CHRONIC MORBIDITIES IN PERINATALLY HIV-ACQUIRED ADOLESCENTS ON ANTIRETROVIRAL THERAPY

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Declaration

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in the Department of Paediatrics and Child Health, Faculty of Health Sciences, University of Cape Town.

The work included in this thesis is original research and has not, in whole or in part, been submitted for another degree at this or any other university. The contents of this thesis are entirely my own work or, in the case of multi-authored papers, constitutes work for which I was the lead author.

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publications in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publications:

- Mahtab S, Lawrenson J, Jamieson-Luff N, Asafu-Agyei NA, Meiring A, Lemmer-Hunsinger C, Myer L, Zar HJ, Zühlke LJ. Echocardiographic findings in a cohort of perinatally HIV-infected adolescents compared with uninfected peers from the Cape Town Adolescent Antiretroviral Cohort. Journal of the American Society of Echocardiography 2020; 33(5):604-611. https://doi.org/10.1016/j.echo.2019.12.010

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- Mahtab S, Jao J, Myer L, Phillips N, Stein DJ, Zar HJ, Hoare J. Mental health and its association with metabolic outcomes in youth living with perinatally acquired HIV in the Cape Town Adolescent Antiretroviral Cohort (CTAAC). AIDS Care 2021. https://doi.org/10.1080/09540121.2021.1950605

- Mahtab S, Frigati L, Nyathi M, Asafu-Agyei NA, Myer L, Zar HJ, Jao J. The determinants of elevated pathobiological determination of atherosclerosis in youth risk score in perinatally HIV-infected adolescents in South Africa. Submitted to European Heart Journal (13 January 22).

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Published manuscripts form part of this thesis. The role played by the student and co-authors is outlined below.

- Mahtab S, Lawrenson J, Jamieson-Luff N, Asafu-Agyei NA, Meiring A, Lemmer-Hunsinger C, Myer L, Zar HJ, Zühlke LJ. Echocardiographic findings in a cohort of perinatally HIV-infected adolescents compared with uninfected peers from the Cape Town Adolescent Antiretroviral Cohort. Journal of the American Society of Echocardiography, 2020. https://doi.org/10.1016/j.echo.2019.12.010

I collected the associated data, did the data analysis and result interpretation, and wrote the manuscript. NL and AM performed the echocardiogram, LZ read and interpreted the echocardiograms. JL provided expert advice on the interpreting echocardiogram findings. HZ, LM, NAAA, LZ, JL and CH reviewed the manuscript and added conceptual and intellectual comments. HZ and LM conceived the CTAAC, obtained funding and oversaw the study. All authors read the manuscript before submission and contributed within their area of expertise.

- Mahtab S, Zar HJ, Ntusi NAB, Joubert S, Asafu-Agyei NAA, Luff NJ, Jele N, Zühlke L, Myer L, Jao J. Endothelial dysfunction in South African youth living with perinatally acquired HIV on antiretroviral therapy. Clinical Infectious Diseases, 2020. https://doi.org/10.1093/cid/ciaa396. https://www.ncbi.nlm.nih.gov/pubmed/32285090.

I collected the associated data, did the data analysis and result interpretation, and wrote the manuscript. NL and SJ performed the endoPAT. JJ, HZ, LM, LZ, NN, NAAA and SJ reviewed the manuscript and added conceptual and intellectual comments. HZ and LM conceived the CTAAC, obtained funding and oversaw the study All authors read the manuscript before submission.

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I collected the associated data, did the data analysis and result interpretation, and wrote the manuscript. TM provided the data set and helped with the analysis. CS helped in interpreting the results. HJ, LM, CS NAAA, LF reviewed the manuscript and added conceptual and intellectual comments. HZ and LM conceived the CTAAC, obtained funding and oversaw the study. All authors read the manuscript before submission.

- Mahtab S, Jao J, Myer L, Phillips N, Stein DJ, Zar HJ, Hoare J. Mental health and its association with metabolic outcomes in youth living with perinatally acquired HIV in the Cape Town Adolescent Antiretroviral Cohort (CTAAC). AIDS Care, 2021. https://doi.org/10.1080/09540121.2021.1950605.

I collected the associated data, did the data analysis and result interpretation, and wrote the manuscript. JJ provided expert advice on the metabolic aspect of the manuscript; JH provided expert advice on the mental health aspect of the study. NP helped assist in collecting mental health data. JJ, LM, DS, HZ, JH reviewed the manuscript and added conceptual and intellectual comments. HZ and LM conceived the CTAAC, obtained funding and oversaw the study. All authors read the manuscript before submission.

- Mahtab S, Frigati L, Ntusi N, Nyathi M, Asafu-Agyei NA, Myer L, Zar HJ, Jao J. The determinants of elevated pathobiological determination of atherosclerosis in youth risk score in perinatally HIV-infected adolescents in South Africa. Submitted to European Heart Journal (13 January 2022).

I collected the associated data, did the data analysis and result interpretation and wrote the manuscript. MN provided the data set. JJ, HZ, LM, LF and NAAA reviewed the manuscript and added conceptual and intellectual comments. HZ and LM conceived the CTAAC, obtained funding and oversaw the study. All authors read the manuscript before submission.

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Abbreviations

AA	Abdominal aorta
ABC	Abacavir
ART	Antiretroviral therapy
ATV	Atazanavir
AZT	Zidovudine
BECK	BECK youth inventories
BMD	Bone mineral density
BMI	Body mass index
BMIZ	BMI z-score
BP	Blood pressure
BUA	Broadband ultrasound attenuation
CA	Coronary artery
CBCL	Child behaviour checklist
CBCL-EP	CBCL-externalizing problem
CBCL-IP	CBCL-internalizing problem
CBCL-TCP	CBCL-total competence problem
CBCL-TP	CBCL-total problem
CD4	Cluster of differentiation
CLHIV	Children living with HIV
CMS	Children's Motivation Scale
CTAAC	Cape Town Adolescents Antiretroviral Cohort
CVD	Cardiovascular disease
DXA	Dual-energy X-ray absorptiometry
D4T	Stavudine
ED	Endothelial dysfunction

EFV	Efavirenz
EndoPAT	Endothelial peripheral arterial tonometry
FAC	Fractional area change
FMD	Flow mediated dilatation
FTC	Emtricitabine
HAART	Highly active antiretroviral therapy
HDL	High-density lipoprotein
HIC	High income countries
HIV	Human immunodeficiency virus
HIV-U	HIV-uninfected
HOMA	Homeostatic Model Assessment
HR	Heart rate
Hs-CRP	High-sensitivity C-reactive protein
IR	Insulin resistance
LDL	Low-density lipoprotein
LIC	Low-income countries
LMIC	Low- and middle-income countries
LPV/r	Lopinavir/ritonavir
LV	Left ventricle
LVIDd	Left ventricle internal diameter
LVSF	Left ventricle shortening fraction
mPAP	Mean pulmonary artery pressure
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PDAY	Pathobiological determinants of atherosclerosis in youth

PHIVA	Perinatally HIV-acquired adolescents
PI	Protease inhibitors
QUS	Quantitative ultrasound
RHI	Reactive hyperaemia index
RVIDd	Right ventricle internal diameter
RR	Relative risk
SI	Stiffness index
SOS	Speed of sound
SSA	Sub-Saharan Africa
TAPSE	Tricuspid annular plane systolic excursion
TB	Tuberculosis
TC	Total cholesterol
TDF	Tenofovir disoproxil fumarate
TG	Triglyceride
VL	Viral load
WHO	World Health Organization
YLPHIV	Youth living with perinatally acquired HIV
3TC	Lamivudine

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Abstract

Background: Increasing access to antiretroviral therapy (ART) has dramatically improved the life expectancy of children perinatally infected with HIV, with an increasing number surviving into adolescence, accompanied by the development of chronic comorbidities. However, there is limited knowledge on the spectrum of comorbidities, determinants, and risk factors among youth living with perinatally acquired HIV (YLPHIV) especially in sub-Saharan Africa, with most data from high-income countries. There is a critical need for data on health and chronic comorbidities among YLPHIV from countries with a high HIV prevalence.

Aim: To investigate the spectrum and determinants of HIV-associated comorbidities among YLPHIV on ART in Cape Town, South Africa. Specific objectives were to investigate cardiovascular, musculoskeletal, mental health and metabolic outcomes in YLPHIV compared to HIV-uninfected adolescents.

Method: In a prospective study YLPHIV on ART were enrolled in the Cape Town Adolescent Antiretroviral Cohort (CTAAC) from seven health-care sites in Cape Town, South Africa, between July 2013 – April 2015. Eligibility criteria were adolescents, 9-14 years old, with perinatally acquired HIV, been on ART for at least six months, and who were aware of their HIV status. A control group of HIV-uninfected adolescents' frequency-matched by age and sex was also enrolled. The cohort was longitudinally followed for development or progression of comorbidities with clinical and laboratory measurements. Comorbidities assessed included: (1) cardiovascular health: echocardiography was used to investigate cardiac structure and endothelial peripheral arterial tonometry technique (EndoPAT) was used for endothelial function. The pathobiological determinants of atherosclerosis in youth (PDAY) risk score was used to assess long-term cardiovascular risk for atherosclerotic disease at the coronary artery (CA) and abdominal aorta (AA). A PDAY score ≥1 was regarded as elevated; (2) bone health: quantitative ultrasound was used to evaluate calcaneal stiffness index (SI); (3) mental health: the Child Behavior Checklist (CBCL) and BECK youth inventories were utilised. The association of mental health with metabolic abnormalities was investigated. Statistical analyses included descriptive data and regression modelling analysis, using the software, Stata® 14.2 to 16 (Stata Corp LP. College Station, Texas, USA).

Results: Overall, 515 YLPHIV and 110 HIV-uninfected participants with median age 12.0 years (IQR 11.9, 10.7) and 11.8 years (IQR 11.7, 10.0) were enrolled; YLPHIV with median duration of ART of 7.6 years (IQR: 4.6–9.2), also had a median CD4 cell count of 713 cells/mm^3 (IQR: 561.0–957.5), and 387 (75%) had a viral load (VL) of <50 copies/mL. At enrolment, YLPHIV had higher median z-scores for left ventricle (LV) internal end-diastolic dimension, LV end-systolic posterior wall thickness and end-systolic inter-ventricular septum thickness. YLPHIV had a lower median z-score for right ventricle internal end-diastolic dimension than uninfected adolescents. There was no difference in ejection fraction or diastolic function between groups. Later initiation of ART (after age six years) was associated with an increased risk of LV hypertrophy (OR 2.9, CI 1.3-6.6, p=0.01) compared to those who started ART earlier. YLPHIV with World Health Organization HIV stage IV were at increased risk (OR 2.2, CI 1.0-4.6, p=0.05) of having LV diastolic dysfunction than those with less advanced clinical disease.

Endothelial function at 24 months of follow-up showed that YLPHIV had higher rates of endothelial dysfunction (ED) compared to uninfected adolescents (50% vs 34%, p=0.01); a relationship that continued after adjusting for age, sex, body mass index (BMI) z-score, elevated blood pressure, and hypercholesterolaemia (RR 1.43, p=0.02). Among YLPHIV, CD4 count >500 cell/mm^3 (RR 1.04, p=0.76), VL (RR 1.01, p=0.78) or current ART class (protease inhibitor-based vs non-nucleoside inhibitor-based, RR 0.90, p=0.186) were not associated with ED after adjustment.

At 48 months of follow-up, among YLPHIV, 8% (n=17) had sustained viraemia, and 54% (n=118) had transient viraemia through this period. The median duration on ART was 12 years (IQR 8-14); 57% (n=124) were on a non-nucleoside reverse transcriptase inhibitor-based ART, while the rest received protease inhibitor-based ART. Few YLPHIV met the criteria for hypertension (2%, n=4) or hyperglycaemia (0.5%, n=1). None of the HIV-uninfected youth had hypertension or hyperglycaemia. Fewer YLPHIV smoked compared to the uninfected youth (15.6% vs 11.5%, p=0.50. Elevated PDAY scores for CA (30.3% [n=66] vs 31.3% [n=10], p=0.74) and AA (18.4% [n=40] vs 21.9% [n=7], p=0.20), respectively among YLPHIV and HIV-uninfected adolescents differed slightly but did not reach statistical significance. Among YLPHIV, sustained viraemia [adjusted odds ratio (aOR)=18.4, p<0.01] and transient viraemia (aOR=2.10, p=0.04) compared to sustained virologic suppression and longer ART duration (aOR=1.12, p=0.03) were associated with elevated risk of CA. Male sex was associated with both elevated CA and AA PDAY (aOR=2.14, p=0.02, and aOR=3.43, p=0.01, respectively). Association of sustained viraemia with elevated AA PDAY trended in the same direction as that for CA PDAY but did not reach statistical significance (aOR=3.24, p=0.09).

Bone health at 24 months showed that during puberty, the mean SI increased with Tanner Stages in YLPHIV and uninfected adolescents, but these increases were higher among those who were uninfected; Tanner Stage II-III (96 vs 101, p=0.009) and Tanner Stage IV-V (104 vs 112, p=0.001). Among YLPHIV, 52 (13%) had a low SI. After adjusting for age, sex and Tanner Stage, use of lopinavir/ritonavir (OR=2.31, p=0.012) and VL >50 copies/ml (OR=2.06, p=0.023) were associated with an increased risk of low SI, while the use of efavirenz (OR=0.41, p=0.009) was associated with a decreased risk of low SI.

YLPHIV had more impairment in mental health in several domains: functional competence (40% vs 25%, p=0.02), self-concept (23% vs 9%, p=0.03), higher depression (6% vs 2%, p<0.01), anger (6% vs 2%, p=0.04), and disruptive behaviour (4% vs 0%, p<0.01). Among YLPHIV, higher levels of anger were associated with increased total cholesterol and low-density lipoprotein (LDL) levels (ß=0.010, p=0.041 and ß=0.012, p=0.048, respectively), higher disruptive behaviour with increased LDL levels (ß=0.010, p=0.043), and severer CBCL-internalizing problems with low albumin levels (ß=-0.067, p=0.052) after adjusting for age, sex, and BMI z-score.

Conclusion: YLPHIV are at higher risk of having subclinical cardiac structural abnormality and ED compared to uninfected adolescents. Both groups had a substantial proportion with high PDAY scores reflecting an increased aggregate atherosclerotic risk. Bone health was worse among YLPHIV. HIV-related factors such as ART initiation at an older age, advanced clinical disease, and specific ARTs were significant risk factors for these conditions. Mental health impairment was common and associated with increased lipid concentration in YLPHIV. These data highlight a high prevalence of chronic comorbidities in YLPHIV,

specific risk factors associated with these and provide information for strengthened strategies to prevent or monitor HIV-associated illnesses.

Format of thesis

Five of the chapters (2–6) are presented as published or submitted manuscripts in this thesis.

Chapter 1 – comprises the introduction, including the literature review and methodology.

Chapter 2 – cardiac structural and functional abnormalities are described using an echocardiogram to locate the determinants among perinatally HIV-infected adolescents and compare these findings with those of HIV-uninfected adolescents (published manuscript).

Chapter 3 – the peripheral endothelial function using the EndoPAT technique among adolescent youth living with perinatally HIV infection is described, and risk factors observed; these findings are compared with the HIV-uninfected group (published manuscript).

Chapter 4 – in this chapter, the pathobiological determinants of atherosclerosis in youth (PDAY) risk score among adolescents living with perinatally infected HIV is described (submitted manuscript).

Chapter 5 – bone health among perinatally HIV-infected adolescents is described, using the HIV-uninfected group as a reference to determine which factors were associated with the bone health outcome in the experimental group (published manuscript).

Chapter 6 – the association of mental health measures with metabolic outcomes among perinatally HIV-infected adolescents is described (published manuscript).

Chapter 7 – the summary of this thesis, along with the discussion, conclusion, strengths, limitations, and recommendations, are included in this final chapter.

Notes on terminology and sample size

Throughout the thesis (including the title), the abbreviation for youth living with perinatally-acquired HIV may differ according to journal preferences. The abbreviation of adolescents living without HIV was also according to the journal preference. The student acknowledges that this may not always align with the current preferred terminology.

The term ‘adolescent’, defined by the World Health Organization (WHO) as a person between 10 and 19 years old, is used throughout. However, the authors acknowledge that some participants were nine years old at study enrolment.

Sample size and follow-up time for each chapter also differ. For each specific objective, a different number of participants had results available for the specific tests required for that objective.

Chapter 1: Introduction

1. Epidemiology

Youth living with perinatlly acquired HIV (YLPHIV) are a growing population that face unique health challenges because of lifetime exposure to HIV and antiretroviral therapy (ART). Globally, HIV is now considered a chronic condition because of early diagnosis, advances in treatment, and early initiation of ART. As a result, chronic comorbidities are increasingly prevalent in YLPHIV. The largest population of YLPHIV are living in sub-Saharan Africa (SSA), but limited data on the prevalence and course of HIV-related chronic cardiovascular, musculoskeletal, or neurological comorbidities in these adolescents are available.

Adolescence, the transition from childhood to adulthood, is a vital phase of human development, characterised by significant biological, psychological, and social changes. In combination with HIV, these may pose specific health challenges during this time. Timing of ART initiation may also influence the long-term health and exposure to the virus, with ART possibly impacting comorbidities.[1] As per the WHO guidelines, ART should be started in all children as early as possible irrespective of their CD4 count and clinical status.[2] In some studies from SSA, a drastic decrease in disease progression and mortality after early ART initiation in infants has been shown.[3-5] Furthermore, it is well-known that children living with HIV (CLHIV) experience deficits in growth and delayed puberty.[6-7] However, the relationship between developmental delays and chronic disease sequelae are not well understood. Several factors, for instance, chronic inflammation, metabolic disorders, and chronic comorbidities may be associated with impaired development and HIV.[8]

The growing burden of cardiovascular disease (CVD), metabolic complications, and mental health disorders among YLPHIV from Africa provide an opportunity to investigate these disorders, their determinants, and complications. In high-income countries (HIC), several cohort studies of YLPHIV have been done for example the Adolescent Master Protocol of the Pediatric HIV/AIDS Cohort Study (PHACS), the European Pregnancy and Paediatric HIV Cohort Collaboration (EPPICC), or the Pediatric Pulmonary and Cardiac Complications of Vertically Transmitted HIV Infection (P^2C^2 HIV) multicentre study. In Asia, a collaborative observational cohort is the Therapeutic Research, Education and AIDS Training Asia Pediatric HIV Observational Database (TApHOD). However, there are much more limited data from SSA, where the predominant burden of HIV resides. The Cape Town Antiretroviral Cohort (CTAAC), a unique South African cohort that followed YLPHIV on ART, has added to limited data that are obtainable from SSA or low- and

middle-income countries (LMIC). Under the following sub-headings, the epidemiology of YLPHIV will be discussed worldwide and, particularly in Africa.

1.1 Global epidemiology of youth living with perinatally acquired HIV

From 2000 to 2005, the number of YLPHIV entering adolescence doubled from 53 000 to 100 000 in five years.[9] Between 2005 and 2015, 100 000 more children living with HIV entered adolescence each year with successful access to ART.[9] In 2016, there were approximately 770 000 perinatally-acquired HIV adolescents between 10 and 14 years old,[10] with the shift of a once fatal disease into a manageable chronic condition through ART. Globally, in 2020, approximately 1.7 million children were living with HIV, with nearly 90% of them in SSA, according to UNAIDS.[11-12] Several factors have dramatically enhanced the life expectancy of these young people, particularly early, improved, and increased availability of ART.[13]

The human immunodeficiency virus is considered the eighth leading cause of death among adolescents worldwide and the fourth leading cause of death in some LMIC.[10] However, the peak mortality for AIDS-related deaths in the age group 10-14 years was in 2010, after which the mortality rate declined in this population group.[10] The mortality rate is still increasing in the 15-19-year-old age range.[10] Among these older adolescents, aged 15-19 years, deaths occur mainly in those who acquired HIV perinatally and not among horizontally infected adolescents.[14]

1.2 The epidemiology of youth living with perinatally acquired HIV in Africa and South Africa

Sub-Saharan Africa accounts for 90% of the world's adolescents living with HIV, and 91% of adolescent AIDS-related deaths occurred in this region.[15] Africa has the largest group of HIV-infected adolescents, with most YLPHIV residing in South Africa,[16] representing 20% of the global burden of HIV among perinatally acquired adolescents.[9] This combination of adolescents either has perinatally-acquired or horizontally-acquired HIV. In a recent National Survey from South Africa, an HIV prevalence of 2.5% among 5-14-year-olds and 7.9% among 15-24-year-olds was reported.[17] Furthermore, the prevalence of HIV in adolescents is higher in females, with 5.8% compared to 4.7% among males between 15 and 19 years old.[18]

A decline in mortality among the 10-14-year-old HIV-acquired population and an increase in the 15-19-year-old age group has been reported.[9] Although the precise number is unknown, more YLPHIV are dying in their late adolescent years, particularly females, as reported in various studies.[19-20] Possible causes for increases among older YLPHIV could be related to treatment failures such as poor adherence or lack of

access to suppressive ART options, predictors of mortality, however, were found to be age, female sex, and rural residence.[19]

2. Spectrum of chronic comorbidities among youth living with perinatally acquired HIV

Children living with HIV are at risk of developing chronic comorbidities and disabilities, which has become a growing realisation.[21-22] Comorbidities may differ according to geographical setting because of the different times ART became available, the average age of ART initiation, and other risk factors being prevalent. ART became available in 1996 in HIC, while in SSA, on 01 April 2004, the national ARV roll-out began for adult and paediatrics. Children were given d4T/lamivudine (3TC) combined with lopinavir/r (LPV/r) if they were under 3 years of age or efavirenz if they were over 3 years old.[23] Therefore, YLPHIV who survived without having access to ART may have been protected against exposure to more toxic ART regimens that were available before 2004 or conversely may have suffered more illness caused by uncontrolled HIV.[23]

HIV-acquired adolescents have high mortality rates, virologic treatment failure rates, and worse retention in care compared to adults and younger children.[15,24-25] However, long-term survival is linked to several HIV-associated chronic complications, including cardiovascular, respiratory, bone, metabolic or mental health impairments. These chronic complications may be associated with ART use, or with ongoing HIV, along with immune activation induced by the virus as well as traditional risk factors for these conditions.[26]

In SSA, diagnosis and initiation of medication occur much later compared to HIC, with 7.1 and 7.9 years being the median age for their first visit and commencing treatment, compared to 0.7 and 0.9 years, respectively in HIC.[27] Therefore, YLPHIV from SSA are at greater risk of long-term complications because of long-standing immune suppression, infection, late initiation of ART, suboptimal ART formulations, and lack of access to care.[28]

Furthermore, perinatally HIV-acquired children and adolescents are likely to be exposed to ART and the residual effects of HIV for the rest of their lives. HIV, along with ART, can contribute to other comorbidities, including cardiovascular complications, metabolic complications, bone health and mental illness. It is critically important to identify, prevent and treat chronic complications where possible.

2.1 Cardiovascular abnormalities

In the pre-ART era, CVD was described among YLPHIV and CLHIV in low- and high-income settings.[29-30] Before ART became available, almost 25% of children with acquired HIV had significant cardiac abnormalities.[30] Common persistent and progressive findings associated with the overall risk of all-cause mortality in YLPHIV, CLHIV, and adults included left ventricle (LV) dilatation, depressed LV systolic function, and ventricular wall thinning on echocardiography.[31] Severe cardiac abnormalities have been described among YLPHIV who started ART at the median age of 15 years in a Zimbabwean study.[32] In an earlier USA study (P2C2), chronic cardiac disease was reported as the underlying cause of death for almost 12% of 93 children with perinatally acquired HIV, while about 52% demonstrated having the disease, despite >90% being on ART.[33] In HIC,[34-35] the prevalence of dilated cardiomyopathy was shown to decline in adults and perinatally infected children, whereas recent studies in LIC showed a significant frequency of cardiac anomalies, ranging from 14-89%.[36-38] While most heart abnormalities persisted after 18 months, individuals were asymptomatic or did not suffer from progressive symptoms, as found in prospective research in Zimbabwe.[39]

A decrease in the incidence of cardiac involvement in HIV-infected adults indicated the cardio-protective effects of ART.[40] In the USA, ART has been reported to be cardio-protective in perinatally HIV-acquired children and adolescents with the preservation of cardiac function in those receiving treatment from infancy and early childhood.[41] Consequently, cardiovascular complications differ from those in the pre-ART and post-ART eras; though, the prevalence of these complications is still higher in the HIV-acquired than in the general population, including atherosclerosis,[42] coronary heart disease, and an increased risk for myocardial infarction.[43] As a result, most post-ART era studies primarily focus on subclinical markers for CVD. However, data determining the importance of cardiac structural and functional abnormalities in the post-ART era from SSA for YLPHIV are very scanty.

YLPHIV on long-term ART are at increased risk for CVD,[44-45] with subclinical vascular[44,46-48] and cardiac dysfunction.[34,41] In another study from a HIC, a link between HIV and ART, particularly protease inhibitors (PI) and subclinical atherosclerosis, was found.[49] As in adults, a few paediatric studies have shown that uncontrolled HIV replication causes substantial inflammation.[50-51] In a study of adolescents in the United States, indicators of inflammation, coagulation, endothelial dysfunction (ED), and metabolic dysfunction were linked to ART and viraemia.[52] An increased HIV viral load (VL), in particular, was linked to the indicators of inflammation and endothelial damage.[53] ED is the inability of the artery to sufficiently dilate

in response to an appropriate endothelial stimulus.[54] As an early predictor of CVD,[55] ED has been shown to be an independent risk factor for a cardiovascular event among HIV-acquired adults with established coronary artery disease.[56] ED is also one of the earliest stages of vessel wall alteration leading to atherosclerosis and, subsequently, CVD.[57]

In several studies, a correlation between ED[46] and early subclinical cardiovascular outcomes with chronic inflammation[48] or immune activation[46] in YLPHIV, similar to adult HIV studies, has also been shown.[58-59] Traditional markers of CVD may not detect pathology in children early,[60] and ED is an early manifestation of atherosclerosis, which is an appropriate surrogate that may be particularly important in HIV-associated cardiovascular disorders.[61] However, no data from SSA are available on endothelial function in YLPHIV.

The early prediction of CVD would be important for YLPHIV. Therefore, the validated pathobiological determinants of atherosclerosis in youth (PDAY) scoring system may be useful for measuring long-term CVD risk in early life by identifying those with current advanced atherosclerosis.[62] The PDAY scoring system was developed with autopsy data from >1 100 individuals, 15 to 34 years old, who died because of external causes. This scoring system estimates the risk of currently having an advanced atherosclerotic lesion in the coronary arteries (CAs) or the abdominal aorta (AA) relative to an individual of the same age and sex without any CVD risk factors.[62] The CA risk score predicted atherosclerosis in several population-based cohorts.[62] Previous studies have shown that a substantial proportion of YLPHIV have high PDAY scores, reflecting an increased aggregate atherosclerotic CVD risk.[63-65] However, there are no data in SSA, despite the need for data from African populations in the context of early and universal ART initiation.[15]

2.2 Metabolic abnormalities

High rates of glucose intolerance, obesity and dyslipidaemia have been described in YLPHIV and are known risk factors for CVD.[66] In addition, different ART regimens may be associated with metabolic complications such as glucose intolerance, dyslipidaemia, osteoporosis, or abnormal fat distribution.[66-68] Many of these abnormalities may be asymptomatic and progress unnoticed, particularly in resource-limited settings where monitoring may be limited.[66]

The association of PI with dyslipidaemia has consistently been shown in children.[67,69] The association between glucose intolerance and PI has been variable, depending on the study.[70-71] Several studies document the relationship between metabolic complications and ART use in adults,[72] but there are only a few in YLPHIV.[69] Before appropriate screening and treatments can be adopted, the prevalence of these

diseases and their associated risk factors must be determined, especially in light of other contending comorbidities that may be more severe and apparent.

2.3 Musculoskeletal abnormalities

During adolescence, bone mineral development is critical, and low bone density during this time can lead to osteoporosis.[73] Recent studies in children have also shown that those on ART have lower bone density.[74-75] Furthermore, reduced bone mineral density (BMD) and increased risk of fractures among YLPHIV have been reported in several studies.[75-77] Risk factors for reduced BMD in YLPHIV include delayed growth and puberty, low body mass, altered inflammatory markers and hormone levels, vitamin D deficiency, poor nutrition, and physical inactivity.[73] HIV-related factors contributing to reduced BMD in YLPHIV include advanced HIV disease, uncontrolled viraemia and ART exposure.[78]

Dual-energy X-ray absorptiometry (DXA) is the gold standard for assessing bone density. However, it can overestimate BMD in tall individuals and underestimate it in stunted persons. Therefore, consideration should be given to YLPHIV because of delayed puberty and possible stunting. In low-resource settings, quantitative ultrasonography (QUS) is utilized, and while it is less accurate than DXA, it correlates with DXA findings in CLHIV and YLPHIV.[79] Among Zimbabwean CLHIV who started ART after eight years of age, at least a one-standard deviation poorer size-adjusted lumbar spine bone density was found.[80] In another study comparing the lifetime fracture history of CLHIV and YLPHIV, fracture incidence was higher in CLHIV under six years old than HIV-exposed uninfected children of a similar age, though, in older children and adolescents, this was not reported.[81]

A USA study of 236 YLPHIV aged 7-24 years on ART showed that males had significantly lower BMD at Tanner Stage V than HIV-uninfected males.[82] Another study from Thailand showed that YLPHIV with WHO stage IV had 3.4 times increased risk of low BMD and having a low height-for-age Z-score increased the risk of low BMD (OR=6.2).[83] A study of YLPHIV showed that protease inhibitors, specifically lopinavir/ritonavir (LPV/r) and tenofovir, were associated with lower BMD.[82] This association has also been verified in an adult randomized trial that showed BMD loss in participants receiving either tenofovir- or abacavir-containing ART. However, BMD loss was significantly higher in participants receiving tenofovir.[84] Tenofovir was also associated with an annual hazard ratio for osteoporotic fracture of 1.12 (95% CI 1.03-1.21) among HIV-infected adults.[84] In adults, it has been shown that bone loss occurs after

ART initiation, and a better BMD is associated with less cumulative ART exposure,[85] suggesting a negative BMD effect of ART in general.

A study of YLPHIV showed that tenofovir use decreased median Z-scores of BMD of the lumbar spine, femoral neck, and total hip from baseline at 48 weeks and then remained stable by 96 weeks.[86] The decreased BMD was higher in younger children than in older adolescents (10.2 vs 13.2 years, p-0.003).[86] On the other hand, a 12-month study of substituting stavudine (D4T) with tenofovir showed no change in BMD.[87] In a randomized trial, there was no significant difference between participants in the tenofovir and non-tenofovir arms. However, the tenofovir-arm participants showed more BMD loss than those in the non-tenofovir-arm (18% vs 3%, p-0.1).[88] In a South African study on CLHIV receiving ART, YLPHIV had lower bone mass compared to HIV-uninfected controls. Additionally, better bone mass was associated with efavirenz-based ART than remaining on LPV/r.[89] However, very few studies to assess the bone health of YLPHIV have been done in South Africa.

2.4 Mental health and neurocognition

In the pre-ART period, HIV encephalopathy was the most prevalent manifestation of neurological disease. Beginning ART early in childhood has been shown to enhance neurocognitive outcomes.[91] Conversely, children who begin ART later in life may still suffer cognitive impairments.[90-91] YLPHIV are at higher risk of psychiatric hospitalisations than the general adolescent population,[92-94] and the prevalence of mental health illnesses, such as depression and anxiety, are more common among YLPHIV.[95]

Cognitive, motor and behavioural abnormalities are also more common in HIV-infected than uninfected adolescents.[96] The association of neurocognitive impairment with chronic HIV is well known, but few studies have investigated this in vertically infected children and there has been little research from Africa.[97] The most affected area includes visual perceptual and motor skills, attention, executive functions, memory, and language, while behavioural problems have also been reported in YLPHIV.[98-100] Results from the CTAAC have shown that YLPHIV had higher levels of disruptive behaviour, depression, anger and anxiety and poorer levels of self-concept and motivation than HIV-uninfected adolescents.[101] Also, a recent study from Uganda showed that nutritional deficiency in YLPHIV was associated with neurocognitive impairment.[102]

While some adult studies have linked poorer mental health to adverse metabolic outcomes, patients with poorer mental health have shown a greater risk of premature mortality related to metabolic syndrome.[103] An HIV adult study from Uganda has suggested a significant association between major depressive disorder and pro-inflammatory proteins.[104] The impact of mental health on metabolic outcomes is an understudied topic, and the underlying mechanism for this link is yet not fully understood. It is important to explore the relationship between mental health and metabolic outcomes among YLPHIV, especially from South Africa, a country known to have one of the highest burdens of YLPHIV with mental disorder.

3. Mechanism of comorbidities in YLPHIV

Non-AIDS defining illnesses may be linked to persistent immune activation, which may be triggered or worsened by infection and deficiency of vitamin D, which has been noted among HIV-infected adults.[66,105-106] HIV-associated factors may also be associated with immune activation or infection. Many YLPHIV may have a high VL because of difficulties with lifetime adherence to ART, initial inadequate treatments, and adverse effects. As a result, it is likely that uncontrolled HIV infection will remain a mechanism for acute morbidity in some YLPHIV. Uncontrolled HIV, chronic immunological activation, inflammation, accelerated ageing, or ART toxicity may underlie comorbidities in YLPHIV as described under the following sub-heading.

3.1 Uncontrolled HIV

Adolescents, are more often admitted to hospitals than younger perinatllyHIV-acquired children, and they also have the highest comorbidities and death rates.[107] Data from well-resourced countries and LMIC have shown that many YLPHIV in longitudinal cohorts remain stable on ART and have favourable clinical, immunological, and virological results.[73,108-111]

Virologic suppression in adolescents cannot be compared to that seen in adults starting on similar regimens. Viral suppression rates in adolescents are lower, with substantially higher virological failure rates (characterised as establishing virological suppression with two consecutive VLs of 400 copies/mL) than young adults (8.2 and 5.0 per 100 person-years, respectively) (p 0.001).[112-113] In a sub-analysis comparing YLPHIV to young adults, this was attenuated.[113] Furthermore, at 12, 18, and 24 months on ART, adolescents were 70-75% less likely to have undetectable viral levels.[112] Adolescents who initially were virally suppressed were more likely than adults to have viral rebound.[113]

Some of the potential causes of unsuppressed VL are a higher pre-treatment or baseline HIV RNA level, lower pre-treatment or nadir CD4 T-cell count, advanced stage of AIDS at the time of diagnosis, and incomplete treatment of infections; and some comorbidities, for instance, active substance abuse, depression, and treatment failure.[14] Adherence to ART, related disease progression, and availability of new regimens all determine clinical, immunological, and virological outcomes in individuals who have failed first-, second-, or third-line ART.[14] Moreover, self-reported adherence in YLPHIV from HIC are lower than those reported for adults,[113-121] with similar findings recorded in an SSA cohort at various time points.[122] Adolescents who achieved 100% adherence at six, 12 and 24 months declined from 20.7%, 14.3% to 6.6% compared to adults (40.5%, 27.9%, 20.6%; significance $p<0.01$), respectively at each point in time.[122]

3.2 Persistent immune activation, inflammation, and premature ageing

Immunological activation, characterised by high inflammatory biomarker levels mostly involving monocytes and macrophages in HIV-acquired adults, has been related to chronic comorbidities, and recently, this relationship was also seen in YLPHIV.[123-124] Immunosenescence involves age-related changes on immune system, and chronic viral infections was found to be one of the major contributors towards immunosenescence.[125] Premature immunosenescence and pathologic processes have been identified in adults, and the same mechanisms, along with additional variables, were identified in children.[125-127] Usually, YLPHIV may be more vulnerable to premature ageing and immunological senescence than adults because of higher HIV viraemia and prolonged ART treatment.[128] Alterations in T and B cell profiles, NK cell profiles, and chronic inflammation indicators are the main pathogenic processes. Additionally, in the CTAAC cohort of YLPHIV in South Africa, epigenetic age acceleration was found and linked to neurocognitive impairment.[126,128] The toxicity of ART and continuous inflammation could lead to the development of early CVD and metabolic complications.[129]

3.3 Drug toxicity and adverse events

Drug toxicity and serious adverse effects have been reported among YLPHIV, with permanent lipodystrophy and lipoatrophy in some of them. Drug toxicity has also caused chronic bone, cardiac, and metabolic comorbidities, while BMD may be reduced by some ART regimens such as TDF (tenofovir disoproxil fumarate), which can induce bone loss.[130] Furthermore, in a study in adolescents, a prodrug of TDF, tenofovir alafenamide that replaced TDF, initially showed good safety profiles.[131]

After D4T was withdrawn through adult recommendations, the WHO continued recommending its use in children.[132] Together with using the PI-based treatment in children, this might explain why YLPHIV have higher rates of metabolic complications than HIV-infected adults.[133-134] It has been documented in African studies that children on PI regimens have higher cholesterol levels than those on non-nucleoside reverse transcriptase inhibitors (NNRTI).[69]

Alternative regimens such as new generation PI and or NNRTI have shown evidence of improved dyslipidaemia in children and adolescents, which are particularly significant for YLPHIV, who will be exposed to ART for the rest of their lives. Atazanavir (ATV), a newer generation PI, is less likely to cause dyslipidaemia and can be used daily.[135] ATV has been boosted with ritonavir among children who had treatment experience. ATV, with boosted ritonavir, has been shown to improve the cholesterol level, which will influence long-term CVD.

However, exposure to perinatal NNRTI and then PI are reasons for metabolic implications in developing children and PHIVA in sub-Saharan Africa.[134] Nucleoside reverse transcriptase inhibitors (NRTIs) and PI can cause insulin resistance by direct inhibition of the insulin-responsive facilitative glucose transporter isoform.[136-137]

Although the newer generation of ART regimens have fewer side effects, monitoring adverse events is still necessary, particularly during fast scale-up. Dolutegravir's introduction was accompanied by concerns of greater weight gain when compared with conventional ART and might have implications for YLPHIV, who suffer from underlying comorbidities and subclinical metabolic problems.[138]

3.4 Social challenges

Social challenges YLPHIV experience include disclosure, stigma, and adherence among other things.

Disclosure – Not disclosing their HIV status could influence YLPHIVs' adherence to medication and their knowledge of medication issues. Lack of knowledge on the benefits of taking ART could also reduce adolescents' adherence.[139-140] Disclosing adolescents' HIV status was found to improve their retention in care.[140] In a study that investigated the impact of adolescent disclosure to friends, stressors of disclosure ranked second to those of medication.[141] Disclosing their status to many friends was associated with an increase in their CD4 count but without any changes in VL.[141]

Stigma – Little is known about the stigma of HIV among YLPHIV. However, it has been reported that they experience physical, social and mental handicaps, putting them at increased risk of the stigma associated

with HIV.[149] Although HIV-related stigma could have unfavourable treatment outcomes, this was not found in our cohort.[142] Furthermore, YLPHIV were not affected by HIV disclosure, and they did not perceive any discrimination affecting their self-worth.[142] Poor mental health associated with the socio-economic environment could impact on these adolescents and their caregivers' uptake of being stigmatised.

Adherence – Non-adherence can lead to disease progression.[143] Reasons for becoming non-adherent include lack of information related to ART, depression, and being older.[143] Poor adherence rates were found to be more prevalent in LIC and rural settings.[143] Sohn and Hazra,[141] noted that adherence among adolescents was complicated for various reasons, including socio-economic burden, neurocognitive insufficiency because of chronic and severe HIV infection, including the stigma and discrimination towards those living with HIV. Although adolescents in a USA cohort had access to third- and fourth-line ART, their lack of adherence and prior resistance led to poor VL responses.[144] As YLPHIV mature, better ways of achieving adherence and remaining on treatment need to be developed, particularly in LMIC.[141] Adhering to ART is particularly important for viral suppression and preventing HIV transmission.[145]

4. Conclusion

The health of YLPHIV has been dramatically improved with early diagnosis and the use of ART. However, long-term HIV infection and use of ART and chronic inflammation may lead to changes in the structure and function of organ systems, such as the cardiovascular, musculoskeletal, and central nervous systems. On-going exposure to ART can cause metabolic derangements over time, with particular implications for cardiovascular risk.

There are limited data from SSA on the determinants and spectrum of chronic comorbidities in YLPHIV on ART. Existing data, limited by small sample sizes, the absence of HIV-negative adolescents for comparison, and information mostly coming from HIC, where the burden of YLPHIV is much less than in SSA and other resource-limited settings, comprise the most important limitations. Defining the spectrum, determinants, and burden of chronic comorbidities in YLPHIV in SSA is needed in developing strengthened strategies for the prevention and management of this growing population. Therefore, we aimed to investigate the spectrum and determinants of HIV-associated comorbidities among YLPHIV on ART in Cape Town, South Africa. Specifically, cardiovascular, musculoskeletal, mental health and metabolic outcomes in YLPHIV compared to HIV-uninfected adolescents.

5. Study Aim and Objectives

5.1 Overall aim

To investigate the spectrum of HIV-associated chronic disease in YLPHIV on ART in Cape Town.

