

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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Supplementary materials

Supplementary methods

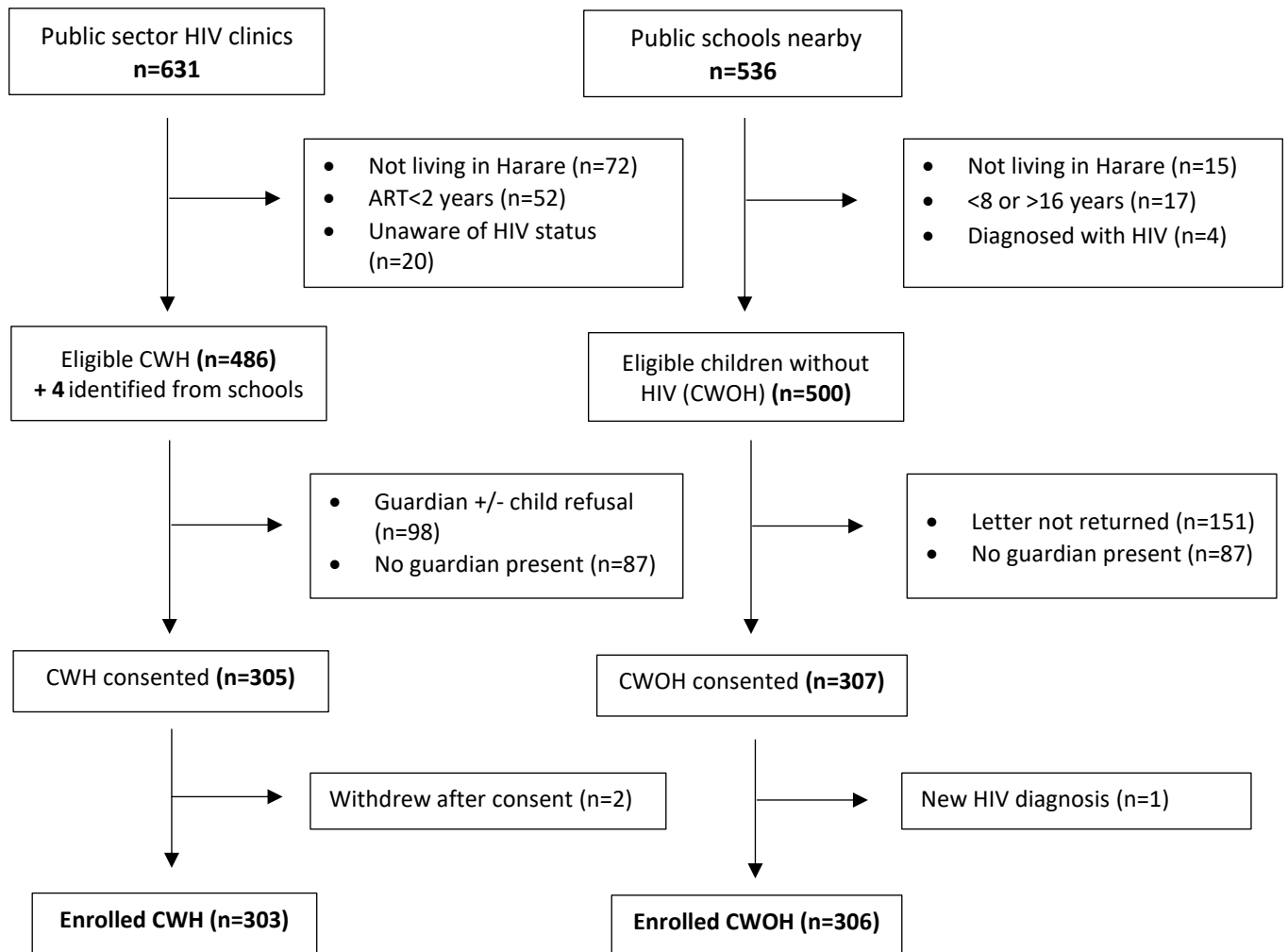
Measurement of lean muscle mass

DXA scans were repeated in a subgroup (n=30) to calculate reproducibility. The precision error was root mean square-standard deviation (RMS-SD) of 0.36 kg for total body lean mass with an RMS-coefficient of variation (%CV) of 1.05%. Daily quality assurance was conducted using the manufacturer-provided spine phantom.

