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Perinatal HIV infection is associated with deficits in muscle function in children and adolescents: a cross-sectional study in Zimbabwe

Short title: HIV and muscle function in children

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Abstract

Objectives

To determine how muscle strength, power, mass, and density (*i.e*. quality) differ between children living with HIV (CWH) and those uninfected, and whether anti-retroviral therapy (ART) regime is associated with muscle quality.

Design

A cross-sectional study in Harare, Zimbabwe.

Methods

The study recruited CWH aged 8-16years, taking ART for ≥2years, from HIV clinics, and HIV-uninfected children from local schools. Muscle outcomes comprised grip strength measured by hand-held Jamar dynamometer, lower-limb power measured by standing long-jump distance, lean mass measured by dual-energy X-ray absorptiometry, and muscle density (reflecting intramuscular fat) by peripheral quantitative computed tomography. Linear regression calculated adjusted mean differences (aMD) by HIV status.

Results

Overall, 303 CWH and 306 without HIV, had mean(SD) age 12.5(2.5) years, BMI 17.5(2.8), with 50% female. Height and fat mass were lower in CWH, mean differences(SE) 7.4(1.1)cm and 2.7(0.4)kgs, respectively. Male CWH had lower grip strength (aMD 2.5[1.1,3.9]kg, p<0.001), long-jump distance (7.1[1.8,12.5]cm, p=0.006), muscle density (0.58[0.12,1.05]mg/cm^3, p=0.018, but not lean mass 0.06[-1.08,1.21]kg, p=0.891) versus boys without HIV; differences were consistent but smaller in females. Mediation analysis suggested the negative effect of HIV on jumping power in males was partially mediated by muscle density (p=0.032). CWH taking tenofovir disoproxil fumarate (TDF) had lower muscle density (0.56[0.00,1.13]mg/cm^3, p=0.049) independent of fat mass, than CWH on other ART.

Conclusions

Perinatally-acquired HIV is associated, particularly in males, with reduced upper and lower-limb muscle function, not mass. Intra-muscular fat (poorer muscle quality) partially explained reductions in lower-limb function. TDF is a novel risk factor for impaired muscle quality.

Keywords: Muscle; HIV; Grip Strength; DXA; pQCT; TDF; children/adolescence

Introduction

The global roll-out of anti-retroviral therapy (ART) has dramatically improved survival in children with HIV (CWH) [1], so that increasing numbers are now reaching adolescence. However, there is growing recognition of multisystem morbidities among children growing-up with perinatally acquired HIV [2], including impaired musculoskeletal growth, particularly if ART initiation is delayed [3]. Whilst the adverse effects of HIV and some ART drugs, such as tenofovir disoproxil fumarate (TDF) on bone development, are recognised [4, 5], less is known about effects on muscle.

Skeletal muscle is the largest organ system comprising 40-50% of body weight. Bone and muscle are closely inter-related, anatomically and physiologically. Bones adapt to mechanical forces imposed by muscle ('mechanostat theory') [6], and to muscle-secreted myokines (e.g., IGF-1 and FGF2) during bone growth [7], whilst a range of bone-derived osteokines exert endocrine effects on muscle. Studies of HIV in adults report impaired muscle function [8, 9]; however, few studies, particularly in Africa, have investigated muscle function in CWH. Those reported are often small, lack a control population [10, 11], or focus on treatment-naïve individuals [12]. A recent systematic review of just five cross-sectional studies (none in Africa), totalling 197 adolescents (age 16-18 years) living with HIV, identified reduce muscle strength and physical fitness compared with uninfected peers [13]. In adults, findings are mixed, for example raltegravir-based therapy has been associated with skeletal muscle toxicity [14], whilst tenofovir alafenamide (TAF) has been associated with greater muscle mass in men [15].

We investigated the effect of HIV infection on muscle mass and function in peripubertal children established on ART and to what extent any identified deficits could be explained by impaired muscle quality. We further explored whether any ART regimen is associated with muscle quality.

Methods

Study design and participants

A cross-sectional study was conducted using baseline measurements from the prospective cohort study of the impact of vertical HIV infection on child and adolescent musculoskeletal development (IMVASK) in Harare, Zimbabwe [ISRCTN12266984], as per published protocol [16]. CWH aged 8-16 years and established on ART for at least two years, were recruited from outpatient HIV clinics at the two large public-sector general hospitals in Harare (Parirenyatwa and Harare Central Hospitals). 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. 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 in the same communities 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. A random number sequence applied to school registers was used to select participants using the same quota-based sampling approach targeting 50 males and 50 females in each of the three age strata (8-10, 11-13 and 14-16 years). Children underwent HIV testing after enrolment; those testing positive and not in care were referred to HIV services.

