

**Trajectories of Emotional Regulation and Behavior Outcomes and related Brain Regions And  
Intrinsic Networks (TERBO BRAIN)**

**with**  
**Aortic Stiffness and Chronic Comorbidities in Youth and Young Adults with Perinatal HIV  
Infection or Exposure (ASTRO) Nested Substudy**

**Protocol Number: PH600**

**A Multi-Center Study of the Pediatric HIV/AIDS Cohort Study (PHACS)**

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National Institute of Neurological Disorders and Stroke (NINDS)  
National Institute on Deafness and Other Communication Disorders (NIDCD)  
The National Heart Lung and Blood Institute (NHLBI)  
National Institute of Mental Health (NIMH)  
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National Cancer Institute (NCI)  
National Institute on Alcohol Abuse and Alcoholism (NIAAA)

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## LIST OF ABBREVIATIONS/TERMS

ABCD	Adolescent Brain Cognitive Development
ACASI	Audio Computer-Assisted Self-Interview
ACEs	Adverse Childhood Experiences
AES	Advanced Encryption Standard
AFNI	Analysis of Functional Neuro Images
AMP	Adolescent Master Protocol
AMP Up	Adolescent Master Protocol for Participants 18 Years of Age and Older
AMY	Amygdala
ART	Antiretroviral Therapy
ARV	Antiretroviral
ASEBA	Achenbach System of Empirically Based Assessment
ASR	Adult Self-Report
BASC-2	Behavior Assessment System for Children, Second Edition
BMI	Body Mass Index
BRIEF-2	Behavior Rating Inventory of Executive Functioning, Second Edition
BRIEF-A	Behavior Rating Inventory of Executive Functioning, Adult Version
BRS	Brief Resilience Scale
CBCL	Child Behavior Checklist
CDI-2	Children's Depression Inventory 2
CDQ	Client Diagnostic Questionnaire
CEN	Central Executive Network
cfPWV	Carotid-femoral Pulse Wave Velocity
CFR	Code of Federal Regulations
CNS	Central Nervous System
ComBat	Combined Association Test
CRF	Case Report Form
CRP	C- reactive protein
CYRM-R	Child and Youth Resilience Measure-Revised
dACC	Dorsal Anterior Cingulate Cortex
DEERS-P	Difficulties in Emotion Regulation Scale – Parent Report
DHHS	Department of Health and Human Services
DICOM	Digital Imaging and Communications in Medicine
dIPFC	Dorsolateral Prefrontal Cortex
DMC	Data Management Center
DMN	Default Mode Network
DSMB	Data Safety Monitoring Board
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion Tensor Imaging
DUA	Data Use Agreement
EDC	Electronic Data Capture
EN-back	Emotional N-back
EP	Emotional Processing
ER-40	Penn Emotion Recognition Test
ERQ	Emotion Regulation Questionnaire
ERQ-CA	Emotion Regulation Questionnaire for Children and Adolescents
ESC	Epidemiological and Statistical Methods Core
FA	Fractional Anisotropy
FDA	Food and Drug Administration

FDR	False Discovery Rate
FSF	Frontier Science Foundation
fMRI	functional Magnetic Resonance Imaging
FSL	Functional Magnetic Resonance Imaging of the Brain Software Library
FS-LDDMM	FreeSurfer-initiated Large-Deformation Diffeomorphic Metric Mapping
GAD-7	Generalized Anxiety Disorder-7
GAM	Gambling
GCP	Good Clinical Practice
GEE	Generalized Estimating Equation
GHAC	General Health Assessment for Children
HCP	Human Connectome Project
HCP-YA	Human Connectome Project Young Adults
HIPAA	Health Insurance Portability and Accountability Act
HIPP	Hippocampus
HIV	Human Immunodeficiency Virus
HLC IRB	Harvard Longwood Campus IRB
HSPH	Harvard T. H. Chan School of Public Health
HTTPS	Hyper Text Transfer Protocol Secure
HUU	HIV-Unexposed, Uninfected
ICA	Independent Component Analysis
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IFO	Inferior Fronto-Occipital Fasciculus
INS	Insula
IPAQ	International Physical Activity Questionnaire – Short Form
IRB	Institutional Review Board
LAR	Legally Authorized Representative
LEGACY	Longitudinal Epidemiologic Study to Gain Insight into HIV/AIDS in Children and Youth
LPC	Laboratory Processing Chart
MID	Monetary Incentive Delay
MNPP	Manual of Network Policies and Procedures
MRI	Magnetic Resonance Imaging
MOP	Manual of Procedures
NAFLD	Nonalcoholic Fatty Liver Disease
NB-WM	N-back Working Memory
NCI	The National Cancer Institute
NHLBI	The National Heart Lung and Blood Institute
NIAAA	The National Institute of Dental and Craniofacial Research
NIAID	The National Institute of Allergy and Infectious Diseases
NICHD	The <i>Eunice Kennedy Shriver</i> National Institute of Child Health and Human Development
NIDA	National Institute on Drug Abuse
NIDCD	The National Institute of Deafness and Other Communication Disorders
NIDCR	The National Institute of Dental and Craniofacial Research
NIH	National Institutes of Health
NIMH	The National Institute of Mental Health
NINDS	The National Institute of Neurological Disorders and Stroke
NRTI	Nucleoside Reverse Transcriptase Inhibitor

NURIPS	Northwestern University Research Image Processing System
OD	Office of the Director, National Institutes of Health
OFC	Orbitofrontal Cortex
OHRP	Office of Human Research Protection
ORARC	Office of Regulatory Affairs and Research Compliance
PASS	Power Analysis & Sample Size
PEARLS	Pediatric Adverse Childhood Experiences and Related Life Events Screener
PDS	Pubertal Development Scale
PHACS	Pediatric HIV/AIDS Cohort Study
PHEU	Perinatal HIV Exposure who are Uninfected
PHI	Protected Health Information
PHIV	Perinatally Acquired HIV
PHQ-9	Patient Health Questionnaire-9
PI	Principal Investigator
PID	Participant Identification Number; also known as Patid
PIN	Personal Identification Number
PNS	Peripheral Nerve Stimulation
PRIME-MD	Primary Care Evaluation of Mental Disorders
PSQI	Pittsburgh Sleep Quality Inventory
PWV	Pulse Wave Velocity
QA	Quality Assurance
QoL	Quality of Life
QNS	Query and Notification System
RAVLT	Rey-Auditory Verbal Learning Test
RIAS	Reynolds Intellectual Assessment Scales
ROI	Region of Interest
rs	Resting-State
rsFC	Resting-State Functional Connectivity
SCARED	Screen for Child Anxiety Related Disorders
SD	Standard Deviation
SEM	Structural Equation Modeling
SES	Study Enrollment System
SID	Study Identification Number
sIRB	Single Institutional Review Board
SMARTT	Surveillance Monitoring for ART Toxicities
SMR	Standardized Mortality Ratio
SN	Salience Network
SSL	Secure Sockets Layer
SST	Stop Signal Task
STI	Sexually Transmitted Infection
SUD	Substance Use Disorders
T1	T1-weighted Magnetic Resonance Imaging
T2	T2-weighted Magnetic Resonance Imaging
TBSS	Tract Based Spatial Statistics
THAL	Thalamus
TERBO BRAIN	Trajectories of Emotional Regulation and Behavior Outcomes and related Brain Regions And Intrinsic Networks
UPS	Uninterruptible Power Supply
URL	Uniform Resource Locator
U.S.	United States

vIPFC	Ventrolateral Prefrontal Cortex
vmPFC	Ventromedial Prefrontal Cortex
vs.	versus
vSTR	Ventral Striatum
WAIS-IV	Wechsler Adult Intelligence Scale, Fourth Edition
WIHS	Women's Interagency HIV Study
WISC-IV	Wechsler Intelligence Scale for Children, Fourth Edition
XNAT	Extensible Neuroimaging Archive Toolkit
YAHUU	Young Adults who are HIV-Unexposed, Uninfected
YAPHEU	Young Adults with Perinatal HIV Exposure who are Uninfected
YAPHIV	Young Adults with Perinatally Acquired HIV
YPHEU	Youth with Perinatal HIV Exposure who are Uninfected
YPHIV	Youth with Perinatally Acquired HIV
YHUU	Youth who are HIV-Unexposed, Uninfected

### TERBO BRAIN PROTOCOL SYNOPSIS

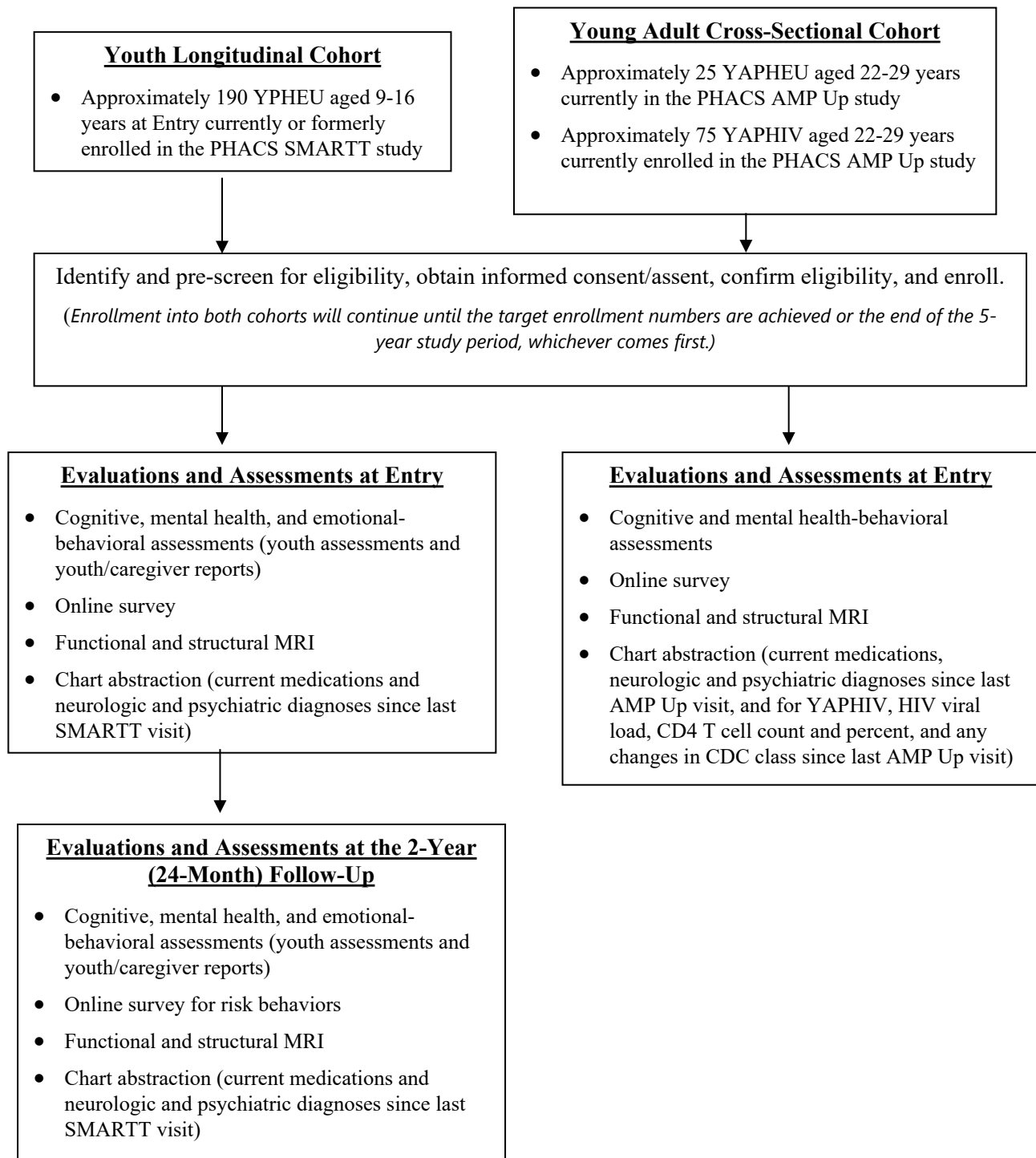
<p><b>Study Description:</b></p>	<p>This is a longitudinal investigation of neurodevelopmental consequences and underlying mechanisms of perinatal exposures to human immunodeficiency virus (HIV) and antiretrovirals (ARVs). Brain networks, mental health, cognition, and risk-taking behavior in children and adolescents with perinatal HIV exposure who are uninfected, hereafter referred to as YPHEU, will be assessed using functional and structural neuroimaging (i.e., magnetic resonance imaging (MRI), cognitive, emotional-behavioral, and computer-based assessments over two years. In addition, a cross-sectional sample of young adults with perinatal HIV exposure who are uninfected (YAPHEU) and young adults with perinatally acquired HIV (YAPHIV) will have a range of outcomes explored such as cognition, mental health, and risk behaviors, as well as adult milestones (e.g., education and employment) using neuroimaging, cognitive, mental health, and behavioral assessments.</p>
<p><b>Study Aims:</b></p>	<ul style="list-style-type: none"> <li>• Aim 1: To longitudinally assess brain network development underlying emotional regulation and relate it to mental health, cognition, and risk-taking behaviors in YPHEU compared to a population-based cohort without known perinatal HIV exposure.</li> <li>• Aim 2: To assess the longitudinal impact of perinatal ARV exposure as well as vulnerability and protective factors on brain network development underlying emotional regulation in YPHEU.</li> <li>• Aim 3 (Exploratory): To cross-sectionally assess brain network integrity and cognitive, social, mental health, and behavioral outcomes during the adult transition period in YAPHEU and YAPHIV compared to one another and to a population-based cohort without known perinatal HIV exposure.</li> </ul>
<p><b>Study Population:</b></p>	<p>YPHEU aged 9-16 years who are current or former participants of the Pediatric HIV/AIDS Cohort Study (PHACS) Surveillance Monitoring for ART Toxicities (SMARTT) study will be enrolled and followed longitudinally for two years (Aims 1 and 2). YAPHEU and YAPHIV aged 22-29 years who are current participants of the PHACS Adolescent Master Protocol for Participants 18 Years of Age and Older (AMP Up) study will be enrolled and complete a one-time evaluation (Aim 3).</p>
<p><b>Sample Size:</b></p>	<ul style="list-style-type: none"> <li>• Youth longitudinal cohort: target 190 YPHEU</li> <li>• Young adult cross-sectional cohort: target 100 total with the ratio between YAPHEU and YAPHIV approximately 1:3.</li> </ul>
<p><b>Study Assessments:</b></p>	<p><u>Youth Longitudinal Cohort at Entry and Year 2:</u></p> <ul style="list-style-type: none"> <li>• Cognitive, mental health, and emotional-behavioral assessments (youth assessments and youth/caregiver reports)</li> <li>• Online survey of substance use behaviors (youth self-report only)</li> <li>• Functional and structural MRI</li> <li>• Chart abstraction (current medications and neurologic and psychiatric diagnoses since last SMARTT visit)</li> </ul>

	<p><u>Young Adult Cross-Sectional Cohort at Entry:</u></p> <ul style="list-style-type: none"> <li>• Cognitive and mental health-behavioral assessments</li> <li>• Online survey</li> <li>• Functional and structural MRI</li> <li>• Chart abstraction (current medications, neurologic and psychiatric diagnoses since last AMP Up visit, and for YAPHIV, HIV viral load, CD4 cell count and percent, and any changes in CDC class since last AMP Up visit)</li> </ul>
<b>Study Duration:</b>	This study is expected to be open for accrual and follow-up for up to five years.
<b>Participant On-Study Duration:</b>	YPHEU enrolled in the longitudinal cohort will be on the study for two years with one visit at Entry and a second visit two years later. YAPHEU and YAPHIV in the cross-sectional cohort will have a single study visit at Entry.
<b>Study Monitoring:</b>	<p>The TERBO BRAIN Protocol Team will monitor implementation of this study at clinical sites.</p> <p>Monitoring of any adverse impact of the study will rely on the PHACS Protocol Query and Notification System (QNS), which is a real-time, web-based interactive reporting system. Sites will also record and enter in the study database all untoward effects associated with study participation, which will be reviewed by the Protocol Team.</p>

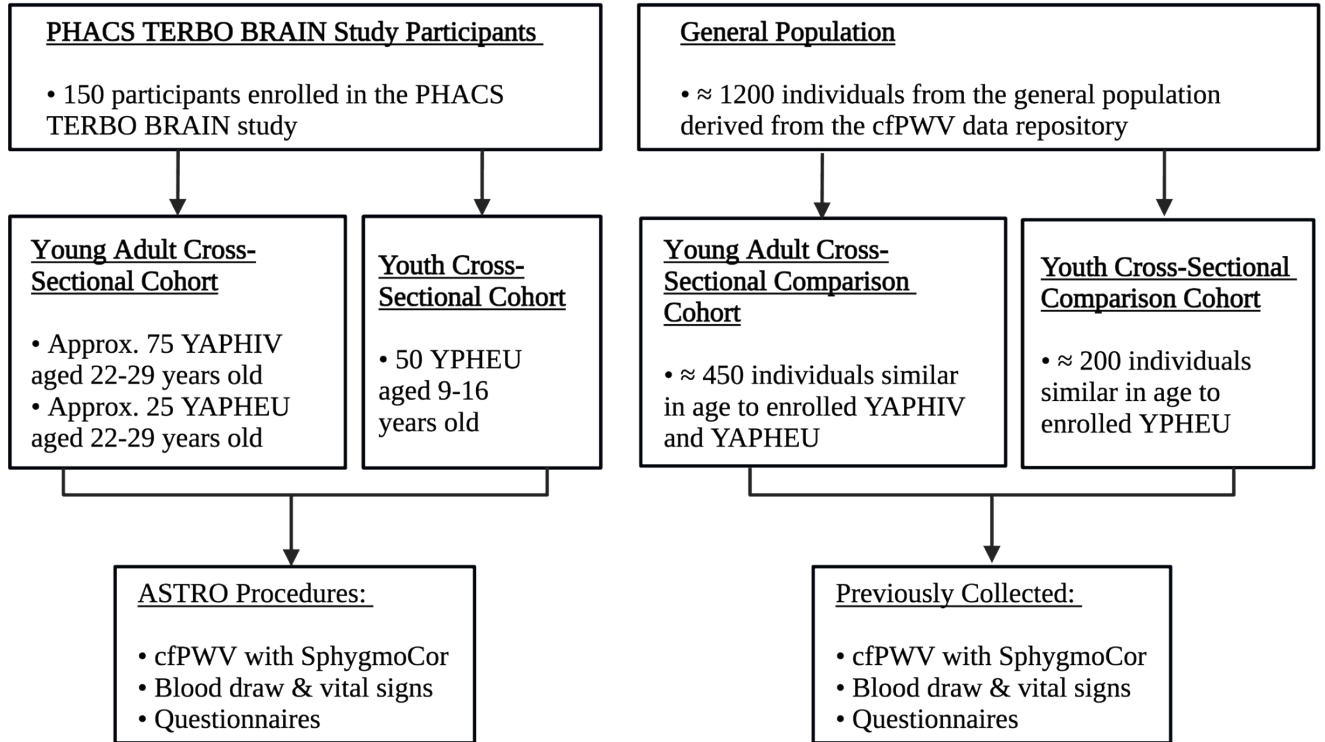
### ASTRO NESTED SUBSTUDY SYNOPSIS

<b>Nested Substudy Description:</b>	The ASTRO substudy is a cross-sectional study to investigate aortic stiffness and its contribution to end-organ damage in YPHEU (9-16 years old) as well as YAPHEU and YAPHIV (22-29 years old). Aortic stiffness will be compared between study groups as well as to similar-age individuals from the general population. Lastly, the relationship of aortic integrity with metabolic, immune, and neurocognitive parameters within PHIV and PHEU groups will be delineated.
<b>Nested Substudy Aims:</b>	<ul style="list-style-type: none"> <li>• Aim 1: To investigate the impact of in utero HIV exposure and perinatal HIV infection on aortic stiffness in children and young adults.</li> <li>• Aim 2: To evaluate the role of aortic stiffness in the pathogenesis of neurocognitive dysfunction in individuals with PHIV and PHEU.</li> </ul>
<b>Nested Substudy Population:</b>	A subset of the participants enrolled in the TERBO BRAIN study will also consent to participate in the ASTRO nested substudy and will complete a single visit at Entry.
<b>Nested Substudy Sample Size:</b>	<ul style="list-style-type: none"> <li>• 50 YPHEU, approximately 100 YA in about a 1:3 ratio of YAPHEU and YAPHIV</li> </ul>
<b>Nested Substudy Assessments:</b>	<ul style="list-style-type: none"> <li>• Carotid-femoral pulse wave velocity (cfPWV)</li> <li>• Vital signs and anthropometrics: weight, height, blood pressure, and mid-waist and hip circumferences</li> <li>• Interviewer-administered questionnaires: education of primary caregiver (young adult participants only) and physical activity</li> <li>• Fasting blood: metabolic and immune serologic markers and eicosanoid profiling</li> </ul>
<b>Nested Substudy Duration:</b>	The ASTRO nested substudy is expected to be open for accrual until the target sample sizes are achieved or the TERBO BRAIN study is completed, whichever occurs first.
<b>Participant On-Study Duration:</b>	Participants in the ASTRO nested substudy will complete a single study visit at Entry.
<b>Nested Substudy Monitoring:</b>	<p>The ASTRO Protocol Team will monitor implementation of the ASTRO nested substudy at clinical sites.</p> <p>Monitoring of any adverse impact of the study will rely on the PHACS Protocol Query and Notification System (QNS), which is a real-time, web-based interactive reporting system. Sites will also record and enter in the study database all untoward effects associated with study participation, which will be reviewed by the Protocol Team.</p>

## TERBO BRAIN PROTOCOL SCHEMA



### ASTRO NESTED SUBSTUDY SCHEMA



## 1.0 TERBO BRAIN STUDY INTRODUCTION

### 1.1 Study Background

Tremendous successes in providing pregnant and breastfeeding women living with human immunodeficiency virus (HIV) with antiretroviral therapy (ART) have significantly reduced new pediatric HIV infections. Consequently, there has been a substantial increase in the number of children and adolescents with perinatal HIV exposure who are uninfected, hereafter referred to as YPHEU, estimated at nearly 15 million globally [1]. Although studies from Pediatric HIV/AIDS Cohort Study (PHACS) and other cohorts are encouraging with regard to the safety of *in utero* antiretroviral (ARV) exposure, the potential for risk remains a concern, given the developing brain's vulnerability to neurotoxic effects and heavy dependence on mitochondrial integrity [1, 2]. However, the clinical course and long-term neurodevelopmental consequences of early exposures in these youth are currently unknown, particularly as they reach older adolescence and young adulthood [3].

Studies of YPHEU suggest that they are at risk for mental health problems [4], including psychiatric disorders [5], and challenges to cognitive, academic and adaptive skill development [6, 7], at rates often similar to youth with perinatally acquired HIV (YAPHIV) [4, 6-14]. Poor mental health is linked to risk-taking behaviors (e.g., condomless sex, substance use) among YPHEU [15], which in turn can increase risk for poor health outcomes such as acquisition of HIV/sexually transmitted infections (STIs). Mental health and neurocognitive challenges can also negatively impact life skills as YPHEU reach adulthood [16, 17], impeding intervention efforts aimed at improving long-term functioning and well-being. Also, many YPHEU live in under-resourced communities and are exposed to adversities (violence, racism, discrimination, family death) that may affect neurodevelopment independently or interact with factors related to perinatal HIV exposure [3, 16]. Several neuroimaging studies show disrupted brain development in early childhood [18, 19], but the mechanisms underlying these neurodevelopmental consequences in YPHEU, particularly as they age, are not fully understood.

Relatedly, poor emotional regulation is thought to underlie childhood psychopathologies, including internalizing and externalizing disorders [20, 21]. Impairments in emotion processing and regulation are associated with structural and functional abnormalities of brain networks, particularly those involving reward and emotional salience processing and regulation of affective states and behavior [22]. Dysfunction of these networks has been linked with risk-taking behavior in youth [23], yet we know little regarding the neural mechanisms linking emotional regulation and behavioral outcomes in YPHEU, despite increased risk behaviors in this population.

This longitudinal investigation of neurodevelopmental consequences and underlying mechanisms of perinatal exposures to HIV and ARVs, assessing baseline brain networks, mental health, cognition, and risk-taking behavior in approximately 190 YPHEU at ages 9-16 years, utilizing functional and structural neuroimaging, clinical, and computer-based assessments will address these gaps. The social and structural factors that may interact with prenatal exposures will also be examined. A key hypothesis is that disrupted networks supporting emotional regulation are critical mechanisms underlying neurodevelopmental consequences in YPHEU. A few studies suggest that young adults with perinatal HIV exposure who are uninfected (YAPHEU) have challenges related to cognition, mental health, sexual and substance use risk behaviors [3, 16, 24, 25], and also experience incarceration and homelessness at rates similar to young adults with perinatally acquired HIV (YAPHIV) [16, 26]. Using neuroimaging and cognitive/behavioral assessments, these outcomes and adult milestones (e.g., education and employment) will be explored in a cross-sectional sample of approximately 100 total of YAPHEU and YAPHIV in about a 1:3 ratio.