Measurement of muscle density

DXA scans were repeated in a subgroup (n=30) to calculate reproducibility. The RMS-SD was 0.36 mg/cm³ for muscle density with an RMS-coefficient of variation (%CV) of 0.49%. All pQCT scan slices and scout views were qualitatively graded by a single radiographer. Movement artefacts were graded 0 to 3: (0) none, (1) slight, (2) medium streaking and (3) scan unusable. Grade 3 images were excluded from analysis. A phantom was scanned daily for quality assurance.

Supplementary Figure 1: Flow diagram showing recruitment of study participants



Supplementary Table 1: Anthropometric characteristics by HIV status in males and females

Anthropometry, mean (SD)	Males N=303			Females N=306		
	CWH	Without HIV	p-value	CWH	Without HIV	p-value
	(n=152)	(n=151)		(n=151)	(n=155)	
Height, m	1.40 (0.13)	1.48 (0.15)	<0.001	1.40 (0.13)	1.47 (0.12)	<0.001
Height-for-age Z-score	-1.80 (1.16)	-0.65 (0.95)	<0.001	-1.49 (1.06)	-0.53 (1.08)	<0.001
Weight, kg	32.8 (7.7)	38.5 (11.5)	<0.001	35.3 (10.7)	41.9 (12.6)	<0.001
Weight-for-age Z-score	-1.65 (1.23)	-0.70 (1.03)	<0.001	-1.29 (1.12)	-0.32 (1.12)	<0.001
Body Mass Index (BMI), kg/m ²	16.5 (1.5)	17.2 (2.2)	0.0043	17.4 (2.7)	18.9 (3.6)	<0.001
BMI-for-age Z-score	-0.79 (0.93)	-0.48 (1.03)	0.0068	-0.57 (0.95)	-0.06 (1.12)	<0.001
TBLH fat mass, kg	5.8 (1.7)	7.3 (3.0)	<0.001	8.5 (4.2)	12.2 (6.0)	<0.001

Missing values: height (2 males); weight (1 male); BMI (3 males); fat mass (15 males, 19 females).

TBLH = total body less head

Supplementary Table 2: Characteristics associated with missing outcome and covariate data

193 participants had at least one variable missing, the vast majority (n=159) were missing pQCT measured muscle density as the machine was offline for a time during the data collection period. Below participants with complete data for every variable are compared against any participant missing one or more variables.

Characteristic ^a	Males N=303			Females N=306		
	Complete data	Missing data	p-value	Complete data	Missing data	p-value
	N=211	N=92		N=205	N=101	
Mean age (SD), years	12.4 (2.5)	12.5 (2.5)	0.76	12.4 (2.5)	12.8 (2.6)	0.19
Pubertal stage, n (%)						
Tanner stage 1	73 (35)	29 (32)		61 (30)	24 (24)	
Tanner stage 2	58 (27)	15 (16)		36 (18)	19 (19)	
Tanner stage 3	24 (11)	22 (24)		41 (20)	21 (21)	
Tanner stage 4	50 (24)	12 (13)		55 (27)	19 (19)	
Tanner stage 5	6 (3)	3 (3)	<0.001	12 (6)	11 (11)	0.002
Socio-economic status (SES), n (%)						
Tertile 1 (low)	57 (27)	35 (38)		65 (32)	46 (45)	
Tertile 2 (middle)	70 (33)	35 (38)		69 (34)	29 (29)	
Tertile 3 (high)	84 (40)	22 (24)	0.02	71 (35)	26 (26)	0.06
Orphanhood, n (%)	48 (23)	23 (25)	<0.001	47 (23)	25 (25)	0.001
Physical activity level, n (%)						
Low, <600 MET mins/week	88 (42)	34 (37)		109 (53)	31 (31)	
Moderate, 600-3000 MET mins/week	54 (26)	31 (34)		52 (25)	28 (28)	
High, >3000 MET mins/week	69 (33)	27 (29)	0.35	44 (21)	42 (42)	<0.001
Animal source foods consumed at least 3 times per week, n (%)						
Low, 0 – 1 per week	69 (33)	33 (36)		79 (39)	39 (39)	
Moderate, 2 per week	95 (45)	31 (34)		74 (36)	38 (38)	
High, 3 – 4 per week	47 (22)	28 (30)	0.14	52 (25)	24 (24)	0.93
Dietary vitamin D intake, n (%)						
Very low, <4.0 µ/day	10 (5)	5 (5)		13 (6)	4 (4)	
Low, 4.0-5.9 µ/day	117 (55)	52 (57)		113 (55)	63 (62)	
Moderate, 6.0-8.0 µ/day	84 (40)	35 (38)	0.94	79 (39)	34 (34)	0.42
History of tuberculosis, n (%)	16 (8)	15 (16)	0.04	14 (7)	6 (6)	0.19