5.2 Specific objectives

- To describe the spectrum and determinants of cardiovascular abnormalities among YLPHIV compared to uninfected adolescents (controls).
- To characterise the status of musculoskeletal among YLPHIV compared to uninfected adolescents.
- To investigate mental health outcomes and their association with metabolic outcomes among YLPHIV compared to uninfected adolescents.

6. Study Methodology

6.1 Rationale and study design

In a follow-up study, the Cape Town Antiretroviral Cohort (CTAAC), a South African cohort of YLPHIV was investigated over eight years and longer to understand the health concerns and challenges of this unique, vulnerable group of adolescents. In this prospective study, we enrolled adolescents at the Research Centre on Adolescent and Child Health (REACH), Red Cross War Memorial Children's Hospital, Cape Town.

There are many comorbidities among YLPHIV; however, specific comorbidities were selected that have not been well described in the CTAAC cohort, such as CVD, bone health, and mental health. These specific comorbidities are addressed in this thesis, whereas other comorbidities, such as diabetes, insulin resistance, and respiratory disease, have been covered by other researchers in the CTAAC study.

6.2 Study population and measures

In 2013, 515 YLPHIV and 110 age- and sex-matched HIV-negative adolescents were enrolled and followed for four years to investigate cardiovascular, musculoskeletal and mental health. We recruited YLPHIV participants from seven different HIV-care Cape Town clinics and controls from the Masiphumelele Youth Centre. Investigations were done on YLPHIV at six-month intervals while they continued receiving care at their primary clinics or hospitals.

Echocardiogram analysis was done on baseline data for those with an echocardiogram (n=474 YLPHIV and 109 youth without HIV. Mental health assessment on a subgroup of participants who were 9-11 years old

included 203 YLPHIV and 44 HIV uninfected youth. Bone health and EndoPAT analyses were done at the 24-month visit data – for bone health, we had complete data for 407 YLPHIV and 92 HIV uninfected youth – for EndoPAT 431 YLPHIV and 93 HIV uninfected youth. The PDAY score analysis was done on the 48-month data, with 218 YLPHIV and 32 HIV uninfected youth who were eligible for the analysis – Figure 1.1.

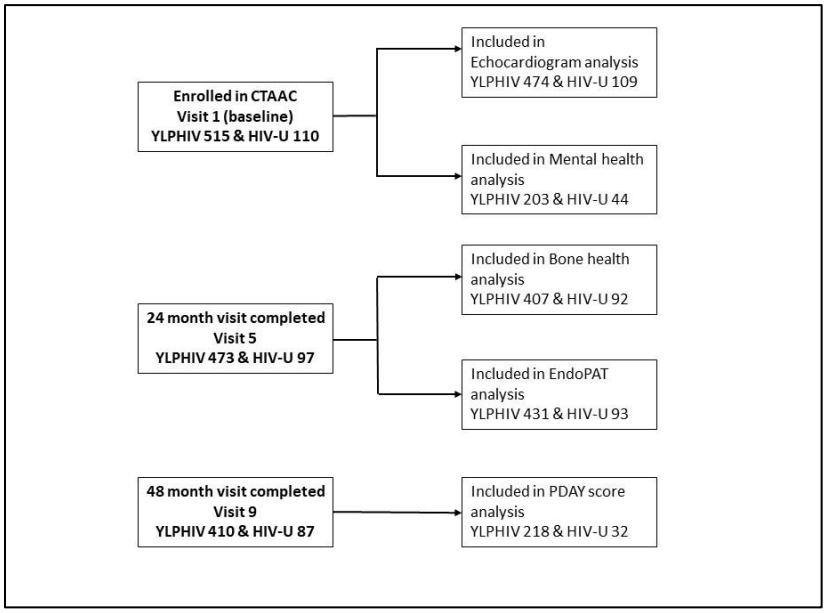

Figure 1.1. CTAAC study sampling and measures at different time points

Legend: YLPHIV – youth living with perinatally acquired HIV; HIV-U – HIV uninfected youth; PDAY – pathobiological determinants of atherosclerosis in youth; EndoPAT - endothelial peripheral arterial tonometry.

6.3 Inclusion criteria

For the CTAAC study, eligibility criteria were Cape Town residents who were perinatally HIV acquired - adolescents, 9-14 years old, had been on ART for a minimum of six months and were aware of their HIV status. Participants had to be willing to provide informed consent and assent to participation. Controls were healthy matched adolescents and had provided informed consent and assent.

6.4 Routine care and follow-up

Enrolled adolescents received ongoing HIV clinical care at the site where they were recruited. Standard protocols were followed, with medication refills every 2-3 months, monthly to three-monthly clinical assessments, and annual CD4+ cell counts and VLs or more frequent laboratory assessments depending on each specific situation. At each routine study clinical care visit, site personnel completed the

adolescent's clinical case record, recording the patient visit, regimen, adherence, inter-current medical events, and most recent laboratory results.

7. Data Sources

7.1 CTAAC measures

In addition to the interview and clinical examination, adolescents enrolled in the CTAAC study had detailed neurological, cardiovascular, and other laboratory investigations. These included electrocardiograms, Endo-PAT, and bone ultrasound. In a sub-sample of adolescents, a set of neurocognitive and neuropsychiatric screening tests was done to assess mental health. The control group had the same assessments and were retested for HIV annually. The measurements taken are summarised below.

7.2 General investigations

Demographic assessment: Detailed demographic data were obtained on all adolescents, including the family and household structure; social and caregiver history; caregiver and living arrangements; educational history; and socio-economic status.

Medical history: A detailed medical history was obtained from all adolescents that was extracted from their primary care site folders at the time of enrolment. The information recorded included, date of initial HIV diagnosis; previous hospital admissions; diagnoses of opportunistic infections (including TB) and use of prophylaxis; TB contacts; and ART initiation, regimens, adherence, and HIV staging at the time of diagnosis. Updated information on ART was obtained from the Provincial Database of the Department of Health.

Family medical history: Detailed family medical history, including TB, HIV, CVD, lung disease, arthritis, and neurological disorders, were obtained for all participants.

Risk behaviours: Risk-taking behaviours were explored as part of the annual interview. These behaviours included smoking, alcohol consumption, exposure to other substances, and relationship status.

Clinical examination: A structured physical examination, including a general and system-specific review of key symptoms and signs; Tanner staging; weight, height; head, waist, hip and mid-upper-arm circumferences; BP; heart rate (HR); oxygen saturation; standard assessment of ART toxicities; and skinfold thicknesses (biceps, triceps, subscapular, supra-iliac, thigh and calf) was performed. Examinations were conducted by the study doctor.

Blood test: VL and CD4 count were done annually on HIV+ participants. The test was performed by the National Health Laboratory Services using the Abbott Molecular Real Time HIV-1 assay (Abbott Molecular, Illinois, USA) and Beckman Coulter FC500 MPL analysers, USA, respectively. Full blood count, platelets, liver function, albumin, urea, creatinine, and electrolyte were performed on the HIV+ and control participants.

7.3 Cardiovascular investigations

Echocardiograms: These were performed at enrolment by a trained research echocardiographer working on either a Philips IE33 or CX50 using standardized measurements.[146-147] Studies were recorded onto the hard drive of the relevant machines, transferred to the Echopac archiving workstation and subsequently a protected server. Echocardiograms assess heart function and detect and follow the progression of heart disease and cardiac masses. It can help diagnose atherosclerosis, cardiomyopathy, congenital heart disease and cardiac disease. Echocardiography is considered gold standard for the diagnosis heart diseases. It is an accurate, non-invasive, nonionizing, painless, and relatively inexpensive diagnostic tool.

EndoPAT: is a new approach and standard method for assessing endothelial function.[148] This procedure was done at the 24-month visit using the EndoPAT system (Itamar Medical Ltd, Casesurea, Israel), which provides a plethysmographic recording of finger arterial pulse-wave amplitudes before and during reactive hyperaemia. In studies that used the EndoPAT method, the reactive hyperaemia index (RHI) score revealed nitric oxide bioavailability.[149] The RHI score is associated with endothelial vasodilator function in the CAs. Individuals with lower scores tend to present with higher CVD rates and other associated conditions.[149] This non-invasive method to measure endothelial reactivity and vascular status through peripheral arterial tonometry is easy to use and FDA-approved. The EndoPAT technique to measure ED in YLPHIV is also described in Chapter 3 of this thesis.

PDAY score: The pathobiological determination of atherosclerosis in youth (PDAY) risk score was used to measure the risk of developing CVD in YLPHIV. One-point increases in the PDAY score is associated with the odds ratio of developing atherosclerotic lesions in the CAs and AA at 1-year age intervals.[150] Selection of the PDAY risk score to calculate the long-term CVD risk among YLPHIV in the CTAAC cohort. This score is described in detail in Chapter 4 of this thesis.

7.4 Musculoskeletal health investigations

Bone Mineral Quality: BMD was evaluated by the calcaneus stiffness index (SI) QUS at the 24-month visit and is a reliable, affordable, and non-invasive method that can be used to screening purposes.[151-154]

QUS: Quantitative ultrasonography is a non-invasive, cost-effective, safe and easy procedure to assess the BMD status in adolescents. The device is portable and a suitable instrument for YLPHIV and CLHIV as it is free from radiation exposure and have shown a correlation with gold standard DEXA.[155] In Chapter 5, the QUS method is further explained.

7.5 Mental health investigations

Neurocognitive assessment: Scales used to assess the mental health of YLPHIV were the BECK youth inventories, Children behaviour checklist (CBCL), Children's Motivation scale (CMS), and the Conners' Parent rating scale (CPRs). Each participant in the neuro sub-study was assessed using a battery of neuropsychological tests that have been well-validated in this setting.[101] Test instructions were translated and back-translated into isiXhosa (most participants' mother-tongue is isiXhosa).

The BECK youth inventory is a self-reporting scale with five inventories to assess an individual's experience of depression, anxiety, anger, disruptive behaviour, and self-concept. This inventory has been used successfully in a few SSA studies, and scores were standardized according to the manual reported norms for gender and age.[156] The CMS is a 16-item instrument used to measure an adolescent's motivation levels.[157] This scale produces a raw score that is interpreted along a spectrum. The CPRs, a popular rating scale, is used for parent rating scales to diagnose ADHD.[158] This scale can be interpreted along a spectrum.

The CBCL is a 113-item instrument and one of the widely used psychometrically sound measures to measure child behavioural and emotional problems and psychopathology.[159] In our study, this instrument was used to measure internalizing and externalizing problems experienced by the adolescents. Parents were also involved in answering questions regarding their children's mental health. This instrument has successfully been used to measure adolescent and child behaviour in South Africans.[156]

7.6 Metabolic investigations

High-sensitivity C-reactive protein (hs-CRP): hs-CRP was measured using the Roche Cobas Tina-quant system (range, 0.1-20 mg/L).

Insulin and glucose: Insulin was measured using an electrochemiluminescence immunoassay (Cobas 6000, Roche USA) and glucose using the enzymatic method (Roche Cobas 6000, Roche USA) at baseline.

Fasting lipogram: An annual lipogram was done on serum samples collected after an eight-hour fast. The lipogram measurement included total cholesterol, lipoprotein sub-fractions and triglycerides and was analysed by the National Health Laboratory Service.

8. Ethical Considerations

This study received Ethics approval from the Faculty of Health Sciences Human Research Ethics Committee, University of Cape Town (HREC ref 101/2019). The overall CTAAC received ethical approval from the Faculty of Health Sciences Human Research Ethics Committee, University of Cape Town (HREC Ref: 051/2013).

Written informed consent was obtained from a parent or legal guardian and assent from the participant by a trained study counsellor. Participants who had permanently relocated to another province, died, or specifically requested to be removed from the study were considered withdrawn from the cohort study. For confidentiality, study participants were assigned unique study codes, while data on hard copies were stored in a locked cabinet with restricted access. Electronic records were password-restricted.

9. Data Analysis

9.1 Chapter 2: To describe the prevalence of echocardiogram abnormalities among YLPHIV and HIV-uninfected adolescents

The outcomes for this analysis were:

(i) The prevalence of the echocardiogram structural and functional abnormalities.

(ii) To identify the predictors of LV hypertrophy, LV diastolic dysfunction and right ventricle systolic dysfunction among YLPHIV.

The sample size for this analysis was limited by those who had echocardiogram results available (n=474 YLPHIV and n=109 HIV- adolescents). The groups were compared according to enrolment visit variables using t-tests, Wilcoxon, and chi-square tests as appropriate. Multivariate logistic regression was used to assess the predictors for LV hypertrophy, LV diastolic dysfunction and right ventricle systolic dysfunction among YLPHIV. Each model was adjusted for confounders, including age, sex, body mass index (BMI), triglycerides, and low-density lipoprotein (LDL).

9.2 Chapter 3: To compare peripheral endothelial function between YLPHIV and HIV-U using the EndoPAT technique

The outcomes for this analysis were:

(i) The prevalence of peripheral ED among YLPHIV and HIV-U.

(ii) To identify the predictors of ED among YLPHIV.

The sample size for this analysis was limited by the number of participants who had completed the 24-months follow-up and had undergone the EndoPAT, which was done at the 24-month visit (n=524, YLPHIV 431 and HIV-U 93). We measured ED using an EndoPAT device (Itamar Medical Ltd). This device is a non-invasive method to assess the reactive hyperaemia index induced by cuff occlusion of the arm, which is validated to evaluate peripheral vascular endothelial function. A reactive hyperaemia index of 1.35 or less was defined as ED. Baseline variables were compared between groups using t-tests, Wilcoxon, and chi-square tests as appropriate. We used modified Poisson regression models to assess the adjusted association of YLPHIV with ED, using a forward selection approach. Among YLPHIV, we tested CD4 count, log VL, and current ART as specific predictors of interest for the association of each exposure with ED in separate models for sub-group analyses.

9.3 Chapter 4: To investigate the progression of atherosclerosis among YLPHIV by the use of validated PDAY scores and compare them with HIV-U

The outcomes for this analysis were:

(i) The prevalence of high PDAY scores among YLPHIV and HIV-U.

(ii) To identify the predictors of a high PDAY score among YLPHIV.

The sample size for this analysis was limited to the number of participants who had completed the 48-month follow-up, were aged ≥15 years at the 48-month visit and had all the covariates required for the PDAY score calculation (n=251, YLPHIV 219 and HIV-U 31). A separate analysis was conducted for the coronary arteries and abdominal arteries PDAY scores. Demographic and HIV-specific characteristics were compared among YLPHIV with low (≤0) and high (≥1) scores at the 48-month visit by using the Wilcoxon rank-sum test or the Fisher exact test as appropriate. Univariate and multivariate logistic regression models were performed to identify significant predictors of a high-risk score.

9.4 Chapter 5: To describe the prevalence and predictor of bone health evaluated by measuring the calcaneus stiffness index

The outcomes for this analysis were:

(i) Comparing the prevalence of low calcaneus SI between YLPHIV and HIV-U.

(ii) Identifying the risk factors for low calcaneus SI among YLPHIV.

The sample size for this analysis was limited by the number of participants who had a calcaneal ultrasound (n=499, YLPHIV 407 and HIV-U 92) done at 24-months follow-up of the study. Bone health was measured

through the SI. The low SI in YLPHIV was defined as a z-score less than 2 SDs. We compared baseline variables between groups, using t-tests, Wilcoxon, and chi-square tests as appropriate. Among YLPHIV, multiple logistic and linear regression was used for examining the adjusted association between low SI and HIV-related and traditional risk factors.

9.5 Chapter 6: To investigate the association between mental health and metabolic outcomes in YLPHIV

The outcomes for this analysis were:

(i) To assess the difference in mental health among YLPHIV and HIV-U.

(ii) To identify the association of mental health measures with metabolic outcomes in YLPHIV.

The sample size for this analysis was limited to the number of participants who had a mental health assessment and metabolic investigation done at the enrolment visit (n=247, YLPHIV 203 and HIV-U 44). We compared baseline variables and mental health measures between groups, using the Student's t-test or Wilcoxon rank-sum for continuous variables and $\chi 2$ or Fisher's exact tests for categorical variables. Among YLPHIV, generalised linear models were used to assess the unadjusted and adjusted association of mental health measures with each metabolic outcome. Each adjusted model was separately adjusted for age, gender and body mass index z-score (BMIZ) ß was presented as one SD change in the mental health measure.

All data were analysed using Stata version 14.2 to 16.1 StataCorpInc. College Station, Texas USA.

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Chapter 2:

Echocardiographic findings in a cohort of perinatally HIV-infected adolescents compared with uninfected peers from the Cape Town Adolescent Antiretroviral Cohort

ECHOCARDIOGRAPHY AND HIV-INFECTIONS

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Abstract

Background: Little is known about the cardiac health of perinatally HIV-infected (PHIV+) adolescents on antiretroviral therapy (ART) in sub-Saharan Africa. The authors examined cardiac structure and function in PHIV+ adolescents on ART compared with HIV-uninfected (HIV–) adolescents.

Methods: Echocardiography was performed on PHIV+ and age- and sex-frequency-matched HIV– adolescents enrolled in the Cape Town Adolescent Antiretroviral Cohort. Participants were eligible if they were 9 to 14 years of age and had been on ART for ≥6 months.

Results: Overall, 474 PHIV+ adolescents (median age, 12 years; 51% boys; mean age at ART initiation, 5 years) and 109 HIV– adolescents (median age, 11.8 years; 45% boys) were included. The mean duration on ART was 7 years, with 37% starting treatment before 2 years of age. Compared with HIV– adolescents, PHIV+ adolescents had higher median *Z* scores for left ventricular (LV) internal end-diastolic dimension, LV end-systolic posterior wall thickness, and end-systolic interventricular septal thickness. PHIV+ adolescents had a lower median *Z* score for right ventricular internal end-diastolic dimension as compared with HIV– adolescents. There was no difference in ejection fraction or diastolic function between groups. Later initiation of ART (after 6 years) was associated with increased risk for LV hypertrophy (odds ratio, 2.9; 95% CI, 1.3–6.6; P = .01) compared with those who started ART earlier. PHIV+ adolescents with World Health Organization stage IV HIV infection were at increased risk (odds ratio, 2.2; 95% CI, 1.0–4.6; P = .05) of having LV diastolic dysfunction compared with those with less advanced clinical disease.

Conclusions: This study revealed subtle differences in echocardiographic parameters between PHIV+ and HIV- adolescents. Although these were not clinically significant, starting ART at an older age was a significant risk factor for LV hypertrophy, while more advanced clinical disease was associated with LV diastolic dysfunction.

INTRODUCTION

Globally, there are an estimated 2.1 million adolescents living with HIV infection.[1] Before the availability of antiretroviral therapy (ART), up to 25% of HIV-infected children manifested significant cardiac abnormalities.[2] Common findings on echocardiography included left ventricular (LV) dilatation, depressed LV systolic function, and ventricular wall thinning.[2,3] These findings were persistent and progressive and associated with overall risk of all-cause mortality in HIV-infected children, adolescents, and adults.[3] The advent of widespread access to ART in the 1990s decreased the prevalence of cardiac pathology in HIV-infected children in Europe and North America. In the United States, ART has been reported to be cardioprotective for perinatally HIV-infected (PHIV+) children and adolescents, with preservation of cardiac function in those children who received ART from infancy and early childhood.[4]

Despite having the greatest burden of PHIV+ children and adolescents, access to ART has lagged in sub-Saharan Africa, and the few cardiac studies from Africa reflect limited access to and late commencement of treatment.[5] For example, one Zimbabwean study of PHIV+ children reported high levels of LV hypertrophy. Fewer than three-quarters of these children were receiving ART, with a median duration of ART of <2 years.[6] However, given the rapid global expansion of ART access coupled with the World Health Organization (WHO) guidelines for universal ART eligibility for all HIV-infected children, these findings are unlikely to represent the "cardiac outcome" in PHIV+ children and adolescents in the coming decades.

There is a clear need for data from African populations on cardiac disease in PHIV+ children and adolescents in the era of early and universal ART initiation. This is particularly important because, despite progress in treatment and earlier use of ART, patients on therapy are probably never completely free of the risk of cardiac disease.

To address this, we investigated echocardiographic abnormalities and their determinants in a group of PHIV+ adolescents and compared these findings to HIV-uninfected (HIV–) adolescents.

METHODS

Study Design

Data for this cross-sectional analysis come from the Cape Town Adolescent Antiretroviral Cohort, who were participants in a prospective study of the long-term health of PHIV+ receiving ART. Between July 2013 and March 2015, 515 PHIV+ children and adolescents 9 to 14 years of age were enrolled from public sector health facilities around Cape Town, South Africa. These children had been on ART for ≥6 months. In addition, 110 age- and sex-frequency-matched HIV– participants were recruited from similar communities in Cape Town.

For all children, sociodemographic data, family history of cardiovascular disease, and other comorbidities were collected using standardized questionnaires. Clinical examination including Tanner staging, blood pressure, resting heart rate, transcutaneous oxygen saturation, and anthropometry were performed at enrollment by a trained research clinician. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Blood pressure was measured using an electronic sphygmomanometer (Spot Vital Signs; Welch Allyn, Skaneateles Falls, NY). Hypertension was defined per the clinical practice guidelines of American Academy of Paediatrics.[7]

Medical records were reviewed for all PHIV+ adolescents, and information such as time of HIV diagnosis, ART history, and WHO HIV staging at the time of diagnosis was extracted. Laboratory measures performed at enrollment included HIV viral load (cobas AmpliPrep, Roche, Mannheim, Germany) and CD4 cell count (FC 500 M.P.L.; Beckman Coulter, Brea, CA). For all subjects, highly sensitive C-reactive protein was measured using the Roche cobas Tina-quant system (range, 0.1–20 mg/L). Fasting lipid levels included total cholesterol, triglyceride (TG), high density lipoprotein and low-density lipoprotein (LDL; cobas 6000; Roche, South San Francisco, CA) and were measured in all subjects. Abnormal lipid levels were defined as per Hazra *et al*.[8]

Ethical approval was obtained from the Human Research Ethics Committee, Faculty of Health Sciences, at the University of Cape Town (051/2013) and Stellenbosch University (X13/07/016). All legal guardians gave informed consent, and assent was obtained from all study participants.

Echocardiographic Assessment

Echocardiographic examinations were performed by two trained research echocardiographers using either a Philips iE33 or CX50 echocardiograph (Philips Medical Systems, Andover, MA) using standardized techniques. Recorded studies were transferred to an archiving workstation. All echocardiograms were reviewed by a single cardiologist (L.J.Z.) and a random subset of 10% of the echocardiograms was also read by a second cardiologist (Norman Silverman). Discrepancies were resolved by consensus, and both cardiologists were blinded to HIV status of the participants.

LV shortening fraction was measured using M-mode echocardiography, and ejection fraction was derived using standard methods. The ejection fraction was also measured using the modified Simpson's method.[9] LV diastolic function was measured using Doppler assessment of mitral inflow. Tissue Doppler techniques were used to measure mitral annular velocity. Tricuspid annular plane systolic excursion was measured using M-mode echocardiography.[9] Pulmonary artery pressures (systolic and diastolic) were estimated using standard continuous and pulsed-wave Doppler methods. Cardiac dimensions were assessed using either direct measurement of two-dimensional images or M-mode recordings.

Body surface area (BSA) was estimated using the Mosteller formula.[10] Echocardiographic structural parameters were expressed as raw median as well as a deviation from the BSA-corrected median (*Z* score), based on normal values.[11] LV mass was also indexed to BSA.[12] Normal values for structural and functional parameters were sourced from various publications.[9,13-19]

The following definitions were used to characterize abnormal findings.

- LV dilatation: *Z* score of LV internal diameter in diastole > 2.
- LV hypertrophy: LV mass > 88 g/m^2 for girls and > 102 g/m^2 for boys.[9,19]
- Dilated cardiomyopathy: LV shortening fraction < 25% and *Z* score of LV internal diameter in diastole > 2.[13]
- Right ventricular (RV) dilatation: *Z* score of RV internal diameter > 2.
- LV systolic dysfunction: LV shortening fraction ≤ 25%.[9]
- LV diastolic dysfunction: E/A ratio normal range was calculated according to age per Eidem *et al*.[14]
- RV systolic dysfunction: TAPSE *Z* score < -2.[15]
- RV fractional area change ≤ 34% was considered abnormal.[16]
- Mean pulmonary arterial pressure was estimated using the equation of Chemla *et al*.[17,18] mean pulmonary arterial pressure = [0.61 × systolic pulmonary arterial pressure] + 2 mm Hg), and mean pulmonary arterial pressure < 25 mm Hg was considered normal.[13]

Statistical Analysis

Statistical analysis was performed using Stata 14.2 (StataCorp, College Station, TX). Baseline variables were compared between groups by using Student's t-test or Wilcoxon rank sum test for continuous variables and $\chi 2$ or Fisher exact test for categorical variables. Structural dimensions of echocardiographic parameters were adjusted using *Z* scores according to BSA[11] and are presented as median and interquartile range using the Wilcoxon rank sum test. Multiple logistic regression analyses were performed for PHIV+ adolescents to identify predictors of LV hypertrophy, LV diastolic dysfunction, and RV systolic dysfunction (using the definition of TAPSE). Each model was adjusted for age, sex, BMI, TG, and LDL.

RESULTS

Clinical Characteristics

Overall, 583 participants (474 PHIV+ adolescents, 109 HIV– adolescents) underwent echocardiography and were included in the analysis. HIV– participants had a higher mean BMI (18.7 kg/m^2) than PHIV+ participants (17.2 kg/m^2). Overall, a larger proportion of PHIV+ adolescents had high total cholesterol, high TG, high LDL and low high-density lipoprotein compared with HIV– adolescents. For highly sensitive C-reactive protein, the percentage of high risk (4–5.99 mg/L) and acute inflammation (>6 mg/L) was higher in the PHIV+ group than the HIV– group (*P*<.01) (Table 2.1).

Table 2.2 demonstrates the HIV disease severity measures among PHIV+ adolescents. The median CD4 count was 712 cells/μL (interquartile range, 571–959 cells/μL) and 12% had viral load > 1000 copies/mL. More than three quarters of the participants were at WHO stage III and IV at the time of HIV diagnosis. (By convention, WHO staging does not change during the course of illness in patients despite a positive response to treatment).

All participants were receiving a nucleoside reverse transcriptase inhibitor, typically abacavir or zidovudine (75% and 16% of participants, respectively). A small proportion of adolescents were receiving didanosine or stavudine (7% and 2% of participants, respectively).

More than half (60%) of PHIV+ adolescents were treated with a non-nucleoside reverse transcriptase inhibitor regimen. (Ninety-eight percent of the participants who were taking non-nucleoside reverse transcriptase inhibitors were on efavirenz, and only 2% were on nevirapine). Thirty-seven percent of PHIV+ adolescents were receiving protease inhibitors (lopinavir and ritonavir in combination was used in all of these patients).

Echocardiography Findings

Tables 2.3 and 2.4 compare echocardiographic findings between PHIV+ and HIV– adolescents. Structurally, end-diastolic interventricular septal (IVS) thickness and end-diastolic LV posterior wall thickness were higher in HIV– than PHIV+ patients. The difference was no longer significant after adjustment for BSA (as reflected by *Z* score). After adjustment for BSA by *Z* scores, end-diastolic LV internal diameter and end-systolic LV posterior wall thickness were higher in PHIV+ adolescents compared with HIV–. The end-systolic IVS thickness *Z* score was higher in the HIV– adolescents.

RV internal dimension was higher in HIV– adolescents than PHIV+ participants (*P*<.01), and the difference remained significant after adjustment for BSA as reflected by *Z* score (*P*= .02).

Among the variables for which there were statistically significant differences, it was noted that the values of end-diastolic IVS thickness, end-systolic IVS thickness *Z* score, end-diastolic LV internal dimension *Z* score, end-systolic LV posterior wall thickness *Z* score, and those for end-diastolic RV internal dimension, and RV internal dimension *Z* score for both groups were in the normal range.

RV systolic dysfunction (low TAPSE and fractional area change), LV diastolic dysfunction, and LV hypertrophy were the most common findings in this cohort; however, both PHIV+ and HIV– adolescents had almost identical percentages of abnormal subjects. In all the participants who had LV hypertrophy, all the other echocardiographic measures were within normal ranges, with the exception of one PHIV+ adolescent who had LV diastolic dysfunction and low TAPSE *Z* score.

Table 2.5 shows the results of logistic regression modelling to identify predictors of echocardiographic findings in PHIV+ children. Each model for a risk factor was adjusted for age, sex, BMI, TG, and LDL. The only significant predictor of LV hypertrophy was later initiation of ART. Those who started ART between 6 and 14 years of age had a 2.86-fold increased risk of having LV hypertrophy compared with those who started between 0 and 5 years of age. For LV diastolic dysfunction, the only significant predictor was being in WHO HIV stage IV at diagnosis; those who have been classified as stage IV had a 2.16-fold increased

risk for LV diastolic dysfunction compared with those in WHO stage less than IV. No significant factors were found for RV systolic dysfunction (TAPSE).

DISCUSSION

We have demonstrated a low prevalence of abnormal echocardiographic findings in a large group of PHIV+ South African adolescents treated with ART from a relatively early age. Subtle echocardiographic differences were noted between PHIV+ and HIV– adolescents. These differences were not clinically significant, however, and few HIV-specific factors remained significant after adjustments were made. This study differs from prior studies in that all PHIV+ adolescents were on ART and had been on ART for a relatively long period. (The mean duration on ART was 7 years).

With the initiation of ART at a mean age of 5 years, our results are similar to those from a smaller cross-sectional multicenter pediatric HIV/AIDS cohort study in the United States that showed that ART appears to be cardioprotective and that cardiac abnormalities (in the post-ART era) in PHIV+ participants were mostly mild, and subjects were asymptomatic.[4] In a similar report from Spain, there were no major differences between PHIV+ subjects and control subjects. In the Spanish study, the PHIV+ adolescents had 10 years of cumulative exposure to non-nucleoside reverse transcriptase inhibitors.[20] LV systolic dysfunction and LV dilatation were rare in our study; similar findings were reported in the multicentre National Heart, Lung, and Blood Institute-funded Cardiac Highly Active Antiretroviral Therapy study, in which therapy was introduced early in patients' lives.[21]

These results differ from those of previous studies of African children and adolescents with limited access to ART, which reported higher prevalence of LV dilatation and systolic dysfunction. A prevalence of 8% of LV dilatation and 5.5% of LV systolic dysfunction were reported in a Zimbabwean study.[6] Thirty three percent of subjects in a Nigerian cohort had systolic dysfunction.[22] These cohorts were characterized by a higher mean age at diagnosis of HIV as well as delayed initiation of ART; participants had generally been

receiving ART for only a short period. Moreover, the participants who received ART for short duration appear to have received little benefit from the therapy compared with those who had not received any treatment.[23]

The prevalence of diastolic dysfunction (simple measurement of mitral inflow velocities alone) was present in 7.6% PHIV+ adolescents versus 5.5% HIV– adolescents in our study. In a study from Cameroon, 36% of participants had LV diastolic dysfunction, but the mean age at the start of ART was 7.6 years, and duration on ART was 4 years; participants who were naïve to ART were included as well.[13] An Indian study reported that 64% of patients had diastolic dysfunction. Only 86% of these children were on ART. LV diastolic dysfunction was not evaluated during the Pediatric Pulmonary and Cardiac Complications of Vertically Transmitted HIV Infection study and Cardiac Highly Active Antiretroviral Therapy study.[21,24]

Among PHIV+ adolescents, we found an association between E/A ratio and being in WHO HIV stage IV at initiation of ART. This finding may support the hypothesis that cardiac dysfunction is related to the severity of HIV before starting therapy. Danbauchi *et al*.,[25] from Nigeria, for example, found diastolic dysfunction to be present in 30% of patients affected by late stage of HIV/AIDS. Singh *et al*.[26] used the more complex measurement of E/e' ratio to assess diastolic function, and 64% of their population had abnormal findings. Fifty percent of the participants who were in late WHO HIV stages had diastolic dysfunction.

LV hypertrophy (measurement of LV mass indexed to BSA), was present in 6.7% PHIV+ adolescents and 5.5% HIV– adolescents in our study, and among PHIV+ adolescents, LV hypertrophy was associated with the late initiation of ART. These figures are lower than those reported in the Zimbabwean study, in which 67.2% of the cohort had LV hypertrophy, but the duration on ART in the Zimbabwean cohort was much lower than (1.6 years)[6] than in our cohort. Our findings are consistent with the recent follow-up report of the Adolescent Master Protocol study of the Pediatric HIV/AIDS Cohort Study network, which reported that a highly active ART-exposed group had lower LV mass compared with an ART-unexposed group after

11 years of follow-up. LV mass was shown to be negatively associated with the duration of ART exposure.[21] Our findings support the Adolescent Master Protocol study conclusion that early initiation of ART is cardioprotective[4] and that it is important to start ART as soon as possible.

RV systolic dysfunction was the most frequent abnormality in this cohort. However, only one PHIV+ adolescent had both RV systolic dysfunction and high pulmonary pressure. The groups did not differ in terms of TAPSE (2.0 in PHIV+ adolescents vs 2.0 in HIV– adolescents). The high prevalence of abnormal TAPSE *Z* scores in both the PHIV+ (24.7%) and HIV– (22%) groups is difficult to explain. An Italian study in a small group of adolescents and young adults showed that HIV-infected subjects on therapy may have slightly lower mean TAPSE compared with control subjects (2.0 ± 0.2 vs 2.3 ± 0.3 cm); RV free wall longitudinal strain proved to be a better indication of RV dysfunction in the Italian patients.[27] It is also recognized that TAPSE and fractional area change measurements are more useful as measurements of trends in specific patient groups rather than techniques for measurements at discrete points in time.[28,29]

Limitations

Echocardiographic reference ranges used were derived from either American or European cohorts because of a paucity of "normal value" publications originating from sub-Saharan Africa. Further studies are needed of large groups of African children to provide appropriate normal ranges. Furthermore, controversy exists regarding normal values in growing children, even for apparently simple estimations such as LV hypertrophy.[30] Although two-dimensional ultrasound and current Doppler techniques are easily accessible, it would be preferable to have used more sophisticated techniques such as speckle-tracking[31] or cardiac magnetic resonance imaging or to measure strain and strain rate, but these were not available during the study. Cardiac magnetic resonance imaging is an important modality for detailed tissue characterization[32] and has shown that subtle myocardial inflammation and fibrosis are present in a cohort of asymptomatic HIV+ adults.[33] Another limitation of the study is the cross-sectional design,

precluding the ability to draw causal inferences or monitor progression of disease; ongoing study of the Cape Town Adolescent Antiretroviral Cohort will enable us to perform longitudinal long-term follow-up and determine whether long-term ART remains effective in preserving cardiac function.

CONCLUSION

In this large population of children with AIDS who have been treated for an extended period of time (following early initiation of therapy in the majority) the prevalence of overt echocardiographic cardiac abnormalities is gratifyingly low. When echocardiographic abnormalities were present, they were associated with later onset of therapy and more severe HIV disease at the time that therapy was initiated.

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Highlights

- There is low prevalence of echo abnormalities among PHIV+ adolescents on ART from early age.
- Starting ART at older age was a risk factor for LV hypertrophy in PHIV+ adolescents.
- Advanced HIV stage at the onset of ART was associated with LV diastolic dysfunction.

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Table 2.1. Clinical characteristics of study population

Characteristics	PHIV+ (*n* = 474)	HIV– (*n* = 109)	*P*
Age, y	12.0 (10.7-13.3)	11.8 (10.0-13.4)	.28*
Sex, male	242 (51.1)	49 (44.9)	.25†
Growth measures			
Height, cm	140.5 (132.5-147.6)	142.8 (136.5-155)	<.01*
Weight, kg	33.9 (28.6-40.7)	39.4 (32.2-47.9)	<.01*
BMI, kg/m^2	17.2 (15.9-18.9)	18.7 (16.8-21.5)	<.01*
BSA, m^2	1.2 (1.1-1.3)	1.3 (1.1-1.4)	<.01*
Blood pressure, mm Hg			
Systolic	105.0 ± 11.3	109.6 ± 11.5	<.01‡
Diastolic	67.2 ±9.0	68.8 ± 8.6	.02‡
High BP	64 (13.5)	19 (17.4)	.29†
Transcutaneous O_2 saturation, %	98.6 ± 0.9	98.8 ± 0.5	.08‡
Family History of CVD	30 (6.3)	2 (1.8)	.06†
Shortness of breath	5 (1.1)	2 (1.8)	.51†
Lipids, mmol/L			
Total cholesterol	4.1 (3.6-4.6)	3.8 (3.4-4.3)	<.01*
Elevated	56 (12.2)	3 (2.8)	<.01†
TG	0.9 (0.7-1.1)	0.7 (0.5-0.8)	<.01*
Elevated	36 (7.7)	2 (1.9)	.03†
LDL cholesterol	2.2 (1.8-2.6)	2.0 (1.6-2.4)	.01*
Elevated	25 (5.4)	3 (2.8)	.25†
HDL cholesterol	1.5 (1.2-1.7)	1.5 (1.2-1.7)	.57*
Low	31 (6.7)	1 (0.9)	.02†
hs-CRP, mg/L§			
<1	192 (41.6)	69 (65.1)	<.01†
1-3.99	171 (37.0)	30 (28.3)	
4-5.99	27 (5.8)	3 (2.8)	
≥6 n	72 (15.6)	4 (3.8)	

BP, Blood pressure; *CVD*, cardiovascular disease; *HDL*, high-density lipoprotein; *hs-CRP*, highly sensitive C-reactive protein; *LDL*, low-density lipoprotein; SOB, shortness of breath. Continuous variables expressed as median (interquartile range) or mean ± SD and categorical variables as number (percentage).

*Rank sum test.

†Chi-square test.

‡Student's t test.

§Twelve missing values for PHIV+ adolescents and three for HIV– adolescents.

Table 2.2. HIV disease severity measures among PHIV+ adolescents (N = 474)

Clinical features	Value
CD4 count (cells/μ,L)*	
<200	10 (2.1)
200–499	61 (12.9)
500–1,000	300 (63.7)
>1,000	100 (21.1)
Viral Load (copies/mL)†	
<50	369 (77.8)
50–1,000	46 (9.7)
1,001–10,000	32 (6.8)
>10,000	26 (5.5)
WHO HIV stage‡	
I	34 (7.2)
II	47 (9.9)
III	266 (56.1)
IV	105 (22.2)
Age at initiation of ART, y§	5.0 ± 3.5
0–2	173 (36.5)
3–5	124 (26.2)
6–14	169 (35.7)
Duration on ART, y	7.0 ± 3.0
Current HAART§	
Two different NRTIs + NNRTI	282 (59.5)
Two different NRTIs + PI	175 (36.9)
Others	9 (1.9)

HAART, Highly active ART; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.
Data are expressed as number (percentage) or as mean 6 SD.
*Three missing values.
†One missing value.
‡Twenty-two missing values.
§Eight missing values.

Table 2.3. Echocardiographic parameters

	PHIV+ (*n* = 474)	HIV–(*n* = 109)	*P**
Structure			
IVS thickness, end-diastolic, mm	7.7 (6.7 to 8.7)	8.1 (7.3 to 8.8)	<.01
IVS thickness, end-diastolic, Z Score	0.9 (0.3 to 1.4)	1.0 (0.4 to 1.4)	.51
IVS thickness, end-systolic, mm	10.3 (9.5 to 11.3)	10.5 (9.7 to 11.5)	.29
IVS thickness, end-systolic, Z score	0.7 (0.2 to 1.1)	0.6 (0.1 to 1.0)	.04
LV internal dimension, end-diastolic, mm	41.2 (38.6 to 43.9)	40.7 (39 to 43.9)	.60
LV internal dimension, end-diastolic, Z score	–0.1 (-0.7 to 0.3)	–0.4 (–1.0 to 0.0)	<.01
LV internal dimension, end-systolic, mm	26.2 (24.5 to 28.3)	26.2 (25 to 28.4)	.65
LV internal dimension, end-systolic, Z score	0.05 (–0.5 to 0.6)	–0.04 (–0.5 to 0.4)	.10
LV posterior wall thickness, end-diastolic, mm	6.8 (6.1 to 7.5)	7.2 (6.5 to 7.8)	<.01
LV posterior wall thickness, end-diastolic, Z score	0.8 (0.2 to 1.3)	0.9 (0.4 to 1.2)	.43
LV posterior wall thickness, end-systolic, mm	10.3 (9.4 to 11.1)	10.6 (9.6 to 11.2)	.31
LV posterior wall thickness, end-systolic, *Z* score	–0.4 (–0.9 to 0.01)	–0.6 (–1.1 to -0.2)	.04
RV internal dimension, end-diastolic, mm	20.3 (18.0 to 22.8)	21.9 (19.4 to 24.4)	<.01
RV internal dimension, end-diastolic, *Z* score	0.3(–0.2 to 0.8)	0.4 (0.0 to 0.8)	.02
LV mass, g/m^2			
Sex			
Male	77.0 (68.0 to 90.0)	82.0 (67.0 to 90.0)	.55
Female	69.0 (61.0 to 78.0)	65.0 (59.0 to 74.0)	.10
Function			
LV ejection fraction, two dimensional assessment, %	65.8 (62.5 to 69.2)	64.8 (62.0 to 68.6)	.17
LV ejection fraction, Simpson method (%)	64.0 (61.0 to 66.3)	64.0 (60.7 to 67.0)	.97
LV shortening fraction, %	36.2 (33.2 to 39.3)	35.5 (32.7 to 38.9)	.16
Mitral valve E/A ratio	2.0 (1.7 to 2.5)	2.2 (1.8 to 2.5)	.26
TAPSE, cm	2.0 (1.8 to 2.2)	2.0 (1.8 to 2.2)	.27
TAPSE, Z score	–0.57 (–1.85 to 0.74)	–0.93 (–0.1.75 to 0.40)	.35
RV fractional area change, %	44.0 (39.0 to 48.0)	43.0 (39.0, 48.0)	.46
Pulmonary artery pressure at end-systole, mm Hg	23.0 (21.0 to 26.0)	23.0 (21.0 to 26.0)	.37
Mean pulmonary artery pressure, mm Hg	16.0 (14.8 to 17.9)	16.0 (14.8 to 17.9)	.37

Data are expressed as median (interquartile range).