### **1.1.1 Limited Neuroimaging Studies on YPHEU**

Few neuroimaging studies have been conducted in individuals with perinatal HIV exposure who are uninfected (YPHEU). Findings show white matter growth appears stunted, reflecting disrupted brain network development [18, 19, 27, 28]. At 9 years, YPHEU show neuronal damage in the basal ganglia as compared to HIV-unexposed children [29]. Limited neuroimaging studies in YPHIV indicate reduced grey matter volume [30-32] and alternations in white matter integrity [33-36] in the brain. Studies on participants with perinatally acquired HIV (PHIV) aged 10-22 years from PHACS found disruptions in the salience network (SN), central executive network (CEN), and default mode network (DMN), with gray matter loss apparent throughout regions of the networks including ventral striatum, thalamus [37], rostral middle frontal gyrus, and superior frontal cortex [38]. Resting-state (rs) network disruption between DMN and CEN was related to impaired processing speed [39] and observed gray matter abnormalities [37], as well as lower fractional anisotropy (FA, a measure of white matter integrity) of the inferior fronto-occipital fasciculus (IFO) connecting DMN and CEN [36], which were associated with higher peak viral load. Further, poorer working memory was related to higher disease severity, which was partially mediated by lower IFO FA values [36]. However, there have been no published longitudinal neuroimaging studies in YPHEU with appropriate comparison groups.

### **1.1.2 Knowledge Gap in Mechanisms of Neurodevelopment in YPHEU**

A systematic review of 11 studies of children with PHEU under age 8 found high prevalence of cognitive (up to 31%) and motor (up to 39%) delays [40]. In YPHEU, PHACS studies have reported greater language impairment (37%) versus (vs.) the United States (U.S.) average (16%) [12] and executive functioning deficits [8, 10, 41]. Impairment in executive functioning compared to youth who are HIV-unexposed, uninfected (YHUU) has also been reported [42]. PHACS and other studies have also shown that while YPHEU perform better than YPHIV in some aspects of prospective memory [43], their visual recognition memory [9], executive functioning [8, 41], math and adaptive functions [6, 13] are often similar to those of YPHIV [44, 45]. However, there have been limited longitudinal studies to examine causal pathways.

Mental health problems often emerge in adolescence, affecting 10-20% of youth in general [46]. Vulnerable populations such as YPHEU often exhibit higher-than-average rates of poor mental health [4, 46-49] including psychiatric disorders [5]. Although, prevalence estimates among YPHEU for having one or more diagnoses of neurodevelopmental disorder [48] or mental health/psychiatric disorders [47, 49] vary widely depending on the study (30-70%) and age group (4-22 years). Recent studies revealed more internalizing problems (e.g., depression, anxiety) in YPHEU compared to YHUU [42], and rates of poor mental health similar to YPHIV, with causes unknown. YPHEU also show increased rates of risk behaviors (condomless sex, alcohol, and substance use) [16, 50]. A high percentage of 7-15 year old YPHEU report initiation of substance use and sex, including condomless sex in 50% of those who were sexually active [25]. Alcohol (42% lifetime) and marijuana (19%) use are also frequent among YPHEU and YPHIV enrolled in PHACS [51]. In other youth populations, psychiatric problems, in particular mania and externalizing disorders, have been associated with sexual risk behaviors [52, 53], and substance use has been shown to mediate the relationship between psychological distress and sexual risk behaviors [50]. In YPHEU, similar to YPHIV, poor mental health predicts risk-taking behaviors [25, 54], and substance use can increase the odds of unprotected sex [55, 56], placing them at risk for their own HIV infection.

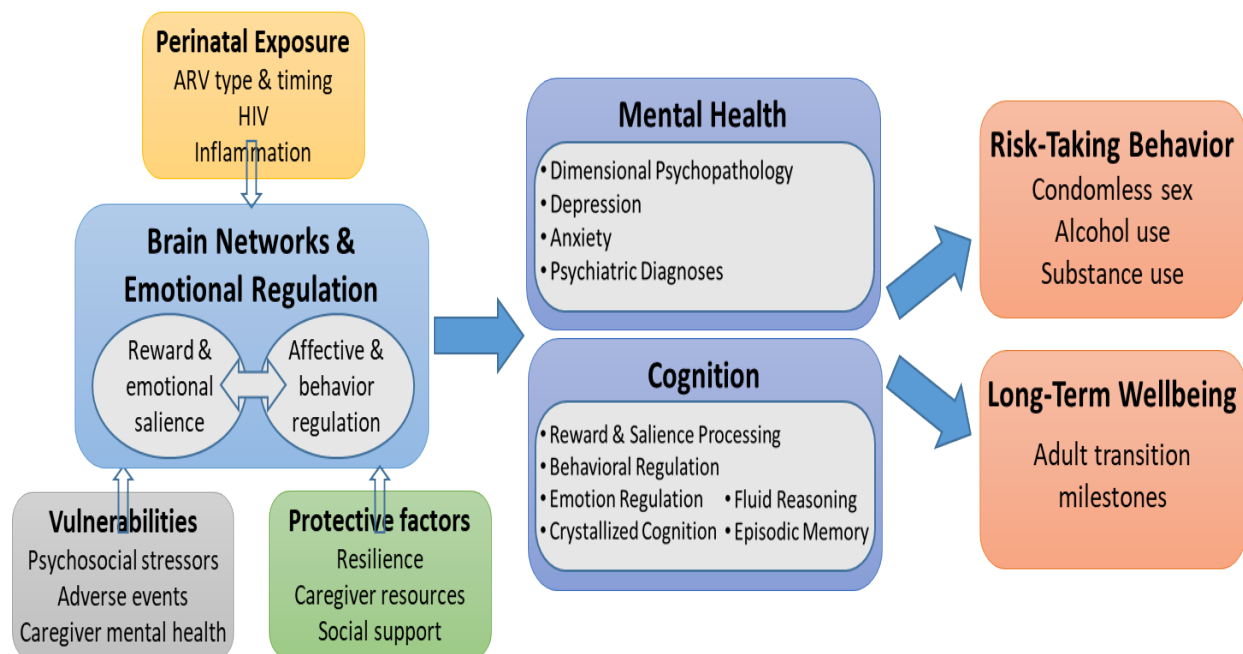
There exists a need to understand emotional regulation and brain network dysfunction as a driving mechanism in YPHEU. Poor emotional regulation underlies many child mental health problems, including internalizing and externalizing disorders [20, 21]. During adolescence, executive functioning, particularly cognitive control involved in the self-regulation of emotion and behavior, is consolidating [57].

Developmental biases toward reward pursuit during this stage, while they underlie age-typical exploration, may limit the role of cognitive processes in regulating emerging behaviors, including sexual and substance use risk behaviors [23, 42]. Disturbances in the balance of rapidly developing capacities, manifesting as abnormalities in the structure and function of underlying brain networks, have been associated with impairments in emotion processing and regulation, leading to a rise in risk-taking [23]. Complex cognitive processes associated with emotional regulation are supported by interconnected large-scale brain networks [58-62]. Impaired interactions among three major networks, SN, CEN, and DMN, play a central role in mental disorders [63, 64], including emotional dysregulation across a spectrum of psychiatric disorders [22]. The SN supports detection of emotional and reward saliency and reward learning [64], as well as directing attention toward relevant interoceptive and emotional information to guide behavior [64]. The CEN supports inhibitory control, working memory, and decision-making to guide goal-directed behavior [65-68]. The DMN is a system for self-referential mental processes including self-related and social cognitive processes [69, 70], value-based decision making [71] and emotion regulation [72]. Complex cognitive processes also require interaction and integration across these networks [63]. For example, emotional and cognitive functions involve goal-oriented executive functions such as working memory and selective attention, which requires the SN to increase CEN activities while suppressing DMN activities [73-76]. In the unifying “triple network model” of SN, CEN, DMN, dysfunctions in one core network impact information flow and integration across all networks [63]. This has been demonstrated across psychopathologies [63] including emotion dysregulation [22, 72]. In children and adolescents, disruptions of these networks have been linked to risk-taking behaviors and impaired cognition [23].

Emotion processing and regulation are associated with reward and emotional salience processing and regulation of affective states and behavior [22]. Reward and salience processing are closely linked processes that are important for goal directed behaviors and adaptive behavioral responses to stimuli where salience guides reward processing [77, 78]. Relevant brain regions are the ventral striatum, insula, thalamus and orbitofrontal and medial prefrontal cortex of the SN and DMN [79]. Behavioral self-regulation is crucial for controlling impulses [80]. Relevant brain regions are the ventrolateral prefrontal cortex and anterior cingulate cortex of the CEN. Attentional control of emotional interference associated with viewing emotional stimuli is a measure of emotional reactivity and emotion regulation. It requires working memory and response inhibition [81]. Relevant brain regions are the dorsolateral, ventrolateral, and ventromedial prefrontal cortex, amygdala, hippocampus, and ventral striatum of the SN, CEN, and DMN.

Longitudinal investigations of brain network, mental health, cognition, and risk-taking behavior are critical for understanding mechanisms underlying neurodevelopmental consequences. The proposed mechanism is that disrupted networks supporting emotional regulation underlie mental health and cognitive impacts of YPHEU that increase risk-taking behaviors and other negative long-term outcomes (Figure 1). This hypothesis is based on the extant developmental, cognitive, and clinical neuroscience literature, and on findings from cross-sectional studies in YPHEU.

**Figure 1. Model for Proposed Mechanism**



### 1.1.3 Impact of Perinatal HIV and ARV Exposure on Brain Development in PHEU

Given the developing brain's vulnerability to neurotoxic effects and heavy dependence on mitochondrial integrity, the impact of perinatal HIV and ARV exposure on brain development in PHEU is a concern [1, 2]. HIV-related chronic inflammation can persist despite suppressive ARVs [82]. Poor neurological and neurodevelopmental outcomes have been observed in infants and children with PHEU [6, 7, 11, 13, 14, 83], which may be related to mitochondrial toxicity induced by nucleoside reverse transcriptase inhibitors (NRTIs) [84]. ARVs when taken in pregnancy can cross the blood-brain barrier, causing pathological microglial activation and other neurotoxic effects [2, 85], which in turn can disrupt neurodevelopment and have long-term developmental consequences [86, 87]. Perinatal exposures to HIV and ARVs may also affect neurodevelopment unrelated to mitochondrial function or increase the risk of birth defects [88]. Although generally considered safe, several studies, including ones from PHACS, have shown that *in utero* exposure to efavirenz and atazanavir is associated with congenital anomalies and early-life neurodevelopmental, language and socio-emotional deficits [13, 89-96]. To date, findings on the effects of *in utero* exposure to specific ARV treatments have been mixed; this study allows examination of unique *in utero* ARV exposure effects on YPHEU as they age.

Insults during specific gestational periods may lead to central nervous system (CNS) developmental consequences [88]. Maternal infections during the first trimester are related to global brain abnormalities [97] while second-trimester insults are more likely associated with neuropsychiatric abnormalities [98]. Third-trimester exposure to zidovudine has been associated with mitochondrial dysfunction [99]. *In utero* exposure to HIV and maternal inflammation could also have neurodevelopmental consequences. A study of children (1-9 years) with PHEU found that HIV type 1-related immunosuppression predicted cognitive impairment [100]. In YPHEU in PHACS Adolescent Master Protocol (AMP), inflammatory markers measured at a mean age of 11.4 years were related to decreased processing speed [101]. In animal studies, offspring of mothers with maternal immune activation show disinhibited behavior and impairments in

emotion regulation and social behavior [102]. A neuroimaging study of infants 40-44 weeks old revealed that functional connectivity between SN, DMN, and fronto-parietal networks was correlated with maternal immune activation markers collected during the third trimester [103], which in turn was associated with 14-month cognitive development. These studies suggest that *in utero* ARV and HIV exposures, and related maternal inflammation, may affect the developing brain. However, long-term effects on brain development in YPHEU are not understood.

#### **1.1.4 Influence of Social and Structural Factors on Brain, Mental Health, and Cognitive Developmental Outcomes in YPHEU and YAPHEU**

Concomitant social and structural factors are known to have deleterious effects across all developmental stages of youth affected by HIV, individually and via complex interactions. YPHEU in the U.S., who are disproportionately African-American or Black and Latinx, are also more likely to experience structural barriers to well-being, such as adverse community circumstances and exposure to poverty [104]. These factors often co-occur with and/or are related to other potentially harmful exposures, including community violence, suboptimal educational systems, toxic environmental exposures, limited access to quality healthcare, inadequate nutrition sources, racism, and racial and ethnic inequities. The cumulative impact of these determinants may increase the likelihood of high rates of drug use, low employment, family instability, and poor cognitive, emotional, and health outcomes among youth across developmental stages [105]. For example, higher exposure to neighborhood disorder, including violence, and other life stressors were associated with mental health problems among YPHEU and YPHIV [104, 106]. Caregiver factors such as cognitive function and caregiver-child relationship are strongly associated with behavioral functioning among YPHEU and YPHIV [107]. Caregivers living with HIV, who are also subject to the adverse exposures described above, experience higher rates of psychiatric and substance use disorders (SUD) than the general population, which may have indirect and direct effects via genetics and caregiving behavior [108, 109]. Socio-emotional health of caregivers is a determinant of child well-being, including distress, hopelessness, positive future orientation, self-esteem, and quality of life (QoL) in children and adolescents [110]. Substance use by caregivers/adults in the home is strongly associated with increased risk of use of alcohol or marijuana in the children living in the home. In YPHEU, maternal psychiatric diagnosis conferred additional risk for recent child substance use [51].

Childhood stressors such as traumatic adverse experiences, including housing or food insecurity, exposure to violence, illness or death of a caregiver, physical or verbal abuse, are also related to impaired learning, emotional regulation, and cognition in childhood [111, 112]. Neuroimaging studies have shown that childhood stressors may lead to impaired hippocampal neurogenesis [113], resulting in hippocampal volume reduction throughout development with greater reductions in adults compared to children [114]. Further, childhood stressors are associated with abnormal task functional magnetic resonance imaging (fMRI) activation in the reward regions of the ventral striatum (poor reward processing and depressive symptoms) [115, 116] and orbitofrontal cortex (childhood maltreatment) [117-119]. They are also associated with poor working memory, as well as volume reductions in the cognitive control regions of lateral prefrontal cortex [120] and anterior cingulate cortex [114, 121-124]. Neuroimaging studies have documented that parental care and social support have positive effects on the development of the amygdala, hippocampus, and prefrontal cortex [125-128], with more pronounced effects in younger children [129]. These studies suggest that social and structural factors have deleterious effects on the mental health, cognition, and behavior of YPHEU, and they negatively impact brain development in the general youth population, particularly in brain networks related to emotional regulation, but perhaps moderated by protective factors. However, how these stressors and protective factors affect brain development in YPHEU is unknown.

### 1.1.5 Transition into Adulthood in YAPHEU

YAPHEU may exhibit poor adult transition milestones in educational achievement and employment with unknown pathways of causality [16]. In the transition into adulthood, adolescents have increased opportunity for independence, with decreased parental supervision and increased financial, vocational, and other adult stressors. YPHEU during this period face added risk for psychiatric disorders and SUD that can lead to worse long-term outcomes [3]. Limited longitudinal studies of YPHEU suggest that mental health deteriorates and risk behaviors increase during this transition. While alcohol and other SUD are relatively rare in adolescence [25], by young adulthood, prevalence of SUD rises to 33% [3]. Compared to YHUU, YPHEU showed increases in mood disorder prevalence over an 18-month period [5, 47], and the odds of unprotected sex doubled over time, with stronger association between alcohol use and unprotected sex than in YPHIV [56]. Internalizing problems in YPHEU can also progressively increase over time compared with YHUU [42].

During young adulthood, studies show that YAPHEU continue to exhibit below-average cognitive functioning [5, 16, 26], with some improvements over time, while YAPHIV, especially those with a history of more severe HIV disease, are less likely to improve [26]. YAPHEU also show higher rates of psychiatric disorders (27-39%) and SUD (31%) compared to norms, with rates similar to YAPHIV [3, 16, 24]. During this period, sexual and substance use risk behaviors persist in YAPHEU [3, 16, 24, 25], with high prevalence of condomless sex (41%) [16], and sexual risk often associated with SUD and behavioral disorders [24]. As YPHEU transition into adulthood, they may encounter additional stressors that may exacerbate poor outcomes [16, 17]. YAPHEU show high prevalence of incarceration (14%) and homelessness (15%) similar to YAPHIV [16, 26, 130]. Extant studies do not include neuroimaging or ARV exposure data, potentially critical factors for understanding pathways of causality [131].

## 1.2 Study Rationale

There is and will continue to be a wave of YPHEU and YAPHEU in the U.S. and globally, particularly in low- and middle-income countries, potentially requiring substantive health, mental health, and social system interventions. Following cohorts of YPHEU with the potential to compare to other cohorts from the general population as well as to YAPHIV is important to inform long-term prevention/intervention needs of the staggering numbers of these youth globally. This study offers a time-sensitive and unique opportunity to examine key neural mechanisms underlying health and behavioral health outcomes and their interactions with other predictors in one of the largest National Institutes of Health (NIH)-sponsored cohorts of YPHEU for whom data on prenatal HIV and ARV and other exposures have been collected during childhood, and into adolescence and adulthood. Together with PHACS data, as well as Adolescent Brain Cognitive Development (ABCD) and Human Connectome Project Young Adults (HCP-YA) studies, this study provides a unique opportunity to move the needle forward on prevention/intervention programming for YPHEU before they reach young adulthood in the millions globally.

This study will be the first to use longitudinal neuroimaging to understand the long-term effects of perinatal HIV and ARV exposures, including specific ARV drugs or classes, on YPHEU as they age, and identify specific mechanisms that drive mental health, cognitive functioning, and behavioral domains that may be impacted. It will provide critical knowledge for potential prevention/intervention strategies to minimize long-term negative consequences by targeting specific mechanisms during periods of rapid and vulnerable brain development. There are evidence-based mental health and behavioral risk reduction interventions that could be adapted for YPHEU, and evidence-based cognitive training programs. A critical first step for intervention research is to identify key mechanisms and predictors to ensure that interventions are most appropriate and efficacious with target populations. Other drivers of neurodevelopmental and behavioral risk common to PHEU populations (e.g., socioeconomic and maternal factors) emphasize the need for

rigorous studies to identify HIV- and ARV-exposure specific contributions and their mechanisms in order to target prevention and intervention efforts.

## **2.0 ASTRO NESTED SUBSTUDY INTRODUCTION**

Individuals who are living with PHIV and PHEU have been found to have increased adiposity [132, 133], insulin resistance [134, 135], dyslipidemia [136, 137], and inflammation [138-140], likely due to the interplay of chronic infection, ART, and effects on fetal programming. In the general population, increased aortic stiffness is an early consequence of metabolic dysfunction and immune activation that results in excess transmission of pulsatile pressure to the microvasculature with resultant end-organ damage [141]. As a low-resistance organ, the brain is particularly vulnerable to pressure and pulsatility induced by increased arterial stiffness [142], which in turn has been linked to neurocognitive impairment [143, 144]. Nonetheless, whether aortic stiffness is increased or contributes uniquely to the pathogenesis of multi-systemic comorbidities in individuals with PHIV and PHEU remains a critical gap in knowledge. This nested substudy will leverage TERBO BRAIN to investigate aortic stiffness and its contribution to end-organ damage in youth and young adults born to mothers with HIV. The central hypothesis of this nested substudy is that the abnormal metabolic and pro-inflammatory milieu in individuals with PHIV and PHEU will promote aortic stiffness, which in turn will predispose to altered brain structure and function as key indicators of end-organ damage. To test this hypothesis, carotid-femoral pulse wave velocity (cfPWV) will be ascertained as a low-cost and reproducible index of aortic stiffness among participants enrolled in TERBO BRAIN. First, differences in aortic stiffness between those living with PHIV and PHEU, as well as similar-age individuals from the general population, will be investigated. Furthermore, associations of metabolic and inflammatory parameters with aortic stiffness will be assessed in people with PHIV and PHEU to uncover novel mechanisms of aortic dysfunction. Second, the role of aortic stiffness in the pathogenesis of altered brain structure and function in those with PHIV and PHEU will be delineated by assessing relationships of cfPWV with neuroanatomic and neurocognitive outcomes identified in TERBO BRAIN. This nested substudy could uncover increased aortic stiffness as an as-yet unrecognized sequela of perinatal HIV infection and in utero HIV exposure that may serve as a harbinger for adverse health outcomes. Moreover, this pilot study will inform the development of targeted interventions to attenuate aortic stiffness as an early and modifiable risk factor in these populations.

### **2.1 Nested Substudy Background and Significance**

Multiple studies of children and young adults with PHIV have shown a high prevalence of metabolic and inflammatory comorbidities including insulin resistance [134, 145, 146], hypertriglyceridemia [136], altered fat distribution [132], and increased immune activation [138, 140]. Individuals with PHEU similarly manifest metabolic and immune perturbations, including higher rates of obesity [133], hypertension [147], and inflammation [148], as well as lower insulin sensitivity [134, 135], compared to HIV-unexposed, uninfected (HUU) individuals. ART, persistent viral infection, and effects of an altered intrauterine milieu on fetal programming have been posited to contribute to the pathogenesis of these findings. In the general population, metabolic and inflammatory abnormalities predispose to increased aortic stiffness as a key mechanism of end-organ damage [141]. Nonetheless, despite the high prevalence of metabolic dysfunction and immune activation in the PHIV and PHEU populations, the integrity of the large arterial system and its contribution to multi-systemic disease remains a critical gap in knowledge.

As the largest and most distensible blood vessel in the body, the aorta buffers the systemic circulation from the high pulsatile pressures generated by the heart. In particular, the elastic nature of this vessel allows it to expand to accommodate blood that enters the arterial system during systole and to recoil to expel blood to the peripheral tissues during diastole, thereby maintaining steady non-pulsatile blood flow within small

blood vessels throughout the cardiac cycle [149, 150]. Increased aortic stiffness augments the transmission of pulsatile pressure from the heart to peripheral tissues, which in turn has deleterious impacts on multiple organs including the heart, kidney, and brain [149]. High arterial stiffness has been implicated in coronary ischemia [151, 152], heart failure [153, 154], and arrhythmia [155, 156]. Longitudinal studies have further shown a bidirectional causal link between aortic stiffness and hypertension [157, 158]. Given the role of aortic dysfunction in the pathogenesis of chronic comorbidities, identifying high aortic stiffness would expose a critical vulnerability in the long-term health of the PHIV and PHEU populations.

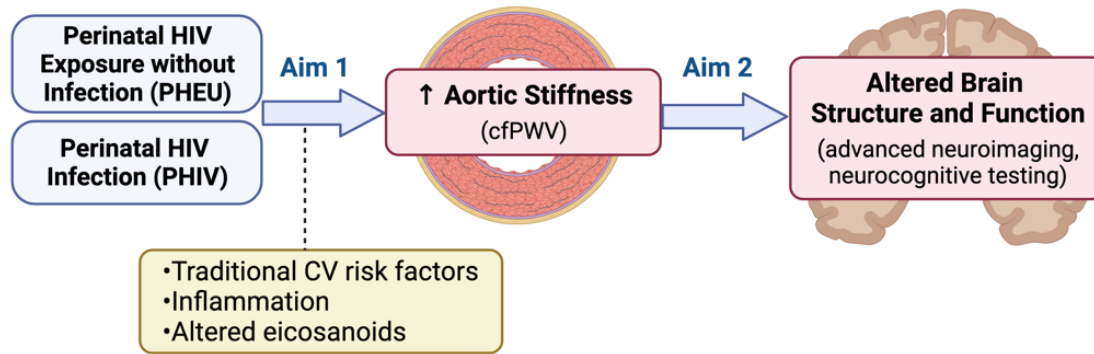
Various pathologic stimuli may result in abnormal stiffening of the aortic wall, resulting in impaired cushioning [141]. Among the general population, aortic dysfunction has been linked to traditional cardiovascular risk factors including obesity, insulin resistance, hypertension, and dyslipidemia [141]. The Bogalusa Heart Study found that young adults with a greater number of features of metabolic syndrome at baseline had steeper rises in aortic stiffness over time [159]. Conversely, attenuation of metabolic dysfunction such as with weight loss, exercise, antagonism of the renin-angiotensin-aldosterone system, statins, or omega-3 fatty acid supplementation was found to restore normal aortic elasticity [160]. In addition to traditional cardiovascular risk factors, systemic inflammation also has been recognized as a key risk factor for arterial dysfunction [141]. In particular, individuals with autoimmune conditions including rheumatoid arthritis and inflammatory bowel disease were found to have elevated aortic stiffness [161, 162], which declined following immunomodulatory therapy [163]. Additionally, inflammatory markers such as C-reactive protein (CRP) have been shown to positively predict higher aortic stiffness in healthy individuals [164], including in our own data in young adults. Based on the high burden of metabolic and immune abnormalities in those with PHIV and PHEU, there are strong imperatives to evaluate the integrity of the arterial system in these populations. By delineating relationships of metabolic and immune indices with aortic function, this nested substudy may inform the rational design of tailored interventions to restore aortic integrity in these groups, to be explored in future work.

The brain is a densely vascularized organ that requires high blood flow to meet its metabolic requirements. Increased aortic stiffness results in excess transmission of pulsatile pressure to its microvasculature, which in turn predisposes to altered tissue structure and function [149]. White matter hyperintensities are a late complication of increased aortic stiffness, which are preceded by microstructural changes to neuronal tract fibers that can be detected using advanced magnetic resonance imaging (MRI) techniques [165, 166]. Gray matter atrophy has also been observed among individuals with increased arterial stiffness, including in brain regions such as the thalamus and cortex [166, 167]. Underlying these structural changes, higher aortic stiffness has been correlated with impaired autoregulation of cerebral blood flow both at rest and during activity, as measured by fMRI [168]. In addition to these radiographic abnormalities, aortic stiffness has been linked to decline in neurocognitive performance, particularly in the domains of executive function, memory, and global cognition [142-144]. Notably, these findings in older adults resonate with our own preliminary data focused on young adults as well as studies linking hypertension to neurocognitive impairment in children and adolescents [169]. Given our hypothesis that individuals living with PHIV and PHEU may be prone to heightened aortic stiffness, there is a pressing need to examine the contribution of aortic stiffness to altered brain structure and function in these patient populations.