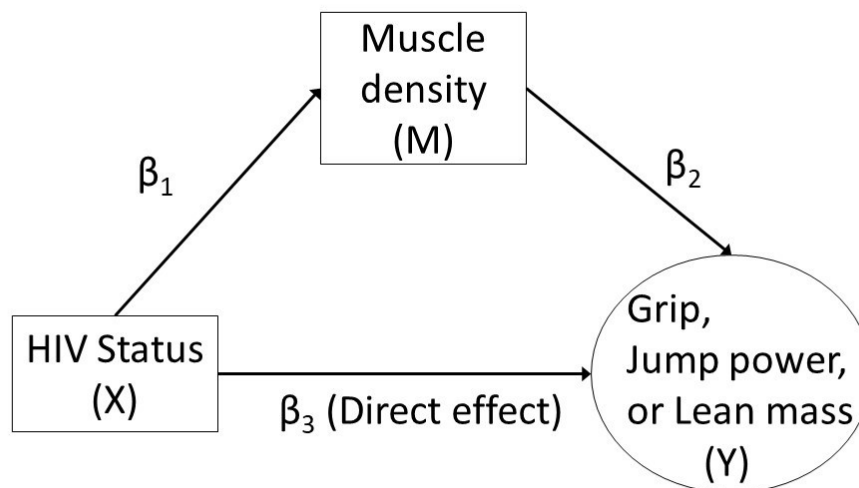
^a Missing values: Tanner stage (11 males and 7 females); orphan-hood (9 males and 7 females); history of tuberculosis (2 females); Grip strength (1 male); Long jump (3 males and 4 females); TBLH lean mass (15 males and 19 females); Muscle density (74 males and 85 females).

Supplementary Table 3: HIV-related factors associated with muscle strength, power, mass and density

