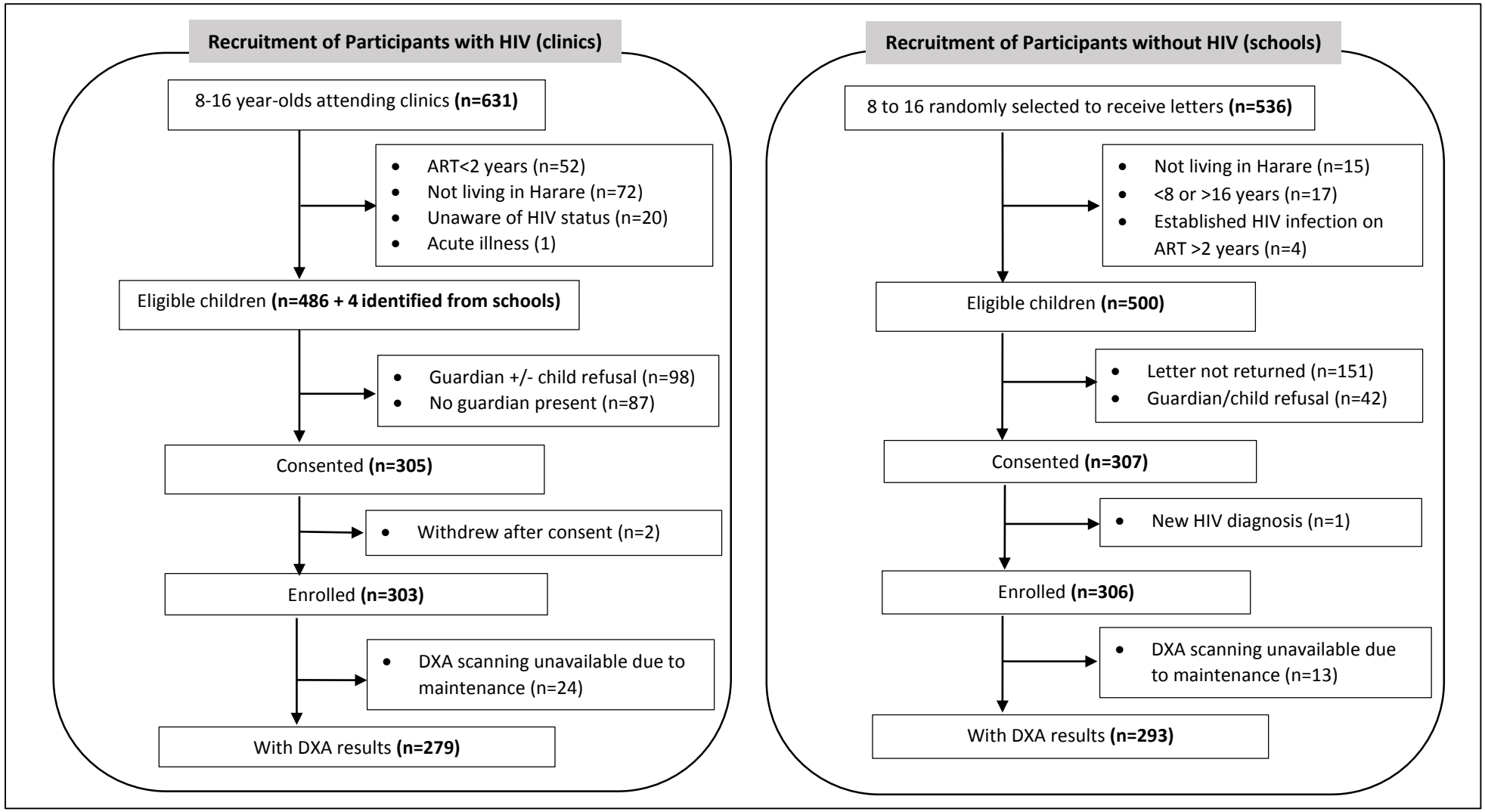
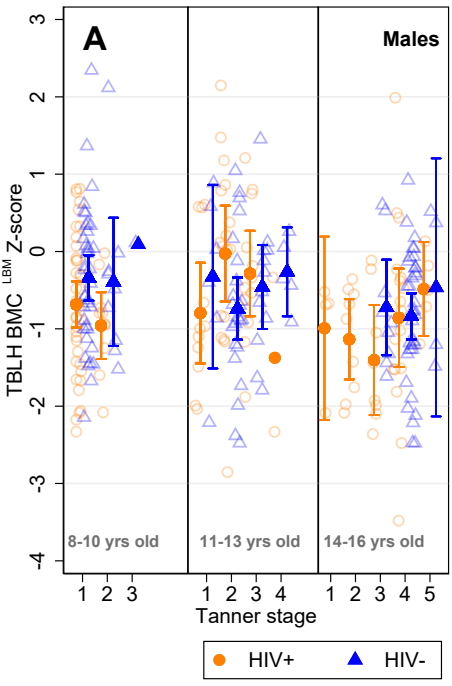
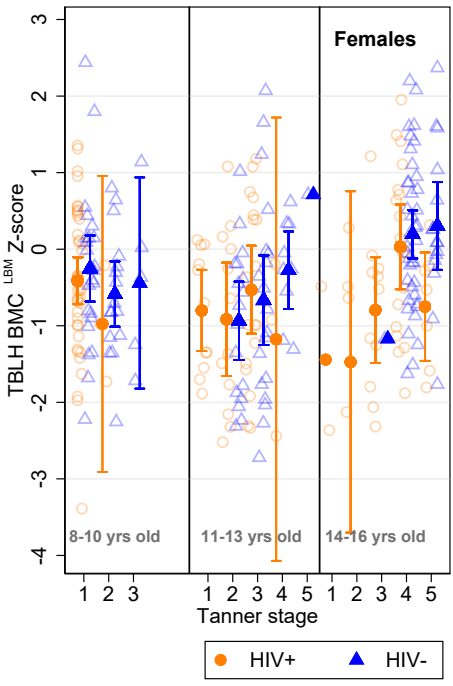