Procedures

An interviewer-administered questionnaire was used to collect sociodemographic and clinical data including a history of tuberculosis, smoking status, alcohol and glucocorticoid use, and HIV history (age at HIV diagnosis and ART initiation, current ART regimen) in CWH. The International Physical Activity Questionnaire (IPAQ) [17], validated in multiple countries including South Africa, was used to assess physical activity as multiples of the resting metabolic rate in MET-minutes (metabolic equivalent of task). Diet and nutrition were assessed using a tool based on a validated dietary diversity and food frequency tool from India and Malawi [18], and adapted to the local context using international guidelines [19]. This tool estimated dietary protein based on the number of animal-source food types (eggs, dairy, fish and meat) consumed ≥3 times per week. The tool also quantified dietary vitamin D intake, with adaptations reflecting

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.

In CWH, CD4 cell count was measured using an Alere PIMA CD4 machine (Waltham, Massachusetts, USA) and HIV viral load using the GeneXpert platform (Cepheid, California, USA), with viral suppression defined as <1,000 copies/μl (per WHO guidelines).

Anthropometric measures

Standing and sitting height, were measured to the nearest 0.1 cm (Seca 213 stadiometer; Seca, Hamburg, Germany), and weight, to the nearest 0.1 kg (Seca 875 weight scales). Measurements were taken independently by two trained research staff. If height and weight measurements differed by more than 0.5 cm or 0.5 kg respectively, a third reading was taken and final values were taken as means of the measurements [20]. Tanner pubertal staging was used to assess pubertal development [21]; pubertal delay was defined as Tanner stage 2 or less in girls aged ≥13 years, and in boys aged ≥14 years [22].

Measurement of forearm muscle strength

Maximal forearm grip strength was measured using a Jamar analogue hand-held dynamometer (Patterson Medical, UK) with participants seated, elbow by their side and flexed to a right angle with a neutral wrist position, whilst the forearm rested on the arm of the study chair. Starting with the right hand and the dynamometer handle (in position 2) alternating recordings were made in each hand three times. The greatest of the six strength measurements was used for analysis.

Measurement of jump power

Standing long jump, a measure of lower-limb muscle power was assessed on a hard even surface [23]. After instruction and demonstration by the researcher nurse/assistant, the children were asked to jump as far (not high) as possible. The children had to land with both feet together and halt the jump without further advancement. Three jumps were performed with the longest jump distance from the heel of the closest foot to the starting line was used for analysis.

Measurement of lean muscle mass

To quantify total body less-head (TBLH) fat and fat-free (*i.e*. lean) mass, dual energy X-ray absorptiometry (DXA) scans were performed on a Hologic QDR Wi densitometer (Hologic Inc., Bedford, MA, USA) using a standard protocol, using Apex Version 4.5 software for scan acquisition analysis.

Measurement of muscle density

Non-dominant tibial peripheral quantitative computed tomography (pQCT) scans were performed using an XCT 2000™ (Stratec Medizintecknik, Pforzheim, Germany), with voxel size 0.5 x 0.5 mm and slice thickness 2 mm (Scan speeds: CT 30mm/s, scout view 40mm/s). Tibial length was measured from the distal medial malleolus to the tibial plateau with a metal ruler. Scans were acquired at 66% of the tibia length. To allow for consistency of scan site, the exact position was determined by scout view placement of a reference line bisecting the medial border of the growth plate, or the end plate for those with fused growth plates [24]. The calf muscle was identified (excluding bones, bone marrow contents and subcutaneous fat area) (Figure 1) and muscle density measured in mg/cm^3, with lower density reflecting more intra-muscular fat and thus 'muscle quality' [25]; normal density is <100 mg/cm^3. The manufacturer's software (version 6.20) was used for image acquisition, processing and analysis. DXA and pQCT scans were repeated in a subgroup (n=30) to calculate reproducibility (Supplementary methods).