Building upon the nested substudy protocol team's expertise in endocrinology, cardiology, infectious diseases, epidemiology, neuroimaging, and neurodevelopment, the integrity of the large arterial system in those with PHIV and PHEU vs. similar-age individuals from the general population will be evaluated for the first time, along with its role in the pathogenesis of chronic comorbidities (Figure 2). The central hypothesis is that metabolic and inflammatory abnormalities in the PHIV and PHEU populations will lead to increased aortic stiffness, which in turn will predispose to altered brain structure and function as key indicators of end-organ damage. This hypothesis is supported by findings in the general population juxtaposed with evidence of an extensive burden of metabolic and inflammatory derangements in people

with PHIV and PHEU. Based on this work, aortic stiffness may come to be used as a simple tool to identify individuals with PHIV and PHEU who are at high risk for adverse health outcomes and who thus require intensive monitoring. Findings from this nested substudy may guide the development of interventions to target aortic stiffness as an early and modifiable risk factor on the path to end-organ damage.

**Figure 2: Nested Substudy Conceptual Framework of Specific Aims**



## 2.2 Nested Substudy Rationale

In the general population, aortic stiffness is an early sequela of metabolic dysfunction and immune activation that predisposes to multi-systemic morbidity including cardiac, renal, and neurologic disease [142-144, 149]. While those living with PHIV and PHEU have a high prevalence of traditional cardiovascular disease risk factors [133, 145, 147, 170] and immune abnormalities [138, 140, 148], the integrity of the large arterial system in these individuals has not been previously well evaluated in comparison to individuals from the general population. Nonetheless, we have previously shown that youth with PHIV in South Africa have endothelial dysfunction of the microvasculature [171, 172]. Furthermore, in a prior PHACS analysis, children with PHIV were found to have higher circulating biomarkers of endothelial dysfunction vs. controls in association with greater abdominal adiposity and HIV infection severity [173]. As endothelial dysfunction is intimately tied to abnormalities in aortic stiffness [149, 174], these data stress the urgency to further delineate the vascular phenotype among those with PHIV and PHEU within PHACS. Consistent with the mission of PHACS to improve the lives of people with PHIV and PHEU, the findings from this nested substudy may illuminate cfPWV as a simple and noninvasive tool to identify individuals who are at high risk of chronic comorbidities and thus require intensive long-term monitoring. Moreover, this pilot nested substudy will inform the development of targeted interventions to attenuate aortic stiffness as an early and modifiable risk factor in these populations, which can be further explored in future work.

## 3.0 TERBO BRAIN STUDY AIMS AND HYPOTHESES

### 3.1 Aim 1

To longitudinally assess brain network development underlying emotional regulation and relate it to mental health, cognition, and risk-taking behaviors in YPHEU compared to a population-based cohort without known perinatal HIV exposure.

Hypotheses:

- 1a. YPHEU will exhibit atypically attenuated growth in brain networks supporting emotional regulation as they age when compared to a population-based cohort.
- 1b. Brain network growth will be more strongly related to poor mental health and impaired cognition in YPHEU than in the comparison cohort.
- 1c. Attenuated brain network growth will be associated with risk-taking behaviors, mediated by mental health and cognition, after accounting for social and structural vulnerabilities and protective factors.

### **3.2 Aim 2**

To assess the longitudinal impact of perinatal ARV exposure as well as vulnerability and protective factors on brain network development underlying emotional regulation in YPHEU.

Hypotheses:

- 2a. Within YPHEU, attenuation of brain network growth will be related to type and timing of perinatal ARV exposure (e.g., efavirenz and atazanavir).
- 2b. Social and structural vulnerabilities and protective factors will each impact brain network growth.

### **3.3 Aim 3 Exploratory**

To cross-sectionally assess brain network integrity and cognitive, social, mental health, and behavioral outcomes during the adult transition period in YAPHEU and YAPHIV compared to one another and to a population-based cohort without known perinatal HIV exposure.

Hypotheses:

- 3a. Compared to young adults without HIV exposure, YAPHEU and YAPHIV will show disrupted brain networks underlying emotional regulation, with more severe disruption in YAPHIV.
- 3b. In YAPHEU and YAPHIV, disrupted brain networks will be related to risk-taking behaviors and deficits in adult transition milestones (e.g., education and employment), mediated by cognition and mental health, considering the potential impact of HIV infection, ARV exposure, social and structural vulnerabilities, and protective factors separately within YAPHEU and YAPHIV.

## **4.0 ASTRO NESTED SUBSTUDY AIMS AND HYPOTHESES**

The ASTRO nested substudy will leverage the TERBO BRAIN study to investigate aortic stiffness and its contribution to end-organ damage among youth (9-16 years old) and young adults (22-29 years old) born to mothers with HIV.

### **4.1 Aim 1**

To investigate the impact of in utero HIV exposure and perinatal HIV infection on aortic stiffness in children and young adults.

Hypotheses:

1a: YAPHIV will have the highest aortic stiffness, followed by YAPHEU, followed by similar-age individuals from the general population. YPHEU will have elevated aortic stiffness as compared to similar-age individuals from the general population.

1b: In YAPHIV, YAPHEU, and YPHEU, alterations in traditional cardiovascular risk factors, immune markers, and pro-inflammatory eicosanoids will be associated with higher aortic stiffness.

## 4.2 Aim 2

To evaluate the role of aortic stiffness in the pathogenesis of neurocognitive dysfunction in individuals with PHIV and PHEU.

Hypotheses:

2a: Among YAPHIV, YAPHEU, and YPHEU, higher aortic stiffness will be associated with alterations in brain structure and function including reduced white and gray matter integrity and altered functional connectivity.

2b: In YAPHIV, YAPHEU, and YPHEU, higher aortic stiffness will be associated with worse neurocognitive outcomes, particularly in global cognition, executive function, and memory.

## 5.0 TERBO BRAIN STUDY DESIGN

This study will follow a longitudinal cohort of approximately 190 YPHEU and a cross-sectional cohort of approximately 100 total of young adults with about 1:3 ratio between YAPHEU and YAPHIV. The longitudinal design for Aims 1 and 2 allows the study to examine developmental trajectories and mechanisms underlying brain-behavior relationships in YPHEU. The cross-sectional design for exploratory Aim 3 uses an available cross-sectional sample to examine long-term outcomes in YAPHEU and YAPHIV. For Aims 1 and 3, comparisons with large-scale population-based data (see Section 5.3) and appropriate statistical analyses allow the study to identify mechanisms specific to the study populations. The aims of this study will address the gap in published longitudinal neuroimaging studies and help elucidate brain development in YPHEU, and consequently, to better understand the mechanisms of the neurodevelopmental trajectories in YPHEU. The study will also explore disruption in brain networks among YAPHIV and YAPHEU and the association of brain networks with other outcomes during the adult transition period. Tables 1 and 2 present the conceptual domains assessed for each study component and the measures that contribute to each domain. Descriptions of the measures are provided in Section 8.0. Administration details can be found in Section 10.0.

<b>Table 1. YPHEU assessment protocol: Domain constructs and measures for Aims 1 and 2</b>	
<b>Cognition</b>	
Reward & Salience Processing	Monetary Incentive Delay (MID) Task <sup>a</sup>
	Cash Choice Task
	Delay Discounting Task <sup>b</sup>
Behavioral Regulation	Stop Signal Task (SST) <sup>a</sup>
	Flanker Inhibitory Control and Attention
	Behavior Rating Inventory of Executive Functioning, Second Edition (BRIEF-2)
Emotion Regulation	Emotional N-back (EN-back) <sup>a</sup>
	Emotion-Word/Emotion-Face Stroop Task <sup>b</sup>
	Emotion Regulation Questionnaire for Children and Adolescents (ERQ-CA)
	Difficulties in Emotion Regulation Scale – Parent Report (DERS-P)

Fluid Reasoning Composite	Composite of Flanker Inhibitory Control and Attention, List Sorting, Dimensional Change Card Sort, Pattern Comparison, Picture Sequence (from NIH Toolbox)
Crystallized Cognition Composite	Composite of Picture Vocabulary, Oral Reading Recognition (from NIH Toolbox)
Episodic Memory	Rey-Auditory Verbal Learning Test (RAVLT)
<b>Mental and Behavioral Health</b>	
Dimensional Psychopathology	Achenbach System of Empirically Based Assessment (ASEBA) Child Behavior Checklist (CBCL)
Depression	Children’s Depression Inventory-2 (CDI-2)
Anxiety	Screen for Child Anxiety Related Disorders (SCARED)
Psychiatric Diagnoses	Child/youth psychiatric diagnoses in medical records and caregiver screening diagnoses from Client Diagnostic Questionnaire (CDQ) data from the SMARTT database
<b>Risk Behaviors</b>	
Substance Use	Substance use questions (adopted from SMARTT Audio Computer-Assisted Self-Interview (ACASI) and modified to include select ABCD questions)
<b>Vulnerabilities</b>	
Childhood Adversity	Pediatric Adverse Childhood Experiences and Related Life Events Screener (PEARLS)
Stress and Trauma	Life Events Checklist (adopted from AMP)
Family Resources	Demographics Questionnaire
<b>Protective Factors</b>	
Resilience	Child and Youth Resilience Measure-Revised (CYRM-R)
	Prosocial Behavior Survey (Brief ABCD version)
Social Support	Emotional Support Fixed Form - NIH Toolbox Emotion Measures (Ages 8-17 years)
<sup>a</sup> fMRI task activation and behavior such as reaction time, accuracy, d-prime. For details on fMRI see Section 8.7. <sup>b</sup> The ABCD study administered these measures at the one-year follow-up visit, a year after the imaging.	

**Table 2. YAPHEU and YAPHIV assessment protocol: Domain constructs and measures for Aim 3.**

<b>Cognition</b>	
Reward & Salience Processing	Gambling Task <sup>a</sup>
	Delay Discounting Task
Behavioral Regulation	N-back Working Memory (NB-WM) <sup>a</sup>
	Flanker Inhibitory Control and Attention
	Behavior Rating Inventory of Executive Functioning-Adult Version (BRIEF-A)
Emotion Processing	Emotional Processing <sup>a</sup>
	Penn Emotion Recognition Test (ER-40)
	Emotion Regulation Questionnaire (ERQ)
Fluid Reasoning Composite	Composite of Flanker Inhibitory Control and Attention, List Sorting, Dimensional Change Card Sort, Pattern Comparison, and Picture Sequence (from NIH Toolbox)
Crystallized Cognition Composite	Composite of Picture Vocabulary and Oral Reading Recognition (from NIH Toolbox)
Episodic Memory	Rey-Auditory Verbal Learning Test (RAVLT)

<b>Mental and Behavioral Health</b>	
Dimensional Psychopathology	ASEBA Adult Self-Report (ASR)
Depression	Patient Health Questionnaire (PHQ-9)
Anxiety	Generalized Anxiety Disorder-7 (GAD-7)
Emotion	Negative Affect, Psychological Well-being, Social Relationships, and Stress and Self-Efficacy (from NIH Toolbox Emotion Measures)
Adult Transition Milestones	Adult Transition Milestone Questions (adopted from AMP Up)
Psychiatric Diagnoses	Psychiatric diagnoses in medical records and Client Diagnostic Questionnaire (CDQ) data from the AMP Up database
Sleep Hygiene	Pittsburgh Sleep Quality Inventory (PSQI)
<b>Risk Behaviors</b>	
Substance Use	Substance Use Questions (adopted from AMP Up with additional questions to harmonize with HCP-YA)
Sexual Risk Behaviors	
<b>Vulnerabilities</b>	
Childhood Adversity	Adverse Childhood Experiences (ACEs)-Revised Questionnaire for Adults
Stress and Trauma	Stressful Life Events (adopted from AMP Up QoL measure)
Household and Financial Resources	Information to be obtained through the online survey
<b>Protective Factors</b>	
Resilience	Brief Resilience Scale (BRS)
Social Support	Emotional Support, Instrumental Support Fixed Forms - NIH Toolbox Emotion Measures (Ages 18+)
<sup>a</sup> fMRI task activation and behavior such as reaction time, accuracy, d-prime. For details on fMRI see Section 8.7.	

## 5.1 Study Population

YPHEU aged 9-16 years who are current or former participants in the Surveillance Monitoring for ART Toxicities (SMARTT) study will be enrolled and followed longitudinally for two years. YAPHEU and YAPHIV between aged 22-29 years who are current participants in the PHACS Adolescent Master Protocol for Participants 18 Years of Age and Older (AMP Up) study will be enrolled and complete a one-time evaluation. (See Appendix IV for participating site requirements.)

## 5.2 Sample Size

Approximately 190 YPHEU and 100 total of YAPHEU and YAPHIV with about 1:3 ratio will be enrolled in the study.

## 5.3 Comparison Populations

After all YPHEU have been enrolled, comparison participants for YPHEU will be drawn from the ABCD study [44], frequency matched on sex, race, ethnicity, household income, and pubertal stage. ABCD data include neuroimaging, mental and physical health, cognition, risk behaviors, environment, and education collected from over 14,000 children enrolled at ages 9-10 and followed up to 10 years. The overall goal of the ABCD study was to identify risk factors for emerging substance user behavior in the general population

of this age group. It is conducted at 21 sites across the US. Because of the large sample size of the ABCD study and its diverse study population, we are confident that we will be able to obtain a comparison sample that will match the demographic and socioeconomic characteristics of our YPHEU cohort with a 3 to 1 or higher ratio in sample sizes. The ABCD study publicly releases its quality-controlled data as they are collected.

Comparison participants for YAPHEU and YAPHIV will be drawn from the HCP-YA study [175], frequency matched on sex, race, ethnicity, household income, and age, after all YAPHIV and YAPHEU have been enrolled. The HCP-YA study was designed to characterize brain connectivity and function in healthy young adults and has publicly released quality-controlled datasets from 1,200 participants aged 22-35 including neuroimaging, mental health, cognition, risk behaviors, physical health, education, and emotion. The HCP-YA study is completed and has publicly released all of its quality-controlled data.

#### **5.4 Study Duration**

This study is expected to be open for accrual and follow-up for up to five years and at least through the grant cycle. Enrollment into the Youth Longitudinal cohort will continue until the target enrollment number is achieved or through year 3 of the 5-year study period in order to allow sufficient time for completion of the 2-Year (24-Month) Follow-Up visit. Enrollment into the Young Adult Cross-Sectional cohort will continue until the target enrollment numbers are achieved or the end of the 5-year study period, whichever comes first

#### **5.5 Participant On-Study Duration**

YPHEU enrolled in the longitudinal cohort will be on the study for two years with one visit at Entry and a second visit two years later. YAPHEU and YAPHIV in the cross-sectional cohort will have a single study visit at Entry. It is recommended that each study visit be completed in two separate sessions (cognitive/behavioral and neuroimaging) occurring on two separate days.

### **6.0 ASTRO NESTED SUBSTUDY DESIGN**

The ASTRO nested substudy is a cross-sectional study that will evaluate differences in aortic stiffness among young adults (22-29 years old) in three groups: 100 young adults in about a 1:3 ratio of YAPHIV and YAPHEU, and similar-age individuals from the general population ( $n \approx 450$ ) and among youth (9-16 years old) in two groups: YPHEU ( $n = 50$ ) and similar-age individuals from the general population ( $n \approx 200$ ). Participants enrolled in the TERBO BRAIN study who consent to participate in the ASTRO nested substudy will undergo several brief procedures for the nested substudy at a single time point at Entry. Furthermore, their data collected in TERBO BRAIN will be incorporated into the analyses of this nested substudy to address novel research questions, which in turn will maximize the insights to be gained from existing efforts in PHACS.

#### **6.1 Nested Substudy Population**

YPHEU aged 9-16 years and YAPHEU and YAPHIV aged 22-29 years who are current participants in the TERBO BRAIN study will consent to participate and complete a one-time evaluation at Entry.

## **6.2 Nested Substudy Sample Size**

Approximately 150 participants (50 each of YPHEU, and approximately 100 young adults in about a 1:3 ratio of YAPHEU and YAPHIV) will consent to participate in the study.

## **6.3 Nested Substudy Comparison Populations**

Youth and young adult participants will be compared with similar-age individuals from the general population (~ 200 youth and ~ 450 young adults). General population comparison groups will be derived from the repository of cfPWV data that has been previously curated by Dr. Elaine Urbina.

## **6.4 Nested Substudy Duration**

This nested substudy is expected to be open for accrual and follow-up until the targeted sample size is achieved or completion of the TERBO BRAIN study, whichever occurs first.

## **6.5 Participant On-Study Duration**

Participants who consent to participate in this nested substudy will undergo several brief procedures at a single time point at Entry. Nested substudy procedures may be completed during the TERBO BRAIN Entry visit to enhance the likelihood of adequate participant recruitment and retention.

## **7.0 SELECTION AND ENROLLMENT OF TERBO BRAIN AND ASTRO NESTED SUBSTUDY PARTICIPANTS**

### **7.1 Inclusion Criteria**

#### **7.1.1 Longitudinal Cohort - YPHEU**

To be considered eligible for enrollment, an individual must meet all the criteria listed below:

- PHEU as documented in the medical record;
- Between 9 and 16 years of age at time of informed consent/assent, inclusive;
- Currently or formerly enrolled in the PHACS SMARTT study;
- Willing to provide access to existing medical records; and
- Willing to participate and provide legal consent and assent, if required.

#### **7.1.2 Cross-Sectional Cohort – YAPHEU and YAPHIV**

To be considered eligible for enrollment, an individual must meet all the criteria listed below:

- PHEU or PHIV as documented in the medical record;
- Between 22 and 29 years of age at time of informed consent/assent, inclusive;
- Currently enrolled in the PHACS AMP Up study;
- Willing to provide access to existing medical records; and

- Willing to participate and provide legal consent, and assent if required.

### **7.1.3 ASTRO Nested Substudy**

To be considered eligible for participation, an individual must meet all the criteria listed below:

- Enrolled in the TERBO BRAIN study; and
- Willing to participate in the ASTRO nested substudy and provide legal consent and assent, if required.

## **7.2 Exclusion Criteria**

### **7.2.1 Longitudinal Cohort – YPHEU**

To be considered eligible for enrollment, an individual must not meet any of the criteria listed below:

- Living with non-perinatally acquired HIV;
- Active untreated psychotic disorders that would interfere with participation in study procedures as determined by the clinical site Principal Investigator (PI) or designee;
- Extreme claustrophobia based on self- or caregiver-report (the scanning environment is a small space);
- Contraindications for magnetic resonance imaging (MRI) including irremovable metal (braces, permanent retainers, tattoos with metallic pigments, implants such as pacemakers, and other metals which may be dangerous and can drastically impact signal to noise in MRI);
- Self-reported traumatic brain injury that resulted in loss of consciousness for 30 minutes or longer anytime in the past;
- Motor, sensory, cognitive, or other impairments that preclude participation in study assessments as determined by the clinical site PI or designee;
- Although there are no known risks from exposure to magnetic fields (MRI) to pregnant people or fetuses, participants who think they are or may be pregnant will not be enrolled;
- Active substance use of a severity to interfere with participation in study procedures based on the judgement of the clinical site PI or designee;
- Non-English speaking (however, English-speaking individuals for whom English is not their primary language may participate); or
- Currently incarcerated or pending incarceration.

### **7.2.2 Cross-Sectional Cohort – YAPHEU and YAPHIV**

To be considered eligible for enrollment, an individual must not meet any of the criteria listed below:

- Living with non-perinatally acquired HIV;
- Active untreated psychotic disorders that would interfere with participation in study procedures as determined by the clinical site PI or designee;

- Extreme claustrophobia based on self or caregiver report (the scanning environment is a small space);
- Contraindications for MRI including irremovable metal (braces, permanent retainers, tattoos with metallic pigments, implants such as pacemakers, and other metals which may be dangerous and can drastically impact signal to noise in MRI);
- Self-reported traumatic brain injury that resulted in loss of consciousness for 30 minutes or longer anytime in the past;
- Motor, sensory, cognitive, or other impairments that preclude participation in study assessments as determined by the clinical site PI or designee;
- Although there are no known risks from exposure to magnetic fields to pregnant people or fetuses, participants who think they are or may be pregnant will not be enrolled;
- Active substance use of a severity to interfere with participation in study procedures based on the judgement of the clinical site PI or designee;
- Non-English speaking (however, English-speaking individuals for whom English is not their primary language may participate); or
- Currently incarcerated or pending incarceration.

### **7.3 Protocol Registration**

Prior to implementation of this study, the Harvard Longwood Campus Institutional Review Board (HLC IRB), the single IRB (sIRB) of record for this study, will approve the study protocol, including template informed consent and assent forms. Subsequently, the local IRBs at participating sites will cede review of this study to the HLC IRB through the execution of a reliance agreement. All site-specific participant-facing materials including informed consent form (ICF) addendums (to incorporate local IRB requirements), fact sheets, and recruitment materials must then be reviewed and approved by the HLC IRB. Finally, sites must receive protocol registration approval from Westat. Confirmation of protocol registration must occur before any participant is enrolled in the protocol. Original approved regulatory documents must be maintained at the site. This study will follow the PHACS procedures for protocol registration which are outlined in the PHACS Manual of Network Policies and Procedures (MNPP). The MNPP chapter pertaining to protocol registration can also be found on the PHACS website (<https://my.phacsstudy.org>).

Prior to implementation of ASTRO nested substudy, the HLC IRB will approve the TERBO BRAIN study protocol with the ASTRO nested substudy embedded (version 1.4 or later), as well as the ASTRO nested substudy ICF addendum and assent addendum. All site-specific participant-facing materials including fact sheets and recruitment materials that will be used for the ASTRO nested substudy must be reviewed and approved by the HLC IRB.

### **7.4 Participant Recruitment**

For the Youth Longitudinal Cohort, the Protocol Team will provide a list of potential participants selected from the pool of participants currently or formerly enrolled in PHACS SMARTT to each participating site. For the Young Adult Cross-Sectional Cohort, sites will recruit all available YAPHEU participants and recruit YAPHIV participants to match on age and sex. Site research staff will pre-screen each participant to determine their eligibility and approach the participant, or caregiver and participant, if the potential

participant is under 18 years of age or lacks the capacity to consent, who are potentially eligible to provide an overview of the study and gauge their interest in participating in the study.

The clinical site team will follow their standard practices and local institutional guidelines for reviewing patient records to pre-screen participants for eligibility based on the list provided by the Protocol Team. Potential participants with significant cognitive impairments that would render them unable to complete study assessments (as determined by the clinical site PI or designee) should not be enrolled in the study. Site staff should consult with the Protocol Team via the Protocol Query and Notification System (QNS) if they have any concerns regarding a participant's eligibility for the study. Site staff will not perform any study assessment or collect any data for the study until after informed consent/assent has been obtained.

To improve the feasibility of frequency matching between YAPHEU and YAPHIV cohorts by age and sex, the Protocol Team will review enrollment data when approximately 50% (i.e., 50 participants) of the targeted accrual has been achieved and will provide guidance to sites, as needed, for further enrollments.

For recruitment in the ASTRO nested substudy, participants being enrolled in TERBO BRAIN will be informed about the ASTRO nested substudy. Those interested and willing to participate in the ASTRO nested substudy will be consented. The ASTRO nested substudy will be open for accrual until the targeted sample sizes are achieved or the TERBO BRAIN enrollment is complete, whichever occurs first.

## **7.5 Informed Consent**

Once the participant is pre-screened eligible for the TERBO BRAIN study, informed consent will be obtained from the participant or the participant's legally authorized representative (LAR) with assent from the participant, as applicable, prior to enrollment and before conducting any study assessments. The informed consent process may occur in-person using paper ICF/assent form or occur remotely through web-based electronic ICF/assent. Study details, including risks and benefits, the information to be collected and assessments to be completed will be discussed with the potential participant and/or their LAR, and all questions will be answered. A copy of the signed ICF and assent form, if applicable, will be provided to the participant and/or their LAR.

For participation in the ASTRO nested substudy, informed consent to participate in the nested substudy will be obtained from the participant or the participant's LAR with assent from the participant, as applicable, using the ASTRO ICF addendum and assent addendum.

If remote consenting using web-based electronic ICF should occur, research staff will be available for phone consultation to address any questions or concerns the participant and/or their LAR may have. The web-based electronic ICF will include verification of comprehension and require participants or their LARs to acknowledge that they have read and agree to the consent form by checking a box following each section of the consent. Verification of identification during the consent process will be confirmed using a consent ID number provided to the participant or their LAR by the clinical site at the time of consent. Security questions may be employed for further verification.

## **7.6 Enrollment Procedures**

When a participant is eligible for the TERBO BRAIN study and informed consent has been obtained, the site will use the Study Enrollment System (SES) at Frontier Science, the Data Management Center (DMC) for PHACS, to enter participant and eligibility information. Participants will continue to use the PHACS participant identification number (PID) they were assigned from their participation in the SMARTT or

AMP Up study. Once confirmed eligible and enrolled, the SES will generate a study identification number (SID). The SID will also serve as the participant's protocol-specific Personal Identification Number (PIN) that will be used as the participant identifier in TERBO BRAIN online assessments. Note that the same SID assigned to the participant for the TERBO BRAIN study will also be used for the ASTRO nested substudy.

## **7.7 Co-Enrollment Guidelines**

Enrollment of TERBO BRAIN and ASTRO participants in other studies (with or without similar goals/data collection as TERBO BRAIN and ASTRO) is at the discretion of the clinical site PI and the TERBO BRAIN and ASTRO Protocol Co-Chairs. The clinical site PI must take into account any issues that enrollment in the additional study may require and which may compromise the participant's ability to fulfill the requirements of the TERBO BRAIN study and/or the ASTRO nested substudy.