*P value derived from rank sum test.

Table 2.4. Number of abnormal echocardiographic findings in HIV-infected and uninfected adolescents

	PHIV+ (*n*= 474)	HIV–(*n*=109)	*P*
LV dilatation	2 (0.4)	0	.50
LV hypertrophy*	31 (6.7)	6 (5.5)	.65
LV systolic dysfunction†	1 (0.2)	0	.63
LV diastolic dysfunction	36 (7.6)	6 (5.5)	.45
RV dilatation	0	0	
RV systolic dysfunction (TAPSE)	117 (24.7)	24 (22.0)	.56
RV systolic dysfunction (FAC) ‡	45 (9.5)	11 (10.1)	.85
Pulmonary hypertension§	2 (0.5)	0	.48
Dilated cardiomyopathy	0	0	

FAC, Fractional area change.
Data are expressed as number (percentage).
*Ten missing values for PHIV+.
†Two missing values for PHIV+ and one missing value for HIV–
‡One missing value for PHIV+.
§Forty-three missing values for PHIV+.

Table 2.5. Regression models for key predictors of LV hypertrophy, LV diastolic dysfunction and RV systolic dysfunction among PHIV+ adolescents

	Multivariate Analysis					
	LV hypertrophy		LV diastolic dysfunction		RV systolic dysfunction	
Variable	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Blood Pressure*						
Systolic†	1.01 (1.0-1.1)	.50	0.98 (0.9-1.0)	.14	1.00 (0.9-1.0)	.65
Diastolic†	0.99 (0.9-1.0)	.50	0.97 (0.9-1.0)	.12	1.01 (0.9-1.0)	.64
hs-CRP*						
Low risk	Reference		Reference		Reference	
Moderate risk	1.14 (0.5-2.5)	.73	0.92 (0.4-2.0)	.83	1.11 (0.7-1.7)	.63
High risk	1.08 (0.2-5.0)	.92	1.53 (0.4-5.6)	.52	0.69 (0.3-1.9)	.47
Active infection	0.95 (0.3-3.0)	.93	1.43 (0.6-3.6)	.45	1.23 (0.7-2.3)	.50
Viral load, copies/mL*						
≤1,000	Reference		Reference		Reference	
>1,000	1.47 (0.5-4.1)	.46	1.80 (0.7-4.5)	.13	1.38 (0.7-2.6)	.31
CD4 count, cells/mL*						
≤499	Reference		Reference		Reference	
≥500	1.90 (0.6-6.6)	.31	0.62 (0.3-1.5)	.28	1.13 (0.6-2.1)	.70
WHO HIV staging*						
Less than stage IV	Reference		Reference		Reference	
Stage IV	1.15 (0.5-2.9)	.76	2.16 (1.0-4.6)	.05	0.85 (0.5-1.5)	.54
Age at initiation of ARTs, y*						
0-5 years	Reference		Reference		Reference	
6-14 years	2.86 (1.3-6.6)	.01	0.89 (0.4-1.9)	.78	0.91 (0.6-1.5)	.72
Current HAART*						
Two different NRTIs + NNRTI	Reference		Reference		Reference	
Two different NRTIs + PI	1.57 (0.7-3.6)	.29	1.16 (0.5-2.5)	.70	0.81 (0.5-1.3)	.58

hs-CRP, Highly sensitive C-reactive protein; HAART, highly active ART; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor.

*All models adjusted for age, BMI, sex, LDL, and TG.

†Continuous variable.

Chapter 3:

Endothelial dysfunction in South African youth living with perinatally acquired human immunodeficiency virus on antiretroviral therapy

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Abstract

Background: Human immunodeficiency virus (HIV) and antiretroviral therapy (ART) confer cardiovascular disease (CVD) risk in adults with HIV. Few studies have assessed endothelial dysfunction (ED), an early marker of subclinical CVD risk, in youth living with perinatally acquired HIV (YLPHIV).

Methods: Using peripheral arterial tonometry, we compared ED in YLPHIV and age-matched youth without HIV. A reactive hyperemic index ≤1.35 was defined as ED. Eligible participants included those aged 9–14 years and on ART ≥6 months at enrollment.

Results: Overall, 431 YLPHIV and 93 youth without HIV with a median age of 14.1 versus 13.9 years, respectively, were included. YLPHIV had a lower BMI *z* score (BMIZ; −0.2 vs 0.4; *P* < .01) but higher proportion of hypercholesterolemia (10% vs 1%; *P* = .01) than youth without HIV. Among YLPHIV, mean log viral load (VL) was 4.83 copies/mL with 21.7% having a CD4 count <500 cell/mm^3; median duration on ART was 9.8 years with 38% initiating at <2 years of age. YLPHIV had higher rates of ED than youth without HIV (50% vs 34%; *P* = .01); this relationship persisted after adjusting for age, sex, BMIZ, elevated BP, and hypercholesterolemia (RR, 1.43; *P* = .02). Among YLPHIV, CD4 count >500 cell/mm^3 (RR, 1.04; *P* = .76), VL (RR, 1.01; *P* = .78), and current ART class (protease inhibitor based vs nonnucleoside inhibitor based: relative risk, 0.90; *P* = .186) were not associated with ED after adjustment.

Conclusions: Even after adjusting for physiologic differences, YLPHIV appear to be at increased risk of ED compared with age-matched youth without HIV. These findings have important implications for the life course of YLPHIV who may be at increased risk of premature CVD and complications.

INTRODUCTION

Globally, an estimated 2.1 million adolescents are living with human immunodeficiency virus (HIV),[1] most of whom live in sub-Saharan Africa. Before the availability of antiretroviral therapy (ART) up to 25% of children living with HIV manifested significant cardiac dysfunction including left ventricular systolic dysfunction, left ventricular hypertrophy, and left atrial dilation.[2] The advent of effective ART has dramatically reduced overall mortality, with death now predominantly due to comorbid illnesses [3]. Among these comorbidities, CVD and metabolic diseases are among the leading cause of death in adults living with HIV.[3-4]

Youth living with perinatally acquired HIV infection (YLPHIV) comprise a unique and vulnerable population with potential cardiovascular disease (CVD) risk given their lifetime exposure to both HIV and ART. Evidence of deranged lipids due to ART, upregulated inflammatory pathway, metabolic factors, and immunosenescence play a role in progression of vascular disease and future atherosclerosis.[5-6] Although clinical symptoms of atherosclerosis may not manifest until adulthood, the process of atherogenesis begins early.[7]

Endothelial dysfunction (ED) is one of the earliest stages of vessel wall alteration leading to atherosclerosis and consequent CVD.[8] Endothelial dysfunction denotes the inability of the artery to sufficiently dilate in response to an appropriate endothelial stimulus.[9] Since the endothelium is not confined to the coronary arteries, less invasive techniques can be used to assess peripheral vascular endothelial function.[10-11] One commonly used method for assessing ED is flow-mediated dilation (FMD);[12] however, this method is operator dependent and has poor reproducibility.[13] To overcome these challenges, the endothelial peripheral arterial tonometry (EndoPAT) device allows noninvasive measurement of vasoreactivity without the disadvantages of conventional ultrasound measurement while being feasible and demonstrating excellent reproducibility in adolescents.[14] EndoPAT detects plethysmographic pressure

changes in the fingertips caused by the arterial pulse and translates these into peripheral arterial tone.[15] It is a noninvasive method that measures the reactive hyperemia index (RHI) induced by cuff occlusion of the arm and has been validated to evaluate peripheral vascular endothelial function.[15] EndoPAT has a sensitivity of 80% and specificity of 85% in identifying individuals with ED[16] as well as a strong correlation with FMD.[15, 17, 18]

Few studies have assessed ED in children or youth living with HIV.[19-21] Current published studies have been limited by small sample sizes ranging from 49 to 142, and none have been from sub-Saharan Africa where over 90% of the world's children living with HIV reside.

Our objective was to compare peripheral endothelial function between YLPHIV and youth without HIV using the EndoPAT technique, with the hypothesis that YLPHIV would have poorer ED due to lifelong exposure to HIV and ART.

METHODS

Study Population

The Cape Town Adolescent Antiretroviral Cohort (CTAAC) is an ongoing South African prospective cohort study investigating the long-term health of YLPHIV receiving ART.[22-23] Youth living with perinatally acquired HIV infection were enrolled between July 2013 and March 2015 from 7 HIV clinics in Cape Town, South Africa, including 3 tertiary health facilities and 4 primary health clinics. Youth without HIV were healthy adolescents, frequency matched by age and sex, who were recruited from the Youth Centre, which has a similar socioeconomic background as the sites where YLPHIV were recruited. Eligible YLPHIV enrolled into CTAAC were 9–14 years of age, on ART for at least 6 months, aware of their HIV status, and had confirmed perinatal HIV acquisition from their clinical record.

Study participants were seen every 6 months at the Medical Research Council Unit on Child and Adolescent Health located at the Red Cross War Memorial Children's Hospital in Cape Town, South Africa.

For this study, we only included those who had an EndoPAT measurement available from the 24-month visit. Written informed consent was obtained from a parent or legal guardian, and study participants provided written informed assent. The study protocol was approved by the Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town and Stellenbosch University. Approval for the study was also obtained from the Western Cape Provincial Research Committee.

Primary Outcome

Endothelial dysfunction was measured using an EndoPAT device (Itamar Medical Ltd.). EndoPAT is a noninvasive method that assesses reactive hyperemia induced by cuff occlusion of the arm and has been validated to evaluate peripheral vascular endothelial function.[15] In addition, it is not operator dependent and has demonstrated good reproducibility.[24] The test was performed with the participant lying in a comfortable position with both hands at the same level using an arm rest in a silent and temperature-controlled (21°C-24°C) environment with dim light.

Three sets of 5-minute recordings were taken: baseline testing period, cuff occlusion period, and postocclusion period. The RHI was automatically calculated by the device, as a ratio of the postocclusion to preocclusion peripheral arterial tone amplitude of the tested arm, divided by the postocclusion to preocclusion ratio obtained in the control arm.[15] An RHI of 1.35 or less was defined as ED.[16]

Covariates

Sociodemographic data were collected from the participant and caregiver at the time of enrollment in the study, and the participant's clinical record was reviewed by a study clinician to record ART history and World Health Organization (WHO) HIV staging[25] from the time of HIV diagnosis. By convention, WHO staging does not change during the course of illness in patients despite a positive response to treatment.

Clinical examination including Tanner pubertal staging, blood pressure (BP), and anthropometry was performed by a trained member of the research team at 24 months at the time when EndoPAT was performed. Elevated BP was defined as as greater than the 90th percentile for age, sex, and height.[26] Body mass index (BMI) was calculated, using WHO references, as weight in kilograms divided by height in meters squared (kg/m^2).[27]

Laboratory measures included viral load (VL) using Roche Cobas Ampliprep/COBAS TaqMan HIV-1 Test, version 2.0-standard technique and CD4 cell count measured by Beckman Coulter FC500 MPL analyzers using the PLG Pan Leukocyte Gating method in YLPHIV. Highly sensitive C-reactive protein (hs-CRP) was measured using the Roche Cobas Tina-quant system. Fasting lipid subfractions including total cholesterol (TC), triglycerides (TGs), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured in all participants at the time when EndoPAt was performed. Abnormal lipids were defined as greater than the 95th percentile using the National Health and Nutrition Examination Survey (NHANES).[28] All of these tests were conducted at 24 months at the time of EndoPAT.

Statistical Analysis

Baseline variables were compared between groups using *t* tests, Wilcoxon, chi-square, or Fischer's exact tests, as appropriate. Body mass index z score for age (BMIZ) and height-for-age and weight-for-age z scores were calculated using WHO references.[29] Basic characteristics of participants were also compared between those with and without available EndoPAT measurements (Supplementary Tables S1 and S2). Since there were less than 5% missing data and missing at random, we did not perform imputation for missing data. Modified Poisson regression models were used to assess the adjusted association of perinatally acquired HIV infection with ED using a forward selection approach. In addition, hypercholesterolemia,[30,31] sex,[32] and BMIZ[31] were considered a priori confounders. BMI has been proven as an independent risk factor for ED due to its association with metabolic syndrome,[33] the impaired

production of endothelium-derived nitric oxide under hypercholesterolemia has proven it to be a risk factor for ED,[34] and the association of hypertension with impaired production of reactive oxygen species will result in ED.[35] For subgroup analyses among YLPHIV we chose CD4 count, log VL, and current ART as specific predictors of interest where we tested the association of each exposure with ED in separate models. All analyses were performed in Stata, version 14.2 (StataCorp).

RESULTS

Among the 625 adolescents (n = 515 YLPHIV and n = 110 youth without HIV) enrolled in the CTAAC study, EndoPAT was performed at the 24-month visit; 570 (91%) completed the 24-month visit. Out of 570, 524 (92%) (431 [91%] YLPHIV and 93 [95%] youth without HIV) had available EndoPAT data (Figure 3.1). No differences in age, sex, BMIZ, Tanner stage, or HIV status were found between participants who had completed the 24-month visit versus those who did not complete 24-month visit and no differences were found between those who had EndoPAT versus those who did not at the 24-month visit. (Supplementary Tables S1a and S2a)

Median age (14.1; interquartile range [IQR], 12.8–15.5 years; vs 13.9 [12.1, 15.3] years; *P* = .21) and sex distribution (female: 49.4% vs 57%) were similar between YLPHIV and youth without HIV as per the study design (Table 3.1). Median BMIZ was lower in YLPHIV (–0.21 [IQR, –0.95, 0.58] vs 0.39 [IQR, –0.56, 1.26] kg/m², *P* < .01) but waist hip ratio was higher in YLPHIV (females: 0.84 [IQR, 0.80, 0.89] vs 0.82 [IQR, 0.78, 0.87], *P* = .01; males: 0.89 [IQR, 0.86, 0.92] vs 0.85 [IQR, 0.82, 0.89]; *P* < .01). No differences in Tanner stage were found between groups. Systolic and diastolic BP was also lower in YLPHIV (104 [IQR, 97, 111] vs 108 [IQR, 103, 115] mmHg; *P* < .01; 67 [IQR, 61, 71] vs 70 [64, 74] mmHg; *P* = .01, respectively), but no difference in rates of elevated BP were noted between groups. Only 11 (2.1% YLPHIV vs 2.2% youth without HIV, *P* = .97) reported tobacco use within the last 12 months.

Median hs-CRP (1.1 [IQR, 0.5, 3.5] vs 0.7 [IQR, 0.3, 2.1] mg/L; *P* = .01), TGs (0.9 [IQR, 0.7, 1.2] vs 0.6 [IQR, 0.5, 0.8] mmol/L; *P* < .01), TC (4.0 [IQR, 3.5, 4.7] vs 3.5 [IQR, 3.2, 4.1] mmol/L; *P* < .01), LDL (2.1 [IQR, 1.8, 2.7] vs 1.9 [IQR, 1.5, 2.2] mmol/L; *P* = .01), and rates of hypertriglyceridemia (17.3% vs 1.9%) and hypercholesterolemia (10.1% vs 1.1%, *P* = .04) were higher in YLHIV (Table 3.1).

Among YLPHIV, mean log VL was 4.83 copies/mL, with 21.7% having a CD4 count less than 500 cells/μL. Median duration on ART was 9.8 years, with 38% initiating ART at younger than 2 years of age. There were 347 (84.1%) YLPHIV who had WHO Stage III and IV at the time of HIV diagnosis (Table 3.2).

Over half (55.9%) of YLPHIV were on a nonnucleoside reverse transcriptase inhibitor (NNRTI)-based ART regimen (96.7% of whom were on efavirenz and 3.3% on nevirapine). Approximately one-third of YLPHIV (32.5%) were receiving protease inhibitors (PIs), all of whom were on lopinavir/ritonavir. All YLPHIV received a backbone of nucleoside reverse transcriptase inhibitors (NRTIs). The majority of YLPHIV were on abacavir or lamivudine (68.5% and 83.5% of participants, respectively); smaller proportions of adolescents were receiving tenofovir, zidovudine, emtricitabine, and stavudine (13.7%, 17.9, 13%, and 2.6%, respectively).

Overall, median RHI was lower among YLPHIV (1.36 vs 1.52; *P* = .01) compared with youth without HIV. Youth living perinatally acquired HIV also had higher rates of ED compared with youth without HIV (50% vs 34%; *P* = .01) (Table 3.3); this relationship persisted even after adjusting for age, sex, BMIZ, elevated BP, and hypercholesterolemia (relative risk [RR], 1.43; *P* = .02) (Table 3.4). Among YLPHIV, CD4 count greater than 500 cell/mm^3 (RR, 1.04; *P* = .76), VL (RR, 1.01; *P* = .78), and current ART class (PI vs NNRTI-based ART; RR, 0.90; *P* = .19) were not associated with ED after adjusting each model for age, sex, BMIZ, elevated BP, and hypercholesterolemia (Table 3.5).

DISCUSSION

This is the first study from Africa that demonstrates the pathogenesis of HIV in altering endothelial function among South African YLPHIV. Results are similar to American and European youth,[20, 21] even with different genetic and environmental backgrounds. We found that ED is more prevalent in YLPHIV compared with controls without HIV. The presence of ED in this adolescent cohort without many traditional risk factors for CVD suggests that vascular alterations may already be present from a young age in YLPHIV, representing an early sign of microvascular disease or subclinical atherosclerosis.[8]

Similar to our findings, studies in North America and Europe [20, 21] also reported worse endothelial function in YLPHIV compared with youth without HIV. A French study compared endothelial function in YLPHIV on ART versus ART-naive individuals but did not observe differences between these groups.[20] In our study, all YLPHIV were on longstanding ART, with median duration of 9.8 years. A US study also compared ED between youth with perinatally versus nonperinatally acquired HIV and found worse ED among those with perinatally acquired HIV.[21] In addition to pediatric studies, adult studies have also shown a higher prevalence of ED among individuals living with HIV.[36-37]

Several adult studies have also suggested that HIV infection itself along with ART is associated with both progression of atherosclerosis and cardiovascular events.[38-39] However, the relative impact of HIV, ART, and underlying CVD risk factor profiles remains unclear. These factors are difficult to disentangle in adults because of the interaction between the presence of classic risk factors for atherosclerotic disease and ART with the fact that the timing of HIV infection is often unknown. Among children and youth living with perinatally acquired HIV several factors are likely to contribute to impaired endothelial function such as in utero and early postnatal exposure to both HIV and/or ART, a critical period of development where cells are particularly vulnerable to mitochondrial toxicity and metabolic alterations.[40] Further, lifelong exposure to HIV and ART for YLPHIV may place YLPHIV at risk for inflammation, subclinical immune activation, and

immunosenescence[5, 6] which may play a role in altering metabolic pathways within the endothelium. We have previously reported that YLPHIV have accelerated aging,[41] and the association of accelerated aging with CVD has been shown by a German case control study.[42] Due to these risk factors, children and adolescents with perinatal HIV may be more likely to experience earlier or more significant long-term HIV-related cardiometabolic complications than adults living with HIV.

Previous studies have demonstrated the association of PIs with atherosclerosis and CVD in adults living with HIV.[43] Pediatric studies[19, 21] have also reported associations of ED and impaired FMD with PI use. One study from the United Kingdom reported impaired FMD in children living with HIV who were PI-treated versus those treated with non-PI ART as well as versus those not treated with ART.[19] However, we did not find any differences in ED between PI-treated and non-PI-treated YLPHIV, similar to observations made by Hsue and colleagues.[44] This suggests that perhaps ED is not solely related to PI use, but rather infection by HIV in and of itself plays a role in ED. In fact, a placebo-controlled study of healthy adults without HIV did not find PIs to induce ED,[45] and a Swiss study of adults living with HIV did not find an association between PI use and atherosclerosis.[46]

We found that RHI increased with age and BMI similar to a prior publication.[47-51] While the relationship between RHI and age is difficult to explain, Kelly et al[48] have suggested that the association of younger age with lower RHI, which they were not able to observe with FMD, could potentially be due to a 1-size finger probe, which might not be suitable for younger children. Other studies have suggested that microvascular development may not be complete until late adolescence, with a potential role of estrogen and dihydroepiandrosterone (DHEA) in improving endothelial function, and this may be the reason for a paradoxical age-RHI association in our youth/adolescent population.[49, 51] The inverse relation of BMIZ and BP found in our study was most likely because of the more pronounced ED finding among younger participants, as younger age is associated with lower BMIZ and lower BP. Other pediatric studies have also

reported that low BP is associated with a higher RHI.[48, 50] In addition, an adult study reported this inverse relationship.[52] The mechanism behind this observation remains elusive due to the lack of our understanding on the relationships between BP and actual microvascular function in children.

Currently, there are no recommendations for routine measurement of ED in YPLHIV. Based on the results from CTAAC, it may be important to monitor YLPHIV in sub-Saharan Africa for early risk factors or signs of cardiometabolic disease as well as ED. Noninvasive techniques, such as EndoPAT technology, provide an easy and reliable measure to assess ED. Early detection of this disorder among YLPHIV may have therapeutic and prognostic implications given that ED is potentially reversible.

Limitations

All participants were well established on ART for extended period, but results may not be generalizable to those who have a shorter duration of treatment or no treatment. Our study is limited by its cross-sectional nature, and therefore causal inference cannot be established. The physical activity of participants was not recorded, although both YLPHIV and youth without HIV were recruited from communities with similar socioeconomic backgrounds, with an assumption that their exercise profile would be similar. We also did not have comprehensive data on the lifetime ART use or all VL measurements since birth for all participants, which hampered our ability to more accurately estimate the association between lifetime cumulative viremic burden and ED. In addition, the vast majority of participants were black African adolescents, thus limiting the worldwide generalizability of our findings, but they may still be generalizable in the sub-Saharan African context.

CONCLUSIONS

This is the first study from Africa that demonstrates the pathogenesis of HIV in altering endothelial function among South African YLPHIV with a comparison group of youth without HIV. Our results show that, even after adjusting for physiologic differences, YLPHIV appear to be at increased risk of ED

compared with age- and sex-matched youth without HIV in South Africa, highlighting the role of perinatal HIV in altering endothelial function and potential downstream future CVD risk, even in a population with vastly different genetic, socioeconomic, and environmental background from North America and Europe. Further longitudinal studies are required to explore risk and mechanisms for the development of CVD in YLPHIV.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online [included below]. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Table 3.1. Characteristics of Youth Living with Perinatally Acquired Human Immunodeficiency Virus and Youth without Human Immunodeficiency Virus

	YLPHIV (n=431)		Youth without HIV (n=93)		
	n	Values	n	Values	*P*
Demographics					
Age,[a] years	431	14.1 (12.8, 15.5)	93	13.9 (12.1, 15.3)	.21
Female[b]	431	213 (49.4)	93	53 (57.0)	.19
Tobacco use in last 12 months[b]	427	9 (2.1)	92	2 (2.2)	.97
Growth Measures					
Height-for-age z score[a]	422	–1.25 (–2.03, –0.55)	89	–0.74 (–1.37, 0.06)	<.01
Weight-for-age z score[a]	429	-0.83 (-1.63, -0.03)	92	–0.13 (–0.83, 0.75)	<.01
BMI-for-age z score[a]	421	-0.21 (-0.95, 0.58)	89	0.39 (–0.56, 1.26)	<.01
Waist circumference,[a] cm	428	66 (62, 70)	91	69 (62, 76)	.02
Hip circumference,[a] cm	427	76 (71, 83)	92	84.5 (73.5, 91.5)	<.01
Mid-thigh circumference,[a] cm	425	39 (36, 42)	92	43 (38, 47)	<.01
Midupper arm circumference,[a] cm	429	22 (20, 24)	91	23 (21, 26)	<.01
Waist to hip ratio[b]					
Female	211	0.84 (0.80, 0.89)	51	0.82 (0.78, 0.87)	.01
Male	216	0.89 (0.86, 0.92)	40	0.85 (0.82, 0.89)	<.01
Blood pressure,[a] mmHg					
Systolic	431	104 (97, 111)	93	107.5 (103, 115)	<.01
Diastolic	431	67 (61, 71)	93	69.5 (64, 73.5)	.01
Elevated BP[b]	431		93		
Normal		366 (84.9)		75 (80.7)	.306
High		65 (15.1)		18 (19.4)	
Tanner Stage[b]	416		91		
1		63 (15.1)		12 (13.2)	.75
2		78 (18.8)		16 (17.6)	
3		87 (20.9)		25 (27.5)	
4		103 (24.8)		21 (23.1)	
5		85 (20.4)		17 (18.7)	
Laboratory measures					
Hs-CRP,[a] mg/L	422	1.09 (0.5, 3.5)	91	0.69 (0.3, 2.1)	.01
Triglycerides,[a] mmol/L	417	0.9 (0.7, 1.2)	88	0.6 (0.5, 0.8)	<.01
Hypertriglyceridemia[b]		54 (17.3)		1 (1.9)	<.01
Total cholesterol,[a] mmol/L	417	4.0 (3.5, 4.7)	89	3.5 (3.2, 4.1)	<.01
Hypercholesterolemia[b]		42 (10.1)		1 (1.1)	.01
LDL,[a] mmol/L	417	2.1 (1.8, 2.7)	87	1.9 (1.5, 2.2)	.01
High LDL[b]		14 (4.5)		1 (1.9)	.39
HDL,[a] mmol/L	417	1.4 (1.2, 1.7)	89	1.3 (1.1, 1.7)	.16
Low HDL[b]		35 (8.4)		9 (10.1)	.60

All continuous variables are expressed as medians (interquartile range) or means (SD) and categorical variables as n (%).
Abbreviations: BMI, body mass index; HDL, high-density-lipoprotein cholesterol; HIV, human immunodeficiency virus; hs-CRP, highly sensitive C-reactive protein; LDL, low-density-lipoprotein cholesterol; YLPHIV, youth living with perinatally acquired HIV.
[a]Continuous variable.
[b]Categorical variable.

Table 3.2. Human Immunodeficiency Virus Disease Severity Measures among Youth Living with Perinatally Acquired Human Immunodeficiency Virus

	YLPHIV (n = 431)
Viral load[a, b] copies/mL	
≤50	259 (62.1)
>50	158 (37.9)
Viral load[b,c]	50 (50-100)
Log viral load[*,#]	4.83 (1.8)
CD4 count[a,d] cells/uL	
<200	19 (4.5)
200–499	73 (17.2)
500–1000	274 (64.6)
>1000	58 (13.7)
WHO HIV staging[a,e]	
I	27 (6.5)
II	39 (9.4)
III	246 (59.6)
IV	101 (24.5)
Age at initiation of ART,[c,f] years	4.8 (1.9-7.4)
Age at initiation of ART[a] years	
0–2	161 (37.8)
3–5	125 (29.3)
6–14	140 (32.9)
Duration on ART,[c,f] years	9.8 (6.8,11.6)
Current ART regimen[a]	
2 X NRTI + NNRTI	241 (55.9)
2 X NRTI + PI	140 (32.5)
Others	50 (11.6)

All continuous variables expressed as median (interquartile range) or mean (SD) and categorical variables as number (%).

Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SD, standard deviation; WHO, World Health Organization; YLPHIV, youth living with perinatally acquired HIV.

[a]Categorical variable.

[b]14 missing values.

[c]Continuous variable.

[d]7 missing values.

[e]18 missing values.

[f]5 missing values.

Table 3.3. EndoPAT Measures

Endo-PAT measures	YLPHIV (n=431)	Youth without HIV (n=93)	*P*
RHI[a]	1.36 (1.09, 1.70)	1.52 (1.23, 1.92)	.01
RHI[b]			
Normal	216 (50.1)	61 (65.6)	.01
Low	215 (49.9)	32 (34.4)	
AI at 75 bpm[a,c]	–4 (–14, 11)	–5 (–13, 11)	.86

All continuous variables are expressed as medians (interquartile range) or mean (SD) and categorical variables as n (%).

Abbreviations: AI, augmentation index; bpm, beats per minute; EndoPAT, endothelial peripheral arterial tonometry; HIV, human immunodeficiency virus; RHI, reactive hyperemia index; SD, standard deviation; YLPHIV, youth living with perinatally acquired HIV.

[a]Continuous variable.

[b]Categorical variable.

[c]16 missing values for YLPHIV and none for youth without HIV.

Table 3.4. Regression Model Evaluating the Unadjusted and Adjusted Association between Perinatally Acquired Human Immunodeficiency Virus Infection and Endothelial Dysfunction

Variable	Unadjusted Relative Risk (95% CI)	*P*	Adjusted Relative Risk (95% CI)	*P*
Youth without HIV	Ref		Ref	
Perinatally acquired HIV infection	1.44 (1.08, 1.95)	.01	1.43 (1.06, 1.95)	.02
Age,[a] per 1-year increment	.83 (.78, .87)	<.01	.81 (.77, .86)	<.01
Gender				
Male	Ref		Ref	
Female	.86 (.72, 1.03)	.10	.90 (.75, 1.08)	.26
Elevated BP				
Normal	Ref		Ref	
High	.76 (.57, 1.02)	.07	0.70 (.51, .96)	.03
BMI z-score,[a] per 1-unit increase	.90 (.83, .97)	<.01	.91 (.84, .99)	.03
Total cholesterol				
Normal	Ref		Ref	
High	1.04 (.76, 1.44)	.78	.87 (.64, 1.19)	.38

Abbreviations: BMI, body mass index; BP, blood pressure, CI, confidence interval; HIV, human immunodeficiency virus; Ref, reference.

[a]Continuous variable.

Table 3.5. Regression Models Evaluating Unadjusted and Adjusted Associations between Key Human Immunodeficiency Virus–Related Factors and Endothelial Dysfunction among Youth Living with Perinatally Acquired Human Immunodeficiency Virus

Model	Unadjusted Relative Risk (95% CI)	*P*	Adjusted Relative Risk[a] (95% CI)	*P*
CD4 count, cells/uL				
<500	Ref		Ref	
≥500	1.09 (.86, 1.40)	.46	1.04 (.81, 1.33)	.76
Log HIV viral load[b]	1.01 (.96, 1.06)	.79	1.01 (.96, 1.06)	.78
Current ART regimen				
2 x XNRTI + NNRTI	Ref		Ref	
2 x NRTI + PI	1.05 (.85, 1.28)	.66	.91 (.73, 1.11)	.32
Others	.88 (.63, 1.24)	.47	.82 (.60, 1.10)	.19
WHO HIV staging				
I	Ref		Ref	
II	1.38 (.78, 2.47)	.27	1.14 (.65, 1.97)	.65
III	1.28 (.77, 2.14)	.37	1.05 (.65, 1.69)	.84
IV	1.55 (.92, 2.61)	.10	1.20 (.74, 1.97)	.46

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; CI, confidence interval; HIV, human immunodeficiency virus; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; Ref, reference; WHO, World Health Organization.

[a]Each model separately adjusted for age, gender, BMI z score, elevated blood pressure, and hypercholesterolemia.

[b]Continuous variable.

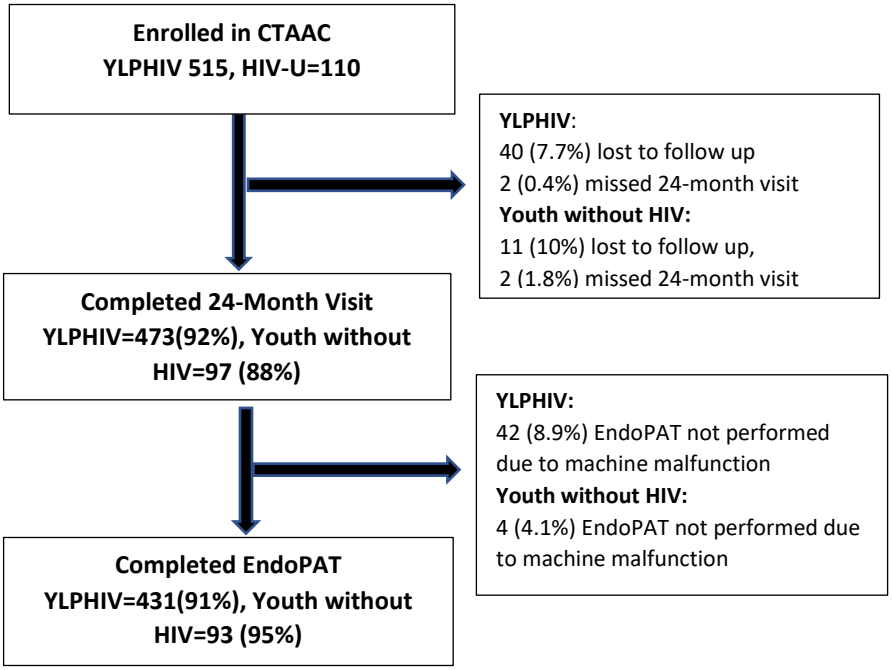

Figure 3.1. Study population flow chart

Abbreviations: CTAAC, Cape Town Adolescent Antiretroviral Cohort; EndoPAT, endothelial peripheral arterial tonometry; HIV, human immunodeficiency virus; YLPHIV, youth living with perinatally acquired HIV.

Supplementary Tables

Table S1a. Characteristics of Youth who had completed Visit 24 months and who did not complete 24 months

	N	Completed 24 month visit (N = 570)	N	Did not complete 24 month visit (N = 55)	P value
Age* (years)	570	11.4 (1.6)	55	11.6 (1.7)	0.45
Female[$]	570	285 (50.0)	55	28 (50.9)	0.90
HIV status	570		55		
YLPHIV		473 (83.0)		42 (76.4)	0.22
Youth without HIV		97 (17.0)		13 (23.6)	
BMI for age z score*	570	-0.12 (1.1)	55	0.07 (1.2)	0.25
Tanner Stage[$]	560		54		
1		265 (47.3)		24 (44.4)	0.85
2		138 (24.6)		14 (25.9)	
3		82 (14.6)		8 (14.8)	
4		50 (8.9)		4 (7.4)	
5		25 (4.5)		4 (7.4)	

Table legend: All continuous variables expressed mean (SD) and categorical variables as number (%), *continuous variable, [$] categorical variable; BMI = body mass index; YLPHIV = Youth living with perinatally acquired HIV.

Table S1b. Characteristics of YLPHIV who had completed Visit 24 months and who did not complete 24 months

	N	Completed 24 month visit (N = 473)	N	Did not complete 24 month visit (N = 42)	P value
Age* (years)	473	11.5 (1.6)	42	11.6 (1.7)	0.54
Female[$]	473	232 (49.1)	42	20 (47.6)	0.86
BMI for age z score*	473	-0.21 (1.1)	42	-0.17 (01.1)	0.81
Tanner Stage[$]	464		41		
1		231 (49.8)		22 (53.7)	0.77
2		109 (23.5)		11 (26.8)	
3		65 (14.0)		5 (12.2)	
4		38 (8.2)		1 (2.4)	
5		21 (4.5)		2 (4.9)	

Table Legend: All continuous variables expressed mean (SD) and categorical variables as number (%), *continuous variable, [$] categorical variable; BMI = body mass index; YLPHIV = Youth living with perinatally acquired HIV.

Table S1c. Characteristics of youth without HIV who had completed Visit 24 months and who did not complete 24 months

	N	Completed 24 month visit (N = 97)	N	Did not complete 24 month visit (N = 13)	P value
Age* (years)	97	11.2 (1.8)	13	11.5 (1.9)	0.58
Female[$]	97	53 (54.6)	13	8 (61.5)	0.64
BMI for age z score*	97	0.38 (1.1)	13	0.87 (1.3)	0.13
Tanner Stage[$]	96		13		
1		34 (35.4)		2 (15.4)	0.77
2		29 (30.2)		3 (23.1)	0.19
3		17 (17.7)		3 (23.1)	
4		12 (12.5)		3 (23.1)	
5		4 (4.2)		2 (15.4)	

Table Legend: All continuous variables expressed mean (SD) and categorical variables as number (%), *continuous variable, [$] categorical variable; BMI = body mass index

Table S2a. Characteristics of Youth who had EndoPAT and who did not have Endopat at the 24-month visit

	N	Endopat done (n = 524)	N	Endopat not done (n = 46)	p value
Age* (years)	524	14.0 (1.6)	46	14.1 (1.8)	0.58
Female[$]	524	266 (50.8)	46	19 (41.3)	0.22
HIV status	524		46		
YLPHIV		431 (82.2)		42 (91.3)	0.12
Youth without HIV		93 (17.7)		4 (8.7)	
BMI for age z score*	510	-0.1 (1.2)	46	-0.4 (1.1)	0.06
Tanner Stage[$]	507		44		
1		75 (14.8)		12 (27.3)	0.06
2		94 (18.5)		6 (13.6)	
3		112 (22.1)		6 (13.6)	
4		124 (24.5)		15 (34.1)	
5		102 (20.1)		5 (11.4)	

Table legend: All continuous variables expressed mean (SD) and categorical variables as number (%), *continuous variable, [$] categorical variable; BMI = body mass index; YLPHIV = Youth living with perinatally acquired HIV.

Table S2b. Characteristics of YLPHIV who had EndoPAT and who did not have Endopat at the 24-month visit

	N	Endopat done (n=431)	N	Endopat not done (n=42)	p value
Age* (years)	431	14.0 (1.6)	42	14.2 (1.8)	0.63
Female[$]	431	213 (49.4)	42	19 (45.2)	0.61
BMI for age z score*	421	-0.2 (1.2)	42	-0.4 (1.1)	0.21
Tanner Stage[$]	416		41		
1		63 (15.1)		12 (29.3)	0.08
2		78 (18.8)		6 (14.6)	
3		87 (20.9)		5 (12.2)	
4		103 (24.8)		13 (31.7)	
5		85 (20.4)		5 (12.2)	
Viral Load[$] (copies/mL)	417		42		
≤50		259 (62.1)		27 (64.3)	0.92
>50		158 (37.9)		15 (35.7)	
CD4 count[$] (cells/uL)	424		41		
<200		19 (4.5)		0	0.39
200-499		73 (17.2)		10 (24.4)	
500-1000		274 (64.6)		25 (61.0)	
>1000		58 (13.7)		6 (14.6)	
WHO HIV Staging[$]	413		41		
I		27 (6.5)		5 (12.2)	0.21
II		39 (9.4)		5 (12.2)	
III		246 (59.6)		26 (63.4)	
IV		101 (24.5)		5 (12.2)	
Age at initiation of ART[$] (years)	426		41		
0-2		161 (37.8)		17 (41.5)	0.11
3-5		125 (29.3)		6 (14.6)	
6-14		140 (32.9)		18 (43.9)	
Current ART regimen[$]	431		42		
2 X NRTI + NNRTI		241 (55.9)		26 (61.9)	0.70
2 X NRTI + PI		140 (32.5)		11 (26.2)	
Others		50 (11.6)		5 (11.9)	

Table legend: All continuous variables expressed mean (SD) and categorical variables as number (%), *continuous variable, [$] categorical variable; ART = antiretroviral treatment; BMI = body mass index; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; WHO = World Health Organization; YLPHIV = Youth Living with Perinatally Acquired HIV.

Table S2c. Characteristics of youth without HIV who had EndoPAT and who did not have Endopat at the 24-month visit

	N	Endopat done (n=93)	N	Endopat not done (n=4)	p value
Age* (years)	93	13.8 (1.8)	4	13.8 (1.9)	0.99
Female$	93	53 (57.0)	4	0	0.04#
BMI for age z score*	89	0.5 (1.2)	4	-0.5 (0.6)	0.13
Tanner Stage$	91		3		
1		12 (13.2)		0	0.69
2		16 (17.6)		0	
3		25 (27.5)		1 (33.3)	
4		21 (23.1)		2 (66,7)	
5		17 (18.7)		0	

Table legend: All continuous variables expressed mean (SD) and categorical variables as number (%), *continuous variable, $ categorical variable; BMI = body mass index

Chapter 4:

The determinants of elevated pathobiological determination of atherosclerosis in youth risk score in perinatally HIV-infected adolescents in South Africa

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Abstract

Background: Youth living with perinatally acquired HIV infection (YLPHIV) may have a higher risk of atherosclerotic cardiovascular disease (CVD) than uninfected adolescents caused by lifetime exposure to HIV infection, chronic immune activation, and long-term antiretroviral therapy (ART).