Statistical analysis

Analyses were conducted using Stata 17.0 (StatCorp, Texas, USA), stratified by sex, given established sex differences in skeletal growth [26]. Height-for-age and weight-for-age Z-scores were calculated using 1990 UK reference data [27]. For analysis of socio-economic status (SES), the first component from a principal component analysis, which combined a list comprising the number of people in the household, age of the head of the household, maternal and paternal education, ownership of the household, monthly income, access to electricity, water and flush toilet, and household ownership of fridge, bicycle, car, television or radio, was split into tertiles.

Groups (those with and without HIV, or those with and without missing data) were compared using independent sample t-tests for means, and chi-squared or Fisher's exact tests for proportions. Marginal mean differences and 95% confidence intervals (95%CIs) by HIV status were calculated using linear regression with robust standard errors (to account for heteroskedasticity with age). Data were further stratified by Tanner stage (stages 1-2 versus 3-5; grouped to maximise group size) and tested for modification of the effect of HIV by Tanner stage on muscle outcomes, within sex strata, generating Wald p-values. The minimally adjusted model (adjusted for height, age [category] and Tanner stage) was compared with a covariate adjusted model (adjusted additionally for SES, orphanhood, dietary animal-source food and vitamin D intakes, physical activity, TBLH fat mass and history of tuberculosis) [4, 26, 28, 29]. All enrolled participants were included in analyses using multiple imputation methods with seven imputed datasets; chained equations allowed for imputation of categorical and continuous data jointly. Imputation models included all outcomes,

plus age, sex, HIV status, pubertal stage, SES, orphanhood, physical activity, dietary animal-source food and vitamin D intakes, and history of tuberculosis.

Associations between HIV-specific risk factors and muscle outcomes were investigated using the same minimally adjusted model restricted to CWH, with additional inclusion of CD4 cell count (<500 or ≥500 cells/μL), HIV viral load (<1000 or ≥1000 copies/ml), and age at ART initiation (<4 years or ≥4 years).

An exploratory complete case analysis explored whether any effects of HIV on grip strength, long jump and lean mass were mediated by 'muscle quality' indicated by muscle density. Structural equation models (*gsem* command) were used for the mediation analysis with sex as a group variable (with unconstrained coefficients), robust standard errors, adjusting for height, age, Tanner stage and fat mass. Confidence intervals for indirect effects, total effects, and the proportion of the total effect mediated were estimated (*nlcom* command). Whether differences in muscle density could be explained by current ART regimen was explored. In CWH (combining males and females), marginal mean differences in muscle density were compared by ART regimens, those that included versus did not include a protease inhibitor (PI) (Atazanavir/Lopinavir), non-nucleoside reverse transcriptase inhibitor (NNRTI) (Efavirenz/Nevirapine), or nucleoside/nucleotide reverse transcriptase inhibitor (NRTI) (Zidovudine, Abacavir or TDF). Models adjusted for sex, height, age, pubertal stage, with and without fat mass (as fat mass may in part explain intra-muscular fat mass and hence muscle density).

Ethical considerations

The study was approved by the Medical Research Council of Zimbabwe (MRCZ/A2494), Parirenyatwa Hospital and College of Health Sciences Joint Research Ethics Committee (JREC/123/19), the Biomedical Research and Training Institute Institutional Review Board (AP150/2019) and the London School of Hygiene and Tropical Medicine (17154) Ethics Committee.

Results

Study population

In total 303 CWH and 306 children without HIV were enrolled (Supplementary Figure 1), 50% female and mean age 12.5 (SD 2.5) years. Of the 303 CWH, 298 (98.3%) were perinatally infected. The median age of HIV diagnosis was 3.0

(IQR 1.2, 5.8) years, with ART initiated at a median age of 3.7 (1.8, 6.9) years. At study enrolment, 114 (75%) had ≥500 CD4 cells/μL and 106 (70%) were virally suppressed.

Compared to those without HIV, CWH were more often in an earlier Tanner stage, orphaned, had lower SES, lower levels of physical activity and more commonly had a history of tuberculosis (Table 1). Pubertal delay was observed in 8 (6.3%) CWH and none in those without HIV. Dietary animal-source food and vitamin D intakes were low in most participants (Table 1). No participant reported smoking, drinking alcohol or use of oral glucocorticoids, all known risk factors for poor bone health.