Enrollment of participants who are already enrolled in other studies of youth and young adults with PHIV and PHEU into the TERBO BRAIN study/ASTRO nested substudy is at the discretion of the Protocol Co-Chairs.

Sites must query the respective Protocol Teams through the QNS for permission to co-enroll participants. The Protocol Team will provide either a "blanket," one-time approval or case-by-case permission for co-enrollment.

## **8.0 TERBO BRAIN STUDY EVALUATIONS DESCRIPTION AND ADMINISTRATION**

Evaluations will assess vulnerabilities and protective factors, mental health, cognition, neuroimaging tasks, and risk behaviors at each visit, in order to collect multi-level data that allow for systems-based analyses and the ability to probe mechanisms.

### **8.1 Cognition**

#### **8.1.1 Computerized Tests**

- Delay Discounting and Cash Choice (YPHEU): The ABCD study included two measures of reward processing and choice, or delay discounting. Due to concerns about task length, ABCD used a one-item Cash Choice Task [23, 176] at baseline, administered verbally by the examiner, as will be done for TERBO. A standard adjusting Delay Discounting paradigm [177] involving 42 choices was included at the one-year follow-up visit. In both tasks, the participant is asked to choose between smaller hypothetical monetary amounts administered immediately, and larger amounts administered later, with the time interval adjusted to determine the participant's "indifference point," indicating future orientation and ability to defer gratification. The Delay Discounting task will be administered using an iPad by the examiner using the ABCD procedures. TERBO BRAIN will include the Delay Discounting task and administer the one-item Cash Choice Task as well to facilitate comparison with ABCD data.
- Emotion-Word/Emotion-Face Stroop (YPHEU): This task [178] examines executive control in the context of distracting emotional information. The participant is presented with words on a computer screen and asked to judge the emotional valence (positive, negative) of the word. This judgment is made in the context of an emotional face behind the word, which either agrees (congruent) or conflicts (incongruent) with the valence of the word. Because congruency typically results in faster responding, response times on the two types of trials are compared. This task will be administered using an iPad by the examiner following ABCD procedures.

- Delay Discounting (YAPHEU and YAPHIV): To harmonize with the HCP-YA study, a different version of the Delay Discounting task will be administered for YAPHEU and YAPHIV [179-181]. This version of the task provides an area-under-the-curve discounting summary measure that indicates how steeply an individual discounts delayed rewards. This task will be completed by the participant using a laptop, with the examiner setting up and initiating the tasks.
- Penn Emotion Recognition Test (YAPHEU and YAPHIV): This task, harmonized with the HCP-YA study, assesses facial emotion recognition ability using images of 4 different emotions (anger, fear, happiness, sadness) in low and high intensity and neutral expressions [182]. This test will be completed by the participant using a laptop, with the examiner setting up and initiating the tasks.

### **8.1.2 NIH Toolbox Cognitive Subtests**

The NIH Toolbox for the Assessment of Neurological and Behavioral Function [183] is a multidimensional set of brief measures that assess cognitive functioning in children, adolescents, and adults. Cognitive subtests evaluate the mental processes involved in gaining knowledge and comprehension, such as thinking, knowing, remembering, judging, and problem-solving. This battery consists of tests to assess Executive Function, Attention, Episodic Memory, Language, Processing Speed and Working Memory and includes the following summary scores, in addition to individual measure scores: Cognitive Function Composite Score, Fluid Cognition Composite Score (includes Dimensional Change Card Sort, Flanker, Picture Sequence Memory, List Sorting, and Pattern Comparison measures), and Crystallized Cognition Composite Score (includes Picture Vocabulary and Reading Recognition measures). The NIH Toolbox was developed and validated with state-of-the-science methodology to be psychometrically sound and appropriate for measuring outcomes in longitudinal studies; scores are based on a nationally representative sample to enable cross-measure comparisons. In addition to using the composite scores, age-corrected standard scores from individual tasks such as the Flanker will contribute to domains listed in Tables 1 and 2.

The following NIH Toolbox cognitive tests will be administered to YPHEU, YAPHIV, and YAPHEU participants by the examiner using the NIH Toolbox App using an iPad.

- Flanker Inhibitory Control and Attention
- List Sorting
- Dimensional Change Card Sort
- Pattern Comparison
- Picture Sequence
- Picture Vocabulary
- Oral Reading Recognition
- Rey-Auditory Verbal Learning Test

### **8.1.3 NIH Toolbox Emotion Domain Questionnaires**

The following brief questionnaire modules from the NIH Toolbox Emotion domain will be administered using an iPad to harmonize with the HCP-YA study [184] for YAPHEU and YAPHIV participants only.

- Negative Affect (Sadness, Fear, Anger)
- Psychological Well-being (Positive Affect, Life Satisfaction, Meaning and Purpose)

- Social Relationships (Social Support, Companionship, Social Distress, Positive Social Development)
- Stress and Self-Efficacy (Perceived Stress, Self-Efficacy)

#### **8.1.4 Behavior Rating Inventory of Executive Functioning**

The Behavior Rating Inventory of Executive Functioning, Second Edition (BRIEF-2) caregiver and self-report questionnaires are standardized paper-and-pencil measures of an adolescent's executive functions or self-regulation in everyday environments. Youth ( $\geq 11$  years old) and parent reports are available and will be used for YPHEU. Two broad indices, Behavioral Regulation and Metacognition, and an overall summary score are included. New standardization data for BRIEF-2, based on a nationally representative sample of 3600 cases, are available for adolescents. Reliability coefficients for the Parent Forms are .90+ and .80+ for the Self-report Form. The BRIEF-2 has concurrent validity with the Child Behavior Checklist (CBCL), Behavior Assessment System for Children, Second Edition (BASC-2), Reynolds Intellectual Assessment Scales (RIAS), Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV), and Wechsler Adult Intelligence Scale, Fourth Edition (WAIS-IV).

The Behavior Rating Inventory of Executive Function-Adult Version (BRIEF-A) [185, 186] will be used for YAPHEU and YAPHIV. Participants will complete the paper-and-pencil questionnaire independently unless assistance is required from the examiner. Two broad indices, Behavioral Regulation and Metacognition, and an overall summary score are provided. Standardization data based on a nationally representative sample of 1136 adults are available. Reliability, validity, and clinical utility are demonstrated.

#### **8.1.5 Emotion Regulation Questionnaires**

Regulation of one's own emotions will be assessed with the Emotion Regulation Questionnaire (ERQ) as self-report for youth and young adults, and the Difficulties in Emotion Regulation Scale – Parent Report (DERS-P) for caregivers to complete regarding youth participants. These questionnaires are administered to harmonize with the ABCD study. The ERQ was developed for use with adults as a 10-item instrument with a 7-point response scale and was designed to reflect an individual's tendency to regulate emotions using cognitive reappraisal or expressive suppression [187]. The standard adult version will be self-administered for YAPHIV and YAPHEU. The ERQ was adapted for children as the Emotion Regulation Questionnaire for Children and Adolescents (ERQ-CA) [188] and the ABCD study instituted a 6-item self-report version of the questionnaire at their Year 3 assessment [189]. A caregiver-completed questionnaire regarding the child or adolescent's emotion regulation, the DERS-P was also added to the ABCD battery at the Year 3 timepoint and will be administered to caregivers of YPHEU participants enrolled in the TERBO BRAIN study at both timepoints. The ERQ-CA has shown good internal consistency and test-retest reliability over 12 months in a sample of 10–18 year-olds. The DERS-P examines difficulties across domains related to nonacceptance, goals, impulses, strategies, awareness, and clarity. The DERS-P was validated in a sample of 11–17 year-olds and has been shown to have good concurrent and convergent validity [190]. The ABCD study selected 29 items from the DERS-P, eliminating 7 items administered in the original study that did not load onto any factors.

## 8.2 Mental Health

### 8.2.1 Achenbach System of Empirically Based Assessment (ASEBA)

Emotional and behavioral problems will be assessed by the Achenbach System of Empirically Based Assessment (ASEBA) [191-193] questionnaire. The ASEBA family of instruments is well validated to study global psychopathology in children and adolescents. They can be completed using paper-and-pencil questionnaires or using a web-based version. The ASEBA CBCL is a questionnaire that assesses a broad range of emotional and behavioral problems in school-age children (CBCL/6-18, for ages 6-18) [194]. It will be completed by the caregiver for all YPHEU and by those YPHEU who are 11 years and older. Caregivers will complete the questionnaire independently with assistance from the examiner as needed and youth will complete the questionnaire as an interview administered by the examiner. Responses are coded as 0 (not true), 1 (somewhat or sometimes true), or 2 (very true or often true). For school-age children, two informants will be used: parent (CBCL/6-18) and child/adolescent (youth self-report, for ages 11-18). In addition to a total score, eight empirically based syndrome scales and six Diagnostic and Statistical Manual of Mental Disorders (DSM)-oriented scales are reported. The eight syndrome scales are: (1) anxious/depressed; (2) withdrawn/depressed; (3) somatic complaints; (4) social problems; (5) thought problems; (6) attention problems; (7) rule-breaking behavior; and (8) aggressive behavior. Some of the eight syndrome scales are grouped into internalizing (anxious/depressed, withdrawn/depressed, somatic complaints) and externalizing (rule-breaking behavior, aggressive behavior) problems. The six DSM-oriented scales are: (1) affective problems; (2) anxiety problems; (3) somatic problems; (4) attention-deficit/hyperactivity problems; (5) oppositional defiant problems; and (6) conduct problems. According to the standardization data of the CBCL, a t-score  $\leq 59$  indicates non-clinical symptoms, a t-score between 60 and 64 indicates that the child is at risk for problem behaviors, and a t-score  $\geq 65$  indicates clinical symptoms. Findings provide strong evidence for the reliability, as well as convergent and discriminative validity, of these scales.

The ASEBA Adult Self-Report (ASR) [195] is a well-validated instrument to assess adult (18-90 years) psychopathology and is used for clinical and research purposes in mental health settings. It will be utilized for YAPHEU and YAPHIV. The ASR is part of the ASEBA taxonomy and consists of items to assess adaptive functioning and problems. The ASR comprises 8 syndrome scales:

- The combination of the syndrome scales Anxious/Depressed (18 items), Withdrawn (9 items), and Somatic Complaints (12 items) results in the broadband scale Internalizing problems.
- The combination of the syndrome scales Aggressive Behavior (15 items), Rule-breaking Behavior (14 items), and Intrusive Behavior (6 items) forms the broadband scale Externalizing problems.
- The other syndrome scales are Attention Problems (15 items) and Thought Problems (10 items).
- Other Problems (21 items) include items that did not qualify for any syndrome.
- The remaining 11 items measure adaptive functioning and are not included in analyses.

The total score on the ASR, based on all problem items (N = 120) represents the Total Problems score for adult psychopathology. The ASR will be self-administered by the participant using a paper-and-pencil questionnaire; the examiner will provide assistance as needed.

### 8.2.2 Children's Depression Inventory-2 (CDI-2)

The CDI-2 [196], to be used for YPHEU, is a brief (28 items) self-report and parent-report questionnaire that helps assess cognitive, affective and behavioral signs of depression in children and adolescents, aged

7 through 17 years. The self-report version will be administered as an interview while the parent-report will be self-administered by the caregiver. The CDI-2 asks about key symptoms of depression, such as a child's feelings of worthlessness and loss of interest in activities. The 28 items of the CDI-2 yield a total score, 2 scale scores (emotional problems and functional problems), and 4 subscale scores (negative mood/physical symptoms, negative self-esteem, interpersonal problems, and ineffectiveness). Each item allows the child/caregiver to respond to 3 options that indicate 3 levels of symptoms: 0 (absence of symptoms), 1 (mild or probable symptoms), or 2 (definite symptoms). The CDI-2 standardization sample is representative of the U.S. population and includes 1100 children aged 7 to 17 years from 26 states in the U.S.; the sample is evenly proportioned in terms of age and gender, with 50 males and 50 females at each age. The racial/ethnic distribution of the sample matches the U.S. census distribution very closely (i.e., all races within 1% of 2000 Census targets). Overall, the normative sample includes a reasonable spread of geographical locations of all four major regions of the U.S.

### **8.2.3 Patient Health Questionnaire-9 (PHQ-9)**

The Patient Health Questionnaire-9 (PHQ-9) [197, 198] is a self-administered version of the Primary Care Evaluation of Mental Disorders (PRIME-MD) diagnostic instrument for common mental disorders. TERBO will administer this paper-and-pencil questionnaire for YAPHEU and YAPHIV during the participant's visit. PHQ-9 consists of nine items measuring depressive symptoms corresponding to the diagnostic criteria for major depressive disorder. Each item is scored on a four-point Likert scale (0–3), including "0" (not at all) to "3" (nearly every day). Total scores range from 0 to 27, with higher scores reflecting greater depression severity. The PHQ-9 has shown good psychometric properties. The PHQ-9 has been strongly supported for its applicability as a screening tool for depression in primary care settings.

### **8.2.4 Screen for Child Anxiety Related Disorders (SCARED)**

The SCARED-P (parent version) and SCARED-C (child self-report) [199-201] are 41 item questionnaires that screen for signs of anxiety disorders in children and will be used for YPHEU. The SCARED-P will be self-administered by the caregiver and the SCARED-C will be administered to the participant as an interview. Participants respond on a 3-point Likert-type scale of "0" (Not True or Hardly Ever True), "1" (Somewhat or Sometimes True), or "2" (Very True or Often True). Prior confirmatory factor analyses suggest that the instrument measures five distinct domains of anxiety. Thus, in addition to total scores, five subscales were examined: generalized anxiety symptoms (nine items), separation anxiety symptoms (five items), social anxiety symptoms (eight items), panic or somatic symptoms (seven items), and school avoidance (three items). A total score of 25 or above has been suggested to indicate the presence of clinically significant anxiety.

### **8.2.5 Generalized Anxiety Disorder-7 (GAD-7)**

The GAD-7 [202] is a valid and efficient tool for screening for generalized anxiety disorder and assessing its severity in clinical practice and research. TERBO will administer this questionnaire for YAPHEU and YAPHIV during the participant's visit using the online survey tool. The GAD-7 consists of seven items measuring worry and anxiety symptoms and has good reliability, as well as criterion, construct, factorial, and procedural validity. A cutoff score of 10 was identified as the optimal point for sensitivity (89%) and specificity (82%). GAD-7 scores are categorized as follows for screening anxiety: minimal anxiety = 0-4, mild anxiety = 5-9, moderate anxiety = 10-14, and severe anxiety 15-21. Increasing scores on the scale were strongly associated with multiple domains of functional impairment [202-204].

### **8.2.6 Client Diagnostic Questionnaire (CDQ)**

The CDQ is a psychiatric screening tool designed and validated for adult populations affected by HIV [205]. The CDQ is a structured interview used to identify current symptoms of psychiatric disorders, including depression, anxiety, post-traumatic stress disorder, and psychosis, as well as alcoholic and nonalcoholic substance abuse. The CDQ is administered to caregivers in SMARTT and to participants in AMP Up. Caregiver CDQ data from the SMARTT database and participant CDQ data from the AMP Up database will be used to obtain information about historical mental health and substance use issues in caregivers of YPHEU and YAPHEU and YAPHIV participants. The CDQ will not be re-administered as part of this study.

### **8.2.7 Pittsburgh Sleep Quality Index (PSQI)**

The Pittsburgh Sleep Quality Inventory will be self-administered by YAPHEU and YAPHIV participants through the online survey with assistance from the examiner, as needed. It is widely used and contains 19 items used to create 7 component scores addressing different aspects of sleep (e.g., sleep latency, subjective sleep quality, sleep duration, and disturbances) and one global score [206].

## **8.3 Risk Behaviors**

Risk behavior assessment for the YPHEU will be conducted using the online survey, with questions adopted from the substance use module in the SMARTT ACASI and modified to include harmonized items from the ABCD study. Questions on substance use, including use of tobacco, alcohol, marijuana, and illicit drugs, will be included. Participants will be asked whether they have used a substance and, if yes, the frequency of use along with other questions about use and consequences of use. On rare occasions, if needed, an examiner can be available to help the participant answer questions or assist with use of the online survey on computer or iPad, if participant agrees.

Risk behavior assessment for the YAPHEU and YAPHIV will also be conducted using the online survey with questions adopted from the AMP Up online survey. The survey will include questions about substance use as well as sexual risk behaviors. Additional questions about past 7-day use of alcohol and tobacco will be administered during the cognitive/behavioral session as a paper-and-pencil questionnaire to harmonize with the HCP-YA study.

## **8.4 Vulnerabilities**

### **8.4.1 Adverse Childhood Events (ACEs)**

The Pediatric Adverse Childhood Experiences and Related Life Events Screener (PEARLS) [207] will be used for report of adverse life events in YPHEU. The PEARLS Child will be used for caregiver report for participants up to 11 years of age and PEARLS Teen Self-Report will be used for self-report for participants 12 years and older. The PEARLS Teen Parent/Caregiver Report will be used for parent or caregiver report of teen's adverse life events.

The ACEs-Revised Questionnaire for Adults will be used for YAPHEU and YAPHIV. This questionnaire is available through the ACEs Aware project and asks whether the participant experienced each of 10 adverse circumstances (e.g., neglect, abuse, loss of caregiver) during their first 18 years. The ACEs-Revised Questionnaire for Adults will be administered through the online survey tool.

#### **8.4.2 Life Events Checklist**

The Life Events Checklist adopted from AMP will be used for YPHEU for self-report of stressful life events. This questionnaire allows adolescents to identify recent stressful life events among 43 possible events and asks youth to identify the positive, negative, or neutral impact of such events. The Life Events Checklist will be administered as an interview using a paper-and-pencil questionnaire.

#### **8.4.3 Stressful Life Events**

For YAPHEU and YAPHIV, the Stressful Life Events Questionnaire [208-210] will be used to identify stressful life events, such as loss of housing or death in the family, during the past 12 months. It was derived from the General Health Assessment for Children (GHAC), a group of age-specific, modular instruments developed for earlier studies of youth affected by HIV [208] and now adopted for young adults. Earlier studies demonstrated that the GHAC has adequate internal consistency, reliability, and validity.

#### **8.4.4 Family and Household/Financial Resources**

The Demographics Questionnaire will be used to identify important family resources information, including family members (caregivers and siblings or others), family size, caregiver education, and family income for YPHEU. The questionnaire will be completed by the caregiver using a paper-and-pencil questionnaire. For YAPHEU and YAPHIV, the participant will provide information on their household and personal financial resources as part of the online survey.

### **8.5 Protective Factors**

#### **8.5.1 Resilience Scale**

The Child and Youth Resilience Measure-Revised (CYRM-R) [211], to be administered for YPHEU, is a measure of social ecological resilience suitable for youth aged 10-23; revised versions of the measures consist of 17 items and can be scored on 3- or 5-point Likert scales. The items in the measures are all positively worded; scoring involves simple summing of responses. In addition to an overall score of resilience, scores can be derived for the two subscales of the measures: Personal Resilience and Caregiver Resilience.

Personal Resilience includes intrapersonal and interpersonal items. Caregiver Resilience relates to characteristics associated with the important relationships shared with either a primary caregiver or a partner or family. These are linked, as both dimensions depend on individuals' social ecologies to reinforce their resilience. The CYRM-R will be self-administered by the participant using paper-and-pencil questionnaires, with assistance from the examiner, as needed.

The Brief Resilience Scale (BRS) [212, 213], to be used for YAPHEU and YAPHIV, is a brief measure of resilience suitable for adults, consisting of 6 items, scored on a 5-point Likert scale. Scoring involves simple summing of responses. The BRS has adequate criterion validity and internal consistency. The BRS will be administered using the online survey tool.

#### **8.5.2 Prosocial Behavior Survey**

The YPHEU participants and their caregivers will complete a brief interview of prosocial behavior, adapted from the Prosocial Behavior Scale of the Strength and Difficulties Questionnaire [214]. Prosocial Behavior,

or the tendency to engage in behaviors to help others, is evaluated in the ABCD cohort, has been studied as part of social competence and resilience in earlier studies of adolescent development, is associated with multiple indicators of mental health and well-being, and has been found to be a protective factor against the development of problem behavior and aggression [215]. The original subscale has 5 items; we will retain three items with the highest factor loadings (e.g., being considerate of other people's feelings, often offering to help others) that are used in the ABCD study. Youth and caregivers rate these behaviors over the past 6 months on a three-point scale ("0=Not True" to "2=Certainly True") [216].

### **8.5.3 Social Support**

Social Support is a concept within the Social Relationships subdomain of the NIH Toolbox Emotion measures. Emotional Support is a self-reported measure of Social Support and refers to the perception that people in one's social network are available to listen to one's problems with empathy, caring, and understanding. This brief, calibrated scale will be administered by iPad for YPHEU youth ages 9-17 years. Instrumental Support refers to the perception that people in one's social network are available to provide information or advice needed to solve problems that arise. Emotional Support and Instrumental Support which each consists of 8 items will be administered by iPad to YAPHIV and YAPHEU participants.

## **8.6 Pubertal Development**

YPHEU will complete the Pubertal Development Scale (PDS) [217], a widely used self-report measure of development of sexual characteristics which has been shown to be correlated with measures of pubertal development derived from physical examination. This measure will be used to match the YPHEU group for maturation with a comparison sample from the ABCD study. There are male and female versions of the PDS, which asks respondents to report their level of development on five indices. Boys are asked whether growth has not begun, barely begun, is definitely underway, or has finished on five dimensions: body hair, facial hair, voice change, skin change, and growth spurt. Girls are asked the same questions about body hair, skin change, breast development, and growth spurt, and are asked whether menses have begun. Responses are coded on 4-point scales (1 = no development and 4 = completed development). For both genders, ratings are then averaged to create an overall score for physical maturation. YPHEU will complete this measure as part of the online survey for privacy; caregivers will complete a paper-and-pencil version.

## **8.7 Neuroimaging**

### **8.7.1 Harmonizing Multisite MRI Scanning Protocol**

For the youth longitudinal cohort, we will adopt the harmonized ABCD Study scanning protocols [79, 218]. This will allow us to compare YPHEU data to the ABCD data. The Northwestern team, led by Dr. Todd Parrish (Protocol Team Member), works directly with site research staff (e.g., clinical site research assistants, technicians at MRI facilities) and oversees the multisite neuroimaging data acquisition and QA. The ~90 minute scanning protocol includes structural MRI, task-based fMRI, rs fMRI, and diffusion MRI. The order of the fMRI tasks will be randomly generated. The 90-minute scanning protocol has been successfully used in the ABCD study for children as young as 9 years old. A short break will be offered following the task-based fMRI. The scanning protocol will be collected by site MRI facility technician, aided by site research staff. The scan itself is approximately 90 minutes (including the break); the participant may be in the scanner for up to 2 hours, and the total visit can take approximately 3 hours. Refer to the TERBO BRAIN Manual of Procedures (MOP) for detailed information on scanner-specific scanning parameters.

The fMRI tasks for the youth longitudinal cohort are:

- Monetary incentive delay (MID) [219]. MID is one of the most commonly used fMRI tasks to measure reward processing.
- Stop Signal Task (SST). SST is among the most frequently used fMRI tasks to measure inhibition of a prepotent response tendency, e.g., demonstrating that reduced cognitive control over emotional stimuli can predict risk-taking in adolescents [220], and poor cognitive control in relapse of depression in young adults [221].
- Emotional N-back (EN-back) [222]. EN-back is a widely used fMRI task to more directly measure emotion regulation and emotional reactivity in the context of cognitive processes by including emotional stimuli, such as emotional faces, and requiring a cognitive component, such as working memory or response inhibition.

For the young adult cross-sectional cohort, we will adopt an abbreviated Human Connectome Project (HCP) neuroimaging protocol that is comparable to the youth protocol. The full HCP protocol is ~4 hours [175, 223]. Our adaptation involves fewer repeating sessions for structural MRI, diffusion MRI and rs fMRI scanning, and select HCP tasks that are relevant to our hypothesis on emotional regulation. This will still allow us to compare HCP-YA data (fewer sessions of HCP data analyzed) with YAPHEU and YAPHIV. The Northwestern team, led by Dr. Todd Parrish (Protocol Team Member), works directly with site research staff and oversees the multisite neuroimaging data acquisition and QA. The ~60-minute scanning protocol includes structural MRI, task-based fMRI, rs fMRI, and diffusion MRI. The order of the fMRI tasks will be randomly generated. The scanning protocol will be collected by site MRI facility technician, aided by site research staff. The scan itself is approximately 70 minutes; the participant may be in the scanner for up to 1.5 hours, and the total visit can take approximately 2.5 hours. Refer to the TERBO BRAIN MOP for detailed information on scanner-specific scanning parameters.

The fMRI tasks for the young adult cross-sectional cohort are:

- Gambling (GAM). HCP adaptation of a card-game task developed by Delgado et al. [224] is used to assess reward processing and decision making [184].
- Emotional Processing (EP). HCP adaptation of the Hariri Hammer Task developed by Hariri et al. [225], is used to assess emotional processes [184].
- N-back Working Memory (NB-WM). HCP adaptation of the N-back task developed by Drobyshevsky et al. [226] is used to assess working memory and executive function [184].

### **8.7.2 Acclimation, Training and Practicing**

It is paramount to assure quality of neuroimaging data. Participants may need to be familiarized with the noisy MRI scanner environment and learn to minimize motion during scanning. We will follow best practices of standardization of data collection and quality practices adapted by the ABCD study [227], which was modeled on the HCP study. These practices include training and scanner acclimation. Practicing of the fMRI task is also required to ensure that participants understand the task instructions. These procedures will be administered by site research staff immediately prior to scanning, lasting approximately 60 minutes.