Methods: We determined the pathobiological determinants of atherosclerosis in youth (PDAY) risk score in coronary arteries (CA) and the abdominal aorta (AA) of ≥15-year-old YLPHIV in the Cape Town Adolescent and Antiretroviral Cohort, matching them against HIV-seronegative youth. YLPHIV on ART were followed every six months since 2013 along with HIV- adolescents. The PDAY score was calculated using: non-high-density lipoprotein (HDL) and HDL cholesterol, hyperglycaemia, hypertension, obesity, and cigarette smoking; a score ≥ 1 was considered elevated. HIV viraemia was categorized as sustained (SV)=VL>50, transient (TV)=mix of VL>50 and ≤50, or sustained virologic suppression (VS)=VL<50 copies/mL throughout the study. Among YLPHIV, log-binomial models were fit to assess factors associated with elevated PDAY scores for CA and AA separately.

Results: Overall, 218 YLPHIV and 31 HIV-seronegative youth (median age 16.8 vs 17.1, p=0.34, male 47% vs 44%, p=0.75) were included. Among YLPHIV, 8% (n=17) had SV, and 54% (n=118) had TV. Median ART duration was 12 (IQR:8-14) years; 57% were on used non-nucleoside reverse transcriptase inhibitor-based ART, while the rest received protease inhibitor-based ART. Among YLPHIV, 30.3% and 18.4% had elevated PDAY for CA and AA, respectively; among HIV-seronegative, 31.3% and 21.9% had an elevated PDAY for CA and AA, respectively. Elevated CA were associated with SV [adjusted odds ratio (aOR)=18.4, p<0.01] and TV (aOR=2.10, p=0.04) compared to VS and ART duration (aOR=1.12, p=0.03) in YLPHIV. Male sex was associated with elevated CA and AA PDAY (aOR=2.14, p=0.02, and aOR=3.43, p=0.01, respectively). The association of SV with elevated AA PDAY trended in the same direction as that for CA PDAY but did not reach statistical significance (aOR=3.24, p=0.09).

Conclusions: A considerable proportion of YLPHIV and HIV-seronegative youth have PDAY scores indicating an increased aggregate risk for atherosclerosis. Among YLPHIV, viraemia, lifetime ART duration and male sex contribute to this risk, highlighting the importance of HIV control, and the need to monitor cardiometabolic health. Future studies are needed to understand how ART impacts atherosclerotic risk in YLPHIV.

INTRODUCTION

Worldwide, an estimated 2.1 million adolescents are living with the human immunodeficiency virus (HIV), with most residing in sub-Saharan Africa (SSA).[1] Global advancement in HIV diagnosis and antiretroviral therapy (ART) has significantly improved the life expectancy of youth living with perinatally acquired HIV infection (YLPHIV).[2-3] The roll-out and expansion of ART for HIV have altered its course from a fatal to chronic infection, with associated comorbidities such as atherosclerotic cardiovascular disease (CVD) and metabolic conditions. In turn, these disorders are leading causes of mortality and morbidity among HIV-positive adults.[4-5] In the current ART setting, extensive literature exists regarding CVD and metabolic outcomes in adults, whereas less is available among the paediatric population. Despite the early use of ART, recent publications suggest that children continue being susceptible to subclinical cardiovascular and metabolic complications at a young age.[6-7]

YLPHIV are a unique and vulnerable population with a potentially increased risk for CVD, given their lifetime exposure to HIV and ART. Findings from the Cape Town Adolescents Antiretroviral Cohort (CTAAC) and other studies have found that YLPHIV are at risk of developing early CVD compared to uninfected peers.[6-7] Also, evidence exists of abnormal lipids attributable to ART, upregulated inflammatory pathways, metabolic risk factors, and immunosenescence contribute to the progression of vascular disease, endothelial dysfunction, and imminent atherosclerosis.[8-9] The atherogenesis process begins early in childhood, though clinical symptoms might not manifest until adulthood.[6,10] Therefore, high-risk adolescents may benefit from early interventions, and identifying the aggregate risk for CVD may be beneficial in monitoring this population by using a scoring system that calculates the risks associated with each risk factor.[11]

The Framingham Risk Calculator (FRC) is a broadly accepted instrument for predicting the overall 10-year risk of CVD and is based on a straightforward, validated algorithm including demographics, lifestyle behaviours, blood pressure and lipid analysis.[12] Though, the FRC has not been validated among adolescents. Using the pathobiological determinants of atherosclerosis in youth (PDAY) scoring system could help measure long-term CVD risk in adolescents through identifying those with advanced atherosclerosis.[13] The PDAY scoring system was

developed with autopsy data from more than 1 100 individuals, ages 15 to 34 years. This score assesses the risk of having an advanced atherosclerotic lesion in the coronary arteries (CA) or the abdominal aorta (AA) comparative to an age- and sex-matched individual without any CVD risk. In several population-based cohorts, using the CA risk score has assisted in predicting coronary artery calcium and carotid artery intima-media thickness in measuring atherosclerosis.[13-14]

Although SSA has the highest prevalence of YLPHIV, limited data are available on the risk of CVD in these populations.[1] Our study objective is to describe the risk of atherosclerosis in YLPHIV and HIV-seronegative youth using PDAY scores and investigate the determinants of high PDAY scores among YLPHIV.

METHODS

Study Population

A South African prospective cohort study, CTAAC, investigated the long-term health of YLPHIV on ART.[15-16] Between July 2013 and March 2015, YLPHIV were recruited from seven HIV clinics in Cape Town, South Africa, of which three tertiary health facilities and four primary health clinics. Those eligible for enrolment were 9-14 years old, taking ART for at least six months, aware of their HIV status, and had confirmed acquiring perinatal HIV.[6,17] HIV-seronegative youth, who served as controls, were healthy, frequency-matched (for age and sex) adolescents. They were recruited from the same communities, thus having a similar socio-economic background as YLPHIV.

Study participants visited the research unit at the Red Cross War Memorial Children's Hospital, Cape Town, South Africa, every six months. Participants included in this analysis had completed 48 months of study follow-up, were at least 15 years old, and had data available on all PDAY score components. Written informed consent was obtained from a parent or legal guardian, and study participants provided written informed assent at every annual visit. The Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, the Stellenbosch University, and the Western Cape Provincial Research Committee approved the study protocol.

Primary Outcome

PDAY scores were assessed, with CA and AA risk scores calculated separately by adding the scores associated with modifiable measures of atherosclerotic risk, including fasting lipids, glucose, blood pressure, smoking, and obesity.[13,18] All participants' fasting lipid sub-fractions, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured using the Cobas 6000 instrument (Roche, South San Francisco, CA). Fasting glucose was determined through the enzymatic method (Roche Cobas 6000, Roche USA). Three blood pressure measurements were taken with an electronic sphygmomanometer (Spot Vital Signs Welch Allyn, Skaneateles Falls, NY), and the mean level was recorded. Body mass index (BMI), calculated as weight in kilograms divided by height in meters squared (kg/m^2), defined obesity. Smoking data, via self-reporting, were collected. Components of the PDAY score, defined as non-HDL cholesterol ≥130 mg/dL, HDL <40 mg/dL, hyperglycaemia (fasting plasma glucose ≥125 mg/dL), hypertension (blood pressure ≥95th percentile for age, sex, and height), obesity (BMI >30 kg/m^2), and cigarette smoking (>1 pack/day in the past three months), were measured at the 48-month study visit.

We considered a PDAY score $\geq$1 as elevated since other studies have demonstrated increased odds for the atherosclerotic disease at the CA and AA for each 1-unit increase in the PDAY risk score, relative to a score of 0.[13,18]

Covariates

At enrolment, clinical and socio-demographic data, including ART history, duration of ART, and the WHO HIV staging[19] at the time of HIV diagnosis, were collected from participants and their caregivers. A trained member of the research team completed the clinical examination, including Tanner pubertal staging and anthropometry in standardized fashion at the 48-month study follow-up. Weight and standing height were measured with a calibrated digital scale and calibrated stadiometer, respectively. Three waist and hip circumference measurements, using a vinyl tape, were taken, of which the mean level was used to calculate the waist-to-hip and waist-to-height ratios. For the skinfold measurements, three readings of which the mean level was recorded were taken with a Vernier skinfold calliper.

Among YLPHIV, the VL was measured through the Roche COBAS Ampliprep/COBAS TaqMan HIV-1 Test, version 2.0-standard technique, and the CD4 cell count by the Beckman Coulter FC500 MPL analysers making use of the Pan Leukocyte Gating (PLG) method. Viraemia was categorized as sustained viraemia (VL>50 copies/mL throughout the study), transient viraemia (mix of VL >50 and ≤50 copies/mL, or sustained virologic suppression (VL<50 copies/mL throughout the study). With the use of the Roche Cobas Tina-quant system, we measured hs-CRP.

Statistical Analysis

We compared baseline variables between groups, using appropriate t-tests, Wilcoxon, Chi-square, or Fischer's exact tests. The BMI z-score was calculated using WHO references,[20] and skinfold z-scores were calculated using National Health and Nutrition Examination Surveys (NHANES).[21] Since missing data were <5% and missing at random, we did not perform multiple imputations. A separate analysis was done for the CA and AA scores. Demographic data and HIV-specific characteristics were compared in YLPHIV and HIV-seronegative youth with low scores (≤0) versus elevated scores (≥1) at the 48-month visit. The cut-off level for low versus high scores was based on the distribution of scores in the study population and consistent with definitions for low risk utilised in previous studies.[14,22-23] Given the small sample size of HIV-seronegative youth, logistic regression models were fit only among YLPHIV to identify predictors of elevated PDAY scores for CA and AA separately. We ran all the analyses in Stata® 16 (StataCorp, Texas, USA).

RESULTS

Among the 625 participants (n=515 YLPHIV and n=110 HIV-seronegative) who enrolled in the CTAAC study, 251 (219 YLPHIV and 31 HIV-seronegative youth) were eligible according to the inclusion criteria (Figure 4.1).

The median age, sex, and Tanner distribution were similar for YLPHIV and HIV-seronegative youth (Table 4.1). YLPHIV had a lower BMI z-score (−0.4, interquartile range [IQR], −0.7, -0.1] vs -0.1 [IQR, −0.5, 0.4] kg/m^2, p=0.08)

but higher TGs (0.8 [IQR, 0.6, 1.0] vs 0.6 [IQR, 0.5, 0.7] mmol/L, p<0.01), TC (3.5 [IQR, 3.1, 4.1] vs 3.2 [IQR, 2.8, 3.9] mmol/L, p=0.04) compared to HIV-seronegative participants (Table 4.1).

Among the YLPHIV, 7.8% (n=17) had sustained viremia and 54.1% (n=118) had transient viremia throughout the 4-year study period. Since the start of the study, 50.5% (n=110) had a CD4 count lower than 500 cells/µL. The median duration of ART was 11.7 (IQR 8.3, 13.9) years, with 27.6% initiating ART at a younger age than two years. Of the YLPHIV, 124 (56.9%) were on NNRTI-based ART, and 43.1% were on PI. At the time of HIV diagnosis, 172 (82.3%) YLPHIV had WHO stages III and IV (Table 4.1).

The distribution of the components of the PDAY scores by HIV status is shown in Table 4.2. Overall, the proportion of elevated CA and AA PDAY scores were similar between groups. Elevated CA scores were associated primarily with low levels of HDL cholesterol. Few YLPHIV were defined as hypertensive (1.8%, n=4) or hyperglycaemic (0.5%, n=1) (Supplemental Table). None of the HIV-seronegative youth had hypertension or hyperglycaemia. Smoking was common among YLPHIV and HIV-seronegative youth (11.5% and 15.6%, respectively). Obesity was higher among HIV-seronegative females compared to YLPHIV females (38.9% vs 12.9%, p=0.01).

Among YLPHIV, sustained viremia [adjusted odds ratio (aOR)=18.4, p<0.01] and transient viremia (aOR=2.10, p=0.04) compared to sustained VS were associated with an elevated CA PDAY score (Table 4.3a). The duration of ART was also associated with an elevated CA PDAY score (aOR = 1.12, p=0.03; Table 4.3a). Female were associated with both CA PDAY score and AA PDAY score $\geq$1 (aOR=2.14, p=0.02 and aOR=3.43, p=0.01, respectively). The relationship between sustained viremia and elevated AA PDAY score trended in the same direction as for CA PDAY score but did not reach statistical significance (aOR=3.24, p=0.09; Table 4.3b)

DISCUSSION

To our knowledge, this is the first study from SSA evaluating the PDAY score among YLPHIV. We found that a considerable proportion of YLPHIV and HIV-seronegative youth have PDAY scores indicating an increased aggregate risk for atherosclerosis. Among YLPHIV, viraemia, lifetime ART duration and male sex were associated

with the increased aggregated atherosclerotic risk, which may be beneficial in identifying and monitoring adolescents at high risk.

Few studies in non-HIV adolescent populations have assessed the PDAY score. The CARDIA study, conducted in healthy adolescents, found a higher prevalence of elevated AA PDAY (53%) than elevated CA PDAY (28%) at year 25 of follow-up.[24] On the other hand, we found an overall higher prevalence of elevated CA PDAY (30.4%) than AA PDAY (18.8%), which to some extent could be explained by the cross-sectional nature of our study compared to the longitudinal CARDIA study. Additionally, the higher prevalence of elevated AA PDAY in the CARDIA study might be because of differences in the age range of enrolled young adults, 18-30 years old, who were followed for 25 years, whereas participants in our study were 15-18 years old. Of note, we observed a higher proportion of those with an elevated AA PDAY in HIV-seronegative youth compared to YLPHIV, which did not reach statistical significance. However, given the small sample size of HIV-seronegative participants in our study, we are cautious against drawing any conclusions.

Previous studies on YLPHIV have also highlighted concerns around increased CVD risk resulting from distributions of individual risk factors in this population.[25-33] In the USA Adolescent Master Protocol (AMP) study within the Pediatric HIV/AIDS Cohort Study (PHACS), YLPHIV were evaluated. Similar to our study, a prevalence of an elevated CA PDAY score doubling that of an elevated AA PDAY (48.5% for CA and 23.6% for AA) was observed.[18] However, the overall prevalence of elevated CA (28.4%) and AA PDAY (13.3%) scores were lower in our study compared to PHACS (48.5% and 23.6%, respectively), likely reflecting baseline population risk for atherosclerotic CVD. In our study, among YLPHIV, the most elevated CA PDAY scores were related to abnormal HDL and non-HDL cholesterol, similar to the PHACS cohort. Low HDL cholesterol levels, often associated with inflammation and endothelial activation, have been reported in adults with untreated HIV infection or AIDS.[34-35] However, we found a lower prevalence of non-HDL cholesterol in contrast to previous studies of YLPHIV.[36-37] Only one YLPHIV had diabetes.

The prevalence of obesity among YLPHIV in our study population was 7.8%, which is lower than the 18% reported among adolescents in the USA,[38] and 13.4% reported among adolescents who participated in the South African NHANES-1.[39] HIV can cause muscle wasting and chronic morbidities, eventually resulting in weight loss,[40] which may explain the differences in obesity rates between our YLPHIV and the general South African adolescent population. Also, we observed a low prevalence of smoking in our study population, which may be attributable to under-reporting. South African adolescents generally report beginning to smoke at about 18 years old.[41]

Among YLPHIV, we found that male participants were more likely to have elevated CA and AA PDAY, similar to other studies.[13,18] Several studies have also evaluated sex differences in cardiometabolic outcomes among YLPHIV. In one study, investigators found significantly higher cholesterol levels in males relative to female participants.[25] In another three studies, no sex differences in lipodystrophy or insulin resistance were reported,[29-30] whereas in others, female participants had a body fat distribution associated with increased CVD risk.[31-32]

Consistent with previous studies,[18,42-43] we demonstrated the independent contribution of detectable VL on cardiovascular risk (particularly CA risk) in our findings. A recent study of youth living with HIV found that for every 1 000 copies/mL increase in VL, there was a 38% increase in the likelihood of having CVD.[44] This risk was independent of ART. However, in this study, an association between CD4 count and CVD risk was not observed, which is similar to our study.[44] Similar trends between VL and an elevated AA PDAY were observed but did not reach statistical significance, likely because of our small sample size. Another explanation could be that the development of atherosclerosis may occur earlier for coronary arteries than for the aorta because of site-specific differences in haemodynamics.[45] The coronary arteries have more turbulent flow than the aorta, which has more laminar flow and lowers shear stress.[45-46]

The duration of ART was also found to be a factor associated with elevated CA PDAY, which partially may be explained by the fact that ART duration is associated with dyslipidaemia and chronic inflammation.[47-49] Persistent immunological activation and systemic inflammation play a key role in HIV pathogenesis, along with CVD risk in

untreated and treated individuals with HIV.[50-51] Additionally, evidence of lipid abnormalities caused by the longer duration of ART, an elevated inflammatory system, metabolic variables, and immunosenescence all contribute to the development of vascular disease and eventually atherosclerosis.[8-9] In the PHACS, researchers also found an association of elevated CA PDAY with the longer duration of PI-based ART.[18] Almost half of our study population was on PI-based ART, and unadjusted models showed a relationship between PI-based ART, though this association did not persist in adjusted models.

The cross-sectional design of our study had limitations, precluding causal inference. Additionally, YLPHIV are unique in their lifetime exposure to HIV, and as a result, have often received ART for most of their lives. Findings in our study may not be generalizable to youth with more recently acquired HIV, shorter duration of ART, or receipt of newer ART. Because of the small sample size of HIV-seronegative adolescents, we could not directly assess whether HIV infection status was associated with CVD risk. Furthermore, we could not validate the PDAY scoring system against non-invasive measures of atherosclerosis or CVD events or in an African adolescent population.

Although the PDAY score possibly underestimates the risk of atherosclerotic lesions in YLPHIV, it is probably a consequence of the independent effects that HIV and ART have in the development of atherosclerosis.[52] Some studies have suggested that risk scores integrating traditional and HIV-specific parameters, including VL, type of ART, and CD4 count in adults with HIV, may better predict CVD risk in HIV populations.[53-54]

CONCLUSION

A substantial proportion of YLPHIV in Cape Town, South Africa, have PDAY scores indicating an increased aggregate risk for atherosclerosis. Viraemia and lifetime ART duration contribute to this risk, highlighting the importance of HIV control and monitoring cardiometabolic health. Also, including future studies to understand how ART impacts atherosclerotic risk in YLPHIV. Validated screening for CVD risk among YLPHIV could be beneficial through implementing a comprehensive assessment and treatment plan.

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Table 4.1. Characteristics of the study population

	HIV-seronegative N = 32	YLPHIV N = 218	P value
Demographics			
Age, years	17.1 (16.2, 17.9)	16.8 (15.9, 17.8)	0.34
Male (n, %)	14 (43.8)	102 (46.8)	0.75
Family history of diabetes/hypertension/hyperlipidaemia (n, %)			
No	4 (12.5)	59 (26.1)	<0.01
Yes	1 (3.1)	80 (36.7)	
Unknown	27 (84.4)	79 (36.2)	
Anthropometric Measures			
BMI z-score	-0.1 (-0.5, 0.4)	-0.4 (-0.7, -0.1)	0.08
Waist: hip ratio	0.9 (0.8, 0.9)	0.9 (0.8, 0.9)	0.08
Waist: height ratio	0.5 (0.4, 0.6)	0.5 (0.4, 0.5)	0.18
Triceps skinfold z-score	-0.4 (0.7, 0.2)	-0.6 (-0.8, -0.2)	0.01
Subscapular skinfold z-score	-0.2 (-0.6, 0.9)	-0.4 (-0.7, -0.03)	0.09
Tanner stage, (n, %)			
I	0	1 (0.5)	0.18
II	0	2 (0.9)	
III	0	22 (10.1)	
IV	4 (12.5)	57 (26.2)	
V	21 (65.6)	115 (52.8)	
Missing	6 (18.8)	21 (9.6)	
Laboratory measures			
hs-CRP (mg/L)	0.9 (0.4, 3.3)	1.4 (0.5, 5.0)	0.14
Fasting lipids			
Triglycerides (mmol/L)	0.6 (0.5, 0.7)	0.8 (0.6, 1.0)	<0.01
Total Cholesterol (mmol/L)	3.2 (2.8, 3.9)	3.5 (3.1, 4.1)	0.04
LDL (mmol/L)	1.6 (1.1, 2.4)	1.8 (1.5, 2.3)	0.27
HDL (mmol/L)	1.3 (1.0, 1.5)	1.3 (1.1, 1.6)	0.38
Fasting glucose (mg/dL)	79.2 (75.6, 84.6)	82.8 (77.4, 88.2)	0.06
Glycaemia (fasting) (mg/dL)			
Normal glucose <100	31 (96.9)	213 (97.7)	0.83
Impaired glucose 100-125	1 (3.1)	4 (1.8)	
Diabetes >125	0	1 (0.5)	
HIV-related laboratory measures			
Age at initiation of ART (years)	--	5.1 (2.9, 8.2)	

Age at initiation of ART (n, %)		
0-2 years	--	59 (27.6)
3-5 years	--	66 (30.8)
6-14 years	--	89 (41.6)
Duration on ART (years)	--	11.7 (8.3, 13.9)
Current ART regimen, (n, %)	--	
2 NRTI + NNRTI	--	124 (56.9)
2 NRTI + PI	--	94 (43.1)
Viral load (copies/mL; n, %)	--	
≤50	--	133 (65.5)
>50	--	70 (34.5)
Missing	--	15
Log_{10} HIV viral load (median, IQR)	--	1.6 (1.6, 2.0)
Peak viral load (copies/mL; n, %) (since enrolment)	--	
≤50	--	86 (39.5)
51-10 000	--	93 (42.6)
>10 000	--	39 (17.9)
Viral suppression	--	
Sustained viral suppression	--	83 (38.1)
Transient viremia	--	118 (54.1)
Sustained viremia	--	17 (7.8)
CD4 count (cells/µL; n, %)	--	
<200	-	11 (5.2)
200-499	--	61 (28.9)
500-1 000	--	124 (58.8)
>1 000	--	15 (7.1)
Missing	--	6
Nadir CD4 count (cells/µL; n, %) (since enrolment)	--	
<200	--	22 (10.1)
200-499	--	88 (40.4)
500-1 000	--	103 (47.3)
>1 000	--	5 (2.3)
WHO HIV staging (n, %)	--	
I	--	16 (7.7)
II	--	21 (10.1)
III	--	125 (59.8)
IV	--	47 (22.5)
Missing	--	9

All continuous variables are expressed as medians (interquartile range) or means (SD) and categorical variables as n (%).
Abbreviations: ART, antiretroviral therapy; BMI, body mass index; HDL, high-density-lipoprotein cholesterol; HIV, human immunodeficiency virus; hs-CRP, highly sensitive C-reactive protein; LDL, low-density-lipoprotein cholesterol; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; WHO, World Health Organization; YLPHIV, youth living with perinatally acquired HIV.

Table 4.2. Distribution of Modifiable Atherosclerotic Risk Factors Included in the PDAY Scoring System

Modifiable risk factor	Coronary artery points	Abdominal aorta points	HIV-seronegative N =32	YLPHIV N = 218	P value
Non-HDL cholesterol, mg/dL					
<130	0	0	30 (93.8)	206 (94.5)	0.06
130–159	2	1	1 (3.1)	9 (4.1)	
160–189	4	2	0	3 (1.4)	
190–219	6	3	1 (3.1)	0	
≥220	8	4	0	0	
HDL cholesterol, mg/dL					
<40	1	0	9 (28.1)	42 (19.3)	0.51
40–59	0	0	16 (50.0)	125 (57.3)	
≥60	-1	0	7 (21.9)	51 (23.4)	
Smoking					
No	0	0	27 (84.4)	193 (88.3)	0.50
Yes	1	4	5 (15.6)	25 (11.5)	
Blood pressure					
Not hypertensive	0	0	32 (100)	214 (98.2)	0.44
Hypertensive	4	3	0	4 (1.8)	
BMI (obesity), kg/m^2					
Male					
≤30	0	0	14 (100)	100 (98.0)	0.60
>30	6	0	0	2 (2.0)	
Female					
≤30	0	0	11 (61.1)	101 (87.1)	0.01
>30	0	0	7 (38.9)	15 (12.9)	
Fasting glucose, mg/dL					
Not hyperglycemic (<125)	0	0	32 (100)	217 (99.5)	0.70
Hyperglycemic (≥125)	5	3	0	1 (0.5)	
Total PDAY score					
PDAY score ≥1 for CA lesion	-	-	10 (31.3)	66 (30.3)	0.91
PDAY score ≥1 for AA lesion	-	-	7 (21.9)	40 (18.4)	0.63

AA, abdominal aorta; BMI, body mass index; CA, coronary artery; HDL, high-density lipoprotein; PDAY, pathobiological determinants of atherosclerosis in youth.

*Adapted from McMahan et al 2005.18 Copyright © 2005, American Medical Association. All rights reserved.

†Smoking was defined as smoking an average of at least one pack per day in the past three months.

‡Hypertensive was defined as an average systolic or diastolic blood pressure ≥95th percentile for age, sex, and height.

§Hyperglycemic was defined as a fasting plasma glucose concentration ≥125 mg/dL.

Table 4.3a. Unadjusted and adjusted odds ratios for factors associated with elevated coronary artery PDAY score among YLPHIV

Variable	Unadjusted odds ratio (95% CI)	P value	Adjusted odds ratio (95% CI)	P value
Age at 48-month visit (years)	1.22 (0.97, 1.55)	0.10	1.14 (0.86, 1.51)	0.36
Sex				
Female	Reference		Reference	
Male	2.45 (1.35, 4.43)	<0.01	2.14 (1.32, 4.05)	0.02
Viral suppression				
Sustained viral suppression	Reference		Reference	
Transient viremia	2.43 (1.22, 4.86)	0.01	2.10 (1.01, 4.82)	0.04
Sustained viremia	16.0 (4.55, 56.4)	<0.01	18.4 (4.40, 76.70)	<0.01
Duration on ART, (years)	1.06 (0.97, 1.17)	0.21	1.12 (1.01, 1.25)	0.03
Current ART regimen				
2 NRTI + NNRTI	Reference		Reference	
2 NRTI + PI	2.13 (1.18, 3.82)	0.01	1.11 (0.55, 2.25)	0.77

ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; YLPHIV, youth living with perinatally acquired HIV.

Table 4.3b. Unadjusted and adjusted odds ratios for factors associated with elevated abdominal aorta PDAY score among YLPHIV

Variable	Unadjusted odds ratio (95% CI)	P value	Adjusted odds ratio (95% CI)	P value
Age at 48-month visit (years)	1.23 (0.93, 1.63)	0.14	1.11 (0.80, 1.53)	0.55
Sex				
Female	Reference		Reference	
Viral suppression				
Sustained viral suppression	Reference		Reference	
Transient viremia	1.28 (0.59, 2.77)	0.53	1.00 (0.41, 2.44)	0.99
Sustained viremia	4.14 (1.32, 13.0)	0.02	3.24 (0.83, 12.7)	0.09
Duration on ART, (years)	1.06 (0.95, 1.19)	0.30	1.08 (0.96, 1.22)	0.18
Current ART regimen				
2 NRTI + NNRTI	Reference		Reference	
2 NRTI + PI	1.80 (0.90, 3.59)	0.10	1.12 (0.55, 2.93)	0.57

ART, antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; YLPHIV, youth living with perinatally acquired HIV.

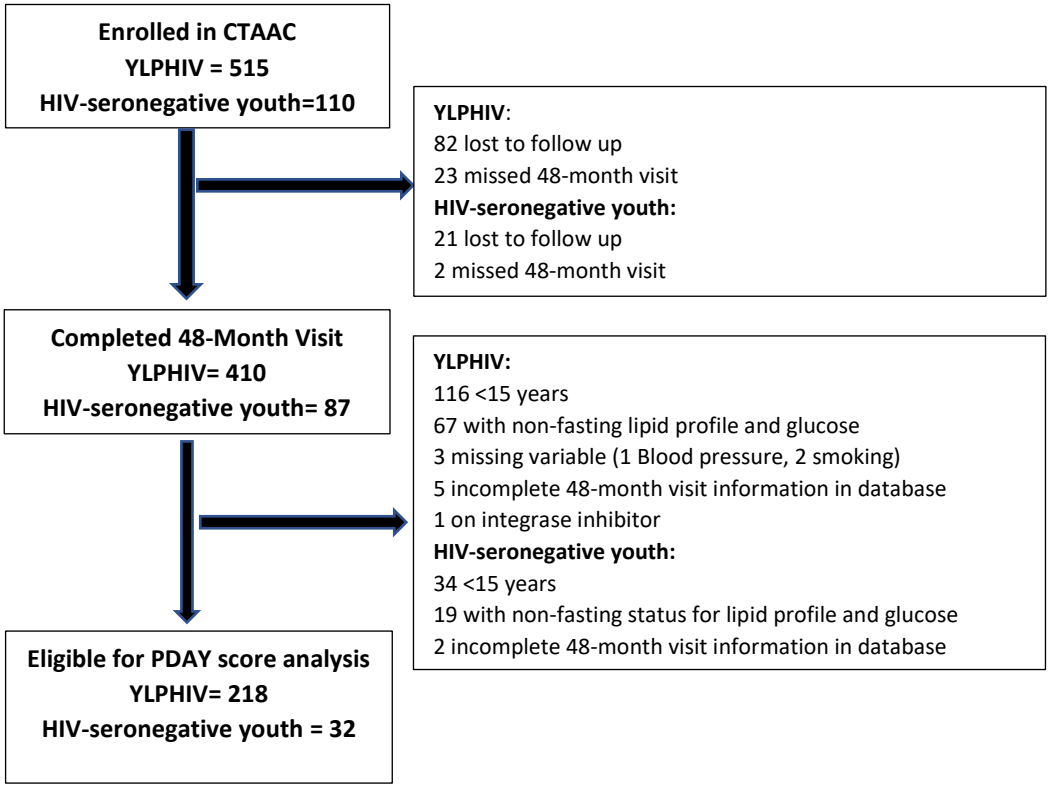

Figure 4.1. Study population derivation

YLPHIV= youth living with perinatally-acquired HIV

Supplemental Table 4.1. Distribution of modifiable atherosclerotic risk factors by PDAY score among YLPHIV

Modifiable risk factor	Total	Coronary artery score		Abdominal aorta score	
	N=218	Low (≤0) N=152	Elevated (≥1) N= 66	Low (≤0) N=178	Elevated (≥1) N=40
Non-HDL cholesterol, mg/dL					
<130	206 (94.5)	152 (100)	54 (81.8)	178 (100)	28 (70.0)
130–159	9 (4.1)	0	9 (13.6)	0	9 (22.5)
160–189	3 (1.4)	0	3 (4.6)	0	3 (7.5)
190–219	0	0	0	0	0
≥220	0	0	0	0	0
HDL cholesterol, mg/dL					
<40	42 (19.3)	0	42 (63.6)	31 (17.4)	11 (27.5)
40–59	125 (57.3)	107 (70.4)	18 (27.3)	107 (60.1)	18 (45.0)
≥60	51 (23.4)	45 (29.6)	6 (9.1)	40 (22.5)	11 (27.5)
Smoking					
No	193 (88.5)	154 (98.1)	51 (82.3)	178 (100)	15 (37.5)
Yes	25 (11.5)	3 (1.9)	11 (17.7)	0	25 (62.5)
Blood pressure					
Not hypertensive	214 (98.2)	152 (100)	62 (93.9)	178 (100)	36 (90.0)
Hypertensive	4 (1.8)	0	4 (6.1)	0	4 (13.3)
BMI (obesity), kg/m^2					
Male					
≤30	100 (98.0)	61 (100)	39 (95.1)	73 (100)	27 (93.1)
>30	2 (2.0)	0	2 (4.9)	0	2 (6.9)
Female					
≤30	101 (87.1)	80 (87.9)	21 (84.0)	93 (88.6)	8 (72.7)
>30	15 (12.9)	11 (12.1)	4 (16.0)	12 (11.4)	3 (27.3)
Fasting glucose, mg/dL					
Not hyperglycaemic (<125)	218 (99.5)	152 (100)	65 (98.5)	178 (100)	39 (97.5)
Hyperglycaemic (>=125)	1 (0.5)	0	1 (1.5)	0	1 (2.5)

BMI, body mass index; HDL, high-density lipoprotein; and PDAY, pathobiological determinants of atherosclerosis in youth.

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†Smoking was defined as smoking an average of at least one pack per day in the past three months.

‡Hypertensive was defined as an average systolic or diastolic blood pressure ≥95th percentile for age, sex, and height.

§Hyperglycaemic was defined as a fasting plasma glucose concentration ≥125 mg/dL.

Chapter 5:

Prevalence and predictors of bone health among perinatally HIV-infected adolescents

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Keywords: antiretroviral therapy, bone health, bone mineral density, calcaneus stiffness index, HIV, perinatally HIV-infected adolescents

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Abstract

Objectives: Long-term complications of HIV including low bone mineral density remain a concern. We studied the prevalence and predictors of low low bone mineral among South African perinatally HIV-infected adolescents (PHIVA) on antiretroviral therapy (ART).

Design: Cross-sectional analysis.

Methods: Bone health was evaluated by measuring the calcaneus stiffness index among PHIVA on ART. Low stiffness index was defined as z-score less than –2 SD compared to age-matched and sex-matched HIV-uninfected adolescents (HIV–).

Result: Overall, 407 PHIVA (median age: 14 years; 50.4% female; median age at ART initiation: 4.2 years) and 92 HIV– (median age: 13.7 years; 54.4% female) were included. Median duration on ART was 9.8 years (interquartile range 6.8–11.5) with 38% initiating ART at 2 years or less of age. Stiffness index was lower in PHIVA (–0.19 vs. 0.43, $P \leq 0.001$), respectively. During puberty, mean stiffness index increased with Tanner Stage in both PHIVA and HIV– but these increases were larger among HIV–; Tanner Stage II–III (96 vs. 101, $P=0.009$) and Tanner Stage IV–V (104 vs. 112, $P=0.001$). Among PHIVA, 52 (13%) had low stiffness index. After adjusting for age, sex and Tanner Stage, use of lopinavir/ritonavir [odds ratio (OR)=2.31, $P=0.012$) and viral load more than 50 copies/ml (OR=2.06, $P=0.023$) were associated with increased risk of low stiffness index, while use of efavirenz (OR=0.41, $P=0.009$) was associated with decreased risk of low stiffness index.

Conclusion: Stiffness index was significantly lower in PHIVA than in HIV–, especially during puberty. Among PHIVA, detectable viral load and use of lopinavir/ritonavir were risk factors for low stiffness index. Further longitudinal studies are important to determine the clinical implications.

INTRODUCTION

Globally, there are an estimated 2.1 million adolescents who were perinatally infected and living with HIV infection.[1] Effective antiretroviral therapy (ART) has dramatically reduced morbidity and mortality for children with perinatal HIV infection, leading to most surviving into adolescence and adulthood.[2] Adolescence is an important period during which a significant amount of bone development happens with more than half of adult bone growth occurring during this time.[3] Lifetime exposure to both HIV infection and ART to perinatally HIV-infected adolescence (PHIVA) may impact on future osteoporosis and related complications.[4, 5] However, there is limited information on PHIVA bone health especially in sub-Saharan Africa which has the largest proportion of HIV-infected adolescents.

Low bone mineral density (BMD) is a common finding in HIV-infected adults.[6, 7] A meta-analysis showed that among HIV-infected adults, 52% had osteopenia and 15% had osteoporosis.[8] The presence of low BMD is well documented in HIV-infected adults. Little data are available for PHIVA, during this period of changing bone physiology and growth. Most studies are from high-income countries and report low BMD among PHIVA compared with uninfected adolescents.[9-11] Therefore, there is a critical need for data on PHIVA bone health from sub-Saharan Africa.

HIV-infected individuals may be at risk for low BMD as a result of chronic inflammation and/or promotion of osteoblast apoptosis and osteoclast proliferation by the HIV envelope glycoprotein.[12, 13] Other HIV-associated risk factors are uncontrolled viral load,[7] duration of HIV infection [14-16] or use of specific ARTs such as protease inhibitors[6, 17] or tenofovir disoproxil fumarate (TDF).[18] In addition, malnutrition, poor intake of calcium and vitamin D can also be a contributing factor.[7]

One of the reasons for limited data on bone health from lower and middle-income countries is the difficulty of measuring BMD. Dual-energy X-ray absorptiometry (DXA) is the gold standard but it is expensive and it requires specialized equipment and resources. Quantitative ultrasound (QUS) is an alternative method for measuring bone

health that is quick and affordable. Many studies have shown good correlation between use of QUS with DXA across all age groups.[19-22] We investigated bone health using QUS in PHIVA on ART and compared them with matched HIV-infected adolescents in a cohort study in Cape Town, South Africa, to determine which factors were associated with bone health outcomes in the PHIVA.

METHOD

Study Population

The Cape Town Adolescent Antiretroviral Cohort (CTAAC) is a South African prospective cohort study investigating the long-term health of PHIVA receiving ART for at least 6 months at the time of the enrollment. Enrollment occurred between July 2013 and March 2015. Participants were enrolled from eight sites in Cape Town together with age and sex frequency-matched HIV-uninfected adolescents (HIV–) as a comparison group.

Covariates

Sociodemographic data were collected from participants and caregivers at the time of enrollment, and each participants' clinical record was reviewed by a study clinician to record ART history, time of HIV diagnosis, WHO HIV staging at the time of diagnosis and other pertinent information.

Clinical examination included Tanner staging. Blood pressure and anthropometry were measured by a trained nurse. Three measurements for waist, mid-upper-arm and mid-thigh circumferences were taken using a vinyl tape and mean of these measurements were used. Weight was measured in kilograms (kg) on a Scales 2000 calibrated digital scale to the nearest 0.1 kg. The standing height was measured in centimeters (cm) using a calibrated stadiometer with a moveable headboard to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2), height and BMI was expressed in z-score based on WHO references.[23]

Laboratory measures included viral load (Roche COBAS Ampliprep/COBAS TaqMan HIV-1 Test, version 2.0-standard technique; Roche, Basel, Switzerland) and $CD4^+$ cell count (Beckman Coulter FC500 MPL analyzers;

Beckman Coulter, Milan, Italy) using the Pan Leukocyte Gating method. Highly sensitive C-reactive protein was measured using the Roche Cobas Tina-quant system (range, 0.1–20 mg/l). Alkaline phosphatase was processed on the Beckman (AU 480; Beckman, Brea, California, USA) using kinetic colour test principle. Fasting lipid sub fractions including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were measured in all participants. Abnormal TC, HDL and LDL were defined as more than 200, less than 35 and more than 130 mg/dl, respectively. Abnormal TG were defined as more than 110 mg/dl if age less than 10 years or more than 150 mg/dl if age at least 10 years.[24]

Bone Health Measurement

Bone health was evaluated on PHIVA and HIV– adolescents using an Achilles EXPII (GE Healthcare Chicago, Illinois, USA), a water-based calcaneal QUS device. Participants placed their foot on the foot pad of the device while in a seated position. Ultrasound waves were transmitted from the water-inflated transducer through the calcaneus to another transducer and the results were analyzed. Two measurements were taken of each foot. Between each measurement the foot was repositioned and the average of the two recordings was used in the analysis. The device generated three ultrasound parameters – speed of sound (SOS), broadband ultrasound attenuation (BUA), and calcaneal stiffness index. SOS is the time taken for ultrasound waves to travel through the calcaneus, whereas BUA is the slope of attenuation of the ultrasound signals. The stiffness index is a composite parameter [(0.67 × BUA) + (0.28 × SOS) – 420] that makes use of the SOS and BUA. Denser bone transmits ultrasound waves faster (indicated by a higher SOS value) and attenuates ultrasound signals at higher frequency (indicated by a higher BUA value), thus resulting in a higher stiffness index value which indicates better bone health. We only captured stiffness index as this is the composite of BUA and SOS which was calculated automatically by the ultrasound machine. All measurements were done by a single trained technician. Quality control calibration was performed at the beginning of each screening session.

Ethics

Written informed consent was obtained from a legal guardian and written informed assent from each participant. The study was approved by the Human Research Ethics Committees (051/2013) of the University of Cape Town and Stellenbosch University. Approval was also obtained from the Western Cape Provincial Health Research committee.

Statistical Analysis

Baseline variables were compared between groups using *t* tests, Wilcoxon, and chi-square tests as appropriate. BMI for age (BMIZ) and height-for-age (HAZ) z-scores were calculated using WHO references.[23] Tanner staging was categorized into three groups (Prepuberty = Tanner stage I, Early Puberty = Tanner stage II and III, Late Puberty = Tanner stage IV and V). Study age and sex frequency-matched HIV− adolescents were used to create a reference stiffness index z-score. Bone health was measured by stiffness index. Low stiffness index in PHIVA was defined as a z-score less than −2 SDs and the coefficient of variance was 0.14. Among PHIVA, multiple logistic and linear regression was used to examine the adjusted association between low stiffness index and both HIV-related and traditional risk factors. All the analyses were performed using statistical software Stata 14.2 (Stata Corp LP, College Station, Texas, USA).