CWH were shorter, lighter and had less fat mass than children without HIV (Supplementary Table 1). Males had greater jump power and forearm grip strength, than females, whilst total body lean mass was similar (Table 1). Females had greater fat mass (Supplementary Table 1) and marginally lower muscle density than males (Table 1).

Outcome data were missing for 0.2% for grip strength, 1.1% for jump power, 6% for lean mass, but 26% for muscle density as the pQCT machine was offline for a period during data collection (Supplementary Table 2). Those with missing data were more likely to be of lower SES, be orphaned, have higher physical activity (in females only), and a history of tuberculosis (in males only). Tanner stage was also associated with missing data.

Muscle outcomes by HIV status

Crude mean values of grip strength, standing long jump distance, lean mass and muscle density were lower among CWH compared to children without HIV (Table 2, Figure 2). After covariate adjustment, mean grip strength, jump power and muscle density remained lower in male children with compared to those without HIV, whilst the difference in lean mass was attenuated (Table 2). In females, CWH showed weak evidence of lower mean grip strength and muscle density, no difference in long jump, and slightly greater lean mass, compared to those without HIV. Puberty appeared to modify the effect of HIV on grip strength, with greater deficits seen in later Tanner stages, although this was more overt in females (interaction p^i-values 0.028 for females, 0.079 for males), similarly an interaction was detected between Tanner stage and HIV on muscle density in females (p^i=0.028), rather than males (p^i=0.504). Tanner stage did appear to modify the effect of HIV on lean mass, with CWH having greater lean mass in early puberty, and less in later

puberty; a pattern which was more overt in males than females (p^i<0.01 for both) (Table 2). This interaction was not observed for jump power.

After adjustment, no association was seen between CD4 cell count or HIV viral load and the outcomes of grip strength, jump power, lean mass and muscle density (Supplementary Table 3). However, delayed ART initiation until age four years or older, was associated with lower lean mass in males and greater grip strength in females, compared to CWH who started ART before the age of four.

Mediation via muscle density in the effect of HIV on muscle function

In males, the effect of HIV on jump power appeared to be partially mediated via (lower limb) muscle density, whilst there was no evidence that muscle density mediated handgrip strength or (total body less head) lean mass, nor any evidence of mediation in females (Supplementary Figure 2 and Table 4). There was a strong relationship between HIV status and the mediator, muscle density. The relationship between muscle density and muscle function was mixed, with clear evidence muscle density was related to jump power in males, and grip strength in females, but with weak evidence of an association with lean mass in both sexes. Evidence for partial mediation was found for long jump in males, where the indirect effect of HIV via muscle density was -1.9 (95%CI -3.6, -0.2 cm, p-value=0.032), the total effect of HIV was -8.2 (95%CI -14.1, -2.3 cm) and the proportion of the total effect mediated via muscle density was 23% (95%CI 0.3, 46%, p-value=0.047).

Effect of current ART regimen on muscle density

All ART regimens had a 2-NRTI backbone with either a ritonavir-boosted PI or an NNRTI**.** Among CWH, there was no evidence of a difference in mean muscle density by any ART drug class Table 3; Figure 1). After adjustment for sex, height, puberty and age the mean muscle density for the 123 participants whose ART regimen *did not* contain TDF was 73.0 (72.7, 73.3) mg/cm^3 and for the 74 participants whose ART regimen contained TDF was 72.2 (71.7, 72.6) mg/cm^3, suggesting lower muscle density among those taking TDF by -0.84 (-1.43, -0.26) mg/cm^3. After additional adjustment for total body fat mass, this mean difference was partially attenuated to -0.56 (-1.13, -0.00) mg/cm^3.

Discussion

This study shows that children growing up with HIV in Zimbabwe have deficits in muscle function. These deficits are more marked in males than females; male CWH have lower grip strength, jump power and muscle density than children without HIV, and these deficits are more apparent in later puberty, when a deficit in lean mass is also seen. In females, clear deficits were seen only in grip strength in later puberty.

The fact that muscle deficits were more apparent in males may reflect their higher absolute values of strength, power and density, with deficits in lean mass seen only in later puberty. Alternatively, pubertal maturation is later in boys, so puberty-associated deficits are more clearly seen. Furthermore, girls experience an earlier surge in endogenous oestrogen, compared to the relatively later surge boys experience in testosterone which may lead to greater vulnerability in males [30]. Oestrogen is anabolic to muscle and may protect female muscle development through puberty. Such sex-specific effects on musculoskeletal growth are seen for example after calcium supplementation [31], and in adipose tissue accumulation [32]. Our findings suggest TDF may impair muscle quality, which in part explains jump power in male CWH. TDF is recommended as a first-line ART in this age-group and hence is widely used. The Zimbabwe national guidelines (at the time this study was undertaken) stipulated that individuals aged 11 years and above or weighing ≥25kg could be started on TDF as part of first line therapy.