As with all study procedures, if a participant becomes upset and does not want to continue with the acclimation, training, and practicing session, the participant can request to stop the session at any time. Refer to the TERBO BRAIN Imaging MOPs for detailed information on scanner acclimation, training, and practicing procedures.

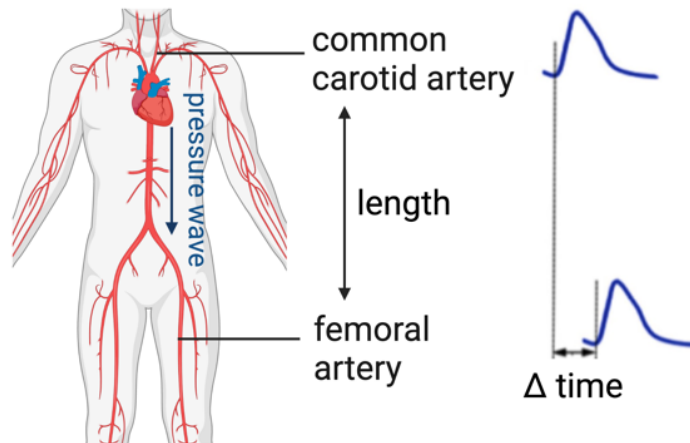
## 9.0 ASTRO NESTED SUBSTUDY PROCEDURES AND ASSESSMENTS

Participants who also consented to participate in the ASTRO nested substudy will complete the procedures described below within the window specified in Section 10.0.

### 9.1 Carotid-Femoral Pulse Wave Velocity

SphygmoCor technology will be used to ascertain cfPWV as the gold standard measure of aortic wall stiffness. The cfPWV describes the speed at which a pressure wave propagates down the aorta with higher values indicating a stiffer vessel. Pressure waveforms at the common carotid and femoral arteries will be assessed transcutaneously in the supine position by trained site personnel. The length between the two body surface sites and the time delay between the waveforms will be used to derive cfPWV (Figure 6). The SphygmoCor device is easy to use with basic training and yields highly reproducible results. Dr. Urbina will oversee the acquisition of cfPWV on study participants across all participating sites.

**Figure 6. cfPWV = Length/ $\Delta$  Time**



### 9.2 Vital Signs and Anthropometrics

Weight, height, and blood pressure will be assessed in light clothing using standard techniques. Mid-waist and hip circumferences will also be measured in triplicate by trained study staff.

### 9.3 Interviewer-Administered Questionnaires

Caregiver education for young adult participants only and physical activity (International Physical Activity Questionnaire [IPAQ] - Short Form) for both youth and young adult participants will be collected via interviewer-administered questionnaires. Responses may be provided by the study participant and/or an accompanying parent/guardian or caregiver [217, 228].

- The primary caregiver education questionnaire is comprised of a single question regarding the highest educational degree held by the participant's primary caregiver while growing up. This question will be asked of young adult participants only. This information is collected for youth participants as part of their TERBO BRAIN participation and the information will be used for the ASTRO nested substudy.

- The IPAQ – Short Form is a 7-item questionnaire that assesses the types of physical activity that the participant was engaged in within the past 7 days.

## **9.4 Fasting Blood Draw**

Fasting blood will be drawn, processed at individual study sites, stored, and shipped to the PHACS Repository per the ASTRO Laboratory Processing Chart (LPC). Approximately 30 mL of blood will be drawn in total for both pre-specified assays (~15 mL) and future studies (~15 mL) as described below.

### **9.4.1 Metabolic and Immune Serologic Markers**

Fasting blood will be drawn for plasma glucose and serum insulin and lipids. Glucose and insulin will be used to calculate HOMA-IR as a measure of insulin resistance [229]. Serum high-sensitivity CRP [230, 231], plasma soluble CD163 (sCD163) [232, 233], and soluble CD14 (sCD14) [234, 235], also will be measured as key immune markers that have been shown to be elevated in both cardiovascular disease and HIV infection. CD4 T cell count and HIV viral load in YAPHIV participants will be ascertained from AMP Up and TERBO BRAIN as measures of HIV infection severity in YAPHIV.

### **9.4.2 Eicosanoid Profiling**

Fasting blood will be drawn for eicosanoid profiling. Eicosanoid profiling (>100 eicosanoids) will be performed on fasting plasma samples using semi-targeted metabolomics techniques in the Einstein Stable Isotopes and Metabolomics Core under the direction of Irwin Kurland, MD, PhD. Samples will be processed, stored locally, and shipped to the PHACS Repository as specified in the ASTRO LPC. Eicosanoids are a complex network of bioactive lipids that play critical roles in biologic processes including metabolism, inflammation, and endothelial function [236]. In collaboration with Dr. Kurland, we have previously shown a distinct eicosanoid signature in infants with PHEU vs. controls in association with metabolic and immune parameters [148]. In the general population, plasma eicosanoids have been shown to strongly correlate with blood pressure [237], which is mechanistically linked to arterial stiffness [141]. Furthermore, supplementation of omega-3 polyunsaturated fatty acids (key eicosanoid precursors) has been found to attenuate arterial stiffness in several small studies [160], possibly due to a favorable shift in the eicosanoid profile [238]. In this nested substudy, relationships of eicosanoids with aortic stiffness among the PHIV and PHEU populations will be investigated. Evidence of novel associations between eicosanoids and aortic stiffness may implicate omega-3 fatty acid supplementation as a potential intervention to attenuate aortic stiffness in these groups, to be explored in future studies.

### **9.4.3 Specimens for Future Studies**

Fasting blood will be drawn and processed for serum and EDTA plasma for storage in the PHACS Repository for yet to-be-determined future studies. Samples will be processed, stored locally, and shipped to the PHACS Repository as specified in the ASTRO LPC. Permission to store specimens in the PHACS Repository for future studies will be obtained from participants as part of the ASTRO nested substudy consent process. Participants who do not agree to the storage of their specimens in the PHACS Repository for future studies may still participate in the ASTRO nested substudy.

## 10.0 SCHEDULE OF EVALUATIONS AND PROCEDURES

The TERBO BRAIN study will assess vulnerabilities and protective factors, mental health, cognition, risk behaviors, and neuroimaging tasks at each study visit in order to collect multi-level data that allow for systems-based analyses and the ability to probe mechanisms.

It is recommended that each TERBO BRAIN study visit be completed in two separate sessions occurring on two separate days.

- The **cognitive/behavioral session** includes cognitive, mental health, and behavioral health assessments, questionnaires for demographic and clinical (e.g., pubertal status; medications) information, and a blood draw if needed to assess viral load and CD4 T cell count and percent in YAPHIV. The assessments are expected to take 2-3 hours and will vary in length depending in part on substance use reported by the participant; breaks will be provided as needed for participants and caregivers.
- The **neuroimaging session** includes training, practice, and MRI scanning and is expected to take 3-4 hours.

The cognitive/behavioral session should be conducted at the first of the two sessions, except where needed to accommodate scheduling constraints. While completing both sessions within a 3-week time period is preferred, scheduling the neuroimaging session can be difficult due to scanner and/or participant availability. Thus, the second visit can occur outside the 3-week window, if needed, to accommodate scheduling constraints. This flexibility is meant to reduce missing data and participant burden and schedule disruption. An extended window does not impact data/specimen fidelity. If/when this occurs, it will be documented with a note to file in the participant's record.

Chart abstraction must be completed on or within 3 months after completion of the first session of the study visit.

**In the unlikely event that technical or unforeseen issues arise a participant may need to return to complete or repeat study assessments, including the neuroimaging session (e.g., unable to complete scans, scan files become corrupted), .** Upon return to complete or repeat scans, reaffirmation/continued agreement from the participant to complete or repeat scans will be obtained prior to the re-scan session. The participant will be compensated for their time and effort completing the re-scan session. A similar reaffirmation should be obtained when other study-related assessments are not able to be completed for technical or unforeseen issues.

The ASTRO nested substudy procedures and assessments should preferably occur anytime from when the consent/assent addenda are signed until 6 months following the final session of the TERBO BRAIN Entry visit. However, the visit may occur outside the 6-month window if needed, to accommodate scheduling constraints. An extended window does not impact data/specimen fidelity but is meant to minimize disruption to and burden on participants. If/when this occurs, it will be documented with a note to file in the participant's record. An optimal time to complete the study procedures from a logistical standpoint may be on the day of the cognitive/behavioral session of the TERBO BRAIN study visit, prior to the TERBO BRAIN evaluation. The nested substudy procedures and assessments are expected to take approximately one hour.

The evaluations outlined below will be performed with each participant, after signed informed consent is obtained, as part of their participation in this study. See Appendices I and II for tabulated summaries of the TERBO BRAIN evaluations described below and their schedule for completion. See Appendix III for

tabulated summaries of the ASTRO procedures and evaluations described below and their schedule for completion.

When participants come off study, contact information will be requested in order to be able to notify participants of important findings, if necessary, or to request participation in future evaluations.

Refer to the TERBO BRAIN Protocol MOP, TERBO BRAIN Assessments Manual, TERBO BRAIN Imaging MOPs, and ASTRO Nested Substudy MOP for additional detailed information on the evaluations and administration procedures.

### **10.1 Youth Longitudinal Cohort at Entry and 2-Year (24-Month) Follow-Up**

Evaluations for YPHEU will be completed at Entry and again at the 2-Year (24-Month) Follow-Up. The evaluations are the same at each of the two study visits and it is recommended that each study visit be completed in two separate sessions (cognitive/behavioral and neuroimaging) occurring on two separate days.

In the time between the two study visits, the site research staff will contact the participant once every six months to check-in on how they are doing and update the family's contact information, if necessary. If the site were already in contact with the participant around the same time, then the phone call can be omitted. Each phone call will take about 5 minutes.

For certain measures, if the measure was completed as part of the SMARTT study within a certain window of the TERBO BRAIN study visit (see below), it does not need to be repeated for the TERBO BRAIN study. In addition to self-report, the caregiver will complete assessments regarding the emotional/behavioral well-being of the participant and report information on behalf of the participant, as needed.

YPHEU participants will be asked a question about use of substances within the past 24 hours by the examiner prior to each cognitive/behavioral session and by the imaging research assistant prior to each neuroimaging session in order to account for acute effects of use that may impact assessment/imaging results.

#### **10.1.1 Cognition and Mental and Behavioral Assessments**

- Computerized Tests (Administer using iPad):
  - Delay Discounting Task
  - Emotion-Word/Emotion-Face Stroop
- NIH Toolbox (Administer using iPad):
  - Flanker Inhibitory Control and Attention
  - List Sorting
  - Dimensional Change Card Sort
  - Pattern Comparisons
  - Picture Sequence
  - Picture Vocabulary
  - Oral Reading Recognition

- Rey-Auditory Verbal Learning Test
- Social Support
- Examiner-Administered
  - Cash Choice Task
- Paper-and-Pencil Questionnaires (Administered as an interview with examiner for youth self-report; self-administered for caregiver-report)
  - BRIEF-2 (Self-report (for youth aged 11 and older only) and caregiver report) (*If not completed as part of SMARTT within the 3 months prior*)
  - ASEBA CBCL (Self- and caregiver-report) (*If not completed as part of SMARTT within the 3 months prior*)
  - CDI-2 (Self- and caregiver-report)
  - SCARED (Self- and caregiver-report)
  - PEARLS (Self-report (for youth aged 12 and older only) and caregiver-report)
  - Life Events Checklist (Self-report only)
  - Demographics Questionnaire (Caregiver-report)
  - CYRM-R (Self-report only) (*If not completed as part of SMARTT within the 3 months prior*)
  - Prosocial Behavior Survey Questions (Self-report only)
  - PDS (Caregiver-report)
  - ERQ-CA (Self-report)
  - DERS-P (Caregiver-report)
- Online Survey
  - Substance Use
  - PDS (Self-report)

### 10.1.2 Neuroimaging Session

- T1-weighted MRI (T1)
- Task fMRI
  - Monetary incentive delay (MID)
  - Stop Signal Task (SST)
  - Emotional N-back (EN-back)
- Diffusion Tensor Imaging (DTI)
- Rs fMRI
- T2-weighted MRI (T2)
- Rs fMRI

### 10.1.3 Chart Abstraction

- Current medications since last SMARTT visit
- Neurologic and psychiatric diagnoses since last SMARTT visit

### 10.1.4 Existing SMARTT Data To Be Utilized

- Demographics, including family resources data
- Medical and medications history
- Caregiver CDQ data
- Birth history, including perinatal ARV exposures

## 10.2 Young Adult Cross-Sectional Cohort

Evaluations for YAPHEU and YAPHIV will be comprised of one study visit at Entry. It is recommended that the study visit be completed in two separate sessions (cognitive/behavioral and neuroimaging) occurring on two separate days. For certain measures, if the measure was completed as part of the AMP Up study within 3 months prior to the TERBO BRAIN study visit, it does not need to be repeated for the TERBO BRAIN study.

Young adult participants will be asked a question about use of substances within the past 24 hours by the examiner prior to the cognitive/behavioral session and by the imaging research assistant prior to the neuroimaging session in order to account for acute effects of use that may impact assessment/imaging results.

### 10.2.1 Cognition and Mental and Behavioral Assessments

- Computerized (Administer using laptop):
  - Delay Discounting Task
  - Penn Emotion Recognition
- NIH Toolbox (Administer using iPad) *(Do not complete the Cognitive battery if it was completed as part of AMP Up within the 3 months prior. Complete the designated surveys from the Emotion domain regardless of whether they were completed as part of AMP Up within the 3 months prior):*
  - Flanker Inhibitory Control and Attention
  - List Sorting
  - Dimensional Change Card Sort
  - Pattern Comparisons
  - Picture Sequence
  - Picture Vocabulary
  - Oral Reading Recognition
  - Rey-Auditory Verbal Learning Test

- Social Support (Instrumental and Emotional Support included within the Social Relationships subdomain)
- Emotional Well-being (Negative Affect, Psychological Well-being, Social Relationships, Stress and Self-Efficacy)
- Paper-and-Pencil Questionnaires (Self-administered; can be interviewer-administered if participant requires assistance)
  - BRIEF-A
  - ASEBA ASR
  - PHQ-9
  - 7-day alcohol and tobacco use
  - ERQ
- Online Survey:
  - GAD-7
  - Adult Transition Milestones
  - Pittsburgh Sleep Quality Inventory
  - Substance Use
  - Sexual Risk Behaviors
  - ACEs-Revised Questionnaire
  - Stressful Life Events
  - Brief Resilience Scale (BRS)
  - Household and Financial Resources

### 10.2.2 Clinical Evaluations

- HIV viral load (*For YAPHIV with no result from within the 3 months prior*)
- CD4 T cell count and percent (*For YAPHIV with no result from within the 3 months prior*)

### 10.2.3 Neuroimaging Session

- T1
- Task fMRI
  - Gambling (GAM)
  - Emotional Processing (EP)
  - N-back Working Memory (NB-WM)
- DTI
- Rs fMRI
- T2

#### **10.2.4 Chart Abstraction**

- Current medications, including ARVs for YAPHIV since last AMP Up visit
- Neurologic and psychiatric diagnoses since last AMP Up visit
- For YAPHIV only: HIV viral load, CD4 T cell count and percent, and any changes in CDC class since last AMP Up visit

#### **10.2.5 Existing AMP Up Data To Be Utilized**

- Demographics, including household and financial resources data
- Medical and medications history
- Participant CDQ data
- Birth history, including perinatal ARV exposures
- For YAPHIV only: HIV disease history, including HIV viral load, CD4 T cell count and percent, and changes in CDC class, and historical and current ARV exposures

### **10.3 ASTRO Nested Substudy**

Evaluations for the ASTRO nested substudy will be comprised of one study visit at Entry, preferably on the same day as the cognitive/behavioral session of the TERBO BRAIN study visit. Evaluations for the ASTRO nested substudy should be completed within approximately 6 months following the final session of the TERBO BRAIN Entry visit (see Section 10.0). Given that the ASTRO nested substudy requires a blood draw in a fasting state (no food except water for at least 8 hours), it is recommended that the ASTRO procedures be completed first. After completion of the ASTRO procedures, the participant will be allowed take a break and eat a meal before proceeding with the TERBO BRAIN visit assessments. Refer to Section 13.7 if participant did not fast for at least 8 hours.

If a participant has an active acute infection (e.g., upper respiratory infection, urinary tract infection) with ongoing symptoms or use of short-course anti-microbial agents including antibiotics, study procedures should be re-scheduled to after the infection resolved.

#### **10.3.1 Clinical Evaluations**

- cfPWV
- Vital signs (height, weight, and blood pressure)
- Anthropometrics (mid-waist and hip circumferences)
- Fasting blood draw (metabolic, immune, and eicosanoid biomarkers)

#### **10.3.2 Survey Assessments**

- Primary caregiver education (young adult participants only)
- IPAQ

### **10.3.3 Existing Study Data To Be Utilized**

- cfPWV data from the general population (from Dr. Urbina)
- Prenatal maternal characteristics (e.g., CD4 T cell count) for YPHEU from SMARTT
- CD4 T cell count and viral load for YAPHIV from AMP Up
- TERBO BRAIN study data

## **11.0 DATA COLLECTION AND MONITORING**

### **11.1 Participant Identification**

Participants must not be identified by name on any case report forms (CRFs), cognitive and behavioral assessments (i.e., questionnaires, online surveys, NIH Toolbox), or neuroimaging files that are part of their research record. The ASTRO nested substudy blood specimens also must not be identified by name. Participants are to be identified only by the PID and SID/PIN numbers assigned by the TERBO BRAIN study. The same PID and SID used in the TERBO BRAIN study will be used for the ASTRO nested substudy. Study research records with PID and SID/PIN numbers must be stored separately from source documents that include personal identifiers.

### **11.2 Neuroimaging Data Management and Quality Assurance (QA)**

Upon completion of each neuroimaging session, site research staff will upload raw Digital Imaging and Communications in Medicine (DICOM) data as well as task behavior data, without protected health information, to the Northwestern University Research Image Processing System (NURIPS), an online collaborative research environment for securely storing, managing, analyzing, and sharing de-identified medical imaging, associated data (behavioral), and results from advanced customized pipelines [239-241]. NURIPS is supported by both Northwestern University Information Technology (NUIT) and Feinberg School of Medicine-Information Technology (FSM-IT) and takes advantage of the Northwestern University high performance computing cluster, Quest. NURIPS is a secure environment that supports the latest Northwestern University policy and procedures for encryption of data during transit and rest, provides granular project level access controls with varying permissions based on user groups, and allows non-Northwestern University collaborators access once they obtain an affiliate network ID. All data are backed up and have restore points that go back for 30 days. Users have access to common data analysis pipelines and the opportunity to create and share their own pipelines. Derived data produced from automated QA and imaging analysis pipelines will be transferred to the PHACS central database. Refer to the TERBO BRAIN MOP for details on transfer site scanning data to NURIPS. A copy of the archived data, as well as derived data, will also be sent from NURIPS to the PHACS DMC at Frontier Science for archival purposes.

For neuroimaging data QA, the following procedures will be employed. Refer to the TERBO BRAIN MOP for details.

- During the scan, site research staff carry out QA procedures as specified in the MOP. This includes a visual inspection to determine whether any scan needs to be repeated before ending of scan session.
- Once data have been uploaded to NURIPS, automated QA pipelines implemented on NURIPS will be triggered to indicate scan sequence parameter compliance/deviation and imaging protocol

completion and will produce metrics for neuroimaging data quality (such as motion). Results will be reviewed by the neuroimaging team (Drs. Lei Wang and Todd Parrish,) and communicated back to the site research staff should attention and correction be necessary.

### **11.3 Online Survey**

The online survey will be administered using a secure cloud-based software tool, that is specifically used for creating online surveys. The online survey can be completed on any device on which the internet can be accessed, including a smartphone. The online survey data will be transferred using Hyper Text Transfer Protocol Secure (HTTPS) connections that adhere to the Food and Drug Administration (FDA) guidelines for secure electronic data capture. The collected data will be stored on a secure cloud server and transferred to the PHACS central database at Frontier Science. Access to the server will be highly restrictive and limited to a small number of technical and project staff members who have been authorized by PHACS Leadership to have access. The DMC will provide information to clinical sites on how to access the online survey for participants.

### **11.4 NIH Toolbox**

The NIH Toolbox data will be stored within the NIH Toolbox app. All data submitted are encrypted and stored on a secure server. Security measures designed to protect against the loss, misuse, or alteration of data are also in place at the physical facilities where servers are housed at Northwestern University in Chicago, IL, and the NIH. Clinical sites must export the Registration Data, Assessment Scores and Assessment Data files for each participant to the DMC through a secure web-service (see TERBO BRAIN MOP for details). The DMC will provide assistance to clinical site staff on how to set up the NIH Toolbox app and transmit the data.

### **11.5 Data Collection – CRFs**

For medical record abstraction and other non-web-based data collection, CRFs will be made available on the PHACS DMC web portal. Whenever possible, sites are encouraged to complete CRFs electronically, including those that are used as source documents, through direct data entry (DDE) into the PHACS central database. The DMC at Frontier Science will provide research staff with instructions about entering study data on electronic CRFs.

### **11.6 Data QA**

Investigators receiving federal funding must adhere to the Code of Federal Regulations (CFR) to protect research participants and produce reliable study information. Clinical sites participating in the TERBO BRAIN study, sponsored by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), will be required to have an internal QA plan that will be employed to identify problems and correct errors in research study records. Clinical sites are responsible for following TERBO BRAIN data QA procedures.

Additional imaging data quality assurance procedures are performed by site research staff and the neuroimaging team (see Section 11.2).

### **11.7 Clinical Site Monitoring and Record Availability**

Clinical site monitoring for protocol and regulatory compliance will be conducted by Westat at each participating TERBO BRAIN site either in-person or via remote monitoring.

The site investigator will make study documents for the TERBO BRAIN study and the ASTRO nested substudy (e.g., consent forms, CRFs, and other study data) and pertinent hospital or clinic records readily available for inspection by the local IRB, the NIH, the Office for Human Research Protection (OHRP), and the site monitors acting on behalf of the NICHD. Site monitors will verify that the informed consent process was provided, the data collected matches source documents, and regulatory compliance is maintained.

**Note:** Participating sites are responsible for specifying these individuals and the PHACS investigators as recipients of private health information in the individual's authorization required under the Health Insurance Portability and Accountability Act of 1996 (HIPAA) Privacy Rule.

## **12.0 STUDY MANAGEMENT**

The TERBO BRAIN study and the ASTRO nested substudy will be conducted in compliance with the protocol, Good Clinical Practice (GCP) Guidelines, and the applicable regulatory requirement(s).

### **12.1 Protocol Query Management**

For the integrity of the study and the welfare of the participants, it is important for the research staff and research participants to have rapid access to the research team. Site research staff will send all queries to the respective protocol team, i.e., TERBO BRAIN or ASTRO, using the QNS accessible via the internal PHACS website. It is expected that the respective Protocol Co-Chairs or designee will respond to queries within 48 working hours of receipt. Queries and replies will be automatically archived by the PHACS webmaster. Those queries deemed relevant to all sites will be posted on the PHACS website, where they will be available to all sites for future reference, as well as emailed weekly to all PHACS staff. Examples of query categories include protocol violations or adverse participant, staff, or community experiences related to the protocol, study management issues requiring clarification, and participant management issues that fall outside the protocol parameters. Queries specific to the ASTRO nested substudy will be its own category within the TERBO BRAIN study and will be directed to the ASTRO nested substudy protocol team.

### **12.2 Data Management**

It is the responsibility of the PHACS DMC to ensure the completeness, quality, and integrity of clinical and laboratory data for each PHACS study. This role extends from protocol development to generation of the final study database. Data for TERBO BRAIN and the ASTRO nested substudy will be entered into the central PHACS database.

This study follows PHACS standards and recommended guidelines for data management. The PHACS DMC will provide site research staff with instructions concerning the collection and recording of study data. The data will be entered into an electronic CRF using an electronic data capture (EDC) system. Each site is responsible for keying the data in a timely fashion according to standards set by the PHACS Network. The EDC system has built-in basic error checking capability so that minor errors can be resolved at the site. The data entered will then be exported to the PHACS central database where additional data checking and processing will take place. Data errors found during the automatic processing and loading of data will be

communicated to the site via daily update reports. The study data manager will perform additional data checks, and any errors found during this process will be communicated via an interactive query mechanism integrated within the EDC system.

Additional imaging data management is performed by the neuroimaging team (see Section 11.2).

### **12.3 Rolling Implementation and New Protocol Versions**

As both the TERBO BRAIN study as well as the ASTRO nested substudy will be implemented across multiple clinical sites, initial implementation of the studies will occur on a rolling basis as each site becomes ready. Furthermore, deployment of surveys, study assessment tools, and other study-related activities may occur on a rolling basis depending on their availability and readiness. It is acknowledged that rolling implementation is no fault of the sites.

The introduction of a new protocol version may result in a period of delay between HLC IRB approval and functional roll-out of the new protocol version to sites in order to allow time for operational changes to be made. In addition, data collection instruments may need to be modified as a result of the approved new protocol and may not be available immediately upon receipt of HLC IRB approval. The Protocol Teams will ensure that all infrastructure-based operational components required for initiating implementation of the new protocol version (including the enrollment system's eligibility checklist and the new data collection instruments) have been aligned with the updated protocol version and are completed. The date this is done is the effective implementation date, which will be specified with the protocol amendment. Sites should not enroll or follow participants under the new protocol version prior to the effective implementation date.