RESULTS

Overall, 499 adolescents (407 PHIVA and 92 HIV−) had stiffness index recorded and were included in this analysis. Median age and sex distribution were similar in both groups (14.0 vs. 13.7 years, *P*=0.22 and female 50.4% vs. 54.4%, *P*=0.49, respectively). Tanner Stage distribution between PHIVA and HIV− were 16.5% vs. 12.2% for prepubertal, 39.5% vs. 45.5% for early puberty and 44.1% vs. 42.2% for late puberty, respectively, *P*=0.46.

Median BMIZ, HAZ, mid-thigh circumference and mid-upper-arm circumference were lower in PHIVA compared with HIV− (−0.19 vs. 0.43, *P*<0.01; −1.31 vs. -0.71, *P*<0.01; 39 vs. 42 cm, *P*<0.01; 22 vs. 23 cm, *P* <0.01, respectively).

Median alkaline phosphatase (ALP) was higher in PHIVA as compared with HIV– (283 U/l vs. 229.5 U/l, *P*<0.01) (Table 5.1).

The median age of ART initiation was 4.2 [interquartile range (IQR) 1.8–7.4] years and the median duration of ART was 9.8 (IQR 6.8–11.5) years, with 38% initiating ART at 2 years of age or less. Most PHIVA had well controlled HIV, with 317 (79%) having a $CD4^+$ count more than 500 cells/µl, whereas only 16 (4%) had a $CD4^+$ count less than 200 cells/µl (Table 5.2). One hundred and forty-eight (37.5%) had a viral load more than 50 copies/ml. There were 93 (23.7%) PHIVA who were WHO Stage IV at the time of HIV diagnosis and the start of ART.

The most common nucleoside reverse transcriptase inhibitors (NRTIs) were abacavir (ABC) and lamivudine (3TC) (68 and 84% of participants, respectively). Two hundred and sixty-five (65%) were on both ABC and 3TC; smaller proportions of adolescents were receiving tenofovir (TDF), zidovudine (AZT), emtricitabine (FTC) and stavudine (D4T) (13, 19, 12 and 2%, respectively). Over half (55.5%) of PHIVA were on a non-NRTI (NNRTI) regimen with most (97%) being on efavirenz (EFV) and only 3% on nevirapine (NVP). Thirty-six percent were receiving protease inhibitors in the form of lopinavir/ritonavir (LPV/r) (Table 5.1).

Mean stiffness index were lower in PHIVA as compared with HIV– (99 vs. 105.1, *P*<0.01) and 13% of PHIVA had low stiffness index. When stratified by Tanner Stage, the mean stiffness index between PHIVA and HIV– were similar (93 vs. 94, *P*=0.832) in Tanner Stage I. However, during puberty, mean stiffness index increased with Tanner Stage in both PHIVA and HIV– but there was more significant increase among HIV–; Tanner Stage II–III (96 vs. 101, *P*=0.009) and Tanner Stage IV–V (104 vs. 112, *P*=0.001) (Table 5.2 and Fig. 5.1).

In males, no difference was found between the PHIVA and HIV– groups in prepuberty and early puberty. However, in late puberty, PHIVA males had low mean stiffness index as compared with HIV– in late puberty stage (103.9 vs. 113.7, *P*=0.02). There were no HIV– females who were prepubertal. Among females in early puberty and late puberty, PHIVA had low mean stiffness index as compared with the HIV– group; (93.5 vs. 103.5, *P*<0.01 and 104.9 vs. 111.8, *P*=0.03, respectively) (Table 5.2).

Among PHIVA, 13% had low stiffness index (Supplementary Table, http://links.lww.com/QAD/B841). Table 5.3 shows the results of logistic regression modeling to identify predictors of low stiffness index in PHIVA children. Univariate analysis identified that having a viral load more than 50 copies/ml or current or ever use of LPV/r were significant risk factors. However, having high ALP, high $CD4^+$ cell count or previous and current use of EFV was associated with better stiffness index.

In multivariate analysis after adjusting for age, sex and Tanner Stage, ever use of LPV/r [odds ratio (OR)=2.31, *P*=0.012] and viral load more than 50 copies/ml (OR=2.06, *P*=0.023) were associated with increased risk of low stiffness index. However, ever use of EFV (OR=0.41, *P*=0.009) and low ALP (OR=0.99, *P* =0.02) were associated with better stiffness index (Table 5.3).

After adjusting each model for age, sex, HAZ and Tanner Stage, ever use of LPV/r (OR=2.18, *P*=0.02) and viral load more than 50 copies/ml (OR=1.96, *P*=0.04) were associated with low stiffness index. However, ever use of EFV (OR=0.40, *P*=0.01), $CD4^+$ count at least 500 cell/µl (OR=0.51, *P*=0.05) and low ALP (OR=0.99, *P*=0.02), were associated with better stiffness index (Table 5.3). Similar results were found when linear regression models were used (Table 5.4).

DISCUSSION

The current study has shown that PHIVA established on ART had lower stiffness index compared with HIV–. Among PHIVA, the use of protease inhibitors especially LPV/r and having uncontrolled HIV were associated with adverse bone health. This is one of the first studies to evaluate the prevalence and predictors of bone health among PHIVA on ART in sub-Saharan Africa.

We found low mean stiffness index among PHIVA compared with age and sex frequency-matched HIV–, similar to previous studies.[25, 26] However, the differences between groups could be due to the influence of bone size on stiffness index measurement, as our PHIVA were shorter and thinner than the HIV–. Thinner adolescents may have lower muscle mass exerting force on bone, resulting in lower bone mass, since the

difference was also arbitrated by weight.[27] Differences in body weight in HIV-infected adults largely explain differences in their bone mass.[28]

During puberty bone growth is at maximum, exposure to both HIV and ART, which may delay bone formation. Our findings of low stiffness index during puberty are similar to other studies from high-income countries,[29] in which no difference between HIV-infected and HIV-uninfected adolescents at Tanner Stages I–II for any skeletal outcome were found but there were differences in late Tanner Stages. They found more pronounced and statistically significant differences in boys as compared with girls. In another study, total body bone mineral content in 5–15-year-old HIV-infected girls was lower than age matched uninfected girls and the effect was more noticeable with increasing age.[9] However, our findings are in contrast with Arpadi *et al*.[30] in which prepubertal perinatally HIV-infected children had low stiffness index compared with uninfected children of similar age and sex, but of different ethnicity.

Association of low stiffness index with advanced or uncontrolled HIV is also consistent with studies that have shown that detectable viral load and low $CD4^+$ count are independent risk factors for low stiffness index.[26, 31, 32] Our results indicate that when there is increased ALP there is decreased risk of low stiffness index. This is a natural phenomenon as bone growth raises serum ALP levels. Therefore, the level of serum ALP activity is 1.5–2.5 times higher in growing children than in normal adults.[33] In general, decreased ALP activity has been observed in children with cessation of bone growth.[33] ALP is often high in HIV-infected patients on ART; however, it is unclear whether it is the result of specific ART or related to comorbidities seen in HIV-infected individuals.[34]

Many studies reported the association of protease inhibitors with increased bone turnover, accelerated bone loss and a higher prevalence of reduced BMD.[6, 35] Our study also showed low stiffness index among those PHIVA who had used LPV/r. This is similar to comparison studies of protease inhibitors vs. NNRTIs which have shown the association of protease inhibitors with a greater loss of BMD.[6, 17] However, there are other studies that have failed to show such a difference.[36, 37] In addition, we found the association of EFV with better stiffness index, similar to

a recent South African randomized trial study in which children who were switched to EFV had a higher BMD compared with those who remained on LPV/r.[38]

LPV/r is generally used in children as it is a highly effective inhibitor of HIV replication and an essential part of treatment in resource limited settings. LPV/r efficacy and safety has been proven by randomized trials among children.[39] Therefore, LPV/r has been part of the first-line ART regimen recommended by the WHO for young children.[40] Despite this, there are several known side effects from long-term use of LPV/r such as dyslipidemia and lipodystrophy [41]. Our results suggest the need to limit the use of LPV/r once viral suppression has been achieved and encourage the use of alternate ART for maintenance.[40, 42, 43]

Previous studies in HIV-infected adults and children have shown TDF was a risk factor for low BMD, but the effect in adolescence is unclear.[18, 35] This was not observed in our cohort, because only 66 (13%) PHIVA had used TDF. This pattern of use is expected because many adolescents in this cohort are relatively young and had not reached the age at which guidelines routinely recommend the transition to TDF.[44]

Our study shows the feasibility of performing QUS to measure stiffness index. Stiffness index is a good indicator of bone structure and several studies have proven high correlation between the stiffness index calculated by QUS and DXA measurements across all age groups from children to older adults.[19-22] Moreover, stiffness index is a better predictor of fracture risk than either BUA or sound of speed (SOS) alone.[45] Stiffness index also showed better correlation with DXA than BUA and sound of speed.[22] In a Chinese study, calcaneal stiffness index calculated by QUS correlated moderately well with total body BMD measured by DXA in both sexes.[46] In addition, studies on HIV-infected adults[47, 48] and children[49] also showed a good correlation between finding of QUS and DXA. Although DXA requires specialized equipment and expertise, stiffness index is relatively easy to measure and can be used longitudinally to evaluate bone development.

Limitations of this study include lack of standard reference ranges from Africa for stiffness index; we therefore derived, reference ranges from age and sex frequency matched HIV– adolescents from similar socioeconomic

backgrounds. However, the sample size of HIV– adolescents was small. Although there is good correlation between QUS and DXA, it is possible for individuals with normal QUS to have low BMD when measured by DXA. Furthermore, we did not capture BUA and SOS measurements separately which makes comparison with other studies difficult, but stiffness index is a composite of these two measurements, so indirectly reflects these. Another limitation is that there were no recordings of bone health prior to ART initiation. We were unable to compare the bone health results obtained via QUS with DXA due to lack of access and cost of DXA. Lastly, this is a cross-sectional study and therefore causal inference cannot be established; longitudinal studies are needed.

CONCLUSION

Differences between PHIVA and HIV– were more pronounced at late puberty. Among PHIVA, detectable viral load and use of LPV/r were risk factors for low stiffness index. This reduction in bone density is a long-term health concern for PHIVA. Longitudinal studies of BMD in a PHIVA cohort are essential to clarify risk factors and periods of greatest risk for poor BMD.

Recommendation

Currently, there are no recommendations for routine measurement of BMD in PHIVA. Based on the results from CTAAC, routine evaluation of BMD in PHIVA is indicated. Noninvasive techniques such as QUS technology provide an easy to use and cost-effective alternative to DXA and allow for changes in bone health to be followed in patients to better evaluate the role of disease-related conditions or treatments which may interfere with bone health during growth.

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Table 5.1. Clinical characteristics of study population

	HIV infected, n=407	HIV uninfected, n=92	P value
Demographics			
Age[a] (years)	14.0 (12.7-15.3)	13.7 (12.0–15.3)	0.22
Female, n (%)	205 (50.4)	50 (54.4)	0.49
African, n (%)	380 (93.4)	92 (100)	0.01
Growth measures			
Height z-score[b]	–1.31 (1.1)	–0.71 (0.9)	<0.01
BMI z-score[b]	–0.19 (1.2)	0.43 (1.2)	<0.01
Waist circumference[a] (cm)	66 (62–70)	68.5 (61–75)	0.08
Mid-thigh circumference[a] (cm)	39 (36–42)	42 (37–46)	<0.01
Mid-upper-arm circumference[a] (cm)	22 (20–24)	23 (21–25)	<0.01
Tanner Stage, n (%)			
Prepubertal (Tanner Stage I)	65 (16.5)	11 (12.2)	0.46
Early Puberty (Tanner Stage II–III)	156 (39.5)	41 (45.6)	
Late Puberty (Tanner Stage IV–V)	174 (44.1)	38 (42.2)	
Laboratory measures			
Calcium[b] (mmol/L)	2.4 (2.3–2.5)	2.4 (2.4–2.5)	0.02
Inorganic phosphate[a] (mmol/l)	1.4 (1.3–1.6)	1.4 (1.2–15)	0.10
Magnesium[a] (mmol/l)	0.83 (0.8–0.9)	0.84 (0.8–0.9)	0.24
Alkaline phosphatase[a] (U/l)	283 (197–371)	229.5 (132–295.5)	<0.01
Calcaneal Stiffness Index[b]	99.0 (13.4)	105.1 (15.2)	<0.01
HIV severity variables			
Viral load (copies/ml, n, %)			
≤50	247 (62.5)		
>50	148 (37.5)		
CD4+ count (cells/µl, n, %)			
<200	16 (4.0)		
200–499	68 (17.0)		
500–1000	263 (65.6)		
>1000	54 (13.5)		
WHO HIV staging, n (%)			
Stage I	25 (6.4)		
Stage II	35 (8.9)		
Stage III	240 (61.1)		
Stage IV	93 (23.7)		
Age at initiation of ART[a]	4.2 (1.8–7.4)		
Age at initiation of ART, n (%)			
0–2 years	152 (37.7)		
3–5 years	118 (29.3)		

6–14 years	133 (33.0)
Duration on ART[a] (years)	9.8 (6.8–11.5)
Current ART regimen, n (%)	
2 NRTI + NNRTI	226 (55.5)
2 NRTI + PI	136 (33.4)
Others	45 (11.1)
Current use of TDF, n (%)	
Yes	53 (13.0)
Ever use of TDF, n (%)	
Yes	55 (13.5)
Current use of EFV, n (%)	
Yes	227 (55.8)
Ever use of EFV, n (%)	
Yes	315 (77.4)
Current use of LPV/r, n (%)	
Yes	143 (35.1)
Ever use of LPV/r, n (%)	
Yes	210 (51.6)

ART, antiretroviral therapy EFV, efavirenz; LPV/r, lopinavir/ritonavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir.

[a]Median and interquartile range

[b]Mean and SD.

Table 5.2. Mean stiffness index difference between HIV infected and uninfected participants by Tanner stage

	n	HIV-infected mean stiffness index (SD)	*n*	HIV-uninfected mean stiffness index (SD)	*P*-value
Whole cohort					
Prepuberty (Tanner Stage I)	65	93.2 (9.3)	11	93.9 (9.0)	0.83
Early puberty (Tanner Stage II and III)	156	95.5 (12.7)	41	101.4 (13.7)	0.01
Late puberty (Tanner Stage IV and V)	174	104.4 (13.5)	38	112.4 (15.1)	<0.01
Male adolescents					
Prepuberty (Tanner Stage I)	55	93.7 (9.6)	11	93.9 (9.0)	0.96
Early puberty (Tanner Stage II and III)	67	98.1 (11.4)	17	98.5 (10.0)	0.89
Late puberty (Tanner Stage IV and V)	74	103.9 (12.7)	12	113.7 (18.1)	0.02
Female adolescents					
Prepuberty (Tanner Stage I)	10	90.5 (7.6)	0	---	---
Early puberty (Tanner Stage II and III)	89	93.5 (13.3)	24	103.5 (15.8)	<0.01
Late puberty (Tanner Stage IV and V)	100	104.9 (14.1)	26	111.8 (13.8)	0.03

Table 5.3. Logistic regression for stiffness index in perinatally HIV-infected adolescents (normal stiffness index vs. low stiffness index)

	Univariate		Multivariate[a]		Multivariate[b]	
Variables	OR [95% CI]	*P*	OR [95% CI]	*P*	OR [95% CI]	*P*
Age[c] (year)	1.18 [0.98, 1,42]	0.08	–		–	
Sex						
Male	Ref					
Female	0.98 [0.55, 1.76]	0.96	–		–	
BMI z-score[c]	0.84 [0.65, 1.08]	0.17	0.97 [0.73, 1.28]	0.82	0.98 [0.75, 1.30]	0.90
Height z-score[c]	0.76 [0.58, 1.00]	0.05	0.85 [0.62, 1.17]	0.33	–	
Waist[c] (cm	1.00 [0.97, 1.03]	0.91	1.01 [0.97, 1.04]	0.74	1.01 [0.97, 1.05]	0.63
Mid-thigh[c] (cm	1.00 [0.95, 1.06]	0.87	1.02 [0.95, 1.08]	0.61	1.08 [0.97, 1.20]	0.16
Mid-upper arm[c] (cm	1.02 [0.94, 1.12]	0.55	1.07 [0.96, 1.19]	0.20	1.02 [0.96, 1.09]	0.29
Tanner Stage, *n* (%)						
I	Ref					
II	1.09 [0.42, 2.81]	0.86	–		–	
III	0.96 [0.37, 2.49]	0.94	–		–	
IV	0.99 [0.39, 2.46]	0.98	–		–	
V	0.51 [0.17, 1.52]	0.23	–		–	
Calcium[c] (mmol/l)	3.11 [0.12, 77.62]	0.49	6.62 [0.23, 189.96]	0.27	11.3 [0.36, 358.90]	0.17
Inorganic phosphate (mmol/l)	0.94 [0.30, 2.90]	0.91	0.96 [0.53, 1.72]	0.89	0.78 [0.15, 1.15]	0.74
Magnesium[c] (mmol/l)	0.32 [0.002, 36.20]	0.64	0.17 [0.001, 26.91]	0.49	0.27 [0.002, 47.07]	0.62
Alkaline phosphatase[c] (U/l)	0.99 [0.994, 0.999]	0.04	0.99 [0.993, 0.999]	0.02	0.99 [0.993, 0.999]	0.02
Viral load, copies/ml						
<50	Ref					
≥50	2.07 [1.15, 3.75]	0.02	2.06 [1.11, 3.83]	0.02	1.96 [1.05, 3.67]	0.04
$CD4^+$ count, cells/µl						
≤499	Ref					
≥500	0.523 [0.28, 1.01]	0.05	0.53 [0.27, 1.05]	0.07	0.51 [0.26, 1.01]	0.05
WHO HIV staging						
Stage I–II	Ref					
Stage III–IV	1.76 [0.67, 4.64]	0.25	1.84 [0.67, 5.04]	0.23	1.71 [0.63, 4.68]	0.30
Age at initiation of ART (year)						

0–5	Ref					
6–14	0.74 [0.38, 1.42]	0.37	0.52 [0.24, 1.10]	0.09	0.49 [0.22, 1.06]	0.07
Current ART regimen						
2 NRTI + NNRTI	Ref					
2 NRTI + PI	1.78 [0.95, 3.37]	0.07	1.62 [0.83, 3.16]	0.16	1.56 [0.80, 3.07]	0.19
Current use of TDF						
No	Ref					
Yes	0.85 [0.35, 2.11]	0.73	0.56 [0.19, 1.64]	0.29	0.65 [0.22, 1.91]	0.43
Ever use of TDF						
No	Ref					
Yes	0.82 [0.33, 2.01]	0.66	0.53 [0.18, 1.52]	0.24	0.59 [0.20, 1.74]	0.35
Current use of EFV						
No	Ref					
Yes	0.59 [0.33, 1.06]	0.08	0.59 [0.32, 1.09]	0.09	0.57 [0.30, 1.06]	0.08
Ever use of EFV						
No	Ref					
Yes	0.45 [0.24, 0.84]	0.01	0.41 [0.21, 0.80]	0.01	0.40 [0.20, 0.78]	0.01
Current use of LPV/r						
No	Ref					
Yes	1.85 [1.03, 3.35]	0.04	1.71 [0.92, 3.20]	0.09	1.74 [0.93, 0.3.28]	0.09
Ever use of LPV/r						
No	Ref					
Yes	2.12 [1.14, 3.92]	0.02	2.31 [1.20, 4.47]	0.01	2.18 [1.13, 4.23]	0.02

ART, antiretroviral therapy; CI, confidence interval; EFV, efavirenz; LPV/r, lopinavir/ritonavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OR, odds ratio; PI, protease inhibitor; TDF, tenofovir.

[a]Continuous variable.

[b]Each model separately adjusted for age, sex and Tanner Stage.

[c]Each model separately adjusted for age, sex, height for age z-score and Tanner Stage.

Table 5.4. Linear regression for stiffness index z-score among perinatally HIV-infected adolescents

	Univariate		Multivariate[a]		Multivariate[b]	
Variables	Coefficient [95% CI]	*P*	Coefficient [95% CI]	*P*	Coefficient [95% CI]	*P*
Age[c] (year)	–0.14 (-0.28, –0.01)	0.04	–		–	
Sex						
Male	Ref		Ref		Ref	
Female	–0.39 (-0.81, 0.03)	0.07	–		–	
BMI z-score[c]	0.24 (0.06, 0.42)	0.01	0.23 (0.03, 0.42)	0.03	0.22 (0.02, 0.41)	0.03
Height z-score[c]	0.27 (0.07, 0.47)	<0.01	0.20 (–0.02, 0.43)	0.08	–	
Waist[c] (cm)	0.01 (–0.01, 0.03)	0.46	0.01 (–0.01, 0.39)	0.28	0.01 (–0.02, 0.04)	0.44
Mid-thigh[c] (cm)	–0.01 (–0.05, 0.03)	0.65	0.01 (–0.04, 0.05)	0.78	–0.01 (–0.05, 0.04)	0.95
Mid-upper arm[c] (cm)	0.02 (–0.05, 0.08)	0.66	0.03 (–0.05, 0.11)	0.45	0.02 (–0.06, 0.10)	0.69
Tanner Stage, *n* (%)						
I	Ref		Ref		Ref	
II	–0.02 (–0.74, 0.70)	0.96	–		–	
III	0.21 (–0.50, 0.91)	0.57	–		–	
IV	0.06 (–0.63, 0.75)	0.86	–		–	
V	–0.01 (–0.72, 0.71)	0.99	–		–	
Calcium[c] (mmol/l)	0.03 (–2.32, 2.38)	0.98	–0.44 (–2.83, 1.95)	0.72	–0.46 (–2.87, 1.94)	0.71
Inorganic phosphate (mmol/l)	–0.46 (–2.87, 1.94)	0.78	0.01 (–0.02, 0.03)	0.60	0.01 (–0.02, 0.02)	0.70
Magnesium[c] (mmol/l)	–0.01 (–0.03, 0.03)	0.98	0.00 (–0.03, 0.04)	0.90	0.01 (–0.03, 0.04)	0.88
Alkaline phosphatase[c] (U/l)	0.01 (0.00, 0.01)	<0.01	0.01 (0.00, 0.01)	0.04	0.01 (0.00, 0.04)	0.04
Viral load, copies/ml						
<50	Ref		Ref		Ref	
≥50	–0.42 (–0.87, 0.02)	0.06	–0.35 (–0.81, 0.11)	0.14	–0.28 (–0.74, 0.18)	0.23
$CD4^+$ count, cells/µl						
≤499	Ref		Ref		Ref	
≥500	0.63 (0.11, 1.16)	0.02	0.68 (0.15, 1.21)	0.01	0.67 (0.14, 1.20)	0.01
WHO HIV staging						
Stage I–II	Ref		Ref		Ref	

Stage III–IV	0.21 (–0.38, 0.81)	0.48	0.20 (–0.43, 0.82)	0.54	0.24 (–0.39, 0.87)	0.46
Age at initiation of ART (year)						
0–5	Ref		Ref		Ref	
6–14	–0.17 (–0.63, 0.28)	0.45	0.02 (–0.48, 0.52)	0.93	0.07 (–0.44, 0.58)	0.79
Current ART regimen						
2 NRTI + NNRTI	Ref		Ref		Ref	
2 NRTI + PI	–0.52 (–0.98, -0.06)	0.03	–0.56 (–1.03, –0.09)	0.02	–0.50 (–0.97, –0.03)	0.04
Current use of TDF						
No	Ref		Ref		Ref	
Yes	0.05 (–0.59, 0.69)	0.87	0.42 (–0.29, 1.14)	0.25	0.18 (–0.55, 0.92)	0.63
Ever use of TDF						
No	Ref		Ref		Ref	
Yes	0.06 (–0.57, 0.69)	0.85	0.43 (–0.27, 1.13)	0.23	0.21 (–0.51, 0.92)	0.57
Current use of EFV						
No	Ref		Ref		Ref	
Yes	0.53 (0.11, 0.95)	0.01	0.60 (0.17, 1.02)	0.01	0.56 (0.13, 0.99)	0.01
Ever use of EFV						
No	Ref		Ref		Ref	
Yes	0.40 (–0.10, 0.90)	0.12	0.42 (–0.10, 0.93)	0.12	0.42 (–0.10, 0.94)	0.11
Current use of LPV/r						
No	Ref		Ref		Ref	
Yes	–0.54 (–0.98, –0.10)	0.02	–0.56 (–1.02, 0.11)	0.02	–0.52 (–0.97, –0.06)	0.03
Ever use of LPV/r						
No	Ref		Ref		Ref	
Yes	–0.46 (–0.87, –0.03)	0.04	–0.55 (–0.98, –0.11)	0.01	–0.48 (–0.92, –0.05)	0.03

ART, antiretroviral therapy; CI, confidence interval; EFV, efavirenz; LPV/r, lopinavir/ritonavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir.

[a]Continuous variable.

[b]Each model separately adjusted for age, sex and Tanner Stage.

[c]Each model separately adjusted for age, sex, height for age z-score and Tanner Stage.

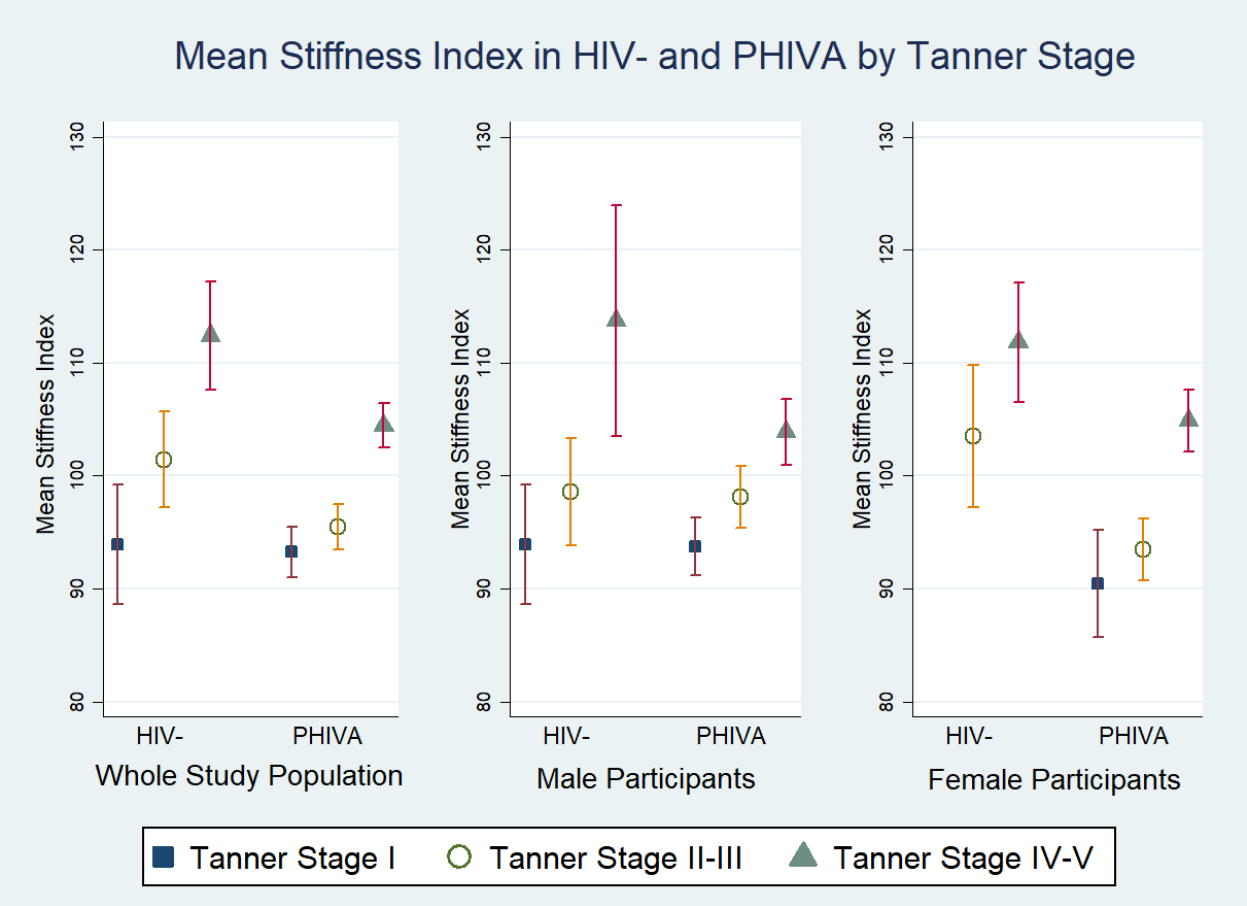

Figure 5.1. Mean stiffness index difference between HIV-infected and uninfected participants by Tanner Stage

Supplementary Table 5.1. Normal vs low stiffness index among PHIVA – (based on our controls as a reference to define normal vs low stiffness index)

	Normal stiffness index (n=355)	Low stiffness index (n=52)	p value
Demographics			
Age*, years	14.0 (12.8-15.1)	14.2 (12.3-16.4)	0.17
Female (n,%)	179 (50.4)	26 (50.0)	0.96
African (n,%)	331 (93.2)	49 (94.2)	0.79
Growth Measures			
Height z score[$]	-1.3 (1.0)	-1.6 (1.1)	0.05
BMI z score[$]	0.15 (1.2)	-0.41 (1.2)	0.17
Waist circumference* (cm)	66 (62-71)	67 (62-70)	0.97
Mid-thigh circumference* (cm)	39 (36-42)	39 (36-41)	0.97
Mid upper arm circumference* (cm)	22 (20-24)	22 (20-24)	0.78
Tanner stage			
Prepubertal (Tanner stage 1)	56 (16.2)	9 (18.0)	0.65
Early Puberty (Tanner stage 2 - 3)	134 (38.8)	22 (44.0)	
Late Puberty (Tanner stage 4 - 5)	155 (44.9)	19 (38.0)	
Laboratory measures			
Calcium* (mmol/L)	2.4 (2.3-2.5)	2.4 (2.4-2.5)	0.51
Inorganic Phosphate* (mmol/L)	1.4 (1.3-1.6)	1.5 (1.2-1.6)	0.83
Magnesium* (mmol/L)	0.8 (0.8-0.9)	0.8 (0.8-0.9)	0.50
Alkaline phosphatase* (U/L)	292 (199-379)	237 (182.5-332.5)	0.04
Viral Load, copies/ml (n, %)			
<50	223 (64.8)	24 (47.1)	0.01
≥50 copies/ml	121 (35.2)	27 (52.9)	
CD4 count, cells/uL (n, %)			
≤499	68 (19.4)	16 (31.4)	0.05
≥500	282 (80.6)	35 (68.6)	
WHO HIV staging (n,%)			
Stage I-II	55 (16.1)	5 (9.8)	0.25
Stage III-IV	287 (83.9)	46 (90.2)	
Age at initiation of ARVs (n,%)			
0-5 years	233 (66.2)	37 (72.6)	0.37
6-14 years	119 (33.8)	14 (27.4)	
Age at initiation of ARVs* (years)	4.2 (1.9-7.2)	4.1 (1.5-7.7)	0.69
Duration on ARVs* (years)	9.7 (6.8-11.3)	10.5 (7.2-11.8)	0.22
Current ARV regimen (n, %)			
2 NRTI + NNRTI	204 (57.5)	22 (42.3)	0.12
2 NRTI + PI	114 (32.1)	22 (42.3)	
Others	37 (10.4)	8 (15.4)	

Current use of TDF (n, %)			
Yes	47 (13.2)	6 (11.5)	0.73
Ever use of TDF (n, %)			
Yes	49 (13.8)	6 (11.5)	0.66
Current use of EFV (n, %)			
Yes	204 (57.5)	23 (44.2)	0.07
Ever use of EFV (n, %)			
Yes	282 (79.4)	33 (63.5)	0.01
Current use of LPV/r (n, %)			
Yes	118 (33.2)	25 (48.1)	0.04
Ever use of LPV/r (n, %)			
Yes	175 (49.3)	35 (67.3)	0.02

Legend: *Median and interquartile range. $Mean and Standard deviation; ART= antiretroviral therapy; BMI = body mass index; EFV= Efavirenz; LPV/r= Lopinavir/ritonavir; NNRTI = non-nucleoside reverse transcriptase inhibitor; NRTI = nucleoside reverse transcriptase inhibitor; PI= protease inhibitor; TDF= tenofovir.

Chapter 6:

The association between mental health and metabolic outcomes in youth living with perinatally acquired HIV in the Cape Town Adolescent Antiretroviral Cohort

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Keywords: youth living with perinatally acquired HIV, mental health, metabolic outcome, HIV, antiretroviral therapy.

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Abstract

Youth living with perinatally acquired HIV (YLPHIV) have been found to have a range of mental disorders. Some adult HIV studies have linked mental health to adverse metabolic outcomes due to dysregulation of the sympathetic nervous system and hypothalamic–pituitary–adrenal axis, but this association has not previously been explored in YLPHIV. We investigated the association of mental health measures with metabolic outcomes in YLPHIV and HIV-uninfected youth (HIV-U) and linear regression was used to assess the adjusted associations. Overall, 203 YLPHIV (median age = 10.7 years; 52% female; mean duration on ART 8 years, 12% CD4 count <500 cells/µL, 18% viral load >50 copies/mL) and 44 HIV-U (median age = 10.3 years; 55% female) were enrolled. YLPHIV had higher median total cholesterol (4.2 vs 3.9 mmol/L, $p = 0.049$) and triglyceride (0.9 vs 0.7 mmol/L, $p < 0.001$) compared to HIV-U. We found higher percentage of poor functional competence (40% vs 25%, $p = 0.02$) and self-concept (23% vs 9%, $p = 0.03$) and higher depression (6% vs 2%, $p < 0.01$), anger (6% vs 2%, $p = 0.04$) and disruptive behaviour (4% vs 0%, $p < 0.01$) in YLPHIV as compared to HIV-U. Among YLPHIV, higher scores of anger were associated with higher total cholesterol and higher low-density lipoprotein (ß = 0.010, $p = 0.041$ and ß = 0.012, $p = 0.048$ respectively) and disruptive behaviour with higher low-density lipoprotein (ß = 0.010, $p = 0.043$) after adjusting for age, sex and BMIZ. This study is one of the first to investigate the association of mental health with metabolic outcomes among YLPHIV. The association of increased anger and disruptive behaviour with increased lipid concentration is a novel finding. Further longitudinal studies are needed to evaluate the causal relationships between mental health and metabolic outcomes.

INTRODUCTION

Globally, an estimated 1.8 million youth are living with HIV,[1] with 229,000 in South Africa alone.[2] Increasing access to antiretroviral therapy (ART) has improved life expectancy and today a growing number of children living with perinatally infected HIV in South Africa and around the world are surviving into adolescence.[3] Adolescents living with HIV face unique health issues, particularly related to chronic complications of HIV and ART.

Youth living with perinatally acquired HIV (YLPHIV) on ART have increased mental illnesses,[4-6] studies have shown the relationships between metabolic outcomes and mental health, in healthy children[7] and in children living with HIV.[8-10] It has been shown that poor mental health is linked to a greater risk of premature all-cause mortality and morbidity compared to the general population across all age groups.[11] Furthermore, the life expectancy of patients with mental illness is affected;[12] individuals with mental illness have worse cardiometabolic outcomes, such as dyslipidaemia or diabetes.[11]

However, the effect of mental health on metabolic outcomes among YLPHIV is an understudied area, and the underlying mechanism for this relationship is not yet fully understood. Allostasis refers to a normal physiological process where the brain activates the sympathetic-adrenal-medullary and hypothalamic-pituitary-adrenal axes and stimulates adrenal glands to release stress hormones and catecholamine to combat against stress,[13] which can cause numerous deleterious effects on metabolic outcomes.[14-15] Additionally, psychologists suggested that long-term exposure to mental illness could alter an individual's perception of need which can associate with metabolic syndrome.[13]

Cape Town Adolescents Antiretroviral cohort (CTAAC) provides a unique opportunity to study the relationship between mental health and metabolic outcomes among YLPHIV.

METHOD

Study Population

YLPHIV and HIV-uninfected youth (HIV-U) were enrolled between July 2013 and March 2015. YLPHIV were recruited from 7 HIV clinics in Cape Town, South Africa.[16] Eligibility criteria for YLPHIV were aged 9–11 years, being aware of HIV status, on ART for at least 6 months at time of enrolment. HIV-U were healthy adolescents, frequency matched by age and sex by convenience sampling, and recruited from the Youth Center, which has a similar socioeconomic background as the sites where YLPHIV were recruited.

Adolescents were enrolled at the Research Centre for Adolescent and Children Health at Red Cross Children's Hospital, South Africa. Written informed consent was obtained from guardians and study participants provided written assent. The study protocol was approved by the Human Research Ethics Committee (051/2013) of the University of Cape Town and Stellenbosch University.

Sociodemographic data, participant's clinical record for ART history, laboratory measures including viral load [Roche COBAS Ampliprep/COBAS TaqMan HIV-1 Test, version 2.0] and CD4 cell count [Beckman-Coulter FC500 MPL Pan-Leukocyte Gating method], clinical examination including Tanner pubertal staging and anthropometry was collected at enrolment.

Metabolic Measures

The Homeostatic Model Assessment (HOMA) (fasting-insulin [mIU/L] x fasting-glucose [mmol/L]/22.5) was used to assess Insulin Resistance (IR) as per previous thresholds in the literature.[17] Fasting lipid subfractions including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), from enrolment.

Mental Health Measures

Mental health assessments were conducted at enrolment within 60 days of metabolic measures using [the] following scales BECK youth inventories, Children behaviour checklist (CBCL), Children's Motivation scale (CMS),

Conners' Parent rating scale (CPRs). These scales have been widely used globally and also have been successfully conducted in the context of South Africa among YLPHIV population.[5] Administration and description of these scales have been provided in a recently published manuscript from the CTAAC study.[5]

Statistical Analysis

Baseline variables were presented as number and percentages for categorical variables and for continuous variables presented as mean and standard deviation (SD) or median and interquartile range (IQR) as appropriate. HOMA was log transformed, abnormal lipids were defined using the NHANES.[18] Baseline variables and mental health measures were compared between groups by using Student's *t*-test or Wilcoxon rank-sum for continuous variables and χ2 or Fisher's exact tests for categorical variables. Missing data for HOMA has been checked; there was no difference in age, sex and BMI of adolescents who had HOMA vs who did not have HOMA. Other than HOMA variables, missing data were <5% and were missing at random, therefore, imputation was not performed.

Among YLPHIV generalized linear models were used to assess the unadjusted and adjusted association of mental health measure with each metabolic outcome. Each adjusted model was separately adjusted for age, gender and BMIZ. ß was presented as one SD change in mental health measure. All statistical analyses were performed using Stata 14.2 (StataCorp LP. College Station, Texas, USA).

RESULTS

Overall, 203 YLPHIV and 44 HIV-U were enrolled, no difference was found between YLPHIV and HIV-U regarding age and sex distribution (Table 6.1). YLPHIV were more likely to have had repeated grades at school (60% vs 41%), and have lost both biological parents (35% vs 14%). On average, YLPHIV were shorter, thinner and had low BMIZ (–0.14 vs 0.14, $p < 0.01$) than the HIV-U. (Table 6.1)

Median duration on ART was 8 years (IQR 5.5, 9.0) with 53% and 43% on non-nucleoside reverse transcriptase inhibitor and protease inhibitor based ART, respectively. Among YLPHIV, 12% had CD4 count <500 cells/uL, and

82% were virally suppressed. YLPHIV had higher median TC (4.2 mmol/L vs 3.9 mmol/L, $p = 0.05$) and TG (0.9 mmol/L vs 0.7 mmol/L, $p < 0.01$) compared to HIV-U. (Table 6.1).

Table 6.2 shows the difference in mental health measure by YLPHIV and HIV-U groups. YLPHIV had higher scores for CPRs total (11 vs 7, $p = 0.02$) and BECK depression (44 vs 40, $p < 0.01$) than HIV-U. However, YLPHIV had lower scores for Children's motivation scale (40 vs 45, $p < 0.01$), CBCL total competence (37 vs 41, $p = 0.01$) and BECK self-concept (47 vs 50.5, $p = 0.01$) than HIV-U.

Figure 6.1 presents the forest plot of adjusted linear regression of mental health measure by one SD for linear metabolic outcome, adjusted and unadjusted ß are presented as Supplementary Table 6.1. Among YLPHIV, higher scores for the BECK Anger Inventory were associated with significantly higher TC and higher LDL (ß 0.132, $p = 0.041$ and ß 0.097, $p = 0.048$, respectively). Higher scores of disruptive behaviour were associated with higher LDL (ß 0.112, $p = 0.043$) among YLPHIV. Furthermore, higher scores for CBCL-TCP were associated with lower log-HOMA (ß = 0.042, $p = 0.05$), all these associations were adjusted for age, sex, and BMIZ.