In older adults, HIV is a recognised risk factor for sarcopenia, characterised by reduced muscle mass, strength and physical performance [33]. Our study adds important findings to a comparatively limited literature in children [34, 35]. Our findings are consistent with a small study of 35 Canadians, age 8-21 years with perinatally acquired HIV who had lower muscle power and force, measured using jump plate mechanography compared to those without HIV [36]. A larger cross-sectional study (n=346) from South Africa found no differences in grip strength between 5-11 year old children with and without HIV; however, this may be explained by early ART initiation (at mean 8.7 months). [37]. In the UK, peak grip strength is reached between age 26-42 years, reaching 31kg and 51kg on average in females and males respectively [38]. Grip strength is lower in African populations with comparative median values of 21kgs and 37kgs reported [39]. Normative data for children in Africa are not currently available, but at 8-16 years, would be expected to be approximately 50% of peak muscle strength [38]. Hence, the 2.4kg and 3.9kg lower grip strengths identified here in CWH (female and male respectively) represent substantial deficits. In adult populations lower grip strength predicts multiple adverse health outcomes, including falls, hospitalisation, functional disability, and all-cause mortality [40].

Reduced muscle function in the context of this study may arise from malnutrition and/or chronic inflammation [12]. In addition, myofiber atrophy occurs in vitamin D deficiency, which is more common in HIV infection [41]. Interestingly, in one study of vitamin D supplementation trial among 81 CWH in the USA, an improvement in jump power was observed, albeit in an exploratory post-hoc analysis [42]. Whether vitamin D supplementation can improve muscle function in African CWH is currently subject to clinical trial [43].

In males, lower muscle density (indicating greater intramuscular fat) partially mediated the negative effect of HIV infection on jump power. Of all the ART regimes examined, only TDF use was associated with greater intramuscular fat, indicative of impaired muscle quality. Intramuscular fat is thought to be an ectopic fat depot similar to abdominal visceral adipose tissue and is associated not only with an increased risk of insulin resistance and metabolic syndrome [44], but also with impaired lower-limb muscle strength and performance in older adults [45, 46]. In older adults, intramyocellular lipid droplets, are associated with slower myofiber contraction, force and power generation of individual fibres [47]. Recent evidence suggests in adult men in the US, HIV is associated with greater fat infiltration of muscle (measured as muscle density), increasing with age [48]. However, any specific effect of TDF on skeletal myofibers is unknown.

Muscle and bone function as an integrated unit; muscle development influences bone development and vice versa [6]. We have previously identified deficits in bone mass [4] and predicted bone strength [49] in CWH, which were more overt in the later stages of puberty, mirroring the findings in this study. This raises the possibility that either HIV-associated bone deficits are (at least in part) a function of muscle deficits, or vice versa.

Strengths of the study are the inclusion of an HIV-uninfected comparison group from broadly the same socioeconomic background, detailed data on risk factors including physical activity, vitamin D and calcium intake using a standardised tool adapted for local context, and access to pQCT. Several limitations should be acknowledged: data are cross-sectional and therefore causality cannot be inferred. While we aimed to collect comprehensive data on factors associated with musculoskeletal growth, there may be residual confounding by other past or current biological or environmental factors. We lacked data on forearm muscle density to investigate associations with grip strength. Evaluation of muscle outcomes at the end of puberty, particularly in boys, was constrained by the age range recruited. We were not able to directly compare e.g., Efavirenz_vs Lopinavir-based regimens, as the numbers in each category

were too small and the analysis would be biased as these drugs were not randomly prescribed. We therefore chose to compare relevant drug classes (PI, NNRTI) as well as individual relevant NRTIs (Zidovudine, Abacavir and TDF).