## **13.0 PARTICIPANT MANAGEMENT**

### **13.1 Data Collection Time Point Management**

All assessments and data collections are to be conducted according to the Schedule of Evaluations in Appendices I, II and III. For the TERBO BRAIN YPHEU cohort, the target date for the 2-Year (24-Month) Follow-Up visit should be the 2-year anniversary of the Entry visit date (approximately +/- 3 months). Both the cognitive/behavioral and the neuroimaging sessions for each study visit must be completed within approximately a 3-week timeframe. Medical record abstraction for current diagnoses and medications should include all data since the participant's last SMARTT (if currently enrolled) or AMP Up visit, and for the past 12 months for those formerly enrolled in SMARTT, and must be completed on or within 3 months after completion of the first session of the study visit.

Assessments completed as part of the SMARTT or AMP Up study within the 3 months prior to the visit, will not be repeated as part of TERBO BRAIN unless specified. For YAPHIV, current HIV disease data (HIV viral load and CD4 T cell count and percent) from within the 3 months prior to the visit should be collected. If not available, sites will perform the tests as part of the TERBO BRAIN study.

According to Human Subjects Protection guidelines, a participant may voluntarily decline any specific protocol assessment or specimen collection during a study visit, and any such missed assessments will not be considered a protocol deviation. Thus, voluntary participant refusal of any research activities does not require HLC IRB notification. The site should document the participant's decline of a specific protocol assessment or specimen collection in the participant's file and on the appropriate CRF.

### **13.2 Enrollment of Participants with Cognitive Impairment**

Potential participants with cognitive impairment who meet study eligibility criteria will not be excluded from enrollment. Enrollment of participants with cognitive impairment is justified given that the cause of the cognitive impairment may be related to the exposures being studied and not enrolling these individuals may introduce bias into the study. TERBO BRAIN and ASTRO are observational studies; therefore, any potential risks or negative impacts on the well-being of these individuals are minimal. Given these conditions, enrollment of participants with cognitive impairment is in line with the U.S. Department of Health and Human Services (DHHS) and FDA regulations.

Clinical sites will evaluate cognitive impairment through multiple means. In many cases, the clinical sites will have extensive experience with and knowledge of the skills and capabilities of the participant including thorough neuropsychological testing results obtained during the individual's previous participation in other studies. All sites have psychologists on staff who can conduct further screenings when appropriate. Potential participants with significant cognitive impairments that would render them unable to complete study assessments (as determined by the clinical site PI or designee) should not be enrolled in the studies. Site staff should consult with the respective Protocol Team through the QNS if they have any concerns regarding a participant's eligibility for a study.

Participants with cognitive impairment will consent on their own behalf if legally able. For individuals with an LAR, LAR permission and participant assent will be obtained. Participating sites will consult with the HLC IRB, or site IRBs if indicated, for guidance when needed. Caregivers of participants with cognitive impairment will answer questions as proxies on behalf of participants but will not be consented and enrolled as study participants themselves.

### **13.3 Participant Retention**

Participant retention will be a challenge and maintaining retention is a high priority. Retention and completion of data collection time points will be monitored carefully.

For participants in the TERBO BRAIN YPHEU cohort, site research staff will schedule the 2-Year (24-Month) Follow-Up visit at the end of the Entry visit. Site research staff will also contact YPHEU cohort participants once every six months during the period between the two study visits to check-in on how they are doing and update the family's contact information, if necessary. Site research staff are responsible for contacting participants to provide reminders about the upcoming study visit as appropriate.

For TERBO BRAIN YPHEU participants who plan to move out of the area between Entry and the 2-Year (24-Month) Follow-Up visit, site research staff will evaluate if the participant can return to the original clinical site for the 2-Year Follow-Up visit. If not possible, the site research staff will determine if there is a TERBO BRAIN participating site in the vicinity of the participant's new location to which the participant can be transferred.

### **13.4 Discontinuing Study Participation**

The Protocol Teams will monitor the rate and reasons for discontinuing follow-up. Participants will be discontinued from the TERBO BRAIN study and/or the ASTRO nested substudy if any of the following occurs:

- The participant withdraws permission;

- The participant fails to comply with the study requirements so as to cause harm to self or seriously interfere with the validity of the study results and the clinical site PI believes that compliance is unlikely to improve;
- The clinical site PI determines that further participation would be detrimental to the participant's health or well-being;
- The study is stopped by a governmental agency, including the NIH or DHHS;
- The clinical site is terminated for significant participant safety concerns, study integrity, poor performance issues, or lack of funding; or
- The HLC IRB decides to withdraw approval for the study due to participant safety concerns.

### **13.5 Participant Compensation**

As approved by the HLC IRB, TERBO BRAIN study participants will receive \$200 remuneration for each of the two sessions for each study visit, totaling \$400 for every study visit. Local IRBs may designate the need for additional remuneration or reimbursement as deemed appropriate. Furthermore, as part of the MID fMRI task, participants may receive an average of about \$20 for completing the task.

Participants may be asked to return to complete or repeat study assessments, including neuroimaging scans (MRI) due to technical or unanticipated issues. If completing or repeating the MRI is necessary, site staff will explain the MRI procedures to the participant again prior to the repeat MRI. Participants will be compensated for their time and effort completing each re-scan session (\$200). Participants will not be asked to repeat the scan more than 2 times.

Participants enrolled in the ASTRO nested substudy will receive \$65 for their time and effort in the completion of the ASTRO nested substudy procedures and assessments.

### **13.6 TERBO BRAIN Test- and Evaluation-Specific Management**

TERBO BRAIN study measures will be administered as described below. Research staff member will monitor participants during and after administration of measures and follow the PHACS Emergency Protocol to address atypical and/or worrisome emotional responses that may occur.

#### **13.6.1 Online Survey**

The online survey consists of a series of web-based questionnaires. Each questionnaire focuses on a specific topic and begins with an introduction explaining the purpose of the questionnaire. Most questionnaires will be structured to allow for completion on any device that a participant might use to access the internet, including a smartphone. Skip patterns will be programmed into the survey, and questions can be skipped by participants if they choose. At the end of the survey, after the participant clicks "submit," their responses will be locked and neither the site research staff nor the parent/caregiver of YPHEU participants will be able to access the responses.

The online survey for YPHEU participants contains questions about substance use. The nature of the substance use questions will be described to the parent or caregiver as part of the informed consent process, however, they will not be able to view the participant's responses. An examiner may assist YPHEU with portions of the online survey if the participant has difficulty reading or responding to the questions.

The online survey for YAPHEU and YAPHIV participants contains questions about substance use and sexual risk behaviors, as well as other areas. Participants with cognitive impairment who cannot complete the online survey independently can either complete survey as an interview with a research staff member or have their caregiver complete the survey. In both formats, substance use and sexual behavior information will not be collected.

The online survey will be completed by the participant in a private space at the clinical site whenever possible. For YAPHEU and YAPHIV participants only, if needed, the online survey may be completed at home or another location outside of the clinic, in which case, the research staff will ensure that the participant is able to complete the survey independently and completes it within 2 weeks after the study visit. Participants will be provided with tips on how to protect their privacy when completing the survey (e.g., use a personal computer or device that is not shared with others when possible, take it in a private space where they are comfortable answering questions, etc.).

### **13.6.2 NIH Toolbox**

The NIH Toolbox was developed for longitudinal epidemiologic studies and prevention or intervention trials to assess cognition, sensation, motor, and emotion domains via streamlined computer-based measures. The TERBO BRAIN study will administer the Cognition domain subtests and one Emotion domain subtest for YPHEU, and the Cognition domain and multiple portions of the Emotion domain for YAPHEU and YAPHIV. The Toolbox uses Item Response Theory and Computer Adaptive Testing to provide a brief, low burden assessment with reliability and validity comparable to psychometrically sound, longer assessments. The assessment will be administered by a centrally-trained examiner and does not require administration by a psychologist.

### **13.6.3 Examiner- and Self-Administered Cognitive, Mental Health and Behavioral Measures**

Administration of all cognitive and mental and behavioral health evaluations/screenings that are not part of the online survey will be conducted by an examiner, or with assistance from an examiner as needed, at the clinical site. The examiner can be a site psychometrist or research staff member who is fully trained on all TERBO BRAIN cognitive/behavioral measures and approved by the TERBO BRAIN Protocol Team to administer the measures, under the supervision of the site psychologist. Refer to the TERBO BRAIN MOP for details. Examiner training will include monitoring for and strategies to minimize participant discomfort, fatigue, or upset.

### **13.7 ASTRO Nested Substudy Blood Draw**

Refer to the ASTRO nested substudy schedule of evaluations in Appendix III for laboratory tests that will be performed as part of the nested substudy. The type of tube/anticoagulant for specific tests is critically important and testing specifications can be found in the ASTRO LPC.

Fasting is required for the ASTRO nested substudy blood draw, unless determined to be unfeasible due to a valid medical condition by the local clinical investigator. The minimum duration of fasting is 8 hours. Water and medications are allowed during fasting.

If a participant did not fast for at least 8 hours, it is advised to not proceed with the blood draw. Instead, the blood draw should be rescheduled for a different day. If a participant cannot return on a different day, a query should be sent to the protocol team via the QNS for guidance.

When the ASTRO nested substudy visit is completed on the same day as a TERBO BRAIN visit session, ASTRO procedures should be done first. Participants should be allowed to rest and eat before proceeding to their TERBO BRAIN visit procedures.

#### **14.0 ADVERSE EVENT (AE) REPORTING**

The TERBO BRAIN study and the ASTRO nested substudy are not therapeutic studies and no medications are prescribed or given as part of these studies. Youth and young adults enrolled in these studies may develop common conditions requiring treatment during the course of the study period. Site study personnel will assist the participants in receiving appropriate care as appropriate to their roles at their site. YAPHIV participants may also experience AEs associated with HIV infection, ART exposure, or other medications. Clinical site PIs are encouraged to use the FDA's MedWatch system to report any events possibly associated with medications clinically prescribed for the participant. The Protocol Team does not anticipate any drug-related AEs as a result of participation in the TERBO BRAIN study and/or ASTRO nested substudy.

#### **15.0 STUDY IMPACT AND SAFETY MONITORING**

The Protocol Teams will monitor participant-, staff-, and community-associated untoward events. Monitoring will consider the impact of the study on the welfare of three groups of people:

- Research participants.
- Site research staff.
- The community in which the research is being conducted.

Reporting of participant or staff-associated negative study impact events to the Protocol Team(s) will result in the re-examination of study procedures and allow changes as necessary to address concerns about participant management, recruitment, enrollment, adequacy of training, and/or the need to modify procedures. Community-associated untoward event reporting will facilitate understanding of the impact of the study on the community and will provide the opportunity to address community-level concerns and to intervene in a timely manner to correct misinformation or perceptions of practices that may cause community concern.

All clinical sites have psychologists, social workers, or other clinical staff qualified to address situations if a participant becomes distressed. In addition, PHACS has developed standard operating procedures for sites to follow in these circumstances (available on the PHACS website at <https://my.phacsstudy.org>).

## 15.1 Grading of Impact

The grading system for events involving study participants or staff will be as follows:

- Minimal Impact: managed at the time of event with no consequences.
- Moderate Impact: managed by referral for supplemental care/counseling.
- Major Impact: needed immediate professional intervention with or without hospitalization.

Community events will not be graded but will be addressed as they occur.

## 15.2 Reporting Requirements

All moderate and major impact events involving study participants or staff are to be reported to the Protocol Team(s) through the QNS by the clinical site. Any event that is deemed to have negatively impacted a participant to a more than minimal extent and is related to the study activity must also be reported to the PHACS Regulatory and Compliance Manager by the study site through the QNS. Reportable events could involve study participants and/or staff members.

Examples of moderate and major impact events for study participants that could be related to the study activity include:

- Disruptive or violent behavior during the scheduled study visit session.
- Information regarding personal harm which is disclosed (e.g., current suicidal or homicidal ideation, physical or sexual abuse, active suicidal/homicidal intent, plan, and/or means).
- Significant visible distress or injury resulting from the research encounter (e.g., emotional response/distress as a result of responding to questions about violence, abuse, etc. or burns related to ferrous metal in the body).
- Breach of confidentiality.

Examples of moderate and major impact events for research staff that could be related to the study activity include:

- Inadequate preparation for management of research-related events.
- Visible distress or injury resulting from the research encounter.

**Note:** The distinguishing feature of moderate and major impact events is the need for enlisting additional support outside the research staff and the research encounter. The online surveys will include information on how and where participants can obtain assistance should they have feelings of anxiety, or other mental health concerns after completing the survey.

Examples of events for the community include:

- Any adverse community feedback received by the institution or the research team concerning the study.
- The study being portrayed adversely in any community forum or in the media.

The PHACS Regulatory and Compliance Manager will be responsible for reporting such events to the HLC IRB. The PHACS Regulatory and Compliance Manager will also work with the sites to ensure that the site's local IRB is notified of the event as required.

### **15.2.1 State Mandated Reporting Requirements**

Laws governing the reporting of certain communicable diseases and illegal behaviors to authorities vary from state to state. Clinical sites are responsible for adhering to the reporting laws in their respective states. Clinical sites will consult with the HLC IRB and their local IRBs for guidance when needed.

### **15.3 Monitoring Plan**

The TERBO BRAIN Protocol Team and ASTRO Protocol Team will each hold regular conference calls to review study progress. These calls will occur at least monthly. More frequent calls or ad hoc calls may occur at the discretion of each study's Protocol Chairs and Co-Chairs if a problem is identified that needs to be addressed immediately.

## **16.0 TERBO BRAIN STUDY STATISTICAL/ANALYTIC CONSIDERATIONS**

### **16.1 Sample Size**

#### **16.1.1 Study Sample**

The TERBO BRAIN study will enroll a longitudinal cohort of approximately 190 YPHEU and a cross-sectional cohort of approximately 100 total of young adults with about 1:3 ratio between YAPHEU and YAPHIV.

#### **16.1.2 Comparison Participants for YPHEU**

After all YPHEU have been enrolled, comparison participants will be drawn from the ABCD study, frequency matched on sex, race, ethnicity, household income, and pubertal stage. The ABCD study has been collecting data from over 14,000 children enrolled at ages 9-10 at 21 sites across the US. Because of the large sample size of the ABCD study and its diverse study population, we are confident that we will be able to obtain a comparison sample with a 3 to 1 or higher ratio in sample sizes for sufficient power that will match the demographic and socioeconomic characteristics of our YPHEU cohort. The ABCD study publicly releases its quality-controlled data as they are collected. See below for power calculations.

#### **16.1.3 Comparison Participants for YAPHEU and YAPHIV**

After all YAPHEU and YAPHIV have been enrolled, comparison participants will be drawn from the HCP-YA study, frequency matched on sex, race, ethnicity, household income, and age. The HCP-YA study was designed to characterize brain connectivity and function in healthy young adults and has publicly released quality-controlled datasets from 1,200 participants aged 22-35 including neuroimaging, mental health, cognition, risk behaviors, physical health, education, and emotion. The HCP-YA study is completed and has publicly released all of its quality-controlled data. We will obtain a comparison sample of a minimal size of 100.

## 16.2 Neuroimaging Data Processing

The procedures described in this section will generate the following for subsequent statistical analysis: a) cortical and subcortical structural region of interest (ROI) volumes; b) beta weights for task activation within each ROI; c) graph theory metrics including density, transitivity, and small-worldness for rs intrinsic network connectivity; and d) FA for white matter tracts connecting the hypothesized ROIs in each network construct – reward & salience processing, behavioral regulation, and emotion regulation.

### 16.2.1 Preprocessing

Harmonized image processing steps and best practices adapted by the ABCD study [227] will be followed in this study. The methods are validated and used for large-scale multisite studies, and include standard routines, e.g., FreeSurfer [242, 243], Functional MRI of the Brain Software Library (FSL) [244], and Analysis of Functional Neuro Images (AFNI) [245]. The processing pipeline used for the current ABCD Data Release 2.0 is publicly available as a self-contained, platform-independent package (<https://collection3165.readthedocs.io/en/stable/pipeline/>).

### 16.2.2 Multimodal Neuroimaging Data Analysis

The primary approach to all neuroimaging analyses will be ROI based. Because this study’s overall hypothesis involves brain networks supporting emotional regulation, with a set of well-documented brain regions and a set of well-established functional tasks that probe these regions, hypothesis-based testing will be constrained to 13 ROIs defined a priori for the following network constructs underlying emotional regulation (Table 3): Reward & salience processing – ventral striatum (vSTR), thalamus (THAL), insula (INS), orbitofrontal cortex (OFC), ventromedial prefrontal cortex (vmPFC) (regions of SN, DMN); Behavioral regulation – ventrolateral prefrontal cortex (vlPFC), dorsal anterior cingulate cortex (dACC) (regions of CEN); Emotion regulation – dorsolateral prefrontal cortex (dlPFC), vlPFC, vmPFC, amygdala (AMY), hippocampus (HIP), vSTR (regions of CEN, SN, DMN). Structural ROI volume, task fMRI ROI activation, white matter integrity and rs intrinsic network measures will be calculated. These measures will each be used in primary statistical analyses (e.g., differences on baseline and change estimates, correlation with non-imaging assessments).

State-of-the-art neuroimaging methods will be applied for secondary analyses. Computational diffeomorphometry [246] will be used to compute brain structural shape for detecting effects that are below volume sensitivity levels. Independent component analysis (ICA) [247], a model-free, data-driven method, will be used to examine interactions between brain networks. Recent advances in dynamic analysis of rs fMRI data (e.g., sliding-window analysis, time-frequency analysis) will be used to capture time-varying properties of rs brain activity [248, 249]. Multivariate pattern analysis [250], multiple kernel learning [251, 252], deep-learning approaches [253] will be utilized for multimodal analysis and collaborate with PHACS Epidemiological and Statistical Methods Core (ESC) on advanced deep-learning methods.

<b>Table 3. Network Constructs</b>	
Based on ABCD Guidelines [77], Literature on Emotional Regulation [22], and Triple-Network Model [63]	
<b>Network Construct</b>	<b>Network ROIs</b>
Reward & salience processing	vSTR, THAL, INS, OFC, vmPFC (SN, DMN)
Behavioral regulation	vlPFC, dACC (CEN)
Emotional regulation	dlPFC, vlPFC, vmPFC, AMY, HP, vSTR (CEN, SN, DMN)

### **16.2.3 Structural Volume**

Validated methods will be used for automated processing of structural MRI, which yield neuroanatomic indices equivalent to those by expert raters but are more transparent and reproducible [242, 243, 246]. Cortical ROI will be obtained with the whole-brain longitudinal-stream FreeSurfer [242, 243]. FreeSurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths [254, 255]. Subcortical ROIs will be generated using automated multi-atlas [256], multi-structure [257], FreeSurfer-initiated Large-Deformation Diffeomorphic Metric Mapping (FS-LDDMM) [257-259] pipeline. FS-LDDMM is an atlas-based mapping algorithm, combining FreeSurfer [242] and high-dimensional large-deformation diffeomorphic metric mapping (LDDMM) [260]. Dr. Wang and collaborators have been part of the development of FS-LDDMM and other computational anatomy [260, 261] and diffeomorphometry [246, 262, 263] tools for nearly two decades, and have applied them extensively in studies of neuropsychiatric disorders in adults [264-270] and youth [271, 272], including YPHIV [37, 38]. These procedures will generate cortical and subcortical structural volumes for statistical analyses.

### **16.2.4 Task fMRI Activation**

Voxel-wide fixed-effects analyses will first be performed on individual runs to produce activation beta estimates associated with each condition and linear contrasts of conditions. For MID and SST analyses, events are modelled as instantaneous; for EN-back, the duration of cues (~3 s) and trial blocks (~24 s) are modelled as square waves convolved with the two-parameter gamma basis function (i.e., block duration). ABCD has partitioned the whole brain into 333 parcellations based on a network atlas [273], and made available beta weights for each of the task conditions and contrasts, averaged within each of the 333 parcellations for each participant. The Gordon et al. atlas [273] will similarly be applied to calculate the beta weights for the parcellations that are within the proposed set of ROIs.

### **16.2.5 Integrity of rs Intrinsic Networks**

Rs fMRI data will be extracted from ROIs, then cross-correlated and standardized, generating a resting-state functional connectivity (rsFC) measure for each ROI pair. A connectivity matrix will be constructed for each participant using the rsFC measures to characterize weighted networks. Unlike binary networks, which consider a connection to be present if it exceeds an arbitrary threshold, weighted networks weigh connections by the strength of the correlation, eliminating the influence of different thresholds. Graph theory metrics will be calculated to describe network integrity outcomes [274] for statistical analyses. Graph-theory based modularity analysis can identify communities of groups of nodes that are highly connected within the same module but less connected across modules [275, 276]. Graph theory metrics will include density, reflecting the total “wiring cost” of a network, transitivity, measuring network segregation into clusters, and small-worldness, or the balance between segregation and integration [277].

### **16.2.6 Integrity of White Matter Regions**

DTI images will be analyzed with Tract Based Spatial Statistics (TBSS) as implemented in FSL [278]. TBSS generates whole brain voxelwise maps of FA - a measure of white matter integrity. Using reference atlases [279] to measure white matter regions that connect the ROIs, FA will be measured, focusing on the white matter tracts connecting the hypothesized ROIs in each of the emotional regulation network constructs (Table 3).

### 16.3 Cognitive/Mental Health/Behavioral Data

For cognitive and mental and behavioral health measures, standardized composite scores will be utilized, where available (e.g., ASEBA index scores, NIH Toolbox Cognitive subtest age-adjusted scores). Standard procedures will be used to generate composite scores for cognitive domains with multiple measures. Standard score ranges will be used to examine clinically relevant groups for mental health measures (e.g., Emotional Problems and Functional Problems (using CDI-2 for YPHEU) and cut-off scores for depression using PHQ-9, and anxiety using GAD-7, for YAPHEU and YAPHIV). As appropriate, additional analyses will explore in depth cognitive and emotional-behavioral functions within specific domains.

### 16.4 Statistical Analyses

#### 16.4.1 Aim 1: To longitudinally assess brain network development underlying emotional regulation and relate to mental health, cognition, and risk-taking behaviors in YPHEU compared to a population-based cohort without known perinatal HIV exposure

**Study Set:** YPHEU and ABCD participants, frequency matched on sex, race, ethnicity, SES, and pubertal stage within cross-classification of all the matching factors.

We will examine whether the enrolled YPHEU are representative of those enrolled in the SMARTT Dynamic cohort in terms of demographic and socioeconomic characteristics and cognitive measures that are also available in SMARTT. If the TERBO BRAIN participants do not seem representative, we will use inverse probability weighting technique to correct for possible selection bias. If an appropriate frequency matched sample between TERBO and ABCD participants is not achievable, we can include a sufficient sample with standardized mortality ratio (SMR) weights to obtain comparability. All models will also adjust for these matching factors.

**Hypothesis 1a:** YPHEU will exhibit atypically attenuated growth in brain networks supporting emotional regulation as they age when compared to a population-based cohort.

- Outcomes: neuroimaging measures at Entry and 2-Year (24-Month) Follow-Up:
  - a) structural MRI - volume from each of the 13 ROIs (Table 3);
  - b) task-fMRI (MID, SST, and EN-back) - activation measured by beta weights per task per ROI;
  - c) rs fMRI - measures of intrinsic functional network integrity based on the ROIs, such as density, transitivity and small-worldness; and
  - d) DTI - mean FA within each of white matter regions that connect ROIs for reward/salience, behavior/self-regulation and emotion regulation network constructs.
- Exposures of interest: cohort, age.
- Covariates and potential confounders: sex, race, ethnicity, baseline PDS, household income, family structure (e.g., single parent household), and other socioeconomic factors at baseline.
- Analysis plan: Each neuroimaging measure at baseline will be summarized using descriptive statistics, including the mean and standard deviation (or median and quartiles, when appropriate) by cohort, and compared between cohorts by a Wilcoxon test. Longitudinal data analyses will be implemented using generalized estimating equations (GEEs), which is relatively robust to departures from normality. The model will assume a linear relationship of each imaging outcome with age, allowing an interaction (difference) between the intercept (mean per given age) or slope (change per year) and cohort, adjusting for covariates/confounding variables. The possible

clustering effect of clinical site will be modelled as a random effect. The Benjamini-Hochberg-Yekutieli [280] procedure will be applied to control the false discovery rate (FDR) across various outcomes within each of the four types of imaging, while considering the correlation among outcomes.

**Hypothesis 1b:** Brain network growth will be more strongly related to poor mental health and impaired cognition in YPHEU than in the comparison cohort.

- Outcomes:
  - a) Cognition – composite scores of each of the six constructs (Table 1): Reward and Salience processing, Behavioral Regulation, Emotion Regulation, Fluid Reasoning, Crystallized Cognition and Episodic Memory; and
  - b) Mental health – internalizing, externalizing composite scores (ASEBA CBCL), depression (CDI-2), anxiety symptoms scores (SCARED), and psychiatric diagnoses.
- Exposures of interest: neuroimaging measures at Entry and 2-Year (24-Month) Follow-Up (ROI volume, beta weights in ROIs during MID, SST and EN-back tasks, network integrity measures from rsFC, FA from DTI).
- Effect modifier: cohort.
- Covariates and potential confounders: sex, race, PDS, socioeconomic status measures, and age at baseline.
- Analysis plan: Each cognition/mental health outcome measured at the Entry visit will be summarized using descriptive statistics by cohort, including the mean and standard deviation (or median and quartiles, when appropriate) for continuous outcomes and proportion for categorical outcomes. They will be compared between cohorts using a Wilcoxon rank sum test or Fisher's exact test as appropriate. Unadjusted associations between each cognition and mental health outcome with each neuroimaging measure will be assessed by Spearman correlation. Associations will then be adjusted for covariates/confounders using GEE models for each imaging type and cognition/mental health construct pair, with an identity link for continuous outcomes and a log or logistic link for categorical outcomes. We will analyze measurements from Entry and the 2-Year (24-Month) Follow-Up visits in longitudinal models, including age and other covariates, at each assessment as time-varying covariates. To assess whether the associations with covariates are different for the YPHEU and ABCD groups, interaction terms with cohort will be added into the models, or the regression analyses will be stratified by cohort. Structural equation modeling (SEM) will be explored to combine various imaging measures, cognition, and mental health constructs in one model.