DISCUSSION

Our study found that YLPHIV have poorer mental health compared to HIV-U. When examining the mental health, YLPHIV had higher scores for CPRs and depression and had poorer scores for self-motivation, functional-competence and self-concept. Among the YLPHIV group greater anger was associated with higher TC and LDL, higher disruptive behaviour was associated with higher LDL, and higher CBCL-TCP was associated with low log-HOMA.

Our findings of poor functional-competence, poor self-concept, depression, anger, and disruptive behaviour among YLPHIV are consistent with previous studies which have found increased prevalence of mental issues among adolescents with HIV.[19-20] As chronic illness can interfere with mental health of youth and make them more vulnerable to psychological problems,[21] HIV in YLPHIV can make them vulnerable to mental illnesses.

The findings of an association between anger and disruptive behaviour with higher TC and LDL are consistent with prior work showing an association between these symptoms and the development of hyperlipidaemia, hypertension, and heightened sympathetic activity—leading to atherosclerosis among adult men and women.[22-24] It has been suggested that dysregulation of hypothalamic-pituitary-adrenal axis underlies both anger and adverse metabolic outcomes.[25] The effect of anger on the hypothalamus causes the release of stress hormones from the adrenal gland.[26] Dyslipidaemia induced by stress involves complex interactions among stress hormones, insulin, adipose tissue metabolism and cytokines.[27] Other research has suggested that an aggressive behaviour and anger is associated with higher TC, whereas a more socially positive mode of anger expression may be related to lower TC and LDL levels.[28] This relationship has not been studied in children living with HIV.

We found that greater CBCL-TCP is associated with lower IR. This conflicts with a prior literature showing a correlation between mental health and IR.[29-30] In adults, mental illness might be associated with poor dietary habits and physical inactivity, which may lead to weight gain and, therefore, having a greater risk of developing IR, diabetes and CVD.[31] However, in young participants and YLPHIV who are very lean this may not occur[32] and the relationships observed in the general adult population may not be the same as in YLPHIV.

The effect of metabolic outcome on mental health has been studied in adults and children, and it has been found that worse metabolic outcome results in worst mental health.[33-34] A study from children living with HIV suggested that metabolic abnormalities such as lipodystrophy in children with HIV lead to subsequent low self-esteem and worse mental health outcomes.[8] Furthermore, studies have shown that metabolic measure also effecting neurocognitive measures.[7] In our study, we explore the effect of mental health on metabolic outcome but as it's cross-sectional, so difficult to comment on causality.

A number of limitations deserve emphasis. First, YLPHIV are well established on ART for extended period, and results may not be generalisable to those who have shorter duration of treatment or no treatment. Second, as our data were cross-sectional, so difficult to comment on causality. Lastly, due to the small sample size of HIV-U,

we were unable to assess for effect modification by HIV-status on the relationships between mental health and metabolic outcome.

In summary, this is one of the first studies to investigate the association of mental health problems with metabolic outcome among YLPHIV in sub-Saharan Africa. YLPHIV with mental illnesses appear to have an increased risk of metabolic risk factors that can result in premature morbidity and mortality. Among YLPHIV, there is limited evidence in our cross-sectional study that greater anger and disruptive behaviour is strongly associated with metabolic outcomes, but further longitudinal studies are warranted to evaluate the long-term effect of mental health on metabolic health.

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Table 6.1. Baseline demographic and clinical characteristics of the cohort

Variable	*N*	YLPHIV	*N*	HIV-uninfected	*p*-value[#]
Demographics					
Age[*] (years)	203	10.7 (9.9, 11.4)	44	10.3 (9.7, 11.1)	0.09
Female[$]	203	106 (52.2)	44	24 (54.6)	0.78
Ethnicity: Black African[$]	203	187 (92.1)	44	44 (100)	0.05
Home language: isiXhosa[$]	203	177 (87.2)	44	41 (93.2)	0.15
Education in years*	203	3.2 (1.1)	44	3.4 (1.4)	0.34
Repeated grades[$]	203	121 (59.6)	44	18 (40.9)	0.02
Orphan-hood[$,a]	203	71 (35)	44	6 (14)	<0.01
Growth measures					
Height for age z score*	203	-1.2 (-1.9, -0.6)	44	-0.6 (-1.2, 0.9)	<0.01
Weight for age z score*	203	-0.9 (-1.4, -0.4)	44	-0.4 (-0.9. 0.5)	<0.01
BMI for age z score*	203	-0.1 (-0.8, 0.5)	44	0.1 (-0.4, 1.1)	<0.01
Waist circumference* (cm)	203	58.5 (56.0, 62.0)	44	59.8 (55.2,69.5)	0.14
Hip circumference* (cm)	203	66.5 (61.0, 71.0)	44	73.8 (66.8, 81.5)	<0.01
Mid-thigh circumference* (cm)	203	37.5 (35.0, 40.1)	44	42 (37.8, 45.3)	<0.01
Mid upper arm circumference* (cm)	202	19.5 (18, 21)	42	20.5 (19, 22)	0.03
Head circumference* (cm)	203	53 (51.5, 54)	44	53 (51.8,55)	0.33
Tanner staging	202		44		
Prepubertal (Stage I)		149 (73.8)		23 (52.3)	0.02
Pubertal (Stages II–V)		53 (26.2)		21 (47.7)	
Laboratory measures					
Log HOMA*	144	0.25 (0.3)	25	0.23 (0.40)	0.79
Insulin resistance	144		25		
Normal		113 (78)		20 (80)	
High		31 (22)		5 (20)	
Total Cholesterol* (mmol/L)	188	4.2 (3.6-4.8)	30	3.9 (3.4-4.3)	0.05
Hypercholesterolemia[$]		30 (16.0)		2 (6.7)	0.18
Triglycerides* (mmol/L)	190	0.9 (0.7-1.2)	30	0.7 (0.5-0.8)	<0.01
Hypertriglyceridemia[$]		36 (19.0)		1 (3.3)	0.03
HDL* (mmol/L)	188	1.5 (1.3-1.8)	30	1.5 (1.3-1.8)	0.88
Low HDL[$]		10 (5.3)		-	0.19
LDL* (mmol/L)	188	2.2 (1.8-2.7)	30	2.1 (1.6-2.5)	0.26
High LDL		13 (7.0)		2 (6.7)	0.96
HIV related variables					
Current ART regimen[$]	203				
2 X NRTI + NNRTI		107 (52.7)		--	--
2 X NRTI + PI		88 (43.4)		--	--
Others		8 (3.9)		--	--
Duration of ART* (years)	201	8.0 (5.5, 9.0)		--	--
Age of initiation* (years)	201	2.9 (1.5, 5.1)		--	--

Viral load[$] (copies/mL)	203			
≤50		167 (82.3)	--	--
>50		36 (17.7)	--	--
CD4 count[$] (cells/µL)	202			
<200		3 (1.5)	--	--
200-499		21 (10.4)	--	--
500-1000		131 (64.9)	--	--
>1000		47 (23.3)	--	--

All continuous variables expressed as median (interquartile range) or mean (standard deviation) and categorical variables as number (%), *continuous variable, [$] categorical variable, [a]orphan-hood was defined as the loss of both biological parents. [#]*p*-value were calculated by using Student's *t*-test or Wilcoxon rank-sum for continuous variables and χ2 or Fisher's exact tests for categorical variables.

ART, antiretroviral treatment; BMI, body mass index; HDL, high-density lipoprotein cholesterol; HOMA, Homeostatic Model Assessment to assess Insulin Résistance (IR); hs-CRP, highly sensitive C-reactive protein; LDL, low-density lipoprotein cholesterol; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; 2X NRTI+NNRTI, 2 nucleoside reverse transcriptase inhibitor with 1 non-nucleoside reverse transcriptase inhibitor; 2X NRTI+PI, 2 nucleoside reverse transcriptase inhibitor with 1 protease inhibitor; YLPHIV, Youth living with perinatally acquired HIV.

Table 6.2. Difference in mental health measure by YLPHIV and HIV-U

Outcome variable	YLPHIV (N=203)	HIV uninfected (N=44)	*P*-value*
Conners Total	11 (5, 23)	7 (2, 16.5)	0.02
Children's motivation scale	40 (33, 45)	45 (39.5, 50)	<0.01
CBCL[a] total competence	37 (32, 43)	41 (34, 47)	0.01
CBCL internalizing problems	58 (50, 66)	54 (50, 63)	0.12
CBCL externalizing problems	53 (46, 60)	51 (44, 57)	0.19
CBCL total problems	55 (49, 63)	53 (46, 59)	0.07
BECK[b] self-concept	47 (41, 52)	50.5 (43.5, 55.5)	0.01
BECK anxiety	54 (45, 63.5)	54 (46.5, 61.5)	0.95
BECK depression	44 (37, 56)	40 (36, 46)	<0.01
BECK anger	42 (35, 53)	40 (36, 47.5)	0.35
BECK disruptive behaviour	41 (38, 49)	41 (38, 49)	0.08

Median values are presented as a whole number and IQR in parentheses.
*Wilcoxon rank-sum test were used to determine the *p*-value.
[a]CBCL, Child Behaviours Checklist parent rated version.
[b]Beck Youth Inventory scale

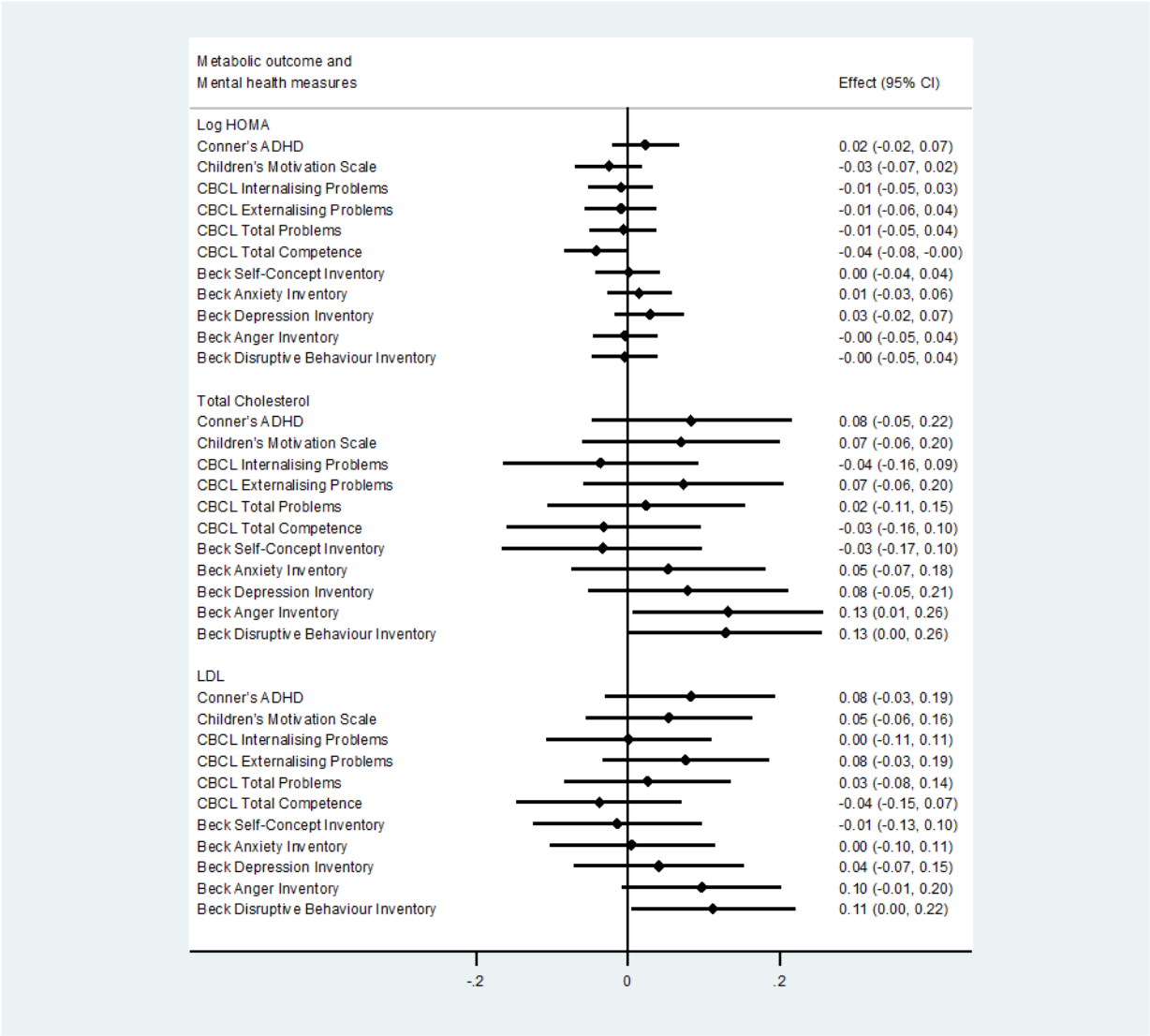

Figure 6.1. Forest plot showing the adjusted association between mental health measures and metabolic outcomes (Log HOMA, total cholesterol, LDL) among youth living with perinatally acquired HIV

ADHD, attention deficit hyperactivity disorder; BECK, Beck Youth Inventory scale; CBCL, Child Behaviours Checklist parent rated version; LDL, low-density lipoprotein. Each model shows the adjusted β coefficient (with 95% confidence interval) of the metabolic outcome (log HOMA, total cholesterol, and LDL) per one standard deviation increase of each mental health measure. All models separately adjusted for age, gender and body mass index z-score

Supplementary Table 6.1. Effect of mental health on biomarkers among YLPHIV

Variable	Unadjusted β [95% CI]	P-value	Adjusted β* [95% CI]	P-value
Log HOMA				
Conner's ADHD	0.0019 [-0.0015, 0.0052]	0.27	0.0016 [-0.0015, 0.0048]	0.31
Children's Motivation Scale	-0.0011 [-0.0066, 0.0044]	0.69	-0.0029 [-0.0080, 0.0022]	0.26
CBCL Internalising Problems	-0.0003 [-0.0047, 0.0040]	0.89	-0.0009 [-0.0050, 0.0032]	0.66
CBCL Externalising Problems	0.0007 [-0.0041, 0.0055]	0.77	-0.0009 [-0.0055, 0.0037]	0.69
CBCL Total Problems	0.0007 [-0.0036, 0.0051]	0.74	-0.0006 [-0.0047, 0.0035]	0.76
CBCL Total Competence	-0.0039 [-0.0097, 0.0019]	0.18	-0.0054 [-0.0108, -0.0001]	0.05
Beck Self-Concept Inventory	0.0014 [-0.0034, 0.0062]	0.56	0.0001 [-0.0046, 0.0046]	0.99
Beck Anxiety Inventory	0.0012 [-0.0025, 0.0049]	0.54	0.0012 [-0.0022, 0.0046]	0.49
Beck Depression Inventory	0.0020 [-0.0022, 0.0062]	0.34	0.0024 [-0.0015, 0.0062]	0.23
Beck Anger Inventory	-0.0006 [-0.0042, 0.0029]	0.73	-0.0003 [-0.0036, 0.0030]	0.84
Beck Disruptive Behaviour Inventory	-0.0010 [-0.0051, 0.0032]	0.65	-0.0004 [-0.0043, 0.0035]	0.83
Total Cholesterol				
Conner's ADHD	0.0046 [-0.0046, 0.0139]	0.33	0.0059 [-0.0034, 0.0153]	0.22
Children's Motivation Scale	0.0104 [-0.0042, 0.0250]	0.16	0.0081 [-0.0070, 0.0231]	0.29
CBCL Internalising Problems	-0.0039 [-0.0161, 0.0084]	0.54	-0.0034 [-0.0157, 0.0089]	0.59
CBCL Externalising Problems	0.0072 [-0.0054, 0.0199]	0.26	0.0071 [-0.0057, 0.0199]	0.28
CBCL Total Problems	0.0020 [-0.0100, 0.0141]	0.74	0.0022 [-0.0099, 0.0144]	0.73
CBCL Total Competence	-0.0017 [-0.0180, 0.0146]	0.84	-0.0041 [-0.0206, 0.0124]	0.63
Beck Self-Concept Inventory	-0.0039 [-0.0178, 0.0101]	0.58	-0.0036 [-0.0180, 0.0107]	0.62
Beck Anxiety Inventory	0.0036 [-0.0065, 0.0138]	0.48	0.0042 [-0.0059, 0.0144]	0.41
Beck Depression Inventory	0.0055 [0.0054, 0.0164]	0.32	0.0066 [-0.0044, 0.0176]	0.24
Beck Anger Inventory	0.0092 [-0.0006, 0.0190]	0.07	0.0103 [0.0005, 0.0201]	0.04
Beck Disruptive Behaviour Inventory	0.0111 [-0.0004, 0.0226]	0.06	0.0117 [0.0001, 0.0233]	0.05
Triglycerides				
Conner's ADHD	-0.0015 [-0.0061, 0.0031]	0.52	-0.0006 [-0.0052, 0.0040]	0.80
Children's Motivation Scale	0.0053 [-0.0019, 0.0125]	0.15	0.0037 [-0.0037, 0.0110]	0.33
CBCL Internalising Problems	-0.0038 [-0.0098, 0.0023]	0.22	-0.0033 [-0.0093, 0.0027]	0.28
CBCL Externalising Problems	0.0003 [-0.0060, 0.0065]	0.94	0.0005 [-0.0056, 0.0067]	0.87
CBCL Total Problems	-0.0015 [-0.0075, 0.0044]	0.62	-0.0012 [-0.0071, 0.0048]	0.70
CBCL Total Competence	0.0018 [-0.0063, 0.0099]	0.66	-0.00002 [-0.0082, 0.0081]	0.99
Beck Self-Concept Inventory	-0.0028 [-0.0097, 0.0041]	0.42	-0.0025 [-0.0094, 0.0045]	0.49
Beck Anxiety Inventory	0.0020 [-0.0030, 0.0070]	0.43	0.0026 [-0.0024, 0.0075]	0.31
Beck Depression Inventory	0.0032 [-0.0022, 0.0086]	0.24	0.0040 [-0.0013, 0.0093]	0.14
Beck Anger Inventory	0.0034 [-0.0015, 0.0082]	0.18	0.0040 [-0.0008, 0.0088]	0.11
Beck Disruptive Behaviour Inventory	-0.0005 [-0.0062, 0.0053]	0.87	-0.0003 [-0.0060, 0.0054]	0.93

HDL				
Conner's ADHD	0.0014 [-0.0030, 0.0058]	0.53	0.0009 [-0.0036, 0.0053]	0.70
Children's Motivation Scale	-0.0011 [-0.0080, 0.0058]	0.75	-0.00003 [-0.0071, 0.0071]	0.99
CBCL Internalising Problems	-0.0011 [-0.0069, 0.0048]	0.72	-0.0014 [-0.0073, 0.0044]	0.63
CBCL Externalising Problems	0.0006 [-0.0054, 0.0066]	0.84	0.0004 [-0.0056, 0.0065]	0.89
CBCL Total Problems	0.0016 [-0.0041, 0.0073]	0.58	0.0014 [-0.0044, 0.0071]	0.64
CBCL Total Competence	-0.0006 [-0.0082, 0.0071]	0.89	0.0006 [-0.0072, 0.0084]	0.88
Beck Self-Concept Inventory	0.0001 [-0.0065, 0.0066]	0.98	-0.0005 [-0.0073, 0.0063]	0.89
Beck Anxiety Inventory	0.0029 [-0.0019, 0.0077]	0.24	0.0025 [-0.0023, 0.0073]	0.30
Beck Depression Inventory	0.0019 [-0.0032, 0.0071]	0.47	0.0015 [-0.0037, 0.0067]	0.57
Beck Anger Inventory	0.0014 [-0.0032, 0.0061]	0.55	0.0011 [-0.0036, 0.0058]	0.65
Beck Disruptive Behaviour Inventory	0.0014 [-0.0041, 0.0069]	0.61	0.0014 [-0.0042, 0.0069]	0.63
LDL				
Conner's ADHD	0.0044 [-0.0034, 0.0123]	0.27	0.0059 [-0.0021, 0.0138]	0.15
Children's Motivation Scale	0.0088 [-0.0036, 0.0211]	0.16	0.0062 [-0.0064, 0.0189]	0.33
CBCL Internalising Problems	-0.0004 [-0.0109, 0.0099]	0.93	0.0001 [-0.0102, 0.0105]	0.98
CBCL Externalising Problems	0.0075 [-0.0033, 0.0182]	0.17	0.0074 [-0.0033, 0.0181]	0.18
CBCL Total Problems	0.0021 [-0.0081, 0.0124]	0.69	0.0024 [-0.0079, 0.0127]	0.65
CBCL Total Competence	-0.0022 [-0.0162, 0.0117]	0.75	-0.0048 [-0.0188, 0.0091]	0.50
Beck Self-Concept Inventory	-0.0022 [-0.0140, 0.0095]	0.71	-0.0015 [-0.0136, 0.0106]	0.81
Beck Anxiety Inventory	-0.0002 [-0.0088, 0.0084]	0.96	0.0004 [-0.0082, 0.0091]	0.92
Beck Depression Inventory	0.0023 [-0.0070, 0.0115]	0.62	0.0034 [-0.0059, 0.0127]	0.47
Beck Anger Inventory	0.0065 [-0.0018, 0.0148]	0.13	0.0076 [-0.0007, 0.0158]	0.07
Beck Disruptive Behaviour Inventory	0.0097 [-0.0001, 0.0195]	0.05	0.0102 [0.0004, 0.0200]	0.04

*each model separately adjusted for age, gender and body mass index z-score.
CBCL=Child Behaviour Checklist, HDL=high density lipo-protein, HOMA=Homeostatic Model Assessment, LDL= low density lipo-protein

Chapter 7:

Summary and Recommendations

1. Major findings

As the aim of this study, we investigated the spectrum and determinants of chronic comorbidities, encompassing (1) cardiovascular health – cardiac structure and function, endothelial function, the PDAY score, (2) bone health, (3) mental health and metabolic outcomes in YLPHIV on ART. A summary of the main findings follows.

1.1 Cardiac structure and function measured by using echocardiography

- At enrolment, total cholesterol, LDL and triglyceride levels were higher in YLPHIV than HIV-uninfected adolescents, probably reflecting the long-term use of ART.
- A low prevalence of cardiac structural and functional abnormalities was found among YLPHIV. Subtle echocardiographic differences were observed between YLPHIV and HIV-uninfected adolescents. These results show similarities with those found in the USA and Spain.[1-2]
- LV systolic dysfunction and LV dilation were rare, showing similar outcomes in the CHAART study.[3] However, these results showed a marked difference from those done in Africa on children and adolescents with low access to ART, reporting a higher prevalence of LV dilation and systolic dysfunction.[4-5] Among YLPHIV, LV hypertrophy was associated with late initiation of ART as reported in a study conducted in Zimbabwe, echoing the importance of starting ART early.[4]
- YLPHIV who had advanced WHO HIV staging at diagnosis were at higher risk of developing cardiac dysfunction. Contributing factors were that some participants might have initiated ART late, while others had untreated HIV since birth or received suboptimal ART formulations; therefore, justifying the need for early ART initiation and clinical care in perinatally infected children.

1.2 Peripheral endothelial dysfunction using the EndoPAT technique

- At the 24-month follow-up, YLPHIV were found to have a lower BMIZ (-0.2 vs 0.4, $p<0.01$) but higher rates of hypercholesterolaemia (10% vs 1%, $p=0.01$) compared with HIV-uninfected adolescents.
- Endothelial dysfunction was more prevalent among YLPHIV than in HIV-uninfected adolescents, similar to those in Europe and America, suggesting that a younger age group of YLPHIV may have presented

with vascular alterations, with a notion of early signs of microvascular disease or subclinical atherosclerosis.[6-7]

- No significant relationship was shown between the HIV-related variables and ED. Pathogenesis of HIV combined with ART played a significant role in altering endothelial function. Compared with HIV studies among adults,[8-9] a high ED prevalence was seen in uninfected children in paediatric studies.[6-7]

1.3 Progression of atherosclerosis using validated PDAY scores

- A substantial proportion of YLPHIV and HIV-uninfected adolescents had elevated PDAY scores showing an increased risk of atherosclerosis.
- Overall, a higher prevalence of elevated CA than AA PDAY scores (30.4% vs 18.8%, respectively) was observed in YLPHIV, in contrast to the CARDIA study.[10] Partially, this could be explained by the cross-sectional versus longitudinal nature and differences in the age range of study participants. The CARDIA study enrolled young adults 18-30 years old and followed them for 25 years, whereas participants in our study were 15-18 years old. PHACS, on the other hand, evaluated YLPHIV, also observing a higher prevalence of elevated CA PDAY and AA PDAY scores, similar to our study.[11] Among YLPHIV, we found that males were more likely to have elevated CA and AA PDAY scores, as reported in other studies.[11-12]
- The most elevated CA PDAY scores were related to abnormal HDL and non-HDL cholesterol among YLPHIV, similar to the PHACS cohort.[11] Adult studies among people living with AIDS or untreated HIV infection found that low levels of HDL cholesterol is often associated with inflammation and endothelial activation.[13-14]
- Detectable VL was associated with an elevated CA PDAY score and is consistent with previous studies.[11,15-17] Similar trends between detectable VL and an elevated AA PDAY were observed but did not reach statistical significance, probably because of our small sample size. Another explanation could be that the development of atherosclerosis may occur earlier in coronary arteries than in the aorta because of site-specific differences in haemodynamics.[18]
- Duration of ART was associated with elevated CA PDAY, which may be partially explained by the fact that the duration of ART is associated with dyslipidaemia and chronic inflammation.[19-21]

1.4 Musculoskeletal health evaluated by measuring the calcaneus stiffness index

- Youth living with perinatally acquired human immunodeficiency virus had a low SI compared with HIV-uninfected adolescents. Low SI is one of the emerging metabolic complications among people infected with HIV.[22] The effects of perinatal transmission of HIV on the bone mass acquisition, mineralisation and

growth in children and adolescents are of concern because of rapid bone growth from childhood to adulthood.[23]

- No difference in SI during pre-puberty among YLPHIV and HIV-uninfected adolescents was observed, but in late puberty, YLPHIV had a lower SI than the HIV-uninfected adolescents. This finding suggests that bone growth is not occurring at the same pace in YLPHIV and HIV-uninfected adolescents.
- Uncontrolled HIV, as evidenced by a high VL and a low CD4 count, was associated with a low SI. It is possible that uncontrolled HIV can increase specific proteins in the body that may contribute to bone loss. Another possibility is that the constant inflammation of the immune system caused by HIV may affect bone health, and HIV-infected cells in bone marrow may cause bone loss.[24]
- In a recent South African randomised control trial where children substituted with EFV had a higher BMD than those who remained on LPV/r, the association of EFV with improved SI was similar.[25]

1.5 Associated mental health and metabolic outcomes

- YLPHIV have poorer mental health compared with HIV-uninfected adolescents as they showed high scores of Conners' Parent rating scale (CPRs) and depression, and poorer scores of self-motivation, functional competence, and self-concept.
- Greater anger was associated with higher TC and LDL levels, high disruptive behaviour with higher LDL, and higher CBCL-TCP was associated with low log-HOMA. The findings between anger and disruptive behaviour were associated with higher TC and LDL levels, which impacted the development of hyperlipidaemia, hypertension and a heightened sympathetic activity leading to atherosclerosis.

Summary of the major findings:

Echocardiogram – "The echocardiographic changes were subtle and similar in prevalence to HIV-uninfected controls. However, these subclinical findings need to be followed, study has shown that echocardiogram subclinical finding are associated with high morbidities and mortality in future.[26] Therefore, echocardiography is a good screening and diagnostic tool and participants with subclinical finding needs to be followed up in clinical care with seral measurement to assess the change over time."

EndoPAT – The increased risk of ED among YLPHIV highlights that they are at risk of developing CVD in future. YLPHIVs need close monitoring for early detection of ED, which can be done with EndoPAT.

PDAY score – Among YLPHIV, the association of viraemia and longer duration on ART with a high PDAY score highlights the importance of HIV control and the association with cardiometabolic health. The PDAY score can easily be calculated by a clinician to detect those with a high PDAY score, who can then be monitored closely for possible interventions to reduce the burden of CVD.

QUS – Among YLPHIV, viraemia and the use of LPV/r were associated with low bone density, which may lead to the development of fractures. This highlights the importance of routine screening for bone health. QUS is an affordable, convenient method for screening, and patients with low bone density can be managed accordingly to reduce the risk of fractures.

Mental health –YLPHIV had a higher prevalence of mental illnesses associated with an increased risk of metabolic derangements. Mental health assessments should be done annually to identify illness that can be treated to prevent the adverse consequences of impaired mental health.

2. Strengths and Limitations

2.1 Strengths

The study participants were followed for an extended period, with high cohort retention. The YLPHIV cohort was on ART for a prolonged period and matched with HIV-uninfected adolescents from the same communities.

Extensive phenotyping was undertaken including echocardiography for cardiac structure and function, a widely used method of diagnosis and assessment.[27] Endothelial function was assessed using the reliable EndoPAT technique.[28] Bone health was investigated through QUS, a good correlation with DXA, which is the gold standard for measuring BMD.[29-32] Measures for mental health were collected using well-validated screening questionnaires, such as the CBCL and BECK youth inventory.[33-34] Additionally, reliable clinical data, including duration of ART and WHO HIV staging, were available at the time of enrolment. Laboratory findings, including HIV markers, such as VL, CD4 counts, and metabolic indicators, also provided additional mechanistic data.

2.2 Limitations

While 2-D ultrasound and current Doppler techniques are easily accessible, it would be preferable to have used more sensitive techniques such as speckle tracking[35] or cardiac magnetic resonance imaging to measure strain and strain rate, but these were not available during the study.

Validation of the PDAY scoring system against non-invasive measures of atherosclerosis or CVD events was lacking. However, the PDAY score may underestimate the risk of atherosclerotic lesions in this population because of the

independent effects of HIV and ART on development and progression of atherosclerosis. Furthermore, the PDAY score has not been validated among African adolescents.

Additionally, standard reference ranges for SI are lacking in Africa. Although our sample size of HIV-uninfected adolescents was comparatively small, we obtained reference ranges from age- and sex-frequency-matched HIV-uninfected youth living in similar socio-economic areas. There is a good correlation between QUS and DXA, though individuals with a normal QUS could have had a low BMD using DXA.

Another limitation of our study was not having measured physical activity levels, which can impact metabolic syndrome. Since participants were recruited from the same socio-economic communities, it was assumed that YLPHIV and HIV-uninfected adolescents' physical activity profiles would be comparable. Also, we could not establish lifetime ART use or all the VL measurements for all participants as data were incomplete. Thus, the accuracy of estimated associations between lifetime cumulative viraemia burden and comorbidities was hampered. A further limitation is that there were no measurements of comorbidities before ART initiation or enrolment in the study.

Because most of the adolescents were black Africans, the global applicability of our results was constrained; however, most YLPHIV in SSA are of black ancestry. Also, HIV-negative youth were enrolled from a youth centre with a similar socio-economic background as HIV-infected participants; however, there may be a bias from different residential locations. Establishing causal inference was limited by the cross-sectional nature of our analysis.

3. Generalizability

Findings cannot be generalized to settings with late ART initiation or where poor access to HIV services and low uptake of ART treatment exists. Priorities may differ in settings with limited access to ART and screening for subclinical disease. In more resource-limited settings in Africa, the early diagnosis of HIV and ART scale-up are prioritised above screening for chronic conditions. However, this is shifting gradually as ART coverage has been increased and people living with HIV are on ART for longer and longer period including children become adolescents as in our CTAAC cohort.

Our adolescent cohort started treatment at an early age, and over time participants were possibly exposed to varied risk factors resulting in differentiated outcomes. Therefore, our findings may be difficult to generalize. Also, results cannot be generalized to YLPHIV, who have a shorter duration on ART in other settings.[36] The cohort was relatively young (median age about 12 years) at enrolment, with a median age of initiation of ART of 4.3 years.

The results may not be generalizable to cohorts where ART is initiated in infancy or where different, current ART regimens may be used. The HIV uninfected adolescent cohort was a small sample, which limits generalizability.

4. Recommendations

- For subtle changes in cardiac structure, more sophisticated techniques, such as cardiac MRI and speckle tracking, are better to use rather than echocardiography for early detection and quick intervention of cardiac diseases in children with HIV disease.
- Screening and monitoring YLPHIV for signs of cardiometabolic disease, including ED, using non-invasive techniques, such as EndoPAT technologies is recommended in SSA, as it provides a reliable and straightforward method in assessing ED. Since ED is potentially reversible, this may have therapeutic and prognostic implications.
- Non-invasive techniques, such as QUS technology, provide a cost-effective alternative to DXA to monitor bone health.
- If possible, use alternate ART once viral suppression has been achieved. Use of LPV/r should be limited, as it is associated with low SI
- Metabolic derangements and hyperlipidaemia screening in YLPHIV is necessary and should occur in early adolescence at least annually. Regular weight measurement and preventive interventions to curb obesity that includes dietary and physical exercise guidelines, should be incorporated into their health-care visits.
- It is recommended that all YLPHIV undergo routine screening for neurocognitive impairment, which ideally should be initiated in early childhood. In the event of being missed earlier, screening for this impairment should be repeated in adolescence. Detecting neurocognitive impairment in childhood would allow early interventions for developing the capability of YLPHIV and encourage optimal adherence to ART.
- All-inclusive care should comprise HIV diagnosis and the management of comorbidities and resulting disabilities. Affordable or at no cost, dedicated HIV services should be available for these children and adolescents in an area neglected in Africa. Services at existing HIV clinics (HIV health platform) should be strengthened to undertake screening for comorbidities and provide management and treatment for these. All carers, guardians, teachers, and others involved in supporting this HIV-acquired population should be included in programmes involving optimal care in developing their full potential. Intervention programmes with meaningful engagement of HIV-acquired adolescents should be developed at health facilities or in the community.

General recommendations

- *Early ART Initiation*: YLPHIV are at a heightened risk of chronic comorbidities compared with age-matched HIV-uninfected adolescents. Prevention and early detection of HIV and other related comorbidities such as CVD, metabolic disorder and mental health and early initiation of ART will reduce the risk of advanced HIV and other related complications.
- *HIV and ART education*: YLPHIV's knowledge and understanding of HIV and the use of ART are of utmost importance. Therefore, they should be literate regarding HIV health and its consequences and ensure continued adherence to ART into adulthood. Parents, partners, guardians, and teachers would also need to be educated to support them throughout this journey.
- *Better monitoring of disease progression*: With the young perinatally HIV-acquired population on the increase and surviving into adolescence and early adulthood, integrated care through the health system should be intensified early on to cater for this vulnerable group.
- *Management of mental health issues*: Mental health has emerged as an important public health concern to monitor in YLPHIV. Thus, it is essential to include mental health screening in routine HIV services for adolescents. A comprehensive metabolic biomarker check-up in YLPHIV with mental health issues is recommended for early diagnosis and treatment.
- *Assessing health*: There is a need for health care workers to perform a comprehensive health assessment on YLPHIV to tailor packages specific to this population group. The existing evidence proposes sufficient effective interventions to promote safe health behaviours among adolescents.
- *Health services*: In South Africa, services have been rolled out within primary health-care settings to treat chronic diseases in adults, including HIV and screening of other diseases, and immediate linkages to treatment. Adaptation of interventions is key to addressing the gaps in health service delivery for adolescents in South Africa and other low-income countries.
- *Health service delivery*: Systematic ways of delivering services, which include adolescent-friendly care and support, should be delivered in conjunction with quality-of-care policies and standards. Understanding of local context is key to ensuring the applicability of health services in various settings. Standardising, assessing, and improving the youth-friendly service package and developing evidence-based training curriculum and service tools to equip the health-care workforce is key to sustainable service delivery.

- *HIV youth support group*: Creating youth-friendly support groups will provide a platform where YLPHIV could share their experiences, seek advice, and receive support from peers who are going through similar burdens. Facilitators who understand their needs should be available to guide them.
- *Monitoring implementation*: Health facilities are currently implementing essential components tailored to the needs of adolescents to scale up comprehensive management, including the psychosocial status and risk profile in this population group with predetermined standards and criteria. Monitoring these services is essential. Integration of HIV in comprehensive service delivery is key to enabling continuous monitoring of chronic comorbidities among YLPHIV in the future.

5. Implications

Implementing the mentioned recommendations will require substantial service and clinical delivery. Thus, there is an urgent need for cardiac, metabolic, and mental health screening, followed by treatment or other interventions. However, it is unknown what ideal screening for cardiac, bone, and mental health in this population entails. Based on our research, EndoPAT and QUS are non-invasive and feasible screening techniques that can be implemented in this population to detect early cardiac and bone changes.

Chronic disease clinics for adults already exist in South Africa, offering screening for common non-communicable diseases. Such screening should also be offered to adolescents at ART clinics. Based on our findings, YLPHIV need close monitoring for early detection of ED, which can be done with the EndoPAT technique. The risk for developing atherosclerosis can easily be calculated by a health care worker using the PDAY score, and youth with a high risk should be monitored closely for possible interventions to reduce the risk for cardiovascular disease. Using QUS will be very helpful for screening patients for low bone density as it is affordable to use, and patients with low bone density can easily be detected. Timely intervention will reduce the risk of fractures and future complications. Because of the high prevalence of mental illness among YLPHIV, annual mental health assessments should be done to identify and treat them to avoid adverse consequences. Metabolic derangement, including dyslipidaemia and insulin resistance among people living with HIV, might be the result of the HIV-specific changes within the body and lifetime exposure of HAART, which could lead to metabolic syndrome with increased CVD which should be monitored closely.

Along the lines of adult model in South Africa of chronic disease clinics, a similar strategy should be developed for adolescents at the clinics where they receive their ART. A factor to consider would be the need to upskill health care workers in the early detection of morbidities and intervention implications in YLPHIV for chronic conditions, as these community workers are mainly trained in HIV care and management only. However, this should be done

in consideration, not to burden already over-burdened health care professionals. Currently, gaps exist in monitoring and documenting basic vitals, such as blood pressure, anthropometry, glucose, and urine analysis; measurements not recorded at busy clinics with a young and 'healthy' patient population.

These kinds of frameworks could be used as an option to introduce a more comprehensive approach to health care. Several opportunities are available for implementing screening and prevention platforms for morbidities in the existing adolescent activities, but these might encounter many challenges. HIV is a health condition leading to frequent health-care visits, thus, many of the clinics initiated attending to health issues of 'adolescent groups'.

In sub-Saharan Africa, many HIV-infected children are reaching adolescence and adulthood. Because ART initiation is delayed, these children are at risk of developing early onset of comorbidities, conditions typically associated with ageing. Presently, HIV care comprises providing and maintaining adherence to ART, while chronic co-existing conditions associated with HIV-infected children tend to be neglected. Overburdened healthcare systems already suffer severe demands, and optimal screening and managerial strategies are vaguely defined, particularly since the COVID-19 outbreak.

In understanding the pathophysiological mechanisms behind HIV- and ART-associated comorbidities, the following steps will need to be identified: First, permanent damage to various organs is possibly caused during the initial often untreated phase of HIV infection. Second, the harmful outcome of multiple organs could be because of ongoing low-grade viral replication and chronic immune activation during ART. Third, although the improved outcome for most organ systems is provided by early and ongoing HIV treatment, various short- and long-term adverse effects have been associated with some ART.

6. Future research

Priority research to identify more sensitive biomarkers of disease is required. Other research studies on factors that influence Viral Suppression and Adherence to HIV treatment are needed in attaining viral suppression among YLPHIV. Further research and documentation of guidelines for standard reference ranges for SI and echocardiogram parameters in children and adolescents are vital in SSA, as there currently is limited scope.

The long-term prognosis of HIV-associated comorbidities is not yet known since perinatally HIV-acquired youth, who have been on lifetime ART, are only in their twenties. The complexity of pathophysiological mechanisms is poorly understood, and most studies have been done in HIV-acquired adults. Therefore, longitudinal cohort studies investigating known and suspected complications of chronic comorbidities in YLPHIV with ART are thus essential.

All ART have long-term toxicities – it's just a matter of what is known and what is selected among all the other factors that go into selecting agents for long-term therapies. Soon, all these adolescents would be on dolutegravir or cabotegravir – what would be the cardiac and metabolic implications – future research is needed to answer the safety and long-term complications of all the ARTs.

Initiating ART and suppressing HIV at the earliest possible can protect YLPHIV from developing chronic complications of HIV. The use of potentially anti-inflammatory drugs could be considered in patients presenting with complications associated with chronic HIV. However, further research should be done to confirm the long-term use of anti-inflammatory drugs in preventing the deterioration of their condition. Efforts should be made to provide adolescent care providers with the necessary knowledge to care and support for this population.

7. Conclusions

Our findings have shown similarities with the PHACS and other cohorts in highlighting signs of chronic comorbidities among YLPHIV. Subtle cardiac structural and functional abnormalities among YLPHIV were associated with late ART initiation and advanced HIV disease. Endothelial dysfunction, the earliest sign of vascular alteration, was more prevalent among YLPHIV than HIV-uninfected adolescents. Importantly, is the high PDAY risk among YLPHIV who are not virally suppressed. Poor bone health was attributed to advanced clinical disease and ART medication, while mental health measures such as excessive anger and disruptive behaviour were associated with increased lipid concentrations in YLPHIV.