Conclusion

In summary, this study shows that perinatally-acquired HIV is associated, particularly in males, with reduced upper and lower-limb muscle strength and power. Intra-muscular fat, measured as muscle density by pQCT, indicative of poorer muscle quality, partially explained deficits in lower-limb power. These data identified TDF as a potential novel risk factor for impaired muscle quality, which requires further study. The HIV-associated deficits in muscle function raise concerns for deficits in physical function persisting into adulthood. Longitudinal studies of muscle function through to acquisition of peak muscle function are warranted.

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Contributors

CLG, RR, RAF 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. AMR and CLG wrote the first draft. All authors contributed to the report and approved the final draft for submission. AMR had full access to all the study data and had final responsibility for the decision to submit for publication.

Declaration of Interests

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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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Figure legends

Figure 1

Cross-sectional pQCT images taken at the 66% right tibia. The tibia, fibula, calf muscle and subcutaneous fat are shown in cross section. Tissue density is indicated by colour grading with normal densities: bone ≥280 mg/cm^3, muscle 70-80 mg/cm^3, fat 0 mg/cm^3. (A) A male age 14.0 years living with HIV and established on an Abacavir-based regime, with high muscle density (76.1 mg/cm^3). (B) A male age 14.2 years living with HIV and established on a TDF-based regime, with low muscle density (68.4 mg/cm^3). A larger muscle cross-section, with higher muscle density is also seen in (A) compared with (B). In addition (B) has substantially greater subcutaneous fat. (The scale is shown in increments of 20mm, and 0.500mm indicates the voxel size of the scan)

Figure 2

Median values for grip strength, standing long jump, total body lean mass measured by DXA, muscle density measured by pQCT, stratified by sex, age, pubertal stage, and HIV status. Unadjusted data are shown. Error bars indicate 25th and 75th percentiles. Raw data are presented as open circles (Children with HIV) and open triangles (Children without HIV).

Table 1: Baseline characteristics stratified by sex and HIV status

	Males	**(n=303)**	**Females**	**(n=306)**
Characteristic[a]	**With HIV**	**Without HIV**	**With HIV**	**Without HIV**
	(n=152)	**(n=151)**	**(n=151)**	**(n=155)**
Mean age (SD), years	12.5 (2.5)	12.4 (2.5)	12.4 (2.6)	12.6 (2.5)
Pubertal stage, n (%)				
Tanner stage 1	57 (37.5)	45 (29.8)	60 (39.7)	25 (16.1)
Tanner stage 2	39 (25.7)	34 (22.5)	20 (13.3)	35 (22.6)
Tanner stage 3	22 (14.5)	24 (15.9)	33 (21.9)	29 (18.7)
Tanner stage 4	19 (12.5)	43 (28.5)	25 (16.6)	49 (31.6)
Tanner stage 5	5 (3.3)	4 (2.7)	7 (4.6)	16 (10.3)
Socio-economic status (SES), n (%)				
Tertile 1 (low)	54 (35.5)	38 (25.2)	61 (40.4)	50 (32.3)
Tertile 2 (middle)	51 (33.6)	54 (35.8)	54 (35.8)	44 (28.4)
Tertile 3 (high)	47 (30.9)	59 (39.1)	36 (23.8)	61 (39.4)
Orphanhood[b], n (%)	60 (39.5)	11 (7.3)	63 (41.7)	9 (5.8)
Physical activity level[c]				
Low, <600 MET mins/week	71 (46.7)	51 (33.8)	77 (51.0)	63 (40.7)
Moderate, 600-3000 MET mins/week	35 (23.0)	50 (33.1)	42 (27.8)	38 (24.5)
High, >3000 MET mins/week	46 (30.3)	50 (33.1)	32 (21.2)	54 (34.8)
Number of animal source foods consumed at least 3 times a week				
Low, 0 – 1	56 (36.8)	46 (30.5)	64 (42.4)	54 (34.8)
Moderate, 2	59 (38.8)	67 (44.4)	52 (34.4)	60 (38.7)
High, 3 – 4	37 (24.3)	38 (25.2)	35 (23.2)	41 (26.5)
Dietary vitamin D intake				
Very low, <4.0 μ/day	8 (5.3)	7 (4.6)	9 (6.0)	8 (5.2)
Low, 4.0-5.9 μ/day	88 (57.9)	81 (53.6)	93 (61.6)	83 (53.6)
Moderate, 6.0-8.0 μ/day	56 (36.8)	63 (41.7)	49 (32.5)	64 (41.3)
Clinical characteristics, n (%)				
History of tuberculosis	29 (19.1)	2 (1.3)	20 (13.3)	0 (0.0)
CD4 cell count ≥500 cells/μL	114 (75.0)	-	116 (76.8)	-
HIV viral load <1,000 copies/ml	106 (69.7)	-	106 (70.2)	-
Age at ART initiation, <4 years	79 (52.0)	-	79 (52.3)	-