Figure 2


A
Males
TBLH BMC LBM Z-score
8-10 yrs old
11-13 yrs old
14-16 yrs old
Tanner stage
HIV+
HIV-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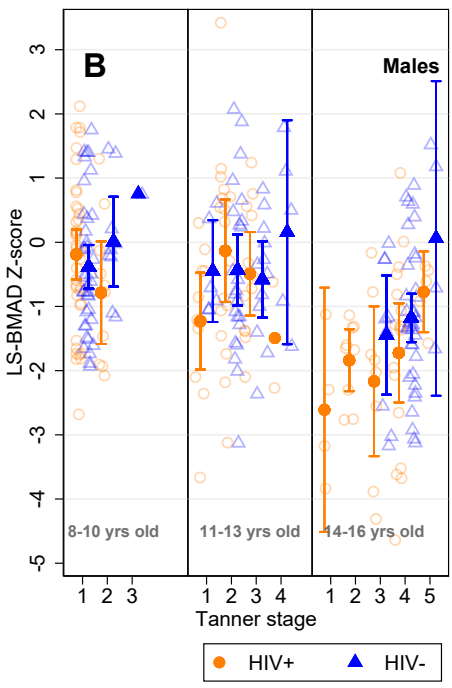
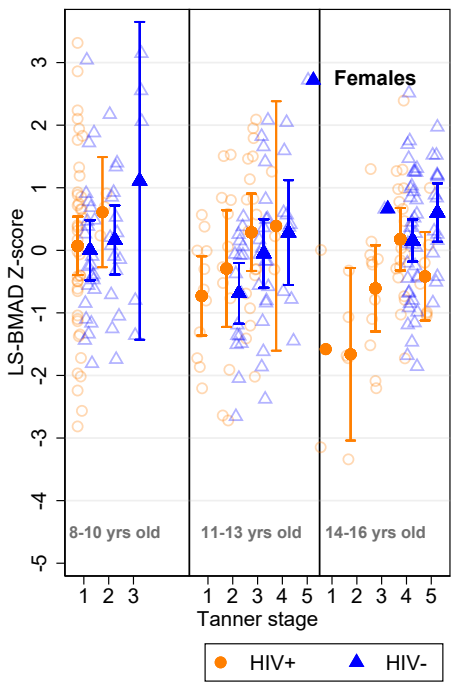
TBLH BMC LBM Z-score
8-10 yrs old
11-13 yrs old
14-16 yrs old
Tanner stage
HIV+
HIV-