					Males				Females	
Outcome	Covariate	Category	n	Mean (SD)	β (95% CI)	p-value	n	Mean (SD)	β (95% CI)	p-value
Grip strength	CD4 cell count	≥ 500	114	19.61 (7.20)	Ref		116	19.12 (6.43)	Ref	
		< 500	34	22.28 (7.27)	0.29 (-1.22, 1.80)	0.705	24	22.44 (8.28)	0.78 (-1.70, 3.26)	0.533
	HIV viral load	<1000	106	20.03 (7.24)	Ref		106	19.53 (6.72)	Ref	
		≥ 1000	29	20.78 (7.19)	-0.92 (-2.61, 0.77)	0.279	27	21.85 (8.20)	0.79 (-1.08, 2.65)	0.402
	Age at ART initiation	< 4 years	79	17.62 (6.06)	Ref		79	16.24 (5.21)	Ref	
		≥ 4 years	73	23.15 (7.58)	0.21 (-1.24, 1.65)	0.777	72	23.64 (6.65)	1.99 (0.42, 3.55)	0.013
Jump power	CD4 cell count	≥ 500	112	128.60 (24.83)	Ref		113	122.15 (22.26)	Ref	
		< 500	34	131.94 (25.39)	-1.37 (-9.31, 6.58)	0.734	23	116.72 (18.09)	-7.50 (-16.18, 1.18)	0.090
	HIV viral load	<1000	104	127.02 (24.47)	Ref		104	120.75 (22.36)	Ref	
		≥ 1000	29	130.63 (27.36)	1.00 (-7.75, 9.75)	0.821	26	122.63 (19.17)	2.30 (-5.08, 9.68)	0.538
	Age at ART initiation	< 4 years	79	125.45 (23.40)	Ref		76	116.82 (21.29)	Ref	
		≥ 4 years	71	133.83 (25.83)	-4.04 (-11.88, 3.79)	0.310	71	125.97 (20.43)	4.79 (-3.98, 13.56)	0.282
Lean mass	CD4 cell count	≥ 500	110	22.58 (6.05)	Ref		106	22.27 (6.31)	Ref	
		< 500	32	24.92 (6.61)	0.70 (-0.85, 2.26)	0.371	22	25.05 (7.17)	1.12 (-0.26, 2.49)	0.109
	HIV viral load	<1000	106	22.35 (5.86)	Ref		102	22.76 (6.26)	Ref	
		≥ 1000	28	24.26 (6.95)	0.84 (-1.25, 2.94)	0.422	26	24.17 (7.40)	0.21 (-0.62, 1.03)	0.622
	Age at ART initiation	< 4 years	76	21.44 (5.45)	Ref		72	20.10 (5.58)	Ref	
		≥ 4 years	69	25.06 (6.82)	-1.39 (-2.68, -0.11)	0.033	65	26.17 (6.07)	-0.08 (-0.91, 0.74)	0.845
Muscle density	CD4 cell count	≥ 500	81	72.90 (2.01)	Ref		82	72.62 (1.80)	Ref	
		< 500	26	73.12 (1.84)	-0.06 (-0.99, 0.86)	0.889	17	71.64 (1.81)	-0.74 (-1.68, 0.21)	0.123
	HIV viral load	<1000	77	72.96 (2.03)	Ref		79	72.69 (1.63)	Ref	
		≥ 1000	22	72.56 (1.79)	-0.29 (-1.17, 0.60)	0.514	19	71.70 (2.35)	-0.52 (-1.33, 0.30)	0.209
	Age at ART initiation	< 4 years	57	72.68 (1.84)	Ref		58	72.49 (2.01)	Ref	
		≥ 4 years	53	73.22 (2.03)	0.14 (-0.72, 1.01)	0.737	48	72.45 (1.53)	0.06 (-0.76, 0.88)	0.884

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), CD4 cell count (4 males and 11 females), HIV viral load (17 males and 18 females) were estimated using multiple imputation models ($n = 7$ datasets). Non-missing n are presented. Notably results were unchanged when using a HIV viral load threshold of 50 copies/ml. Models are adjusted for height in metres, age, and Tanner stage (as five levels), and individually for each HIV-related characteristic.

Supplementary Figure 2 and Table 4: The estimated direct and indirect effects of HIV status on muscle outcomes mediated via muscle density stratified by sex