**Hypothesis 1c:** Attenuated brain network growth will be associated with risk-taking behaviors, mediated by mental health and cognition, after accounting for social and structural vulnerabilities and protective factors.

- Outcomes: substance use: tobacco, alcohol, marijuana, and illicit drugs (Y/N, frequency)
- Exposures of interest: neuroimaging measures at Entry and 2-Year (24-Month) Follow-Up (ROI volume, beta weights in ROIs during MID, SST and EN-back tasks, network integrity measures from rsFC, FA from DTI).
- Effect modifier: cohort.
- Mediators: cognition and mental health.

- **Covariates and potential confounders:** sex, race, PDS, and age at baseline, vulnerabilities (family resources), and protective factors (Prosocial Behavioral Scale).
- **Analysis plan:** The frequency of use for each substance will be categorized and described by cohort and compared using Chi-square tests. Associations between neuroimaging measures and each type of substance use will be assessed using log-binomial models for binary outcomes and generalized linear models for continuous outcomes with GEE methods as described for H1b. For each neuroimaging measure-substance use pair showing an association, the potential mediating effects of cognition or mental health measures will be examined, provided there is an association between the potential mediator (cognition/mental health measure) with both the outcome (substance use) and exposure (neuroimaging measure). Mediation analysis [281] will be conducted both overall (adjusting for cohort) and then stratified by cohort (YPHEU vs. ABCD) to explore the difference in mediation effects of the two cohorts. The controlled direct effect, natural direct effect, and natural indirect effect of the neuroimaging measure on risk behavior will be calculated. The measures of proportion mediated by cognition or mental health will also be evaluated for each cohort. Sensitivity analyses will be conducted to assess the extent of effect of unmeasured confounders between cognition/mental health and substance use, such as calculation of E-value [282], and by possible interaction between neuroimaging and cognition/mental health constructs. Marginal structural models for controlled direct effects with time-varying exposures and mediators will also be explored [281].

#### **16.4.2 Aim 2: To assess the longitudinal impact of perinatal ARV exposure and other factors on brain network development underlying emotional regulation in YPHEU**

**Study Set:** YPHEU with perinatal ARV exposure.

**Hypothesis 2a:** Within YPHEU, attenuation of brain network growth will be related to type and timing of perinatal ARV exposure (e.g., efavirenz and atazanavir).

- **Outcome:** neuroimaging measures at Entry and 2-Year (24-Month) Follow-Up
  - a) ROI volume;
  - b) Beta weights in ROIs during MID, SST, and EN-back tasks;
  - c) Network integrity measures from rsFC; and
  - d) FA from DTI.
- **Exposures of interest:** timing of initiation of perinatal ARV (at conception, trimester 1, trimester 2/3), individual ARVs, ARV classes (protease inhibitors or non-nucleoside reverse transcriptase inhibitors), and age.
- **Potential confounding variables:** sex, race, ethnicity, PDS at baseline, maternal education, and maternal self-reported substance use and HIV disease severity during pregnancy.
- **Analysis plan:** Each neuroimaging measure evaluated at the Entry visit will be summarized using descriptive statistics, including the mean and standard deviation (or median and quartiles, when appropriate) by timing of initiation of perinatal ARV exposure and by ARV classes, and compared using Wilcoxon rank sum tests. GEE models similar to those described for H1a will be used. The effect of ARV classes will be analyzed overall and stratified by timing of ARV initiation if the sample size of each stratum allows. We will pay special attention to ARVs, such as efavirenz and atazanavir, given findings from previous studies. However due to small percentages exposed to specific ARV, such analyses will be exploratory.

**Hypothesis 2b:** Social and structural vulnerabilities and protective factors will each impact brain network growth.

- Outcomes: neuroimaging measures at Entry and 2-Year (24-Month) Follow-Up:
  - a) ROI volume;
  - b) Beta weights in ROIs during MID, SST, and EN-back tasks;
  - c) Network integrity measures from rsFC; and
  - d) FA from DTI.
- Exposures of interest: vulnerability and resilience – composite scores from following constructs (Table 1):
  - a) Vulnerabilities: PEARLS, Life Events Checklist, and family resources; and
  - b) Protective factors: Prosocial Behavior Scale, Social Support, and CYRM-R.
- Potential confounding variables: sex, race, ethnicity, PDS at Entry, maternal education, maternal substance use and HIV disease severity during pregnancy, and ARV classes identified in H2a.
- Analysis plan: Unadjusted associations between each neuroimaging measure and each vulnerabilities/protective factor measure will be assessed by Spearman correlation. Associations will then be adjusted for confounders using GEE models with an identity link for each imaging type, with individual or multiple vulnerabilities/protective factor measures in the same model.

**16.4.3 Aim 3: To cross-sectionally assess brain network integrity and cognitive, social, mental health, and behavioral outcomes during the adult transition period in YAPHEU and YAPHIV compared to one another and to a population-based cohort without known perinatal HIV exposure**

**Hypothesis 3a:** Compared to young adults without HIV exposure, YAPHEU and YAPHIV will show disrupted brain networks underlying emotional regulation, with more severe disruption in YAPHIV.

**Study Set:** YAPHIV, YAPHEU and matching HCP-YA.

If an appropriate frequency matched sample is not achievable, we can include a sufficient sample with SMR weights to obtain comparability.

- Outcomes: neuroimaging measures
  - a) ROI volume;
  - b) Beta weights in ROIs during gambling, emotion processing and N-back working memory tasks;
  - c) Network integrity measures from rsFC; and
  - d) FA from DTI.
- Exposures of interest: cohort - YAPHIV vs. YAPHEU vs. HCP-YA.
- Covariates and potential confounders: sex, race, ethnicity, age, and household income.
- Analysis plan: Each neuroimaging measure will be summarized using descriptive statistics by cohort, and compared among cohorts using Kruskal Wallis tests. GEE models with an identity link will be applied to assess the association of cohort with neuroimaging measures, adjusting for

confounding variables. The possible clustering effect of clinical site will be modelled as a random effect. Given the exploratory nature of this aim with limited sample size, we will report results with and without correction for FDR.

**Hypothesis 3b:** In YAPHEU and YAPHIV, disrupted brain networks will be related to risk-taking behaviors and deficits in adult transition milestones (e.g., education and employment), mediated by cognition and mental health, considering the potential impact of HIV infection, ARV exposure, social and structural vulnerabilities and protective factors separately within YAPHEU and YAPHIV.

#### **Study Set:** YAPHEU and YAPHIV

- Outcomes:
  - a) Risk-taking behaviors, including substance use (Yes/No, frequency) and condomless sex (Yes/No), number of sex partners (numerical); and
  - b) Transition milestones, including education level (categorical), and employment (Full time/Part time/No).
- Exposure of interest: neuroimaging measures (ROI volume, beta weights in ROIs during gambling, emotion processing and N-back working memory tasks, network integrity measures from rsFC, FA from DTI).
- Mediators: cognition and mental health.
- Potential confounding variables: sex, race, ethnicity, age, cohort, vulnerability, and protective factors for both YAPHEU and YAPHIV. Viral load, CD4 T cell count and percent, and specific ARV exposure for YAPHIV.
- Analysis plan: Each risk behavior transition milestone will be summarized by descriptive statistics by cohort, including the mean and standard deviation (or median and quartiles, when appropriate) for continuous outcomes and the proportion for categorical outcomes. They will be compared between YAPHEU and YAPHIV by a Wilcoxon test or Fisher's exact test as appropriate.

Unadjusted associations between each cognition/mental health measure and each neuroimaging measure or each behavior measure will be assessed by Spearman correlation, Kendall Tau correlation or biserial correlation depending on the type of measure. A cognition/mental health measure is identified as a potential mediator if it is associated with both a neuroimaging measure and a behavior outcome. Associations between each behavior and neuroimaging measure will be assessed by log-binomial or logistic regression models for categorical outcomes and generalized linear models for continuous outcomes using GEE methods. For each behavior-neuroimaging pair with significant association and potential mediators, mediation analysis will be performed to evaluate the controlled direct effect, natural direct effect and natural indirect effect of the neuroimaging measure on behavior, using an approach similar to that described for hypotheses H1b and H1c.

## **16.5 Power Calculations**

### Aim 1 and Aim 2

For YPHEU, power calculations considered a target sample of 190 and an assumed 80% 2-year (24-month) follow-up retention, for a final longitudinal sample of approximately 150. Table 4 presents minimal detectable effect sizes in time-averaged difference [283] for Aim 1 with a 1:1 or 1:3 frequency matched for

YPHEU with ABCD youth, and for Aim 2 assuming the proportion with exposure (such as a specific ARV class) is 50% or 30%. The sample sizes provide sufficient power to detect reasonable effect size even with small unadjusted false positive rate ( $\alpha$ ) of 0.5% to allow for adjustment of multiple comparison. To estimate the detectable Spearman correlation between two continuous variables such as for H1b, H2b, with a sample size of 150, simulation results show we can detect a correlation of at least 0.32 with > 80% power and a small  $\alpha=0.5%$  to allow for correction of multiple comparisons.

<b>Table 4: Minimal Detectable Effect Sizes</b>								
Difference in SD for continuous outcome, minimal detectable risk ratio for binary outcome, with 80% power and 0.5% unadjusted false positive rate								
	Aim 1						Aim 2	
Outcome	Continuous		Binary				Continuous	
Minimal Detectable	Difference in SD		Risk Ratio				Difference in SD	
			0.2*		0.4*			
Sample Size	150:150	150:450	150:150	150:450	150:150	150:450	75:75	45:105
$\rho = 0.25$	0.33	0.27	1.74	1.57	1.42	1.34	0.47	0.51
$\rho = 0.50$	0.37	0.30	1.82	1.63	1.46	1.37	0.52	0.56
<p><math>\rho</math>: correlation between 2 assessments within participant  SD: standard deviation  *: 0.2 and 0.4 are example risks within the unexposed for the calculations</p>								

### Exploratory Aim 3

For young adults, using  $\alpha=5%$ , a sample size of 100, 75 and 25 can detect correlations of at least 0.29, 0.34 and 0.57, respectively. If we compare 75 and 25 YAPHIV or YAPHEU with 100 HCP-YA, a mean difference of 0.43 SD and 0.63 SD or larger can be detected, respectively. Power calculations used Power Analysis & Sample Size (PASS) 15.0.4.

## 16.6 Sex as a Biological Variable and Other Common Covariates

This study will aim to recruit equal numbers of male and female participants. Studies have shown that protective effects of maternal care against stress effects on brain development might have greater protective benefits for females [284]. Sex differences will be explored in this study's relatively large youth population. Site and scanner platform will be included as a random effect in all regression models as described above. Possible confounders or covariates are listed above by hypothesis.

## 16.7 Potential Problems and Alternative Strategies

**Attrition:** Although attrition is always a concern, experiences from SMARTT as well as our local 2-year (24 month) study of similar age children (N=190), and our team's experience with longitudinal studies indicate that with good follow-up procedures, we can minimize loss to follow up (e.g., efforts to keep in contact with participants and families in between visits, reminder notes and phone calls about upcoming visits, birthday cards, etc.).

**Data loss:** Issues relating to MRI data loss will be addressed. We will discuss the potential of braces with all participants prior to enrolling, working around orthodontic appointments. In order to minimize data loss due to movement, we will provide simulation and motion compliance training for participants in a mock scanner using ABCD procedures [79]. During scanning, we will provide a weighted blanket if requested. Some participants may not complete all scans. We will schedule additional scanning sessions to complete

missing scans if possible. In case of partial data loss (experience from ABCD indicates that the EN-back has the lowest completion rate at 88% [79]), we will focus on data with higher completion rates (i.e., structural, diffusion, and rs fMRI, 99%-97% [79]) to investigate targeted brain networks.

**Missing data:** Every effort will be made to ensure that the amount of missing data is kept to a minimum as missing data complicates the statistical analyses or results in biased parameter estimates. When data for covariates are missing, missing indicator categories can be used for categorical variables. When data for outcomes are missing, we will describe the reasons for missing outcomes and compare participant characteristics between the group of participants with missing outcome data and those with non-missing data. Assumptions needed to obtain valid statistical inferences in the presence of missing data will be thoroughly investigated. If the assumption of missing at random is reasonable, multiple imputation will be implemented [285]. If the chance of missing is dependent on baseline characteristics or exposures of interest, factors associated with missingness will be identified and included in analyses using missing data methods, such as inverse probability weighting [286] and applied to regression models to correct for selection bias.

**Scanner effects:** A recent ABCD study on neuroimaging data demonstrates that some scanner effects exist in rs fMRI and task-based conditions but task-based contrasts show minimal scanner effects [218]. The authors recommend that further harmonization can be performed using combined association test (ComBat) [287]. ComBat was first developed in genomics research [288] and has recently been applied to harmonize multisite MRI. It is an effective harmonization technique that both removes unwanted variation associated with site and preserves biological associations in the data. We will explore this technique for further harmonization of the multisite neuroimaging data.

## 17.0 ASTRO NESTED SUBSTUDY STATISTICAL/ANALYTIC CONSIDERATIONS

### 17.1 Sample Size

#### 17.1.1 ASTRO Nested Substudy Sample

The ASTRO nested substudy will enroll 50 YPHEU and approximately 100 young adults in about a 1:3 ratio of YAPHEU and YAPHIV.

#### 17.1.2 ASTRO Nested Substudy Comparison Participants

Similar-age individuals from the general population ( $n \approx 650$ ) from the repository of cfPWV data that had been previously curated by Dr. Urbina will be used as comparison groups.

### 17.2 Statistical Analyses

#### 17.2.1 Aim 1: To investigate the impact of in utero HIV exposure and perinatal HIV infection on aortic stiffness in children and young adults

In Aim 1, differences in cfPWV between groups will be assessed, and relationships of cfPWV with metabolic and inflammatory parameters within each group will be examined. Analyses of youth (YPHEU  $n = 50$  vs. similar-age general population  $n \approx 200$ ) and young adults (young adults  $n = 100$  in about a 1:3 ratio of YAPHEU to YAPHIV vs. similar-age general population  $n \approx 450$ ) and will be performed separately.

For comparison groups, all data in the appropriate age ranges from Dr. Urbina's repository involving the general population will be utilized.

To minimize differences between groups, the general population sample will be standardized to the PHIV and PHEU groups on key covariates using probability weighting [289]. Among youth, a propensity score for each participant will be obtained using a logistic regression model with study group (YPHEU, general population) treated as the outcome and rounded age, sex, race, and BMI treated as predictors. All YPHEU will be given a weight of 1, whereas the weight for each individual in the general population will be defined as (propensity score)/(1 - propensity score). Young adults in the general population will be standardized to the combined group of YAPHIV and YAPHEU using similar methods. A sensitivity analysis in which the general population sample is standardized to YAPHIV and YAPHEU individually and compared to each group in separate analyses will also be performed.

For analyses, variables that are not normally distributed and right-skewed will be log-transformed to approximate a normal distribution. **Hypothesis 1a:** The distribution of sociodemographic and clinical variables (including cfPWV) will be described across study groups using the mean (standard deviation, SD) and frequency, as appropriate, before and after weighting. To compare cfPWV between groups, unadjusted and adjusted linear regression models with weighting will be fit using generalized estimating equations (GEE) to obtain the robust variance estimator. The average difference (95% confidence interval, CI) will be reported for each pair of groups. The basic adjusted model will include age, sex, race, pubertal status (in youth only), and potential confounders (e.g., primary caregiver education [290-292], adverse childhood experiences [293-295], and physical activity [296-298]). Analyses stratified by sex and obesity will also be performed to evaluate qualitatively whether differences in cfPWV between groups differ by strata, acknowledging that these analyses will have limited power. **Hypothesis 1b:** Within the YAPHIV, YAPHEU, and YPHEU groups, the relationship of cfPWV with each metabolic and immune parameter (i.e., blood pressure, anthropometrics, serologic markers, and eicosanoids) will be plotted and categorizing the parameter will be considered if there is a deviation from a linear relationship. Unadjusted and adjusted linear regression models using GEE will be fitted to determine the association of each parameter with cfPWV. For analyses involving eicosanoids, a graphical lasso approach will be employed to estimate group-specific eicosanoid networks that are associated with cfPWV, as previously done [299]. Networks will be plotted using the R package igraph in which eicosanoids associated with cfPWV are represented as nodes and correlations between eicosanoids (conditional on all other analytes) are indicated by lines between nodes [300]. As an alternate approach, elastic net regression will also be considered in predictive modeling of all metabolic and immune parameters including eicosanoids. Lastly, to evaluate the impact of the in utero environment on lifelong disease risk, data available in SMARTT will be leveraged to test associations of prenatal maternal characteristics (e.g., CD4 T cell count) with cfPWV in YPHEU.

**Power calculations:** Assuming a mean (SD) of 4.8 m/sec (0.97) in YAPHIV from preliminary data, there is 80% power to detect a difference of at least 0.60 m/sec of cfPWV between YAPHEU and YAPHIV ( $\alpha = 0.05$ , two-sided) and greater power in comparisons to individuals in the general population. For correlations within groups, there is 80% power to detect a correlation between cfPWV and each factor of interest of at least  $r = 0.38$  within the YPHEU,  $r = 0.53$  within YAPHEU and  $r = 0.32$  within YAPHIV. The minimum detectable correlation will be slightly higher when adjusted for confounders.

**Expected Outcomes:** In young adults, it is expected that aortic stiffness will be highest among YAPHIV, followed by YAPHEU, followed by individuals in the general population. Furthermore, YPHEU will have higher aortic stiffness than those in the general population. It is also hypothesized that markers of metabolic and immune dysfunction (including eicosanoids) will directly relate to aortic stiffness within YAPHIV, YAPHEU, and YPHEU.

### **17.2.2 Aim 2: To evaluate the role of aortic stiffness in the pathogenesis of neurocognitive dysfunction in individuals with PHIV and PHEU.**

In Aim 2, relationships of aortic stiffness with neuroimaging and neurocognitive measures within YAPHIV, YAPHEU and YPHEU will be interrogated. Each group will be analyzed separately. Descriptive summaries will be prepared using the same approach described in Aim 1. Unadjusted and adjusted linear regression models will be fitted using GEE to assess associations between cfPWV and each neuroimaging or neurocognitive parameter. A basic model will adjust for age, sex, race, pubertal status (in youth only), and potential confounders (as listed in Aim 1). Analyses will be stratified by sex, obesity, and CRP to determine if key relationships differ by strata, acknowledging that these analyses will have limited power.

**Power calculations:** There is 80% power to detect a correlation of at least  $r = 0.38$  within the YPHEU,  $r = 0.53$  within YAPHEU and  $r = 0.32$  within YAPHIV ( $\alpha = 0.05$ ) unadjusted and a slightly higher correlation when adjusted.

**Expected Outcomes:** In YAPHIV, YAPHEU, and YPHEU, higher aortic stiffness will be associated with reduced white and gray matter integrity (e.g., lower fractional anisotropy; DTI) and gray matter volume (MRI), as well as disrupted brain networks (e.g., reduced resting-state functional connectivity; fMRI). cfPWV also will inversely relate to neurocognitive outcomes (e.g., Executive Function, Episodic/Working Memory, Fluid Cognition; NIH Toolbox).

## **18.0 HUMAN SUBJECTS**

The TERBO BRAIN study and ASTRO nested substudy will be conducted in compliance with the protocol, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) GCP guidelines, and 45 CFR §46.

### **18.1 Participant Confidentiality**

All participants enrolled in the PHACS Network are assigned unique PHACS PID and SID (PIN) numbers as described in Section 7.6. All participants co-enrolled in a PHACS study will use the same PHACS PID number assigned for their first PHACS study. The PID and SID (PIN) numbers will be used for identification purposes on all laboratory specimens, evaluation forms, and reports retained in the research records and generated in the PHACS central database, as well as for online assessments such as the online survey. A list linking the participant names with the PID and SID (PIN) numbers will be stored at the clinical site under double locks, separate from all other research records. All research records will be stored in a secured area in locked files.

All PHACS investigators and research staff persons at the clinical sites are required to sign non-disclosure forms pledging to hold research information in confidence. All collaborators seeking PHACS data are required to sign DUAs as described in Section 12.4.

Research staff will work with participants and/or their legal guardians to record contact information, which may also include the names and contact information of people (friends, family, or others) who may always know the whereabouts of participants. Establishing this list is a voluntary exercise and, if used in the event contact is lost with a participant, only a previously agreed to level of information will be disclosed. When contact is re-established with participants who were lost, willingness to continue study participation will be first ascertained.

## 18.2 Certificate of Confidentiality

As an NIH-funded project using identifiable, sensitive information, TERBO BRAIN is automatically covered by a Certificate of Confidentiality issued from the DHHS. With this Certificate in place, the TERBO BRAIN researchers cannot be forced to turn over identifying information about a study participant in any Federal, State, or local criminal, administrative, legislative, or other proceedings. This Certificate does not prevent a study participant from volunteering to turn over their research information nor does it prevent investigators from providing research-related information to others when requested by the study participant or when required by law such as in cases of suspected or actual harm to or by the study participant.

The clinical site PI will make study documents (e.g., ICFs, CRFs) and pertinent records available for inspection by the local IRB, the HLC IRB, the PHACS site monitors, the NIH, the OHRP, or the sponsor's designee for confirmation of the study data as needed.

## 18.3 Risks and Benefits

### 18.3.1 Risks Associated with Participation in the TERBO BRAIN Study

Participation in this study poses no more harms or discomforts to research participants than they may experience in normal daily life or during routine medical tests. Possible risks resulting from this study include the following:

- The evaluations that are involved in this study require answering questions about mental health, stressful or traumatic events, risk behaviors, and ART adherence. Some of the questions may make participants feel distressed or uncomfortable, and they may decline to respond. Some participants may require triage emergency referrals and treatment for significant suicidal intent and/or behaviors. As the members of the research team have specific and extensive training in dealing with risks of this kind, no additional difficulties are expected beyond those experienced in a typical clinical practice. The PHACS Emergency Protocol provide specific guidance to site staff to address atypical and/or worrisome emotional responses that may occur during a study visit. The online survey tool is designed to reduce the discomfort that some participants may experience in self-reporting some behaviors such as substance use and increase the likelihood of accurate responses.
- The information that participants provide through surveys will not be shared with medical providers without their permission, unless there is serious risk of self-harm or harm to others as specified in the consent and local IRB requirement, in which case, information including ART adherence and mental health information may need to be shared even without the participant's permission.
- Despite the multiple measures taken to protect participant confidentiality, web-based communications may be at risk for hacking, intrusions, and other violations.
- Another potential risk is the inadvertent disclosure of a participant's HIV status to someone who does not yet know about the infection. Additionally, there is the potential risk of inadvertent disclosure of maternal HIV status to YPHEU and YAPHEU who are unaware of their mother's HIV status and their own *in utero* exposure to HIV. Research staff members are required to complete human subjects research training and will be trained on protecting participant/caregiver confidentiality in the TERBO BRAIN study. Personal medical information will not be shared between the participant and caregiver, unless necessary. Research staff members will provide guidance to caregivers of YPHEU on how to maintain confidentiality of their HIV status, as needed. Research staff members will also provide guidance to those who are YAPHIV to help them maintain their confidentiality, including while completing the online survey.

Potential risks from the neuroimaging (MRI) scanning include the following:

- Participants may experience discomfort or anxiety from being in the confined and noisy environment of the MRI scanner. Participants will be able to talk with the experimenters throughout the study and will be able to inform the experimenters immediately if they want to stop the study and exit the scanner.
- This study has the potential to cause peripheral nerve stimulation (PNS) while participants are in the scanner, which would cause a slight discomfort, but the potential for inducing PNS is low. PNS is not dangerous and does not occur with proper operation of the equipment. In addition, it resolves at the conclusion of the scan. The fMRI laboratory includes trained technicians who are aware of this potential risk and take appropriate precautions in following rules and regulations.
- There is a risk that the MRI may reveal something that is already in the participant's brain, such as a tumor. Such a finding might require additional studies and the participant would be referred for further studies. However, the types of scans performed are not very sensitive to many neurological abnormalities. Therefore, it is also possible that any abnormality that the participants currently have will not be revealed by the images obtained for this experiment. If scanning procedures do reveal an abnormality to casual observation of the technician or one of the investigators involved in the project, the clinical site PI will be notified. The clinical site PI will share the information with the participant and their caregiver as appropriate and clinical referrals will be initiated, if needed.
- There are no other known side effects of neuroimaging to participants without contraindications. However, as with any research study, there may be additional risks that are unknown or unexpected.

Risks associated with scanning will be minimized in the following manner: 1) Participants will be screened for contraindications to MRI, including claustrophobia, possible intra-oral, intra-ocular, intracranial, intra-thorax, or intra-abdominal metal or cardiac pace makers; 2) During the scanning procedures, participants will be continually monitored visually and auditorily for any potential problems, and participants will be assured that they can be removed from the scanner at any time if problems should arise or they are experiencing discomfort; 3) Emergency medical equipment and pharmaceuticals are present at all of the MR facilities to be used in this study; and 4) Earphones or earplugs will be used to dampen the sound of the MRI procedure.

### **18.3.2 Benefits Associated with Participation in the TERBO BRAIN Study**

While there is no guarantee of direct benefits to the individuals who participate in this study, benefiting from participating is possible. If the participant or their legal guardian chooses, the information obtained in this study can be made available to their health care providers and it may inform their primary health care. Participants will be contributing to scientific understanding.