Although HIC such as the USA have had their cohorts on treatment for much longer than our South African participants, findings have shown a similar burden of disease despite different genetic and environmental backgrounds. The current adolescents participating in the CTAAC began taking less toxic ART regimens than their American counterparts, which possibly explains this finding.

Our findings imply that monitoring of YLPHIV is needed in following the development of comorbidities over their lifespan. For chronic comorbidities to be delayed or reduced, interventions screening and counselling services need to be implemented and incorporated into routine care for this growing group of adolescents.

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Appendices

Appendix 1: Echocardiogram CRF

BASIC INFORMATION

1. Date of Birth D D / M M M / Y Y Y Y
2. Weight(Kg)
3. Height(cm)
4. BSA (m^2)
5. Blood Pressure (mmHg) /

ECHOCARDIOGRAM

LV dimensions

IVSd			.			mm
LVIDd			.			mm
LVPWd			.			mm
IVSs			.			mm
LVIDs			.			mm
LVPWs			.			mm
AO			.			mm
LA			.			mm
LA:AO			.			ratio
LV mass						g
LV mass indexed						g/m^2
LVOT diameter			.			mm
LVOT VTI			.			cm

LV function

LVEF		.		%	2D assessment
LVEF		.		%	Simpsons
LVSF		.		%	M-Mode

RV function

TAPSE	.		cm	RVEF (tapse)			.	
FAC			%	RVEF (volume)			.	

Valves	Yes	No	If yes, state severity and pathology

MR	☐	☐	☐ Physiologic ☐ Pathologic If Pathologic: ☐ Mild ☐ Moderate ☐ Severe	
MS	☐	☐	☐ Physiologic ☐ Pathologic If Pathologic: ☐ Mild ☐ Moderate ☐ Severe	
AS	☐	☐	☐ Physiologic ☐ Pathologic If Pathologic: ☐ Mild ☐ Moderate ☐ Severe	
AR	☐	☐	☐ Physiologic ☐ Pathologic If Pathologic: ☐ Mild ☐ Moderate ☐ Severe	
PS	☐	☐	☐ Physiologic ☐ Pathologic If Pathologic: ☐ Mild ☐ Moderate ☐ Severe	
PR	☐	☐	☐ Physiologic ☐ Pathologic If Pathologic: ☐ Mild ☐ Moderate ☐ Severe	
TR	☐	☐	☐ Physiologic ☐ Pathologic If Pathologic: ☐ Mild ☐ Moderate ☐ Severe	
TS	☐	☐	☐ Physiologic ☐ Pathologic If Pathologic: ☐ Mild ☐ Moderate ☐ Severe	

Pericardium	Yes	No	If yes, state severity and pathology
Pericardial effusion	☐	☐	

RV

TR max PG:	.		mmHg
TR Vmax	.		m/s

IVC

Est. JVP from IVC	.		mmHg
Est. PAP:	.		mmHg
RVOT VTI	.		cm
PVR	.		ratio
PVR	.		Wood units
PV AT			ms acceleration time
Tei Index	.		

Tissue Doppler and diastolic function LV :

Mitral valve inflow E/A ratio:	.		
E-wave peak velocity:	.		m/s
E-wave deceleration time:			ms
Medial E':	.		cm/s
Lateral E':	.		cm/s
E/E' medial:	.		
E/E' lateral:	.		
Medial MAPSE Trace:	.		cm

Lateral MAPSE Trace: . cm

Tissue Doppler and diastolic function RV:

Tricuspid valve inflow E/A ratio: .
E-wave peak velocity: . m/s
E-wave deceleration time: ms
Medial E': .
Lateral E': .
E/E' medial: .
E/E' lateral: .
TAPSE trace: . cm

Right heart and atrial dimensions:

Parasternal long-axis:

RVIDd (mid) . mm
RVIDs (mid): . mm
RVOTd: . mm
RVOTs: . mm
RV wall thickness . mm

Apical 4-chamber:

RVIDd (base): . mm
RVIDs (base): . mm
TV annulus: . mm
RV length (diastole): . mm
RV length (systole): . mm
RV area (diastole): . cm^2
RV area (systole): . cm^2
RVEDV: . ml
RVESV . ml

RA dimensions

RA length . mm
RA end-systolic area . cm^2

LA dimensions

LA length . mm
MV annulus: . mm
LA end-systolic area . cm^2

Carotid measurements

Left common carotid IMT	.			mm
Left carotid bulb IMT mm	.			
Left internal carotid IMT	.			mm

Right common carotid IMT	.			mm
Right carotid bulb IMT mm	.			
Right internal carotid IMT	.			mm

Formulas

BSA	$((Ht\ (cm) \times Wt\ (kg)) / 3600)^{1}/_{2}$
RVEF (volume)	RVEDV – RVESV / RVEDV x 100
RVEDV	$0.85\ (A^2/L)$
RVESV	$0.85\ (A^2/L)$
FAC	(RVAd – RVAs) / RVAd x 100
PVR ratio	TRVmax/RVOT VTI
PVR (Woods units)	(TRVmax/RVOT TVI) 10 + 0.16.

ECHOCARDIOGRAM REPORT SUMMARY:

Normal Structure: ☐ Abnormal Structure: ☐

Normal function: ☐ Abnormal function: ☐

Referral required: ☐ Details: _____

Date CRF completed D D / M M M / Y Y Y Y

Name of person completing CRF

Signature

Appendix 2: EndoPAT CRF

ENDOTHELIAL PULSE AMPLITUDE TESTING (Endo-PAT) CRF

BASIC INFORMATION

1. Date of Birth D D/ M M M / Y Y Y Y
2. Age
3. Gender
4. Weight(Kg)
5. Height(cm)
6. BMI (kg/m²)
7. Blood Pressure (mmHg) SYS/DIA /

STUDY INFORMATION

Test duration (hh:mm:ss)

: :

ENDO Score

LnRHI (-.--):

HR bpm

Endo-PAT REPORT SUMMARY:

AI:

%

AI@75bpm:

%

AI@75bpm percentile in male population as function of age: --.--
AI@75bpm percentile relative to age and gender matched: --.--

Normal function Abnormal function Comments	

Referral required: Details : _____

Date CRF completed	D D/ M M M / Y Y Y Y
Name of person completing CRF	
Signature	

Appendix 3: Bone density CRF

BONE DENSITY CRF:

BASIC INFORMATION	
1. Date of Birth	DD / MM / YYYY
2. Age at time of scan	_____ years old
3. Sex	Male ☐ Female ☐

STIFFNESS INDEX:

LEFT FOOT 1st reading: _____

2nd reading: _____

RIGHT FOOT 1st reading: _____

2nd reading: _____

Date completed	D D / M M / Y Y Y Y
Name of study staff	
Signature	

Appendix 4: CBCL CRF

Please print **CHILD BEHAVIOR CHECKLIST FOR AGES 6-18**

For office use only
ID#

CHILD'S FULL NAME First Middle Last

PARENT'S USUAL TYPE OF WORK, even if not working now (Please be specific – for example taxi driver, high school teacher, homemaker, laborer, shop keeper, shoe salesman, army sergeant

CHILD'S GENDER
☐ Boy ☐ Girl

CHILD'S AGE

CHILD'S ETHNIC GROUP OR RACE

FATHER'S TYPE OF WORK _____

MOTHER'S TYPE OF WORK _____

TODAY'S DATE
Mo. ____ Date ____ Yr. ____

CHILD'S BIRTHDATE
Mo. ____ Date ____ Yr. ____

THIS FORM FILLED OUT BY: (print your full name)

GRADE IN SCHOOL _____

NOT ATTENDING SCHOOL ☐

Please fill out this form to reflect your view of the child's behavior even if other people might not agree. Feel free to print additional comments beside each item and in the space Provided on page 2.
Be sure to answer all items.

Your gender ☐ Male ☐ Female

Your relation to the child:
☐ Biological Parent ☐ Step Parent ☐ Grandparent
☐ Adoptive Parent ☐ Foster Parent ☐ Other (Specify)

I. Please list the sports your child most likes to take part in. For example: swimming, soccer, rugby, skate boarding, bike riding, fishing etc.

Compared to others of the same age, about how much time does he/she spend in each?

Compared to others of the same age, how well does he/she do in each one?

	Less Than Average	Average	More Than Average	Don't Know	Below Average	Average	Above Average	Don't Know
a. _____	☐	☐	☐	☐	☐	☐	☐	☐
b. _____	☐	☐	☐	☐	☐	☐	☐	☐
c. _____	☐	☐	☐	☐	☐	☐	☐	☐

II. Please list your child's favorite hobbies, activities and games, other than sports. For example: singing, dancing, reading books, playing with dolls, etc. (Do not include listening to the radio or TV.)

Compared to others of the same age, about how much time does he/she spend in each?

Compared to others of the same age, how well does he/she do each one?

		Less Than Average	Average	More Than Average	Don't Know		Below Average	Average	Above Average	Don't Know
a.	_____	☐	☐	☐	☐		☐	☐	☐	☐
b.	_____	☐	☐	☐	☐		☐	☐	☐	☐
c.	_____	☐	☐	☐	☐		☐	☐	☐	☐

III. Please list any organizations, clubs, teams, or groups your child belongs to.

☐ None

Compared to others of the same age, how active is he/she in each?

		Less Active	Average	More Active	Don't Know
a.	_____	☐	☐	☐	☐
b.	_____	☐	☐	☐	☐
c.		☐	☐	☐	☐

IV. Please list any jobs or chores your child has. For example looking after brothers or sisters, making bed, working in store, etc.

☐ None

Compared to others of the same age, how well does he/she carry them out?

		Below Average	Average	Above Average	Don't Know
a.	_____	☐	☐	☐	☐
b.	_____	☐	☐	☐	☐
c.	_____	☐	☐	☐	☐

Be sure you Answered all Items. Then See other side.

Please print. Be sure to answer all items.

V. 1. About how many close friends does your child have? (Do not include brothers & sisters)

☐ None ☐ 1 ☐ 2 or 3 ☐ 4 or more

2. About how many times a week does your child do things with any friends outside of regular school hours? (Do not include brothers & sisters)

☐ Less than 1 ☐ 1 or 2 ☐ 3 or more

VI. Compared to others of his/her age, how well does your child:

	Worse	Average	Better	
• Get along with his/her brothers & sisters?	☐	☐	☐	☐ Has no brothers or sisters
• Get along with other kids?	☐	☐	☐	
• Behave with his/her parents?	☐	☐	☐	
• Play and work alone?	☐	☐	☐	

VII. 1. Performance in academic subjects. ☐ Does not attend school because_____

Check a box for each subject that child takes	Failing	Below Average	Average	Above Average
• Home language (English, Afrikaans, isiXhosa, etc.) – say which language	☐	☐	☐	☐
Other academic subjects - for example: Geography, Arts and Culture, Economic and Management Sciences, etc. (Do not include non-academic subjects like Life Orientation and Technology) • History	☐	☐	☐	☐
• Maths or Maths Literacy (say which one)	☐	☐	☐	☐
• Natural Sciences (Science and/or Biology)	☐	☐	☐	☐
e. _____	☐	☐	☐	☐
f. _____	☐	☐	☐	☐

g. _____ ☐ ☐ ☐ ☐

2. Does your child receive special education or remedial services or attend a special school?
☐ No ☐ Yes - kind of services, class, or school:

3. Has your child repeated any grades? ☐ No ☐ Yes - grades and reasons:

4. Has your child had any academic or other problems in school? ☐ No ☐ Yes - please describe:

When did these problems start? _____
Have these problems ended? ☐ ☐ Yes-when?

Does your child have any illness or disability (either physical or mental)? ☐ No ☐ Yes - please describe:

What concerns you most about your child?

Please describe the best things about your child.

Please print. Be sure to answer all items.

Below is a list of items that describe children and youths. For each item that describes your child now or within the past 6 months, please circle the 2 if the item is very true or often true of your child. Circle 1 if the item is somewhat or sometimes true of your child. If the item is not true, of your child, please circle the 0. Please answer as well as you can, even if some do not always apply to your child.

0 = Not True (as far as you know) **1 = Somewhat or Sometimes True** **2 = Very True or Often True**

0	1	2		• Acts too young for his/her age	0	1	2		32. Feels he/she has to be perfect
0	1	2		• Drinks alcohol without parents approval (describe):_____ _____	0	1	2		33. Feels or complains that no one love him/her
					0	1	2		34. Feels others are out to get him/her
					0	1	2		35. Feels worthless or inferior
0	1	2		• Argues a lot	0	1	2		36. Gets hurt a lot, accident-prone
0	1	2		• Fails to finish things he/she starts	0	1	2		37. Gets in many fights
0	1	2		• There is very little he/she enjoys	0	1	2		38. Gets teased a lot
0	1	2		• Bowel movements outside toilet	0	1	2		39. Hangs around others who get in trouble
0	1	2		• Bragging, boasting	0	1	2		40. Hears sounds or voices that aren't there (describe):_____ _____
0	1	2		• Can't concentrate, can't pay attention for long					
0	1	2		• Can't get his/her mind off certain thoughts or obsessions (describe): _____ _____	0	1	2		41. Impulsive or acts without thinking
					0	1	2		42. Would rather be alone than with others
0	1	2		• Can't sit still, restless, or hyperactive	0	1	2		43. Lying or cheating
0	1	2		• Clings to adults or too dependent	0	1	2		44. Bites fingernails
0	1	2		• Complains of loneliness	0	1	2		45. Nervous, high strung, or tense
0	1	2		• Confused or seems to be in a fog	0	1	2		46. Nervous movements or twitching (describe): _____
0	1	2		• Cries a lot	0	1	2		47. Nightmares
0	1	2		• Is cruel to animals	0	1	2		48. Not liked by other kids
0	1	2		• Cruelty, bullying, or meanness to others	0	1	2		49. Constipated, doesn't move bowels
0	1	2		• Daydreams or gets lost in his/her thoughts	0	1	2		50. Too fearful or anxious
0	1	2		• Deliberately harms self or attempts suicide	0	1	2		51. Feels dizzy or lightheaded
0	1	2		• Demands a lot of attention	0	1	2		52. Feels too guilty
0	1	2		• Destroys his/her own things	0	1	2		53. Overeating
0	1	2		• Destroys things belonging to his/her family or others	0	1	2		54. Overtired without good reason
0	1	2		• Disobedient at home	0	1	2		55. Overweight
0	1	2		• Disobedient at school					56. Physical problems *without known* ***medical cause:***
0	1	2		• Doesn't eat well	0	1	2		• Aches or pains (***not*** stomach or headaches)
0	1	2		• Doesn't get along with other kids	0	1	2		• Headaches
0	1	2		• Doesn't seem to feel guilty after misbehaving	0	1	2		• Nausea, feels sick
0	1	2		• Easily jealous	0	1	2		• Problems with eyes (***not*** if corrected by glasses) (describe):_____
0	1	2		• Breaks rules at home, school, or elsewhere	0	1	2		• Rashes or other skin problems
0	1	2		• Fears certain animals, situations, or places, other than school (describe):_____	0	1	2		• Stomachaches
0	1	2		• Fears going to school	0	1	2		• Vomiting, throwing up
0	1	2		• Fears he/she might think or do something bad	0	1	2		• Other (describe):_____

Please print. Be sure to answer all items.

0 = Not True (as far as you know) **1 = Somewhat or Sometimes True** **2 = Very True or Often True**

0	1	2	• Physically attacks people
0	1	2	• Picks nose, skin, or other parts of body (describe): ____
0	1	2	• Plays with own sex parts in public
0	1	2	• Plays with own sex parts too much
0	1	2	• Poor school work
0	1	2	• Poorly coordinated or clumsy
0	1	2	• Prefers being with older kids
0	1	2	• Prefers being with younger kids
0	1	2	• Refuses to talk
0	1	2	• Repeats certain acts over and over; Compulsions (describe):________________________________
0	1	2	• Runs away from home
0	1	2	• Screams a lot
0	1	2	• Secretive, keeps things to self
0	1	2	• Sees things that aren't there (describe):________________________________
0	1	2	• Self-conscious or easily embarrassed
0	1	2	• Sets fires
0	1	2	• Sexual problems (describe):________________________________
0	1	2	• Showing off or clowning
0	1	2	• Too shy or timid
0	1	2	• Sleeps less than most kids
0	1	2	• Sleeps more than most kids during day and/or night (describe):________________________________
0	1	2	• Inattentive or easily distracted
0	1	2	• Speech problem (describe):________________________________
0	1	2	• Stares blankly
0	1	2	• Steals at home
0	1	2	• Steals outside the home
0	1	2	• Stores up too many things he/she doesn't need (describe):________________________________

0	1	2	84.Strange behavior (describe):____
0	1	2	85. Strange ideas (describe):____
0	1	2	86. Stubborn, sullen, or irritable
0	1	2	87. Sudden changes in mood or feelings
0	1	2	88. Sulks a lot
0	1	2	89. Suspicious
0	1	2	90. Swearing or obscene language
0	1	2	91. Talks about killing self
0	1	2	92. Talks or walks in sleep (describe):____
0	1	2	93. Talks too much
0	1	2	94. Teases a lot
0	1	2	95. Temper tantrums or hot temper
0	1	2	96. Thinks about sex too much
0	1	2	97. Threatens people
0	1	2	98. Thumb-sucking
0	1	2	99. Smokes, chews, or sniffs tobacco
0	1	2	100. Trouble sleeping (describe):____
0	1	2	101. Truancy, skips school
0	1	2	102. Underactive, slow moving, or lacks energy
0	1	2	103. Unhappy, sad, or depressed
0	1	2	104. Unusually loud
0	1	2	105. Uses drugs for nonmedical purposes (***don't*** include alcohol or tobacco) (describe):____
0	1	2	106. Vandalism
0	1	2	107. Wets self during the day
0	1	2	108. Wets the bed
0	1	2	109. Whining
0	1	2	110. Wishes to be of opposite sex
0	1	2	111. Withdrawn, doesn't get involved with others
0	1	2	112. Worries
			113. Please write in any problems your child has that were not listed above:
0	1	2	____
0	1	2	____

Appendix 5: BECK Inventory CRF

Participant Enrolment Number:							
Date:							

BECK Youth INVENTORIES SECOND EDITION

for children and adolescents

Combination Booklet

Please read instructions at the top of each inside page.

Background Information

Name:_____ Date of Birth:_____

Today's Date:_____ Location:_____

Sex: ☐ Female ☐ Male Grade:_____ ID:_____

Parent/Guardian Name:_____

Notes:

[redacted]

Participant Enrolment Number:							
Date:							

Here is a list of things that happen to people and that people think or feel. Read each sentence carefully, and circle the one word (Never, Sometimes, Often, or Always) that tells about you best. THERE ARE NO RIGHT OR WRONG ANSWERS.

		Never 0	Sometimes 1	Often 2	Always 3
1.	I work hard.				
2.	I feel strong				
3.	I like myself				
4.	People want to be with me				
5.	I am just as good as the other kids				
6.	I feel normal				
7.	I am a good person				
8.	I do things well				
9.	I can do things without help				
10.	I feel smart				
11.	People think I'm good at things				
12.	I am kind to others				
13.	I feel like a nice person				
14.	I am good at telling jokes				
15.	I am good at remembering things				
16.	I tell the truth				
17.	I feel proud of the things I do				
18.	I am a good thinker				
19.	I like my body				
20.	I am happy to be me				

BSCI-Y Total RS

Participant Enrolment Number:						
Date:						

Here is a list of things that happen to people and that people think or feel. Read each sentence carefully, and circle the one word (Never, Sometimes, Often, or Always) that tells about you best, especially in the last two weeks. THERE ARE NO RIGHT OR WRONG ANSWERS.

	Never 0	**Sometimes 1**	**Often 2**	**Always 3**
21. I worry someone might hurt me at school				
22. My dreams scare me				
23. I worry when I am at school				
24. I think about scary things				
25. I worry people might tease me				
26. I am afraid that I will make mistakes				
27. I get nervous				
28. I am afraid I might get hurt				
29. I worry I might get bad grades				
30. I worry about the future				
31. My hands shake				
32. I worry I might go crazy				
33. I worry people might get mad at me				
34. I worry I might lose control				
35. I worry				
36. I have problems sleeping				
37. My heart pounds				
38. I get shaky				
39. I am afraid that something bad might happen to me				
40. I am afraid that I might get sick				
	BAI-Y Total RS			

Participant Enrolment Number:						
Date:						

Here is a list of things that happen to people and that people think or feel. Read each 'sentence carefully, and circle the one word (Never, Sometimes, Often, or Always) that tells about you best, especially in the last two weeks. THERE ARE NO RIGHT OR WRONG ANSWERS.

	Never 0	**Sometimes 1**	**Often 2**	**Always 3**
41. I think that my life is bad				
42. I have trouble doing things				
43. I feel that I am a bad person				
44. I wish I were dead				
45. I have trouble sleeping				
46. I feel no one loves me				
47. I think bad things happen because of me				
48. I feel lonely				
49. My stomach hurts				
50. I feel like bad things happen to me				
51. I feel like I am stupid				
52. I feel sorry for myself				
53. I think I do things bad				
54. I feel bad about what I do				
55. I hate myself				
56. I want to be alone				
57. I feel like crying				
58. I feel sad				
59. I feel empty inside				
60. I think my life will be bad				
	BDI-Y Total RS			

Participant Enrolment Number:						
Date:						

Here is a list of things that happen to people and that people think or feel. Read each sentence carefully, and circle the one word (Never, Sometimes, Often, or Always) that tells about you best. THERE ARE NO RIGHT OR WRONG ANSWERS.

	Never 0	**Sometimes 1**	**Often 2**	**Always 3**
61. I think people try to cheat me				
62. I feel like screaming				
63. I think people are unfair to me				
64. I think people try to hurt me				
65. I think my life is unfair				
66. People bully me				
67. People make me mad				
68. I think people bother me				
69. I get mad at other people				
70. When I get mad, I stay mad				
71. When I get mad, I have trouble getting over it				
72. I think people try to control me				
73. I feel people try to put me down				
74. I feel mean				
75. I feel like exploding				
76. I think people are against me				
77. I get angry				
78. When I get mad, I feel mad inside my body				
79. I hate people				
80. I get mad				
	BANI-Y Total RS			

Participant Enrolment Number:							
Date:							

Here is a list of things that happen to people and that people think or feel. Read each sentence carefully, and circle the one word (Never, Sometimes, Often, or Always) that tells about you best. THERE ARE NO RIGHT OR WRONG ANSWERS.

	Never 0	**Sometimes 1**	**Often 2**	**Always 3**
81. I steal				
82. Other people get me into trouble				
83. I think about running away from home				
84. I do mean things				
85. I break into cars, houses, or other places				
86. I fight with others				
87. I like getting people mad				
88. I skip school				
89. I hate listening to other people				
90. I argue with adults				
91. I hurt people				
92. I like being mean to others				
93. I break the rules				
94. I like it when people are scared of me.				
95. I like to hurt animals				
96. I like to bully others				
97. I tell lies				
98. I like to trick people				
99. I break things when I am mad				
100. I swear at adults				
	BDBI-Y Total RS			

For Office Use Only After All Testing Is Complete

When the booklet is returned, ensure that all items are completed. Follow the instructions below to score the inventories.

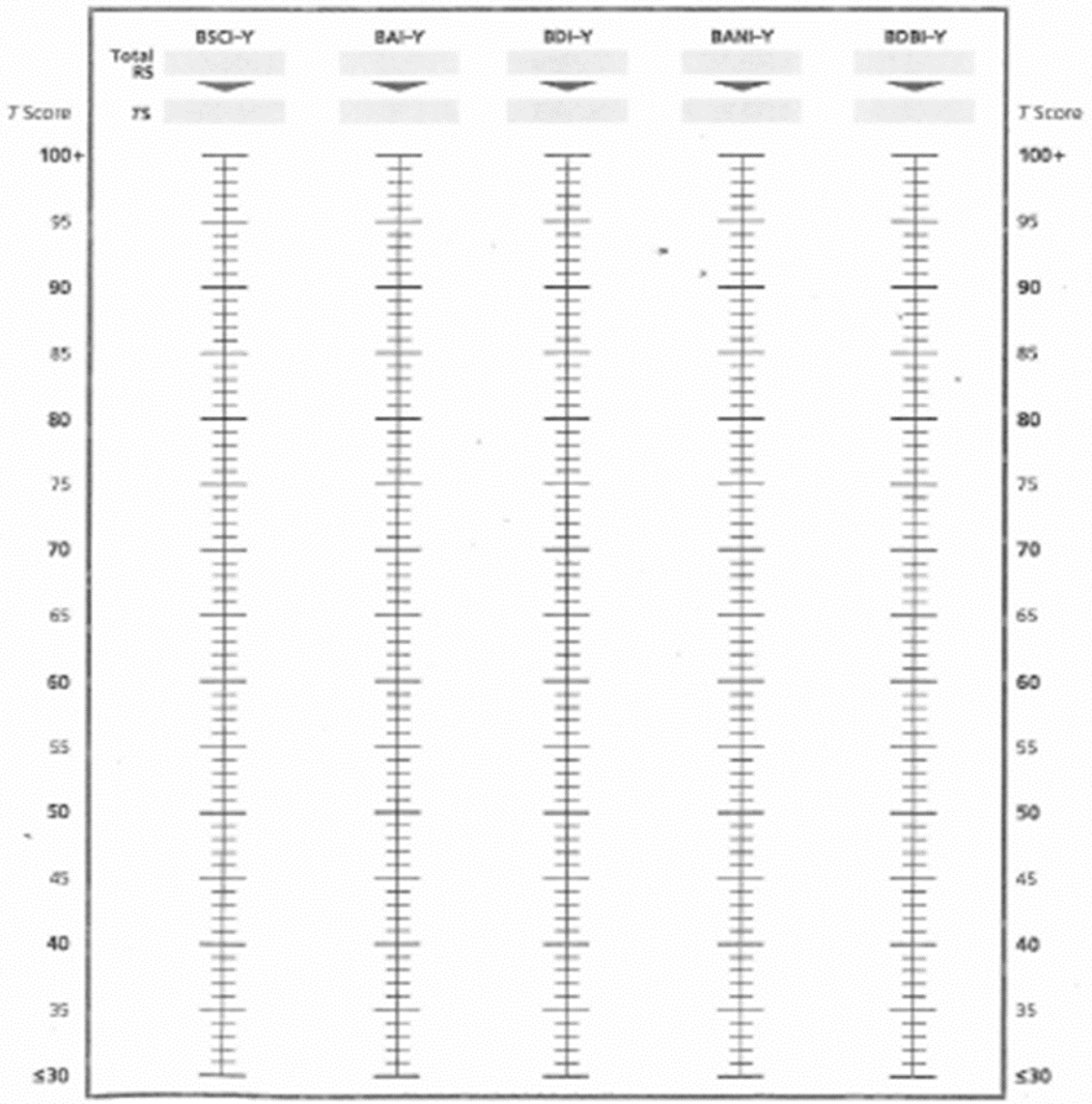
Starting on page 2, total the value of the responses for all 20 items of the inventory. Record the total raw score in the box at the bottom of the page. Repeat this for pages 3–6.

Transfer each total raw score to the total raw score box (in the row labeled Total RS) for the inventory.

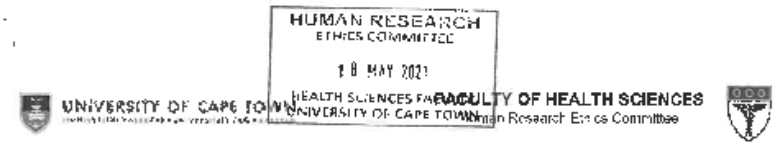
Use Tables A.1–A.3 to convert the raw scores to *T* scores. The tables are presented age-by-sex across the five inventories.

Enter the *T* score for each inventory in the corresponding *T* score box (in the row labeled *TS*). The profile can be plotted after the *T* scores are obtained.

T Score Profile



Appendix 6: CTAAC Ethics Approval



UNIVERSITY OF CAPE TOWN FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30-5-22
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	18/5/21

Note: Please note that: incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	26 April 2021		
HREC REF Number	051/2013	Current Ethics Approval was granted until	30th May 2021
Protocol title	Cape Town Adolescent Antiretroviral Cohort (CTAAC)		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes ✓ No		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	PI: Prof. Heather Zar/co-PI: Prof. Landon Myer		

(Note: Please complete the Closure form (FHS018) once the study is completed within the approval period)

Department / Office Internal Mail Address	Heather.zar@uct.ac.za Department of Paediatrics and Child Health REACH (B11) Red Cross War Memorial Children's Hospital Klipfontein Road Rondebosch 7700

1.1 Does this protocol receive US Federal funding?	✓ Yes	☐ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review **MUST** be submitted on the monthly HREC submission dates (Please send electronic copy for full committee review to hrec-enquiries@uct.ac.za)	☐ Yes	✓ No
If yes in 1.2 please complete section 1.3 below for invoicing purposes		
1.3 Annual Approval for **full committee** review	- R 3450 (inclusive of vat)	
For invoicing purposes, please provide:		
Sponsor's name		
Contact person		
Address		
Telephone number		
Email Address		

2. List of documentation for approval

There have been no changes to any of the documents since an Ethics amendment was granted in September 2020.

3. Protocol status (tick ✓)

✓	Open to enrolment
☐	Closed to enrolment (tick ✓)
✓	Research-related activities are ongoing
☐	Research-related activities are complete, long-term follow-up only
☐	Research-related activities are complete, data analysis only

☐	Main study is complete but sub study research related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS010)

4. Enrolment

Number of participants enrolled to date	715
Number of participants enrolled, since last HREC Progress report (continuing review)	34
Additional number of participants still required	106

5. Refusals

Total number of refusals (participants invited to join the study, but refused to take part)	4

6. Cumulative summary of participants

Total number of participants who provided consent	715
Number of participants determined to be ineligible (i.e. after screening)	85
Number of participants currently active on the study	499
Number of participants completed study (without events leading to withdrawal)	0
Number of participants withdrawn at participants' request (i.e. changed their mind)	30
Number of participants withdrawn by PI due to toxicity or adverse events	0
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	0
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	216
Prior to first enrolment visit at Red Cross Hospital *(unchanged from 2015)*	
5 Relocated to the Eastern Cape	
7 No longer willing	
32 Unable to contact – consent was signed but the first enrolment visit was never completed	
1 Withdrawn by doctor at primary clinic	
7 Insufficient level of disclosure about HIV status	
1 Older than 14 years at time of visit	
2 Participants were aware of HIV positive status but in denial	
1 Control tested positive for HIV (participant was not aware of their status but both parents were on ARVs)	
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	Total: 100 Cases: 127 Controls: 33

Since Enrolment Visit at Red Cross Hospital

38 No longer willing

29 Relocated

84 Unable to contact

5 Death

12 Other reasons

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:

Sites and Enrolment

Research activities are ongoing with participants from the 9 original sites - Gugulethu Community Health Centre, Tygerberg Hospital, Red Cross War Memorial Children's Hospital, Groote Schuur Hospital, Nolungile Community Health Centre, Crossroads Community Health Centre, Mitchell's Plain Community Health Centre and the Masiphumelele Youth Centre. 515 perinatally HIV-infected adolescents and 110 age matched HIV-negative controls completed the initial enrolment visit between July 2013 and March 2015.

The cohort of perinatally HIV-infected adolescents and uninfected controls continue to be followed with a focus on general adolescent development; neurocognition; lung health and cardiometabolic function. Participants are seen 6 monthly at the SAMRC Unit for Child and Adolescent Health at Red Cross War Memorial Children's Hospital. All baseline until 66 month visits have been completed with a retention rate of about 70 per cent. The 72 month and 78 month visits are ongoing. To investigate cardiometabolic outcomes, Cardiac Magnetic Resonance (CMR) is being done. There are currently 499 participants active on the study and 149 CMR scans have been completed at the 72 month visit.

Effect of the COVID-19 Pandemic

In person research activities were suspended on 21 March 2020 as a result of the COVID-19 pandemic. Research activities continued remotely with participants and caregivers completing CRFs over the phone. In all, 83 66-month visits and 183 72-month visits were completed telephonically.

In person visits resumed on 14 October 2020 after permission from HREC and with careful adherence to the UCT approved standard operating procedures for infection control. These procedures include strict observation of social distancing, hand hygiene, staggering of participants, pre-screening of participants and caregivers and universal mask wearing.

The prevalence of antibodies to SARS-CoV2 among participants was included in an amendment which was approved in September 2020.

Neurocognitive Assessment App (NASA)
NASA, an ongoing sub-study began in 2018 in a subset of participants, to improve assessment of neurocognition using an innovative, minimally invasive, automated neurocognitive tablet app. In total, 152 participants have been seen.

Neurocognitive/Neuropsychiatric Substudy
249 participants were enrolled in the NeuroHIV substudy of which 44 were HIV-negative controls. 248 participants underwent neuropsychiatric testing and 220 completed MRI imaging at baseline. Baseline assessments were completed between July 2013 and November 2015 and epigenetic testing was performed on all participants. The 3 year follow up began in June 2016 and 164 participants completed the NeuroHIV substudy follow up by December 2018. Analysis of this data is ongoing.

Revisions
There has been one protocol revision since the last Ethics renewal. This revision was approved on 10 September 2020.

Database
An on line data system, REDCAP Database, hosted on the UCT network, and supported by UCT is now used. The database is protected by user logon and password. The REDCAP database has an internal independent audit trail and access is monitored and recorded for security checks. The data is entered by a trained data entry clerk, data integrity and quality is managed and maintained by a trained data manager.

8. Protocol violations and exceptions (tick ✓ all that apply)

✓	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

☐	No prior amendments have been made since the original approval
✓	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, *italicised* or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
There have been no serious adverse events and/or unanticipated problems since the last progress report. This is an observational, longitudinal study, so no study-related adverse events are anticipated

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
✓ Yes	☐ No	☐ Not applicable
If yes, please describe:		
Unchanged from previous years: Participants found to have any abnormal or incidental findings have been appropriately referred as follows: a. For minor clinical or incidental findings, results are sent back to the referring doctor from the relevant site to manage and follow up, including management of scabies, minor warts, eczema, HIV-related issues etc. that can be managed at primary level. b. For findings that cannot be managed at this level, participants are referred to the appropriate subspecialty service such as Cardiology, Pulmonary, ENT, Rheumatology etc. at RCWMCH or GSH for further management c. For any psychosocial issue, counselling is provided or referral to the outpatient child psychiatry service at RCWMCH or to the Adolescent Psychiatry unit or G22 clinic at Groote Schuur Hospital as appropriate, is arranged. As the study has been ongoing, referral pathways are well developed		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	✓ No	☐ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	✓ No	☐ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	✓ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:	
☐	Increased
☐	Decreased
✓	Shown no change
If there has been a change, please explain:	

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)	
☐ Yes	✓ No
If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):	

14. Signature

My signature certifies that the above is complete and correct.			
Signature of PI	 Heather Zar	Date	22 April 20221

Appendix 7: Long term outcomes of perinatally HIV acquired adolescents on antiretroviral therapy - Ethics Approval

HUMAN RESEARCH ETHICS COMMITTEE
24 MAR 2021

UNIVERSITY OF CAPE TOWN FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.3.22
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	25/3/21
Comments to PI from the HREC			

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	23rd Mar 2021		
HREC REF Number	101/2019	Current Ethics Approval was granted until	30th Mar 2021
Protocol title	Long term outcomes of perinatally HIV infected adolescents on antiretroviral therapy		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes ✓ No		
If yes, could you please provide the HREC Ref's for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Prof Heather Zar		

(Note: Please complete the Closure form (FHS016) if the study is completed within the approval period)

Department / Office Internal Mail Address	Heather.zar@uct.ac.za Department of Paediatrics and Child Health REACH (B11) Red Cross War Memorial Children's Hospital Klipfontein Road Rondebosch 7700

1.1 Does this protocol receive US Federal funding?	☐ Yes	✓ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval? **Note:** Any annual approvals for **Full Committee** review MUST be submitted on the monthly HREC submission dates. (Please send electronic copy for full committee review to hrec-enquiries@uct.ac.za)	☐ Yes	☐ No
If yes in 1.2 please complete section 1.3 below for invoicing purposes		
1.3 Annual Approval for full committee review	- R 3450 (inclusive of vat)	

For invoicing purposes, please provide:	
Sponsor's name	
Contact person	
Address	
Telephone number	
Email Address	

2. List of documentation for approval

There has been no change since last approval

3. Protocol status (tick ✓)

☐	Open to enrolment
✓	Closed to enrolment (tick ✓)
☐	Research-related activities are ongoing
✓	Research-related activities are complete, long-term follow-up only

☐	Research-related activities are complete, data analysis only
☐	Main study is complete but sub-study research-related activities are ongoing
☐	Study is closed → Please submit a Study Closure Form (FHS018)

4. Enrolment

Number of participants enrolled to date	704
Number of participants enrolled, since last HREC Progress report (continuing review)	25
Additional number of participants still required	0

5. Refusals

Total number of refusals (participants invited to join the study, but refused in whole part)	4

6. Cumulative summary of participants

Total number of participants who provided consent	704
Number of participants determined to be ineligible (i.e. after screening)	05
Number of participants currently active on the study	469
Number of participants completed study (without events leading to withdrawal)	0
Number of participants withdrawn at participants' request (i.e. changed their mind)	29
Number of participants withdrawn by PI due to toxicity or adverse events	0
Number of participants withdrawn by PI for other reasons (e.g. pregnancy, poor compliance)	0
Number of participants lost to follow-up. Please comment below on reasons for loss of follow-up.	152
Prior to first enrolment visit at Red Cross Hospital (unchanged from 2015) 5 Relocated to the Eastern Cape 7 No longer willing 32 Unable to contact – consent was signed but the first enrolment visit was never completed 1 Withdrawn by doctor at primary clinic 7 Insufficient level of disclosure about HIV status 1 Older than 14 years at time of visit 2 Participants were aware of HIV positive status but in denial 1 Caregiver tested positive for HIV (participant was not aware of the status but both parents were on ARVs)	
Number of participants no longer taking part for reasons not listed above. Please provide reasons below:	

(Note: Please complete the Closure form (FHS018) if the study is completed within the approval period)

Since first enrolment visit at Red Cross Hospital
29 No longer willing
32 Relocated
45 Unable to contact
2 Withdrawn by doctor at primary clinic
1 Participant requested to de-enrol because they were enrolled in another study
2 Controls tested positive for HIV
1 HIV infected participant committed suicide
3 Deaths of participants

7. Progress of study

Please provide a brief summary of the research to date including the overall progress and the progress since the last annual report as well as any relevant comments/issues you would like to report to the HREC:
All the data has been collected and analyzed. Three manuscripts have been published, fourth has been submitted- working on fifth manuscript.

8. Protocol violations and exceptions (tick ✓ all that apply)

✓	No prior violations or exceptions have occurred since the original approval
☐	Prior violations or exceptions have been reported since the last review and have already been acknowledged or approved
☐	Unreported minor violations that have occurred since the last review, as well as significant deviations not yet reported, are attached for review

9. Amendments (tick ✓ all that apply)

✓	No prior amendments have been made since the original approval
☐	Prior amendments have been reported since the last review and have already been approved
☐	New protocol changes/ amendments are requested as part of this continuing review (See note below)

Note: If new protocol changes are being requested in this review, please complete an amendment form (FHS006).
Specific changes in the amended protocol and consent/assent forms must be **bolded**, italicised or tracked and all changes must include a rationale.

10. Adverse events

10.1 Please provide below or attach a narrative summary of serious adverse events and/ or unanticipated problems since the last progress report. Please indicate changes made to the protocol and informed consent document(s) as a result (if not already reported to the HREC). Please comment on whether causality to any study procedure or intervention could be established.
There have been no serious adverse events and/or unanticipated problems since the last progress report.

10.2 Have participants received appropriate treatment/ follow-up/ referral when indicated (e.g. in the case of abnormal or incidental clinical findings, distress or anxiety)?		
x☐ Yes	☐ No	☐ Not applicable
If yes, please describe:		
Abnormal clinical and incidental findings have all been appropriately referred as follows: a. For minor clinical or incidental findings results are sent back to the referring doctor from the relevant site to manage and follow up, including management of, scabies, minor warts, eczema etc. that can be managed at primary level, and mildly elevated HIV VL or low CD4 counts that affect the routine care participants receive from the primary referral site. b. For findings that cannot be managed at this level, participants are referred to the appropriate subspecialty service such as Cardiology, Pulmonary, ENT, Rheumatology etc. at RCWMCH or GSH for further management		

11. Summary of Monitoring and Audit Activities (tick ✓)

11.1 Was this study monitored or audited by an external agency (e.g. SAHPRA, FDA)?		
☐ Yes	X☐ No	☐ Not applicable

11.2 Did a Data and Safety Monitoring Board publish a report?		
☐ Yes	X☐ No	☐ Not applicable

11.3 If yes, please identify the agency and attach a summary of the findings.					
Agency Name		Report attached	☐ Yes	☐ No	☐ Not applicable
		DSMB report attached	☐ Yes	☐ No	☐ Not applicable

11.4 Has there been any agency, institutional or other inquiry into non-compliance in this study, or any finding of non-compliance concerning a member of the research team?	
☐ Yes	x☐ No
If yes, please explain:	

12. Level of risk (tick ✓)

12.1 In light of your experience of this research, please indicate whether the level of risk to participants has:

(Note: Please complete the Closure form (FHS21:0) if the study is complete within the approval period)

☐	Increased
☐	Decreased
☒	Shown no change

If there has been a change, please explain:

12.2 Please provide a narrative summary of recent relevant literature that may have a bearing on the level of risk.

13. Statement of conflict of interest

Has there been any change in the conflict of interest status of this protocol since the original approval? (tick ✓)

☐ Yes	✓ No

If yes, please explain and if necessary, attach a revised conflict of interest statement (Section #7 in the New Protocol Application Form FHS013):

14. Signature

My signature certifies that the above is complete and correct.