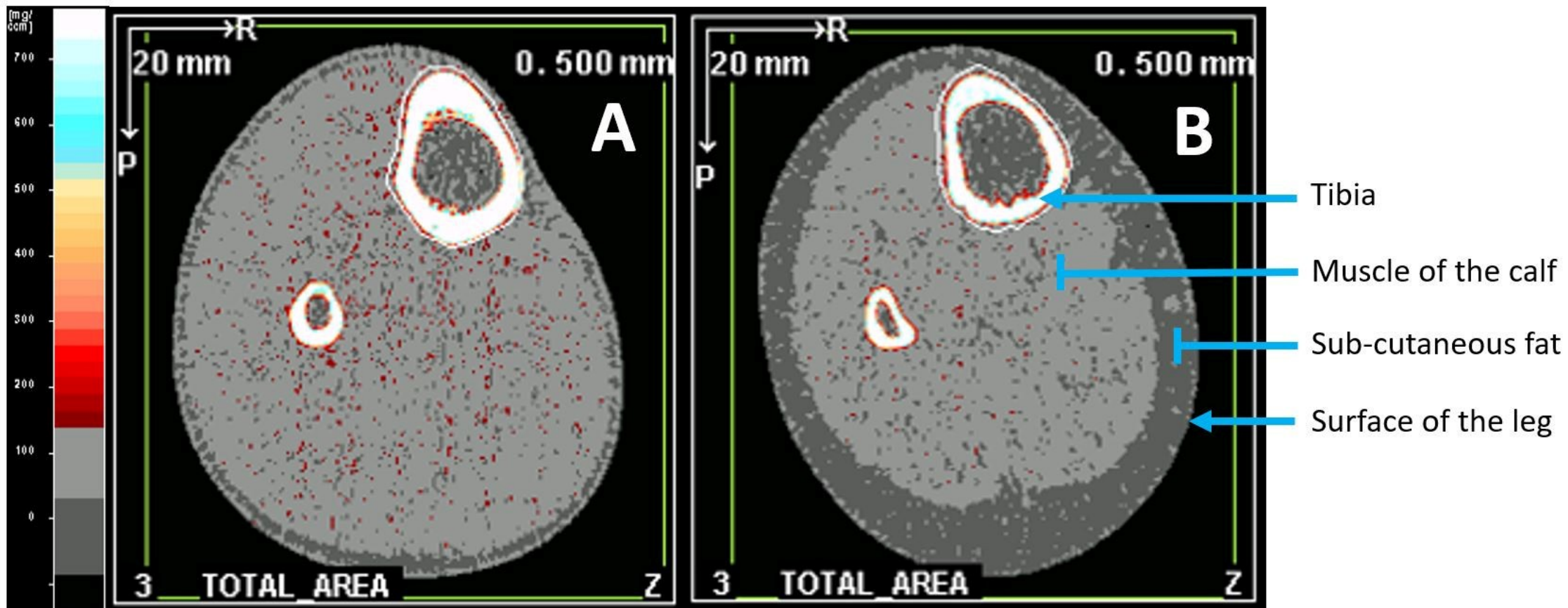


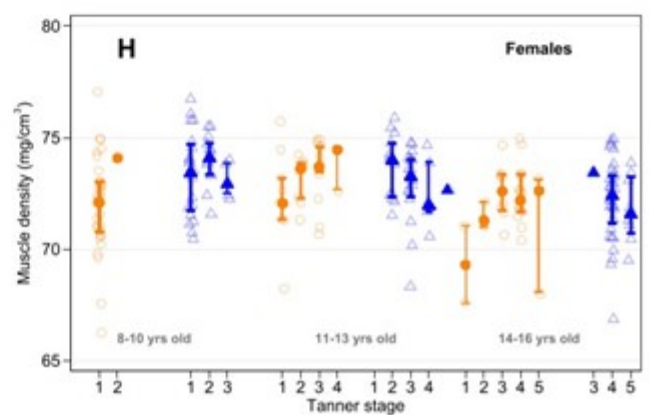
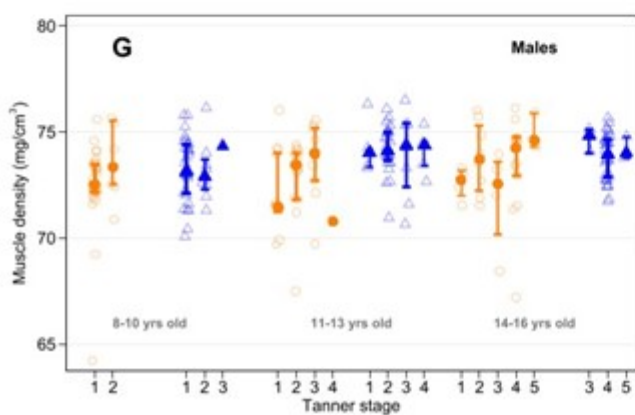
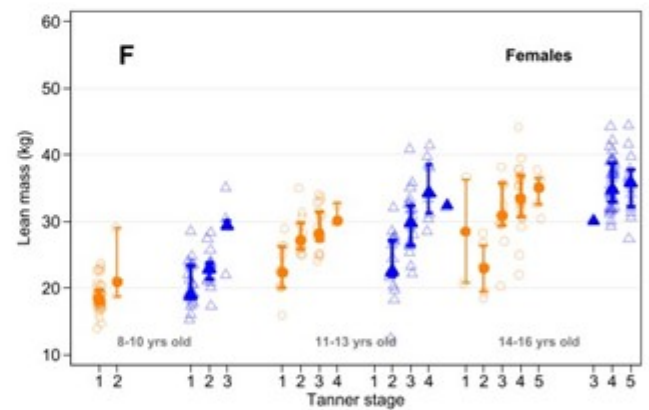
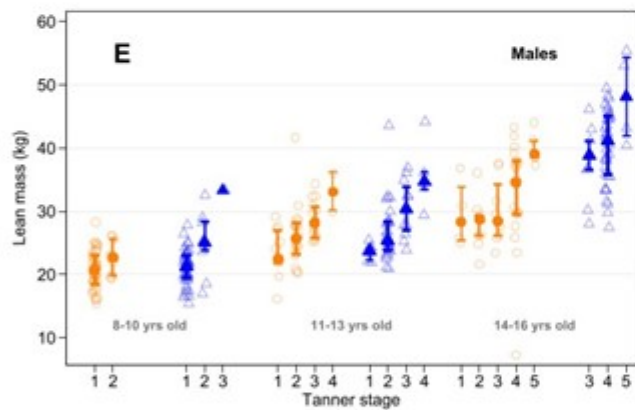