[a] Missing values: Pubertal stage – n=11 males, n=7 females; orphanhood – n=9 males, n=7 females; history of tuberculosis – n=2 females; CD4 cell count – n=4 males, n=11 females; HIV viral load – n=17 males, n=18 females. [b] Defined as the loss of one or both parents. [c] MET – metabolic equivalent of task.

Table 2: Mean differences (95% CI) in muscle strength, power, mass and density, between 8–16-year-olds with and without HIV, stratified by sex; before and after adjustment

Outcome and stratification	N	Mean (SD) in CWH	Mean (SD) in CWOH	Minimally adjusted[a] MD[b] (95% CI)	Covariate adjusted[c] MD[b] (95% CI)	P for interaction between HIV status and Tanner stage in model [c,e]
MALES						
Grip strength, kg[d]	303	20.3 (7.4)	26.7 (10.2)	-2.68 (-3.99, -1.37)	-2.47 (-3.85, -1.09)	0.079
Tanner stage 1-2[e]	175	16.9 (4.7)	19.2 (4.5)	-1.56 (-2.96, -0.17)	-1.55 (-3.01, -0.08)	
Tanner stage 3-5[e]	117	27.4 (7.2)	35.0 (8.2)	-3.89 (-6.01, -1.78)	-3.54 (-5.81, -1.27)	
Jump power, cm[d]	303	129 (25)	142 (26)	-6.4 (-11.6, -1.2)	-7.1 (-12.5, -1.8)	0.576
Tanner stage 1-2[e]	175	121 (20)	130 (22)	-6.4 (-12.9, 0.1)	-7.6 (-14.2, -1.0)	
Tanner stage 3-5[e]	117	146 (27)	156 (23)	-4.2 (-13.8, 5.3)	-4.6 (-14.4, 5.2)	
TBLH lean mass, kg[d]	303	23.2 (6.4)	26.9 (9.0)	-0.13 (-1.28, 1.01)	0.06 (-1.08, 1.21)	0.002
Tanner stage 1-2[e]	175	20.5 (4.4)	20.5 (4.3)	1.14 (-0.0006, 2.28)	1.26 (0.10, 2.42)	
Tanner stage 3-5[e]	117	28.5 (6.5)	34.5 (6.8)	-2.14 (-4.03, -0.25)	-1.84 (-3.74, 0.06)	
Muscle density, mg/cm^3 [d]	303	72.92 (1.92)	73.74 (1.31)	-0.45 (-0.89, -0.008)	-0.58 (-1.05, -0.12)	0.504
Tanner stage 1-2[e]	175	72.78 (1.87)	73.64 (1.41)	-0.52 (-1.09, 0.05)	-0.67 (-1.24, -0.10)	
Tanner stage 3-5[e]	117	73.38 (1.62)	73.95 (1.19)	-0.12 (-0.98, 0.73)	-0.38 (-1.24, 0.48)	
FEMALES						
Grip strength, kg[d]	306	19.8 (7.0)	24.2 (7.9)	-1.06 (-2.29, 0.18)	-1.04 (-2.39, 0.30)	0.028
Tanner stage 1-2[e]	139	15.4 (4.7)	17.3 (3.8)	-0.06 (-1.57, 1.44)	-0.06 (-1.71, 1.58)	
Tanner stage 3-5[e]	159	25.1 (5.6)	28.7 (6.4)	-2.38 (-4.11, -0.65)	-2.29 (-4.17, -0.40)	
Jump power, cm[d]	306	121 (21)	123 (19)	3.6 (-1.0, 8.3)	-1.7 (-6.9, 3.6)	0.742
Tanner stage 1-2[e]	139	116 (20)	121 (19)	-2.5 (-9.7, 4.7)	-3.8 (-11.2, 3.7)	
Tanner stage 3-5[e]	159	127 (22)	124 (19)	4.4 (-2.8, 11.6)	-2.2 (-10.5, 6.1)	
TBLH lean mass, kg[d]	306	23.0 (6.5)	26.0 (6.9)	0.80 (-0.17, 1.78)	1.38 (0.37, 2.39)	0.007
Tanner stage 1-2[e]	139	18.8 (4.5)	19.2 (3.6)	1.64 (0.53, 2.75)	1.85 (0.69, 3.02)	
Tanner stage 3-5[e]	159	28.4 (4.4)	30.4 (4.5)	-0.35 (-1.53, 0.82)	0.46 (-0.72, 1.63)	
Muscle density, mg/cm^3 [d]	306	72.47 (1.80)	72.90 (1.74)	-0.07 (-0.49, 0.36)	-0.58 (-1.04, -0.13)	0.028
Tanner stage 1-2[e]	139	72.14 (1.99)	73.64 (1.58)	-0.97 (-1.66, -0.28)	-1.13 (-1.79, -0.46)	
Tanner stage 3-5[e]	159	72.81 (1.53)	72.42 (1.66)	0.41 (-0.25, 1.07)	-0.28 (-0.91, 0.35)	