Signature of PI	Heather Zar	Date	23rd March 2021

Appendix 8: Consent form

Parent /Legal Guardian/Caregiver Information Sheet and Consent Form

Cape Town Adolescent Antiretroviral Cohort (CTAAC)

Your child is being invited to take part in an extended follow-up of this study because he/she is an HIV-infected adolescent receiving antiretroviral therapy (ART) and has been part of this study for several years. This study will continue to be done at Red Cross Children's Hospital in the School of Child and Adolescent Health, University of Cape Town. Before you decide if you want to be a part of this study, we want you and your child to know more about the study.

This form gives you information about your child's participation in this study. The research staff will talk with you and your child about this information. You are free to ask questions about this study and discuss any concerns with the staff. If you agree to take part in this study, you will be asked to sign this consent form and your child may be asked to sign a separate assent form. You will be given copies of these forms.

WHY IS THIS STUDY BEING DONE?

We want to know how the Human Immunodeficiency Virus (HIV) and antiretroviral therapy (ART) affect HIV-infected adolescents over time. The study will take place over a further 5 years during which we will follow-up your child and monitor his/her health. At any time during this study, if we find that your child has a specific health issue that needs treatment (such as tuberculosis, TB); we will refer you to an appropriate health facility for treatment and care.

WHO CAN TAKE PART IN THE STUDY?

To participate, adolescents must be between the ages of 9 and 14 years at enrolment, and must have been on ART for at least 6 months and must live in Cape Town. Approximately 600 adolescents will take part in this study.

WHAT DO I HAVE TO DO IF I TAKE PART?

Your child will need to attend study visits held at Red Cross War Memorial Children's Hospital every 6 months for the next 5 years, and an additional visit to Groote Schuur Hospital for Cardiac Magnetic Resonance.

Annual (yearly) visits

We will continue to follow your child annually at Red Cross War Memorial Children's Hospital as we have done before. Your child will be asked to undergo the following measurements:

- You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, HIV treatment history, and other aspects of health, both in the past and at present.

- Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting 10 minutes approximately, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic.

- Your child will be asked to give blood. We will collect about 15 millilitres (3 teaspoons) of blood once a year. This blood will be used to test for HIV in the body (viral load), the body's response to HIV infection (CD4 cell count), the fats in the blood (lipogram), the body's degree of health or general inflammation (Full blood count, Liver and Kidney function, C-reactive protein, Calcium, Magnesium Phosphate, Albumin), TB infection and autoantibodies like ANA, anti-cardiolipin antibody and anti-dsDNA. Antibodies are made by the body to help fight infection. Sometimes rather than fighting infection these antibodies attack the body and are called autoantibodies. There are specific autoantibodies that cause specific diseases like Lupus. It is possible to have these autoantibodies even before one gets sick with a disease and these antibodies can sometimes be present without actually causing any disease. If permission is given some of this blood may be stored for future tests and studies.

- Your child will be asked to undergo echocardiography. This is a video of the child's heart. This video is called an echocardiogram or "echo". For this, we will put some gel and a probe on your child's chest. The doctor will see your child's heart move on a screen. A short video of this movement will be recorded. We will also check your heart rate and rhythm (the way in which your heart is beating). We will also look at the arteries in your child's neck to see if they are harder or thicker than usual. This will take less than 5 minutes. We will only do this test at the first visit, at the two year visit and the last study visit.

- Your child will be asked to undergo endothelial pulse amplitude tonometry (Endo-PAT). This is a painless procedure very similar to having blood pressure measured, using a special machine. This will measure the thickness of your child's veins and how easily the blood flows through the veins. This test will be done at the first visit and then yearly.

- Your child will be asked to have a chest x-ray. This is a painless procedure to take a picture of your child's lungs. This helps to check if your child has a lung problem which needs treatment. An X-ray will be done at the first visit and yearly.

- Your child will be asked to provide a urine sample to test for kidney function and to test for substances that may affect his/ her health and if permission is given a sample will be stored for future testing. Urine will be tested for commonly abused drugs and exposure to smoking. If any of these are detected in the urine we will inform you and your child of these results, you will both receive counselling and you will be referred to the Cape Town Drug Counselling Centre for treatment.

- Your child will be asked to give us some stool (kaka) that we will collect in a container. The stool (kaka) is being collected so that we can study it to see what it is made up of and to see if the medicines your child has taken has any effects on the stool (kaka). This can help us to understand how your child is growing and also understand any illnesses that they may have. To collect the stool, we will put a container in the toilet. Your child will sit on the toilet as they usually do to provide the stool. They will leave it in the bathroom and the study nurse will collect it afterwards.

- Your child will be asked to undergo lung function testing. This involves breathing into a special machine to see how the lungs are working. Your child will do 3 different blowing tests. One test measures inflammation in the lungs and takes only 1 or 2 minutes. A second test measures the size of the lungs and how well they are working and involves breathing oxygen for the middle part of the test. The third test measures the flow of air out of the lungs when your child blows as hard and fast as she/he can. If your child cannot blow air well out of their lungs we will give your child an asthma medicine (bronchodilator) to open their chest. After 5 minutes we will repeat the blowing test, to see if this treatment helps your child and what kind of lung problem your child might have.

- Your child will be asked to do a 6 minute walk test to measure their ability to do exercise. This involves your child walking back and forth on a flat surface such as a hallway for 6 minutes. We will monitor their oxygen level and blood pressure before and after the test.

- Your child may be asked to undergo sputum induction to test for TB and other infections. This involves having a nebuliser treatment to open your child's chest and make him / her cough. We will then ask your child to cough some phlegm up into a container or suction him/ her to get phlegm if he/ she cannot cough this up. The phlegm will be sent to the laboratory for tests for TB and other germs.

- Your child will be asked to have a nasal swab done. This procedure involves putting a small stick with cotton wool at the tip into your child's nose to collect mucus. We will test this for bacteria and infection.

- Your child will be asked to undergo a bedside ultrasound, it involves putting a little bit of gel on your child's stomach and chest and a probe that will take a video of your child's organs, this is similar to the ultrasound that is done when you are pregnant and is quick and painless.

- Your child will be asked to undergo another ultrasound of the joints with a bit of gel and the probe on their knuckles, wrist and the heel of their foot. It is also quick and painless though the gel may be cold.

- Your child will be asked to have a hearing test done. This involves your child putting in earphones and listening to beeping sounds at different volumes and telling us when he/she hears the sounds. This will help us to test whether or not your child has a hearing problem. This is a quick and painless test.

- Your child will be asked to have a Cardiovascular Magnetic Resonance (CMR) performed, which is a way of taking pictures of the heart. CMR is very safe, but some people may not be comfortable in the machine. Your child will lie down on a special bed that can move in and out of the centre of the magnet (tube). There will be a loud knocking sound in the room as the machine is preparing and taking the pictures. Your child will be given headphones, so the noise is not so loud. The procedure should take about 60 minutes. It may take a little longer if pictures need to be repeated.

  The CMR will be done at Groote Schuur Hospital; and we will organise transportation for you and your child. There is no preparation needed before the CMR and there will be no medications or injections. There is no radiation with an MRI, and the procedure is not harmful to your child.

Six-monthly visits

Between the yearly study visits your child will be asked to participate in a shorter study visit. This visit will last approximately 1-2 hours and will include the following:

- You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, HIV treatment history, and other aspects of health.

- Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting about 10 minutes, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic.

We will also ask for permission to look at information from your child's health care records at the clinic he/ she currently attends, and other facilities he/she may have attended. We will get information about your child's past health in the past, including whether they attended a clinic or hospital, the treatment received, and laboratory results over time. This information is important to help us understand the results of the tests done as part of this study.

We are not taking over your child's routine health care or ART; he/she should still attend all their regular follow up visits at your local health facility. We will inform your child's doctor of any abnormal results we may find during the study.

We may also ask you to participate in other studies that are related to this research. Participation in this additional research will have a separate consent process and another form, similar to this one.

WHAT ARE THE RISKS OF TAKING PART IN THE STUDY?

Some of the questions asked in the questionnaire may make you or your child feel uncomfortable; this may include questions on smoking, alcohol and drug use and sexual behaviour. Our interviewers are trained in asking sensitive questions and you or your child can choose not to answer any questions if you don't want to.

The clinical examination may make your child feel uncomfortable. The examination will be in a private room, and if your child prefers you or another caregiver can be present.

Some of the study measurements are associated with slight discomfort. Specifically:

- Blood drawing: Your child will experience discomfort from the needle when blood is taken. Where possible this blood test will be done at the same time as other blood tests and using an anaesthetic cream to dull the pain from the needle. Only a small amount of blood will be taken.
- Endo-PAT: This will be similar to having a blood pressure done- that is for 5 minutes, a tight bandage will be placed on the upper part of both arms while measurements are done on the fingers. This is slightly uncomfortable but not painful.
- Echocardiography: It is not painful, but will need your child to lie still for about 15 minutes. Gel will be used to help make the pictures clearer and this will be slightly cold.
- Lung function testing: Lung function testing is not painful. Your child may feel a little dizzy for a short time when they blow out hard and fast. All the testing will be done by a trained technologist who is experienced in guiding young people in these tests. Breathing oxygen feels similar to breathing room air and is safe.
- 6 minute Walk Test: The object of this test is to walk as far as possible in 6minutes. This is a painless test but your child may feel short of breath. We will monitor their oxygen level and blood pressure before and after the test and if your child feels tired they can stop to rest.
- Chest X-ray: This is painless but may cause your child some discomfort as it requires sitting still for less than 5 minutes. An X-ray will be done at the start and at the end of the study, unless your child has an illness for which an X-ray is needed.
- Induced Sputum: Your child may experience minor side effects such as increased coughing, vomiting, wheezing or mild bleeding from the nose. A trained nurse will do this in a special room.

- Nasal swab: This is a painless procedure but may cause some sneezing that will stop quickly.
- Bedside ultrasound: The gel might be slightly cold but it won't hurt.
- Cardiovascular Magnetic Resonance (CMR): CMR is a type of Magnetic Resonance Imaging (MRI). An MRI scan is painless, very safe and has no known side effects. During the MRI scan, your child may feel confined while the pictures of the heart are being taken. If he/she struggles with a fear of small spaces, you should tell the research staff. If during the MRI scan he/she no longer wishes to continue because of this feeling or any other reason, the scan will be stopped. If he/she has any metallic materials within their body like a heart pacemaker, metal implants, metal chips or clips, artificial heart valves, metallic ear implants, bullet fragments or insulin pumps, you must notify the research staff before the MRI scan. It may not be safe for him/her to be scanned with an MRI if there are medical devices inside the body.

WHAT ARE THE BENEFITS OF TAKING PART IN THE STUDY?

Your child's health will be carefully monitored during the study so any new health problems may be picked up early. If we find your child has a problem that needs to be treated, we will refer him/her to an appropriate facility. The information we learn through this study may help to improve the management of HIV-infected children in the future.

WILL I RECEIVE ANY PAYMENT?

You will receive R200 for the visits when you and your child visit the study to compensate you for transport costs and time associated with the study.

CAN I AND/OR MY CHILD REFUSE TO TAKE PART IN THE STUDY?

If you or your child do not wish to participate, you or your child can refuse now or at any time in the future. Your child may also refuse to participate despite you having given consent for him/her participation. Even if you and your child decide not to take part, your child will receive the same health care, including ART, through your local health facility.

CONFIDENTIALITY

Every effort will be made to ensure that your child's information is protected. The study team will keep your child's study information confidential. Your child will be given a study number. The questionnaire and study specimens will be labelled with this study number and not with his/ her name. As a participant in this study it is very important to be able to contact you and therefore we will need to collect detailed tracing information like your address and at least two phone numbers where we might get hold of you. Please take note that even when contacting friends or neighbours we will never give them the reason that we are looking for you.

After the study is completed, you may be contacted to determine if you are interested in participating in future studies.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions about this study you may ask the study staff or you may contact:

Professor Heather Zar at (021) 658 5111 or the study Medical Officer at (021) 658 5520

If you have any questions about your rights as a research participant, you may contact the following member of the Human Research Ethics Committee in the Faculty of Health Sciences at the University of Cape Town:

Prof Marc Blockman at (021) 406 6338

<u>STORAGE OF SAMPLES</u>

If any of the blood, sputum, nasal swabs or urine samples my child has provided for this research project are unused or leftover when the project is completed

□ I give my permission for my child's samples to be stored indefinitely and used in future research of any type which has been properly approved by the Human Research Ethics Committee including genetic testing and other research.

□ I give permission for my child's samples to be stored indefinitely and used in future research but only for research on HIV.

□ I give permission for my child's samples to be stored indefinitely and used in future research except for research about _____.

OR □ I wish for my child's samples to be destroyed immediately.

I have read this consent form (or have had it explained to me), all my questions have been answered, and I agree for my child to take part in this study.

_____ _____ DD/MMM/YYYY at - -H - -

Parent/Legal Guardian/Caregiver (Print) | Parent/Legal Guardian/Caregiver signature/Thumbprint) | Date and Time

_____ _____ DD/MMM/YYY at - -H- -

Study Staff (Print) | Study Staff's Signature | Date and Time

Please complete the Witness page if the participant's Parent/Legal Guardian/Caregiver is unable to read or write.

_____ _____ DD/MMM/YYYY at - -H- -

Witness' Name (Print) | Witness' Signature | Date and Time

Participant Enrolment number: _____

Please complete the following:

□ The caregiver of this child confirms that the biological mother / father (please circle) of this child is alive but does not take ANY responsibility for the care of or decision-making for this child.

Not applicable - neither of the child's biological parents are alive.

Not applicable - the child's biological parent signed consent.

Parent / Legal Guardian/ Caregiver Information Sheet and Consent Form

HIV negative controls

Cape Town Adolescent Antiretroviral Cohort (CTAAC)

Your child is being invited to take part in an extended follow-up of this study that he/she has been a part of for several years. The study will continue to be done at Red Cross Children's Hospital in the School of Child and Adolescent Health, University of Cape Town. Before you decide if you want to be a part of this study, we want you and your child to know more about the study.

This form gives you information about your child's participation in this study. The research staff will talk with you and your child about this information. You are free to ask questions about this study and discuss any concerns with the staff. If you agree to take part in this study, you will be asked to sign this consent form and your child may be asked to sign a separate assent form. You will be given copies of these forms.

WHY IS THIS STUDY BEING DONE?

We want to know how the Human Immunodeficiency Virus (HIV) and antiretroviral therapy (ART) affect HIV-infected adolescents over time. When collecting this type of information about HIV, it is important to compare the findings of the HIV positive group with a control group that are HIV negative, like your child. The study will take place over a further 5 years during which we will follow-up your child and monitor his/her health. At any time during this study, if we find that your child has a specific health issue that needs treatment (such as tuberculosis, TB); we will refer you to an appropriate health facility for treatment and care.

WHO MAY TAKE PART IN THIS STUDY?

You may participate in this study if your child is between the ages of 9 and 14years old at the time of enrolment, HIV negative and living in Cape Town. Approximately 600 adolescents will take part in this study.

HIV testing procedure:

Since your child is a ***HIV negative participant*** in this study, with your permission we would like to do a simple HIV screening test to make sure we are comparing data from HIV positive children to HIV negative children like your child. It is also important for you and your child to know your HIV status to ensure early treatment if you test positive. This test will be done at your first study visit, prior to doing any other investigations.

If your child is confirmed HIV negative, he/she will be enrolled into this study with your permission as parent/legal guardian/ caregiver. If as a result of your participation in this research study your child is initially diagnosed as positive, the results will be given to your child privately. And because your child is older than 12 years they may choose to share this result with you. Your child will then be referred by the study doctor to the appropriate health facility for immediate counseling and appropriate treatment.

Having an HIV test done can cause feelings of anxiety and worry. These kinds of feelings are normal. We will take every step possible to ensure that you are comfortable with having your child take the HIV

test. We will perform an HIV rapid test, which will require your child to have a finger prick for a drop of blood. The test results are immediately available.

As part of this procedure, you and your child will be counseled both prior to taking the test and afterwards, regardless of the test outcome.

If your child has already had a recent HIV test, he/she will not need to redo the test to participate in this study, but with your permission, we will have to get this information from the clinic at which he/she was tested.

WHAT DO I HAVE TO DO IF I TAKE PART?

Your child will need to attend study visits at Red Cross War Memorial Children's Hospital every 6 months for the next 5 years and an additional visit to Groote Schuur Hospital for Cardiac Magnetic Resonance.

Annual (yearly) visits

We will continue to follow your child annually at Red Cross War Memorial Children's Hospital as we have been doing. Your child will be asked to undergo the following measurements:

- You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, and other aspects of health, both in the past and at present.

- Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting 10 minutes approximately, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic.

- Your child will be asked to give blood. We will collect about 15 millilitres (3 teaspoons) of blood once a year. This blood will be used to test for the fats in the blood (lipogram), the body's degree of health or general inflammation (Full blood count, Liver and Kidney function, C-reactive protein Calcium, Magnesium Phosphate , Albumin), TB infection and autoantibodies like ANA, anti-cardiolipin antibody and anti-dsDNA. Antibodies are made by the body to help fight infection. Sometimes rather than fighting infection these antibodies attack the body and are called autoantibodies. There are specific autoantibodies that cause specific diseases like Lupus. It is possible to have these autoantibodies even before one gets sick with a disease and these antibodies can sometimes be present without actually causing any disease. If permission is given some of this blood may be stored for future studies.

- Your child will be asked to undergo echocardiography. This is a video of the child's heart. This video is called an echocardiogram or "echo". For this, we will put some gel and a probe on your child's chest. The doctor will see your heart move on a screen. A short video of this movement will be recorded. We will also check your heart rate and rhythm (the way in which your heart is beating). We will also look at the arteries in your child's neck to see if they are harder or thicker than usual. This will take less than 5 minutes. We will only do this test at the first visit, at the two year visit and the last study visit.

- Your child will be asked to undergo endothelial pulse amplitude tonometry (Endo-PAT). This is a painless procedure very similar to having blood pressure measured, using a special machine. This

will measure the thickness of your child's veins and how easily the blood flows through the veins. This test will be done at the first visit and then yearly.

- Your child will be asked to have a chest x-ray. This is a painless procedure that involves taking a picture of your child's lungs. This helps to check if your child has a lung problem which needs treatment. An X-ray will be done at the start of the study and yearly.

- Your child will be asked to provide a urine sample to test for kidney function and to test for substances that may affect his/ her health and if permission is given a sample will be stored for future testing. Urine will be tested for commonly abused drugs and exposure to smoking. If any of these are detected in the urine you and your child will be informed of these results, you will both receive counselling and you will be referred to the Cape Town Drug Counselling Centre for treatment.

- Your child will be asked to give us some stool (kaka) that we will collect in a container. The stool (kaka) is being collected so that we can study it to see what it is made up of and to see if the medicines your child has taken has any effects on the stool (kaka). This can help us to understand how your child is growing and also understand any illnesses that they may have. To collect the stool, we will put a container in the toilet. Your child will sit on the toilet as they usually do to provide the stool. They will leave it in the bathroom and the study nurse will collect it afterwards.

- Your child will be asked to undergo lung function testing. This involves breathing into a special machine to see how the lungs are working. Your child will do 3 different blowing tests. One test measures inflammation in the lungs and takes only 1 or 2 minutes. A second test measures the size of the lungs and how well they are working and involves breathing oxygen for the middle part of the test. The third test measures the flow of air out of the lungs when your child blows as hard and fast as she/he can. If your child cannot blow air well out of their lungs we will give your child an asthma medicine (bronchodilator) to open their chest. After 5 minutes we will repeat the blowing test, to see if this treatment helps your child and what kind of lung problem your child might have.

- Your child will be asked to do a 6 minute walk test to measure their ability to do exercise. This involves your child walking back and forth on a flat surface such as a hallway for 6 minutes. We will monitor their oxygen level and blood pressure before and after the test.

- Your child will be asked to undergo sputum induction so that we can test for TB and other infections. This involves having a nebuliser treatment which will open your child's chest and make your child cough. We will then ask your child to cough some phlegm up into a container or suction him/ her to

get phlegm if he/ she cannot cough this up. The phlegm will be sent to the laboratory for tests for TB and other germs.

- Your child will be asked to have a nasal swab done. This procedure involves putting a small stick with cotton wool at the tip, into your child's nose to collect mucus. We will test this for bacteria and infection.

- Your child will be asked to undergo a bedside ultrasound, it involves putting a little bit of gel on your child's stomach and chest and a probe that will take a video of your child's organs, this is similar to the ultrasound that is done when you are pregnant and is quick and painless.

- Your child will be asked to undergo another ultrasound of the joints with a bit of gel and the probe on their knuckles, wrist and the heel of their foot. It is also quick and painless though the gel may be cold.

- Your child will be asked to have a hearing test done. This involves your child putting in earphones and listening to beeping sounds at different volumes and telling us when he/she hears the sounds. This will help us to test whether or not your child has a hearing problem. This is a quick and painless test.

- Your child will be asked to have a Cardiovascular Magnetic Resonance (CMR) performed, which is a way of taking pictures of the heart. CMR is very safe, but some people may not be comfortable in the machine. Your child will lie down on a special bed that can move in and out of the centre of the magnet (tube). There will be a loud knocking sound in the room as the machine is preparing and taking the pictures. Your child will be given headphones, so the noise is not so loud. The procedure should take about 60 minutes. It may take a little longer if pictures need to be repeated.

  The CMR will be done at Groote Schuur Hospital; and we will organise transportation for you and your child. There is no preparation needed before the CMR and there will be no medications or injections. There is no radiation with an MRI, and the procedure is not harmful to your child.

Six- monthly visits

Between the yearly study visits your child will be asked to participate in a shorter study visit. This visit will last approximately 1-2 hours and will include the following:

- You and your child will be asked to complete a questionnaire. The questionnaire asks about personal and family circumstances, medical history, and other aspects of health.

- Your child will be asked to undergo a clinical examination. A study doctor or nurse will examine your child, lasting about 10 minutes, to assess his/her general health. This is similar to an examination whenever your child goes to the clinic.

We will also ask for permission to look at information from your child's health care records at the clinic he/ she currently attends, and other facilities he/she may have attended. We will get information about your child's past health, including whether they attended a clinic or hospital, the treatment received, and laboratory results over time. This information is important to help us understand the results of the tests done as part of this study.

We are not taking over your child's routine health care; he/she should still attend all their regular follow up visits at your local health facility. We will inform your child's doctor of any abnormal results we may find during the study.

We may also ask you to participate in other studies that are related to this research. Participation in this additional research will have a separate consent process and another form, similar to this one.

WHAT ARE THE RISKS OF TAKING PART IN THE STUDY?

Some of the questions asked in the questionnaire may make you or your child feel uncomfortable; this may include questions on smoking, alcohol and drug use and sexual behaviour. Our interviewers are trained in asking sensitive questions and you or your child can choose not to answer any questions if you don't want to.

The clinical examination may make your child feel uncomfortable. The examination will be in a private room, and if your child prefers you or another caregiver can be present.

Some of the study measurements are associated with slight discomfort. Specifically:

- Blood drawing: Your child will experience discomfort from the needle when blood is taken. Where possible this blood test will be done at the same time as other blood tests and using an anaesthetic cream to dull the pain from the needle. Only a small amount of blood will be taken.
- Endo-PAT: This will be similar to having a blood pressure done- that is for 5 minutes, a tight bandage will be placed on the upper part of both arms while measurements are done on the fingers. This is slightly uncomfortable but not painful.
- Echocardiography: It is not painful, but will need your child to lie still for about 15 minutes. Gel will be used to help make the pictures clearer and this will be slightly cold.
- Lung function testing: Lung function testing is not painful. Your child may feel a little dizzy for a short time when they blow out hard and fast. All the testing will be done by a trained technologist who is experienced in guiding young people in these tests. Breathing oxygen feels similar to breathing room air and is safe.
- 6 minute Walk Test: The object of this test is to walk as far as possible in 6minutes. This is a painless test but your child may feel short of breath. We will monitor their oxygen level and blood pressure before and after the test and if your child feels tired they can stop to rest.
- Chest X-ray: This is painless but may cause your child some discomfort as it requires sitting still for less than 5 minutes. An X-ray will be done at the start and at the end of the study, unless your child has an illness for which an additional X-ray is needed.
- Induced sputum: Your child may experience minor side effects such as increased coughing, vomiting, wheezing or mild bleeding from the nose. The procedure will be done in a special room by a trained nurse.
- Nasal swab: This is a painless procedure but may cause some sneezing that will stop quickly.
- Bedside ultrasound: The gel might be slightly cold but it won't hurt.
- Cardiovascular Magnetic Resonance (CMR): CMR is a type of Magnetic Resonance Imaging (MRI). An MRI scan is painless, very safe and has no known side effects. During the MRI scan, your child may feel confined while the pictures of the heart are being taken. If he/she struggles with a fear of small spaces, you should tell the research staff. If during the MRI scan he/she no longer wishes to continue because of this feeling or any other reason, the scan will be stopped. If he/she has any metallic materials within their body like a heart pacemaker, metal implants, metal chips or clips, artificial heart valves, metallic ear implants, bullet fragments or insulin pumps, you must notify the research staff before the MRI scan. It may not be safe for him/her to be scanned with an MRI if there are medical devices inside the body.

WHAT ARE THE BENEFITS OF TAKING PART IN THE STUDY?

Your child's health will be carefully monitored during the study so any new health problems may be picked up early. If we find your child has a problem that needs to be treated, we will refer him/her to an appropriate facility. The information we learn through this study may help to improve the management of HIV-infected children in the future

WILL I RECEIVE ANY PAYMENT?

You will receive R200 for the visits when you and your child come to visit the study to compensate you for transport costs and time associated with the study.

CAN I AND/OR MY CHILD REFUSE TO TAKE PART IN THE STUDY?

If you or your child do not wish to participate, you or your child can refuse now or at any time in the future. Your child may also refuse to participate despite you having given consent for their participation. Regardless of whether or not you and your child participate, your child will receive the same health care through your local health facility.

CONFIDENTIALITY

Every effort will be made to ensure that your child's information is protected. The study team will keep your child's study information confidential. Your child will be given a study number. The questionnaire and study specimens will be labelled with this study number and not with his/ her name. As a participant in this study it is very important to be able to contact you and therefore we will need to collect detailed tracing information like your address and at least two phone numbers where we might get hold of you. Please take note that even when contacting friends or neighbours we will never give them the reason that we are looking for you.

After the study is completed, you may be contacted to determine if you are interested in participating in future studies.

WHAT DO I DO IF I HAVE QUESTIONS OR PROBLEMS?

If you have any questions about this study you may ask the study staff or you may contact:

Professor Heather Zar at (021) 658 5111 or the study Medical Officer at (021) 658 5520

If you have any questions about your rights as a research participant, you may contact the following member of the Human Research Ethics Committee in the Faculty of Health Sciences at the University of Cape Town:

Prof Marc Blockman at (021) 406 6338

<u>STORAGE OF SAMPLES</u>

If any of the blood, sputum, nasal swab or urine samples my child has provided for this research project are unused or leftover when the project is completed

□ I give my permission for my child's samples to be stored indefinitely and used in future research of any type which has been properly approved by Human Research Ethics Committee including genetic testing and other research.

□ I give permission for my child's samples to be stored indefinitely and used in future research but only for research on HIV.

□ I give permission for my child's samples to be stored indefinitely and used in future research except for research about _____.

OR □ I wish for my child's samples to be destroyed immediately

I have read this consent form (or have had it explained to me), all my questions have been answered, and I agree for my child to take part in this study.

_____	_____	DD/MMM/YYYY at - -H - -Parent/Legal
Guardian/Caregiver (Print)	Parent/Legal Guardian/ Caregiver Signature/Thumbprint	Date and Time

_____	_____	DD/MMM/YYY at - -H- -
Study Staff (Print)	Study Staff's Signature	Date and Time

Please complete the Witness page if the participant's Parent/Legal Guardian/Caregiver is unable to read or write.

_____	_____	DD/MM/YYYY at - -H- -
Witness' Name (Print)	Witness' Signature	Date and Time

Participant Enrolment number: _____

Appendix 9: Assent form

PATIENT INFORMATION AND ASSENT FORM FOR PARTICIPANTS FOR STUDY:

Cape Town Adolescent Antiretroviral Cohort (CTAAC)

You are invited to take part in an extended follow-up of this study because you are HIV positive on ARVs and you live in Cape Town. HIV is very common in South Africa and can affect different parts of the body in children of your age but very little about these effects in adolescence have been studied, this information will help us improve the treatment of other children your age with HIV in the future.

WHY IS THIS STUDY BEING DONE?

We want to know how the Human Immunodeficiency Virus (HIV) and antiretroviral therapy (ART) affect HIV-infected adolescents over time. The study will take place over a further 5 years during which we will follow you up and monitor your health. Approximately 600 adolescents will take part in this study. At any time during this study, if we find that you have a specific health issue that needs treatment (such as tuberculosis, TB); we will refer you to an appropriate health facility for treatment and care.

WHAT WILL HAPPEN TO YOU IF YOU JOIN THE STUDY?

If you take part in the study the following will happen:

- You will have to attend study visits held at Red Cross War Memorial Children's Hospital every 6 months for the next 5 years.
- The first visit at Red Cross Memorial Children's Hospital will last 4-5 hours. At the first visit and once a year you will be asked to complete a questionnaire. The questionnaire asks you questions about you, your family, where you live, your school, your health and your medication.
- A study doctor or nurse will ask to examine you; this will take more or less 10 minutes, to look at your general health. This is similar to an examination whenever you go to the clinic.
- We will ask to take about 3 teaspoons of blood once a year. We will do several tests on this blood and if you give your permission some of this blood will be kept in a fridge to be tested later on.
- We will ask to take a special video of your heart. It is not painful, but you have to lie still on a bed for about 15 minutes while the picture of the heart is made. We will only do this test at the first and last study visit.
- We will ask to take special pictures of your heart. It is not painful, but you have to lie down on a special bed in a machine that can move in and out of the centre of the magnet (tube). There will be a loud knocking sound in the room as the machine is preparing and taking the pictures. You will be given headphones, so the noise is not so loud.
- We will ask to do another test to look at your veins, this is painless and very similar to having your blood pressure measured, using a special machine.
- We will ask you to have a chest X-ray. This is when we take a picture of your lungs, it is painless and just like the X-rays done in the hospital and clinics.
- We will ask you to breathe into a special machine to see how well your lungs are working.
- We will ask you to walk as fast as you can back and forth on a flat surface like a hallway for 6 minutes.
- We will ask you to give us sputum (phlegm) so that we can test for TB and other infections. If you are unable to cough enough phlegm we will give you a mask to breathe in a water vapour to make you cough.
- We will ask to do a nasal swab, this means we will take a small stick with cotton wool at the tip and insert it into the front part of your nose to test for bacteria and infection.

- We will ask to do an ultrasound, this means we will put a little bit of gel on your stomach and chest and a probe that will take a video of your organs, this is a quick and painless procedure.
- We will ask you to do another ultrasound of your joints with a bit of gel and a probe on your knuckles, wrist and the heel of your foot. It is quick and painless though the gel may be cold.
- We will ask to do a hearing test to test for any hearing problems. This means you will put in earphones and listen to beeping sounds at different volumes and tell us when you hear the sounds. This is a quick and painless test.
- We will ask you to give us some urine (wee) in a bottle to test how your kidneys are working and for commonly abused drugs (like cannabis/weed and 'tik') and other substances and if you give your permission we will store a sample for further testing in the future. If it is found that your urine has tested positive for any of these drugs, we will inform you and your parent/legal guardian/caregiver of these results and we will then refer you to the Cape Town Drug Counselling Centre for counselling and advice on how to stop using drugs.
- We will ask you to give us some stool (kaka) that we will collect in a container. The stool (kaka) is being collected so that we can study it to see what it is made up of and to see if the medicines you take have any effects on the stool (kaka) . This can help us to understand how you are growing and also understand any illnesses that you may have. To collect the stool, we will put a container in the toilet. You will sit on the toilet as you usually do to provide the stool. You will leave it in the bathroom and the study nurse will collect it afterwards.
- We will also ask you to participate in another study, this study will have a separate form similar to this form that will explain the study and ask your permission to include you in the study. You may choose to participate in only one or both of these studies. We will also ask your parent/legal guardian/caregiver for permission to take part in one or both of these studies.
- At the other visits (every 6 months) you will again be asked a few questions about yourself, your family, your house and anything that has changed since your last visit. You will also be asked to be examined again like at your first visit. The 6 monthly visits will be much shorter than the first visit and will take about 1 to 2 hours.

WHAT ARE THE ADVANTAGES OF BEING IN THIS STUDY?

If we find that you have an untreated health problem during, you will be told the result and you will be sent to the appropriate clinic/hospital to give you the right treatment.

We are not taking over your treatment and you should still go to your ARV clinic for your regular clinic visits. We will inform your doctor of any abnormal results we find during the study.

With this new information we learn through this study it will help us to improve the treatment of HIV-infected children in the future.

WILL THIS STUDY HURT OR MAKE ME FEEL BAD?

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Some of the questions we ask you during your visits might be of a personal nature and will include questions on drug abuse, alcohol and tobacco use and sexual behaviour however these questions will be asked in a private room by trained study staff and you may choose to answer these questions alone or with your parent/legal guardian/ caregiver present. You may also choose not to answer these questions. The information we get from these questions will only be revealed to your parent/legal guardian/caregiver if it affects your health in a negative way or endangers your life or the life of another person.

HOW LONG WILL YOU BE IN THE STUDY?

You will be seen at Red Cross Hospital every 6 months for 5 years. That means you will have to come to Red Cross 11 times over 5 years.

DO YOU HAVE TO BE IN THE STUDY?

You may choose to be in this study or not. If you choose not to be in the study then you will get regular treatment as usual at your ARV clinic.

WHAT DO I DO IF I HAVE QUESTIONS?

If you have any questions about this study you may ask the study staff or you may contact:

Professor Heather Zar at (021) 658 5111 or the study medical officer at (021) 658 5520

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STORAGE OF SAMPLES

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□ I give my permission for my samples to be stored indefinitely and used in future research of any type which has been properly approved including genetic testing and other research.

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OR

□ I wish for my samples to be destroyed immediately.

I have read and understood this form. My questions have been answered. I am willing to participate in this study.

I, _____ (please print name) agree to participate in this study.

Participant signature: _____ Date and Time: DD/MMM/YYYY at - -H - -

Participant Enrolment number: _____

Study staff name (print): _____ Study staff signature: _____

Date and Time: DD/MMM/YYYY at - - H - -

PATIENT INFORMATION AND ASSENT FORM FOR PARTICIPANTS FOR STUDY:

Cape Town Adolescent Antiretroviral Cohort (CTAAC)

HIV negative controls

You are invited to participate in an extended follow up of this study that you have been a part of for several years. This study will continue to be done at Red Cross Children's Hospital. Please read this information which explains the project and ask the study staff or doctor any questions you have about the study.

You have been asked to take part in this study because you were HIV negative, between the ages of 9 and 14 years at enrolment and you live in Cape Town. HIV is a very common disease in South Africa and can affect different parts of the body in children of your age but very little about these effects in adolescence have been studied. Comparing information of HIV negative children, like yourself, with information of HIV positive children will help us improve the treatment of other children your age with HIV in the future.

WHY IS THIS STUDY BEING DONE?
We want to know how the Human Immunodeficiency Virus (HIV) and antiretroviral therapy (ART) affect HIV-infected adolescents over time. The study will take place over a further 5 years during which we will follow you up and monitor your health. Approximately 600 participants will take part in this study. At any time during this study, if we find that you have a specific health issue that needs treatment (such as tuberculosis, TB); we will refer you to an appropriate health facility for treatment and care.

WHAT WILL HAPPEN TO YOU IF YOU JOIN THE STUDY?
If you take part in the study the following will happen:

- *HIV testing procedure*

Since you are a HIV negative participant in this study, with your permission we would like to do a simple HIV screening test to make sure we are comparing data from HIV positive children to HIV negative children like yourself. It is also important for you to know your HIV status to ensure early treatment if you test positive.
This test will be done at your first study visit, prior to doing any other tests. If you are confirmed HIV negative, you will be enrolled to take part in the study, with your permission and your parent/legal guardian/caregiver's permission. If you test positive, the results will be revealed to you. You may choose to have your parent/legal guardian/caregiver present when we give you these results. You will then be referred by the study doctor to the appropriate hospital/clinic for immediate counseling and medical treatment.
Having an HIV test done can cause feelings of anxiety and worry. These kinds of feelings are normal. We will take every step possible to ensure that you are comfortable with having the HIV test. We will perform an HIV rapid test, which will require you to have a finger prick for a drop of blood. The test results are immediately available.
As part of this procedure, you will be counseled both prior to taking the test and afterwards, regardless of the test outcome.
If you already had a recent HIV test, you will not need to redo the test to participate in this study, but with your permission, we will have to get the information from the clinic where you were tested.

- You will have to attend study visits held at Red Cross War Memorial Children's Hospital every 6 months for the next 5 years.
- The first visit at Red Cross War Memorial Children's Hospital will last 4-5 hours. At the first visit and twice a year you will be asked to complete a questionnaire. The questionnaire asks you questions about you, your family, where you live, your school, your health and your medication.
- A study doctor or nurse will ask to examine you; this will take more or less 10 minutes, to look at your general health. This is similar to an examination whenever you go to the clinic.

- We will ask to take about 3 teaspoons of blood once a year. We will do several tests on this blood and if you give your permission some of this blood will be kept in a fridge to be tested later on.
- We will ask to take a special video of your heart. It is not painful, but you have to lie still on a bed for about 15 minutes while the picture of the heart is made. We will only do this test at the first and last study visit.
- We will ask to take special pictures of your heart. It is not painful, but you have to lie down on a special bed in a machine that can move in and out of the centre of the magnet (tube). There will be a loud knocking sound in the room as the machine is preparing and taking the pictures. You will be given headphones, so the noise is not so loud.
- We will ask to do another test to look at your veins, this is painless and very similar to having your blood pressure measured, using a special machine.
- We will ask you to have a chest x-ray. This is when we take a picture of your lungs, it is painless and just like the x-rays done in the hospital and clinics.
- We will ask you to breathe into a special machine to see how well your lungs are working.
- We will ask you to walk as fast as you can back and forth on a flat surface like a hallway for 6 minutes.
- We will ask you to give us sputum (phlegm) so that we can test for TB and other infections. If you are unable to cough enough phlegm we will give you a mask to breathe in a water vapour to make you cough.
- We will ask to do a nasal swab, this means we will take a small stick with cotton wool at the tip and insert it into the front part of your nose to test for bacteria and infection.
- We will ask to do an ultrasound, this means we will put a little bit of gel on your stomach and chest and a probe that will take a video of your organs, this is a quick and painless procedure.
- We will ask you to do another ultrasound of your joints with a bit of gel and a probe on your knuckles, wrist and the heel of your foot. It is quick and painless though the gel may be cold.
- We will ask to do a hearing test to test for any hearing problems. This means you will put in earphones and listen to beeping sounds at different volumes and tell us when you hear the sounds. This is a quick and painless test.
- We will ask you to give us some urine (wee) in a bottle to test how your kidneys are working and for commonly abused drugs (like cannabis/weed, 'tik') and other substances and if you give your permission we will keep a sample for future testing. If it is found that your urine has tested positive for any of these drugs, we will inform you and your parent/legal guardian/caregiver of these results and we will then refer you to the Cape Town Drug Counselling Centre for counselling and advice on how to stop using drugs.
- We will ask you to give us some stool (kaka) that we will collect in a container. The stool (kaka) is being collected so that we can study it to see what it is made up of and to see if the medicines you take have any effects on the stool (kaka). This can help us to understand how you are growing and also understand any illnesses that you may have. To collect the stool, we will put a container in the toilet. You will sit on the toilet as you usually do to provide the stool. You will leave it in the bathroom and the study nurse will collect it afterwards.

- At the other visits (every 6 months) you will again be asked a few questions about yourself, your family, your house and anything that has changed since your last visit. You will also be asked to be examined again like at your first visit. The 6 monthly visits will be much shorter than the first visit and will take about 1 to 2 hours.

WHAT ARE THE ADVANTAGES OF BEING IN THIS STUDY?

If we find that you have an untreated health problem during this study, you will be told the result and you will be sent to the appropriate hospital or clinic to give you the right treatment.

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