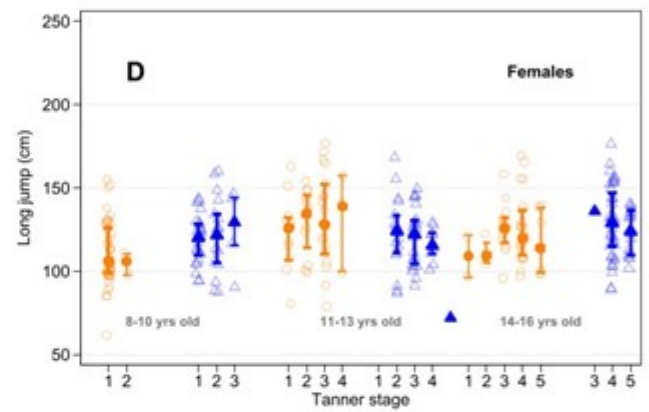
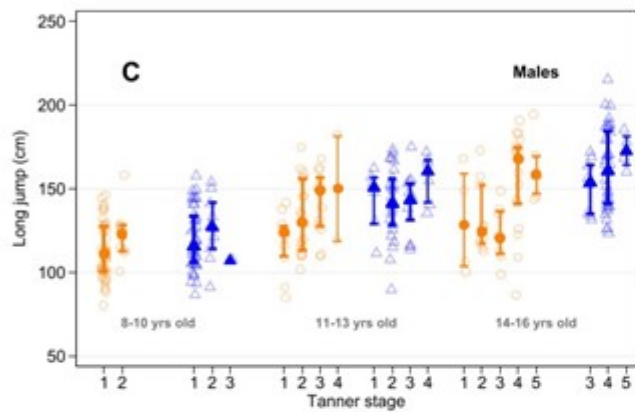
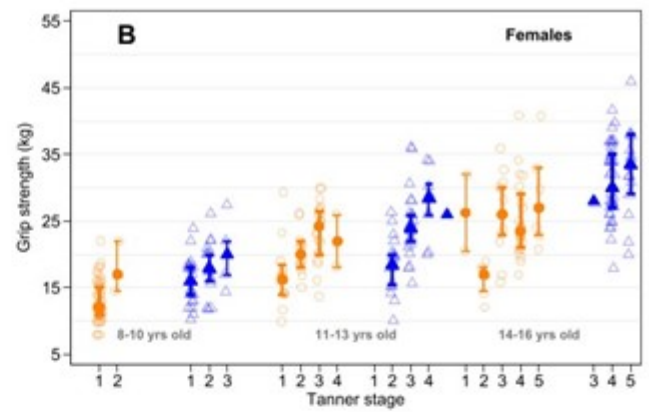
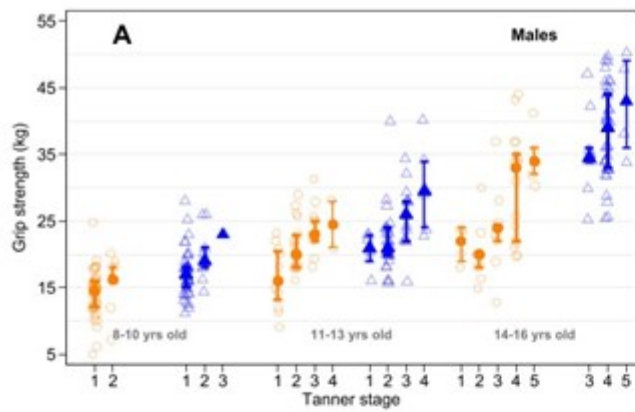
$$\text{Indirect effect} = \beta_1 \times \beta_2$$