Negative mean difference (MD) indicates lower mean values among children with HIV. Mean (SD) columns are generated using non-missing data. Missing data for grip strength (1 male), long jump (3 males and 4 females), lean mass (15 males and 19 females), muscle density, pubertal stage (11 males and 7 females), orphanhood (9 males and 7 females), fat mass (15 males and 19 females) and history of tuberculosis (2 females) were estimated using multiple imputation models and numbers in each pubertal stage stratum varied by imputation dataset (n=7 datasets). Number of participants are shown, with non-missing N provided for Tanner stage. TBLH=Total body less head.

[a] Adjusted for height in metres, age, and Tanner stage (as five levels except in models stratified by Tanner stage). [b] Marginal mean difference (MD) estimated using linear regression with robust standard errors because of heteroskedasticity with age. [c] Adjusted for ([a]) and SES tertile, orphanhood, number of animal source foods consumed, daily dietary vitamin D intake, physical activity, TBLH fat mass in kg and history of tuberculosis. [d] Estimated by fitting a two-way interaction term – sex by HIV status. [e] Estimated by fitting a three-way interaction term – sex by Tanner stage (two levels) by HIV status and all combinations of two-way interactions.

Table 3: Association between ART regimes and muscle density (males and females combined); marginal mean differences by specified ART regimes are shown

ART regimen		N	Marginal mean (95% CI) Model 1	Marginal MD (95% CI) Model 1	p-value	Marginal mean (95% CI) Model 2	Marginal MD (95% CI) Model 2	p-value
Atazanavir or Lopinavir	No	92	72.81 (72.43, 73.19)	Ref		72.77 (72.39, 73.15)	Ref	
	Yes	65	72.55 (72.09, 73.01)	-0.26 (-0.87, 0.35)	0.399	72.61 (72.18, 73.04)	-0.16 (-0.75, 0.43)	0.588
Efavirenz or Nevirapine	No	65	72.56 (72.11, 73.02)	Ref		72.62 (72.19, 73.05)	Ref	
	Yes	93	72.79 (72.41, 73.17)	0.23 (-0.38, 0.83)	0.461	72.75 (72.37, 73.13)	0.13 (-0.46, 0.71)	0.666
Zidovudine	No	68	72.65 (72.21, 73.09)	Ref		72.68 (72.26, 73.10)	Ref	
	Yes	92	72.74 (72.35, 73.12)	0.09 (-0.51, 0.68)	0.774	72.72 (72.34, 73.10)	0.04 (-0.53, 0.61)	0.892
Abacavir	No	113	72.61 (72.25, 72.96)	Ref		72.61 (72.25, 72.96)	Ref	
	Yes	45	72.94 (72.45, 73.44)	0.34 (-0.29, 0.96)	0.290	72.95 (72.48, 73.42)	0.34 (-0.26, 0.95)	0.266
TDF	No	123	73.01 (72.70, 73.32)	Ref		72.90 (72.59, 73.22)	Ref	
	Yes	74	72.17 (71.70, 72.64)	-0.84 (-1.43, -0.26)	0.005	72.34 (71.91, 72.78)	-0.56 (-1.13, -0.00)	0.0495

MD: mean difference compared against referent (ref) group. TDF: tenofovir disoproxil fumarate. Model 1: adjusted for sex, height, puberty and age. Model 2: adjusted for sex, height, puberty, age and TBLH fat mass. Models use available data.

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