B
Males
LS-BMAD Z-score
8-10 yrs old
11-13 yrs old
14-16 yrs old
Tanner stage
HIV+
HIV-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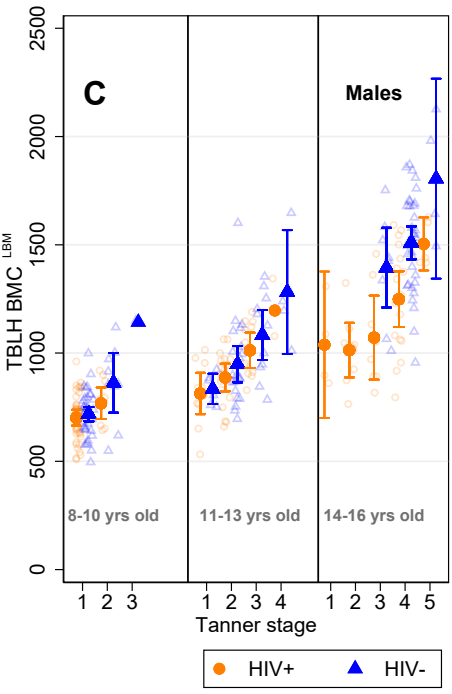
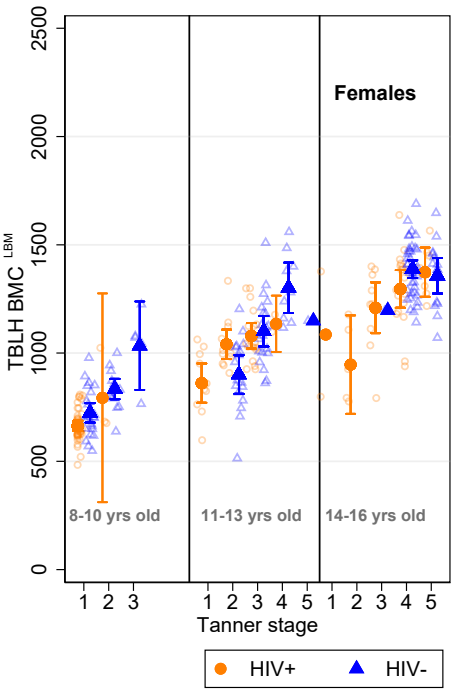
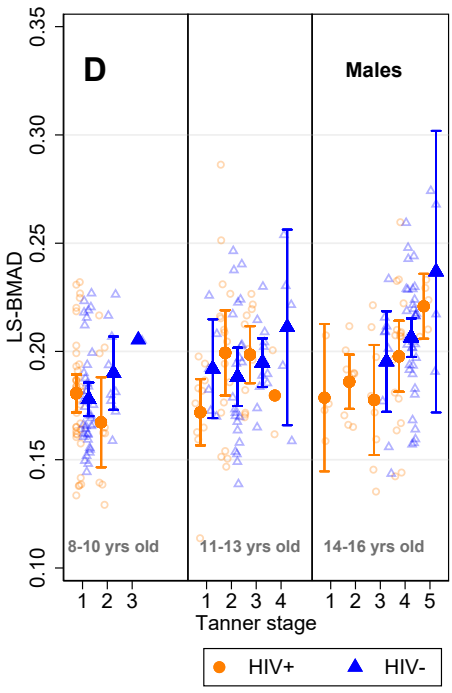
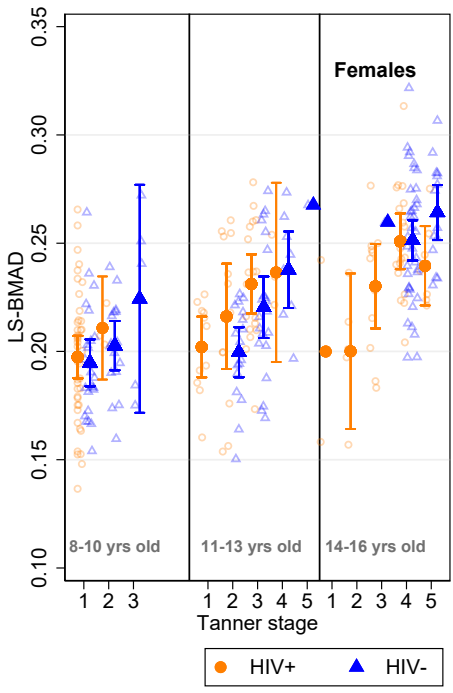
LS-BMAD Z-score
8-10 yrs old
11-13 yrs old
14-16 yrs old
Tanner stage
HIV+
HIV-


C
Males
TBLH BMC LBM
8-10 yrs old
11-13 yrs old
14-16 yrs old
Tanner stage
HIV+
HIV-


Females
TBLH BMC LBM
8-10 yrs old
11-13 yrs old
14-16 yrs old
Tanner stage
HIV+
HIV-


D
Males
LS-BMAD
8-10 yrs old
11-13 yrs old
14-16 yrs old
Tanner stage
HIV+
HIV-


Females
LS-BMAD
8-10 yrs old
11-13 yrs old
14-16 yrs old
Tanner stage
HIV+
HIV-

Supplementary Materials

Click here to access/download

Necessary Additional Data

Rukuni Supplement Tables Revised clean 13_04_2021.docx