Muscle Functional Outcome	β_1 HIV -> Muscle density (95% CI); p-value	β_2 Muscle density -> outcome (95% CI); p-value	β_3 HIV -> outcome DIRECT (95% CI); p-value	$\beta_1 \times \beta_2$ HIV -> Muscle density -> outcome INDIRECT (95% CI); p-value
Males				
Grip strength	-0.78 (-1.20, -0.35); <0.001	0.19 (-0.14, 0.51); 0.264	-1.45 (-2.57, 0.32); 0.012	-0.14 (-0.42, 0.14); 0.316
Jump power	-0.78 (-1.20, -0.35); <0.001	2.44 (0.77, 4.10); 0.004	-6.32 (-11.96, -0.68); 0.028	-1.89 (-3.62, -0.16); 0.032
Lean mass	-0.78 (-1.20, -0.35); <0.001	0.25 (-0.05, 0.53); 0.092	0.65 (-0.31, 1.60); 0.184	-0.19 (-0.45, 0.07); 0.147
Females				
Grip strength	-0.68 (-1.16, -0.20); 0.006	0.30 (0.01, 0.59); 0.042	-0.33 (-1.55, 0.89); 0.593	-0.20 (-0.44, 0.03); 0.091
Jump power	-0.68 (-1.16, -0.20); 0.006	0.72 (-0.76, 2.21); 0.34	-2.40 (-8.55, 3.74); 0.442	-0.49 (-1.61, 0.63); 0.39
Lean mass	-0.68 (-1.16, -0.20); 0.006	-0.13 (-0.27, 0.02); 0.079	1.48 (0.86, 2.10); <0.001	0.09 (-0.03, 0.21); 0.144

Mediation analysis was conducted using complete case analysis. Models were adjusted for height in metres, TBLH fat mass, age, and Tanner stage (as five levels), with sex as a group variable using unconstrained coefficients and robust standard errors.

Interpretation: if all columns show evidence of an effect (p-value<0.05) then evidence for partial mediation is detected. If columns apart from third column (direct effect) show evidence of an effect, then evidence of total mediation is detected. Evidence for partial mediation of jump power in males is shown.





● HIV+ ▲ HIV-