### **18.3.3 TERBO BRAIN Sexual Risk Behaviors and Substance Use**

Information on substance use for YPHEU and sexual risk behavior and substance use for YAPHEU and YAPHIV reported in the confidential online survey will not be disclosed to the site research staff, the clinicians responsible for their health care, or the caregiver or parent of YPHEU. If a participant is unable to complete the online survey independently due to technical challenges, a research staff member may assist with the process as needed. Participants with cognitive impairment who cannot complete the online survey independently will not complete substance use and sexual behavioral questions. Site research staff will maintain participant confidentiality, unless mandated reporting is required by law.

#### **18.3.4 Risks Associated with Participation in the ASTRO Nested Substudy**

The measurements that are involved in ASTRO nested substudy require: cPWV, venipuncture, anthropometric evaluation, and a fasting period (not to exceed 8 hours) for laboratory studies. Possible risks resulting from the study include:

- Measurement of aortic stiffness with SphygmoCor may cause mild discomfort or anxiety as it is an unfamiliar procedure. The procedure will be explained to put the participant at ease.
- Venipuncture to collect blood specimens may cause local discomfort, bleeding, or bruising; rarely a small clot or infection can occur at the blood draw site.
- There are no risks associated with vital signs or anthropometric evaluation.
- Fasting periods is required for 8 hours prior to blood draw and should present little risk. Some participants may feel hunger, irritability, and lightheadedness as a result of fasting.

#### **18.3.5 Benefits Associated with Participation in the ASTRO Nested Substudy**

While there is no guarantee of direct benefits to the individuals who participate in the ASTRO nested substudy, benefiting from participating is possible. Participants may learn health information and contribute to scientific understanding.

#### **18.4 Institutional Review Board Review and Informed Consent**

All participating sites will rely on the HLC IRB as their sIRB of record.

Prior to initiation of study implementation, participating site IRBs will sign Reliance Agreements detailing the roles and responsibilities of the HLC IRB in relation to participating sites. The HLC IRB and the PHACS Regulatory and Compliance Manager will retain copies of all Reliance Agreements and communications and facilitate the process of obtaining HLC IRB approval for this protocol, ICFs and assent forms, and any other participant-facing documents (e.g., fact sheets, recruitment materials, assessment surveys/interviews, etc.). All site-specific study materials (e.g., informed consent addendums, recruitment materials, etc.) will also require review and approval by the HLC IRB prior to study initiation. The HLC IRB Reliance Agreement Specialist and the PHACS Regulatory and Compliance Manager will maintain consistent and regular communications to ensure that participating sites are in compliance with the requirements of the HLC IRB.

This protocol, the informed consent documents, and any subsequent modifications will be reviewed and approved by the HLC IRB. The ICFs will describe the purpose of this study, the procedures to be followed, and the risks and benefits of participation. In accordance with 45 CFR §46.116, a legal informed consent will be obtained from the participant or their legal guardian, or person with power of attorney for participants who cannot consent for themselves. The participant's assent must also be obtained if they are able to understand the nature, significance, and risks of the study.

#### **18.5 Participation of Individuals who are Incarcerated**

The PHACS and NIH have concluded that this protocol does NOT meet Federal requirements governing participation of individuals who are incarcerated in human subjects research and should not be considered

by HLC IRB for the recruitment of individuals who are incarcerated. Participants who become incarcerated after enrollment may not be seen for research evaluations as long as they are incarcerated.

## **18.6 45 CFR §160 and 164 Standards for Privacy of Individually Identifiable Health Information ("Privacy Rule" pursuant to HIPAA)**

Each site is responsible for adherence to their individual institution's HIPAA policies and procedures.

### **18.6.1 Database**

Specific protected health information will be needed to create the TERBO BRAIN study databases. Institutional Privacy Boards (or IRBs serving as such) are requested to add the following persons to the disclosure element of the HIPAA authorization form (45 CFR §164.514):

- PHACS DMC and PHACS Westat site monitors;
- PHACS investigators and their collaborators;
- Participant's primary care provider, if so desired by the participant;
- The NIH; and
- Technical support staff at the institutions hosting the online assessments for the sole purpose of providing technical assistance.

## **18.7 Study Discontinuation**

The study may be discontinued at any time by the NIH.

## **19.0 PUBLICATION OF RESEARCH FINDINGS**

Publication of the results of this study will be governed by PHACS policies as outlined in the PHACS Publication Policy (available on the PHACS website).

Participant summaries of findings will be developed, approved by the HLC IRB, and provided directly to the clinical sites to distribute to participants.

## REFERENCES

1. Slogrove, A.L., et al., *Surviving and Thriving-Shifting the Public Health Response to HIV-Exposed Uninfected Children: Report of the 3rd HIV-Exposed Uninfected Child Workshop*. Front Pediatr, 2018. **6**: p. 157.
2. Robertson, K., J. Liner, and R.B. Meeker, *Antiretroviral neurotoxicity*. J Neurovirol, 2012. **18**(5): p. 388-99.
3. Bucek, A., et al., *Psychiatric disorders and young adult milestones in HIV-exposed, uninfected youth*. AIDS Care, 2019: p. 1-9.
4. Malee, K.M., et al., *Mental health functioning among children and adolescents with perinatal HIV infection and perinatal HIV exposure*. Aids Care-Psychological and Socio-Medical Aspects of Aids/Hiv, 2011. **23**(12): p. 1533-1544.
5. Mellins, C.A., et al., *Rates and types of psychiatric disorders in perinatally human immunodeficiency virus-infected youth and seroreverters*. J Child Psychol Psychiatry, 2009. **50**(9): p. 1131-8.
6. Garvie, P.A., et al., *Discordance of cognitive and academic achievement outcomes in youth with perinatal HIV exposure*. Pediatr Infect Dis J, 2014. **33**(9): p. e232-8.
7. Smith, R., et al., *Impact of HIV severity on cognitive and adaptive functioning during childhood and adolescence*. Pediatric Infectious Disease Journal, 2012. **31**(6): p. 592-598.
8. Malee, K.M., et al., *Impact of Perinatally Acquired HIV Disease Upon Longitudinal Changes in Memory and Executive Functioning*. J Acquir Immune Defic Syndr, 2017. **75**(4): p. 455-464.
9. Nichols, S.L., et al., *Learning and Memory in Children and Adolescents With Perinatal HIV Infection and Perinatal HIV Exposure*. Pediatr Infect Dis J, 2016. **35**(6): p. 649-54.
10. Nichols, S.L., et al., *Executive Functioning in Children and Adolescents With Perinatal HIV Infection and Perinatal HIV Exposure*. J Pediatric Infect Dis Soc, 2016. **5**(suppl 1): p. S15-S23.
11. Nozyce, M.L., et al., *Safety of in utero and neonatal antiretroviral exposure: cognitive and academic outcomes in HIV-exposed, uninfected children 5-13 years of age*. Pediatr Infect Dis J, 2014. **33**(11): p. 1128-33.
12. Rice, M.L., et al., *Language impairment in children perinatally infected with HIV compared to children who were HIV-exposed and uninfected*. J Dev Behav Pediatr, 2012. **33**(2): p. 112-23.
13. Sirois, P.A., et al., *Safety of perinatal exposure to antiretroviral medications: Developmental outcomes in infants*. Pediatr Infect Dis J, 2013. **32**(6): p. 648-55.
14. Williams, P.L., et al., *Neurodevelopment and in utero antiretroviral exposure of HIV-exposed uninfected infants*. Pediatrics, 2010. **125**(2): p. e250-60.
15. Mellins, C., *Behavioral Challenges in Perinatally HIV Youth*, in *Invited paper at symposium at the 20th Conference on Retroviruses and Opportunistic Infections (CROI)*. 2013: Atlanta.

16. Abrams, E.J., et al., *Behavioral Health and Adult Milestones in Young Adults With Perinatal HIV Infection or Exposure*. Pediatrics, 2018. **142**(3).
17. Pearlstein, S.L., et al., *Youth in transition: life skills among perinatally HIV-infected and HIV-exposed adolescents*. J Pediatr Psychol, 2014. **39**(3): p. 294-305.
18. Jankiewicz, M., et al., *White matter abnormalities in children with HIV infection and exposure*. Frontiers in Neuroanatomy, 2017. **11**: p. 9.
19. Tran, L.T., et al., *White matter microstructural integrity and neurobehavioral outcome of HIV-exposed uninfected neonates*. Medicine, 2016. **95**(4): p. 7.
20. Cicchetti, D., B.P. Ackerman, and C.E. Izard, *Emotions and emotion regulation in developmental psychopathology*. Development and Psychopathology, 1995. **7**(1): p. 1-10.
21. Zeman, J., et al., *Emotion regulation in children and adolescents*. Journal of Developmental and Behavioral Pediatrics, 2006. **27**(2): p. 155-168.
22. Phillips, M.L., et al., *Neurobiology of emotion perception II: Implications for major psychiatric disorders*. Biol Psychiatry, 2003. **54**(5): p. 515-28.
23. Luciana, M., et al., *Adolescent neurocognitive development and impacts of substance use: Overview of the adolescent brain cognitive development (ABCD) baseline neurocognition battery*. Dev Cogn Neurosci, 2018. **32**: p. 67-79.
24. Benson, S., et al., *Association Between Psychiatric Disorders, Substance Use, and Sexual Risk Behaviors in Perinatally HIV-Exposed Youth*. J Assoc Nurses AIDS Care, 2018. **29**(4): p. 538-549.
25. Mellins, C.A., et al., *Behavioral health risks in perinatally HIV-exposed youth: co-occurrence of sexual and drug use behavior, mental health problems, and nonadherence to antiretroviral treatment*. AIDS Patient Care STDS, 2011. **25**(7): p. 413-22.
26. Robbins, R.N., et al., *Longitudinal trajectories of neurocognitive test performance among individuals with perinatal HIV-infection and -exposure: adolescence through young adulthood*. AIDS Care, 2019. **0**(0): p. 1-9.
27. Jahanshad, N., et al., *Brain imaging and neurodevelopment in HIV-uninfected Thai children born to HIV-infected mothers*. Pediatric Infectious Disease Journal, 2015. **34**(9): p. E211-E216.
28. Tardieu, M., et al., *Cerebral MR imaging in uninfected children born to HIV-seropositive mothers and perinatally exposed to zidovudine*. AJNR Am J Neuroradiol, 2005. **26**(4): p. 695-701.
29. Robertson, F.C., et al., *Perinatal HIV infection or exposure is associated with low N-acetylaspartate and glutamate in basal ganglia at age 9 but not 7 years*. Frontiers in Human Neuroscience, 2018. **12**: p. 10.
30. Cohen, S., et al., *Cerebral injury in perinatally HIV-infected children compared to matched healthy controls*. Neurology, 2016. **86**(1): p. 19-27.

31. Sarma, M.K., et al., *Regional brain gray and white matter changes in perinatally HIV-infected adolescents*. *Neuroimage-Clinical*, 2014. **4**: p. 29-34.
32. Yadav, S.K., et al., *Altered structural brain changes and neurocognitive performance in pediatric HIV*. *Neuroimage-Clinical*, 2017. **14**: p. 316-322.
33. Ackermann, C., et al., *Early antiretroviral therapy in HIV-infected children is associated with diffuse white matter structural abnormality and corpus callosum sparing*. *American Journal of Neuroradiology*, 2016. **37**(12): p. 2363-2369.
34. Hoare, J., et al., *White matter micro-structural changes in ART-naive and ART-treated children and adolescents infected with HIV in South Africa*. *Aids*, 2015. **29**(14): p. 1793-1801.
35. Li, J., et al., *White matter development is potentially influenced in adolescents with vertically transmitted HIV infections: A tract-based spatial statistics study*. *American Journal of Neuroradiology*, 2015. **36**(11): p. 2163-2169.
36. Uban, K.A., et al., *White matter microstructure among youth with perinatally acquired HIV is associated with disease severity*. *AIDS*, 2015. **29**(9): p. 1035-44.
37. Lewis-de los Angeles, C.P., et al., *Deformed subcortical structures are related to past HIV disease severity in youth with perinatally acquired HIV infection*. *Journal of the Pediatric Infectious Diseases Society*, 2016. **5**: p. S6-S14.
38. Lewis-de los Angeles, C.P., et al., *Lower total and regional grey matter brain volumes in youth with perinatally-acquired HIV infection: Associations with HIV disease severity, substance use, and cognition*. *Brain Behavior and Immunity*, 2017. **62**: p. 100-109.
39. Herting, M.M., et al., *Default mode connectivity in youth with perinatally acquired HIV*. *Medicine*, 2015. **94**(37): p. 10.
40. McHenry, M.S., et al., *Neurodevelopment in Young Children Born to HIV-Infected Mothers: A Meta-analysis*. *Pediatrics*, 2018. **141**(2).
41. Nichols, S.L., et al., *Executive Functioning in Children and Adolescents With Perinatal HIV Infection*. *Pediatr Infect Dis J*, 2015. **34**(9): p. 969-75.
42. Kerr, S.J., et al., *Increased Risk of Executive Function and Emotional Behavioral Problems Among Virologically Well-Controlled Perinatally HIV-Infected Adolescents in Thailand and Cambodia*. *J Acquir Immune Defic Syndr*, 2019. **82**(3): p. 297-304.
43. Harris, L.L., et al., *Prospective memory in youth with perinatally-acquired HIV infection*. *Child Neuropsychol*, 2018. **24**(7): p. 938-958.
44. Jernigan, T.L., S.A. Brown, and G.J. Dowling, *The Adolescent Brain Cognitive Development Study*. *J Res Adolesc*, 2018. **28**(1): p. 154-156.
45. Milligan, R. and K. Cockcroft, *Working memory profiles in HIV-exposed, uninfected and HIV-infected Children: A comparison with neurotypical controls*. *Frontiers in Human Neuroscience*, 2017. **11**: p. 13.

46. Kieling, C., et al., *Child and adolescent mental health worldwide: Evidence for action*. Lancet, 2011. **378**(9801): p. 1515-25.
47. Mellins, C.A., et al., *Prevalence and change in psychiatric disorders among perinatally HIV-infected and HIV-exposed youth*. AIDS Care, 2012. **24**(8): p. 953-62.
48. Piske, M., et al., *Neurodevelopmental outcomes and in-utero antiretroviral exposure in HIV-exposed uninfected children*. AIDS, 2018. **32**(17): p. 2583-2592.
49. Smith, R., et al., *Mental Health Diagnoses, Symptoms, and Service Utilization in US Youth with Perinatal HIV Infection or HIV Exposure*. AIDS Patient Care STDS, 2019. **33**(1): p. 1-13.
50. Elkington, K.S., J.A. Bauermeister, and M.A. Zimmerman, *Psychological distress, substance use, and HIV/STI risk behaviors among youth*. J Youth Adolesc, 2010. **39**(5): p. 514-27.
51. Alperen, J., et al., *Prevalence of and risk factors for substance use among perinatally human immunodeficiency virus-infected and perinatally exposed but uninfected youth*. J Adolesc Health, 2014. **54**(3): p. 341-9.
52. Brown, L.K., et al., *Psychiatric disorders and sexual risk among adolescents in mental health treatment*. J Consult Clin Psychol, 2010. **78**(4): p. 590-7.
53. Tapert, S.F., et al., *Adolescent substance use and sexual risk-taking behavior*. J Adolesc Health, 2001. **28**(3): p. 181-9.
54. Mellins, C.A., et al., *Sexual and drug use behavior in perinatally HIV-infected youth: mental health and family influences*. J Am Acad Child Adolesc Psychiatry, 2009. **48**(8): p. 810-819.
55. Elkington, K.S., et al., *Substance use and sexual risk behaviors in perinatally human immunodeficiency virus-exposed youth: roles of caregivers, peers and HIV status*. J Adolesc Health, 2009. **45**(2): p. 133-41.
56. Elkington, K.S., et al., *Substance use and the development of sexual risk behaviors in youth perinatally exposed to HIV*. J Pediatr Psychol, 2015. **40**(4): p. 442-54.
57. Nigg, J.T., *Annual Research Review: On the relations among self-regulation, self-control, executive functioning, effortful control, cognitive control, impulsivity, risk-taking, and inhibition for developmental psychopathology*. J Child Psychol Psychiatry, 2017. **58**(4): p. 361-383.
58. Bressler, S.L. and V. Menon, *Large-scale brain networks in cognition: emerging methods and principles*. Trends Cogn Sci, 2010. **14**(6): p. 277-90.
59. Goldman-Rakic, P.S., *Topography of cognition: parallel distributed networks in primate association cortex*. Annu Rev Neurosci, 1988. **11**: p. 137-56.
60. McIntosh, A.R., *Towards a network theory of cognition*. Neural Netw, 2000. **13**(8-9): p. 861-70.
61. Mesulam, M.M., *Large-scale neurocognitive networks and distributed processing for attention, language, and memory*. Ann Neurol, 1990. **28**(5): p. 597-613.

62. Sporns, O., et al., *Organization, development and function of complex brain networks*. Trends Cogn Sci, 2004. **8**(9): p. 418-25.
63. Menon, V., *Large-scale brain networks and psychopathology: a unifying triple network model*. Trends Cogn Sci, 2011. **15**(10): p. 483-506.
64. Seeley, W.W., et al., *Dissociable intrinsic connectivity networks for salience processing and executive control*. J Neurosci, 2007. **27**(9): p. 2349-56.
65. Koechlin, E. and C. Summerfield, *An information theoretical approach to prefrontal executive function*. Trends Cogn Sci, 2007. **11**(6): p. 229-35.
66. Miller, E.K. and J.D. Cohen, *An integrative theory of prefrontal cortex function*. Annu Rev Neurosci, 2001. **24**: p. 167-202.
67. Muller, N.G. and R.T. Knight, *The functional neuroanatomy of working memory: contributions of human brain lesion studies*. Neuroscience, 2006. **139**(1): p. 51-8.
68. Petrides, M., *Lateral prefrontal cortex: architectonic and functional organization*. Philos Trans R Soc Lond B Biol Sci, 2005. **360**(1456): p. 781-95.
69. Amodio, D.M. and C.D. Frith, *Meeting of minds: the medial frontal cortex and social cognition*. Nat Rev Neurosci, 2006. **7**(4): p. 268-77.
70. Spreng, R.N., R.A. Mar, and A.S. Kim, *The common neural basis of autobiographical memory, prospection, navigation, theory of mind, and the default mode: a quantitative meta-analysis*. J Cogn Neurosci, 2009. **21**(3): p. 489-510.
71. Rangel, A., C. Camerer, and P.R. Montague, *A framework for studying the neurobiology of value-based decision making*. Nat Rev Neurosci, 2008. **9**(7): p. 545-56.
72. Etkin, A., T. Egner, and R. Kalisch, *Emotional processing in anterior cingulate and medial prefrontal cortex*. Trends Cogn Sci, 2011. **15**(2): p. 85-93.
73. Fox, M.D., et al., *Spontaneous neuronal activity distinguishes human dorsal and ventral attention systems*. Proc Natl Acad Sci U S A, 2006. **103**(26): p. 10046-51.
74. Kelly, A.M., et al., *Competition between functional brain networks mediates behavioral variability*. Neuroimage, 2008. **39**(1): p. 527-37.
75. Sridharan, D., D.J. Levitin, and V. Menon, *A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks*. Proc Natl Acad Sci U S A, 2008. **105**(34): p. 12569-74.
76. Weissman, D.H., et al., *The neural bases of momentary lapses in attention*. Nat Neurosci, 2006. **9**(7): p. 971-8.
77. Cooper, J.C. and B. Knutson, *Valence and salience contribute to nucleus accumbens activation*. Neuroimage, 2008. **39**(1): p. 538-547.

78. Mahler, S.V. and K.C. Berridge, *Which cue to "Want?" Central amygdala opioid activation enhances and focuses incentive salience on a prepotent reward cue.* Journal of Neuroscience, 2009. **29**(20): p. 6500-6513.
79. Casey, B.J., et al., *The Adolescent Brain Cognitive Development (ABCD) study: Imaging acquisition across 21 sites.* Developmental Cognitive Neuroscience, 2018. **32**: p. 43-54.
80. Ochsner, K.N. and J.J. Gross, *The cognitive control of emotion.* Trends in Cognitive Sciences, 2005. **9**(5): p. 242-249.
81. Villemonteix, T., et al., *Attentional control of emotional interference in children with ADHD and typically developing children: An emotional N-back study.* Psychiatry Res, 2017. **254**: p. 1-7.
82. Hileman, C.O. and N.T. Funderburg, *Inflammation, immune activation, and antiretroviral therapy in HIV.* Current Hiv/Aids Reports, 2017. **14**(3): p. 93-100.
83. Malee, K.M., et al., *Mental health functioning among children and adolescents with perinatal HIV infection and perinatal HIV exposure.* AIDS Care, 2011. **23**(12): p. 1533-44.
84. Barret, B., et al., *Persistent mitochondrial dysfunction in HIV-1-exposed but uninfected infants: Clinical screening in a large prospective cohort.* AIDS, 2003. **17**(12): p. 1769-1785.
85. Brinkman, K., et al., *Adverse effects of reverse transcriptase inhibitors: Mitochondrial toxicity as common pathway.* AIDS, 1998. **12**(14): p. 1735-1744.
86. Knuesel, I., et al., *Maternal immune activation and abnormal brain development across CNS disorders.* Nature Reviews Neurology, 2014. **10**(11): p. 643-660.
87. Mottahedin, A., et al., *Effect of neuroinflammation on synaptic organization and function in the developing brain: Implications for neurodevelopmental and neurodegenerative disorders.* Frontiers in Cellular Neuroscience, 2017. **11**: p. 16.
88. Landreau-Mascaro, A., et al., *Risk of early febrile seizure with perinatal exposure to nucleoside analogues.* Lancet, 2002. **359**(9306): p. 583-4.
89. Cassidy, A.R., et al., *In Utero Efavirenz Exposure and Neurodevelopmental Outcomes in HIV-exposed Uninfected Children in Botswana.* Pediatr Infect Dis J, 2019. **38**(8): p. 828-834.
90. Ford, N., et al., *Safety of efavirenz in the first trimester of pregnancy: an updated systematic review and meta-analysis.* AIDS, 2014. **28 Suppl 2**: p. S123-31.
91. Rice, M.L., et al., *Evaluation of risk for late language emergence after in utero antiretroviral drug exposure in HIV-exposed uninfected infants.* Pediatr Infect Dis J, 2013. **32**(10): p. e406-13.
92. Sibiude, J., et al., *Association between prenatal exposure to antiretroviral therapy and birth defects: an analysis of the French perinatal cohort study (ANRS COI/CO11).* PLoS Med, 2014. **11**(4): p. e1001635.
93. Watts, D.H., et al., *Combination antiretroviral use and preterm birth.* J Infect Dis, 2013. **207**(4): p. 612-21.

94. Williams, P.L., et al., *Congenital anomalies and in utero antiretroviral exposure in human immunodeficiency virus-exposed uninfected infants*. JAMA Pediatr, 2015. **169**(1): p. 48-55.
95. Williams, P.L., et al., *Association of maternal antiretroviral use with microcephaly in children who are HIV-exposed but uninfected (SMARTT): a prospective cohort study*. The Lancet HIV, 2019. **0**(0).
96. Yao, T.J., et al., *In Utero Antiretroviral Exposure and Risk of Neurodevelopmental Problems in HIV-Exposed Uninfected 5-Year-Old Children*. AIDS Patient Care STDS, 2023.
97. Hulshoff Pol, H.E., et al., *Prenatal exposure to famine and brain morphology in schizophrenia*. Am J Psychiatry, 2000. **157**(7): p. 1170-2.
98. Stevens, M.C., D.H. Fein, and L.H. Waterhouse, *Season of birth effects in autism*. J Clin Exp Neuropsychol, 2000. **22**(3): p. 399-407.
99. Brogly, S.B., et al., *In utero nucleoside reverse transcriptase inhibitor exposure and signs of possible mitochondrial dysfunction in HIV-uninfected children*. Aids, 2007. **21**(8): p. 929-938.
100. Henry, R.R., et al., *Relationship between cognitive and immune functioning in children born to HIV-1 seropositive women*. Developmental Neuropsychology, 1996. **12**(3): p. 283-298.
101. Kapetanovic, S., et al., *Biomarkers and neurodevelopment in perinatally HIV-infected or exposed youth: a structural equation model analysis*. AIDS, 2014. **28**(3): p. 355-64.
102. Malkova, N.V., et al., *Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism*. Brain Behav Immun, 2012. **26**(4): p. 607-16.
103. Spann, M.N., et al., *Maternal Immune Activation During the Third Trimester Is Associated with Neonatal Functional Connectivity of the Salience Network and Fetal to Toddler Behavior*. J Neurosci, 2018. **38**(11): p. 2877-2886.
104. Kang, E., et al., *Disadvantaged Neighborhood Influences on Depression and Anxiety in Youth with Perinatally Acquired Human Immunodeficiency Virus: How Life Stressors Matter*. J Community Psychol, 2011. **39**(8): p. 956-971.
105. Atkinson, L., et al., *Cumulative risk, cumulative outcome: a 20-year longitudinal study*. PLoS One, 2015. **10**(6): p. e0127650.
106. Mutumba, M., et al., *A Prospective Longitudinal Study of Mental Health Symptoms Among Perinatally HIV-Infected and HIV-Exposed but Uninfected Urban Youths*. J Adolesc Health, 2016. **58**(4): p. 460-466.
107. Hermetet-Lindsay, K.D., et al., *Contributions of Disease Severity, Psychosocial Factors, and Cognition to Behavioral Functioning in US Youth Perinatally Exposed to HIV*. AIDS Behav, 2017. **21**(9): p. 2703-2715.
108. Malee, K.M., et al., *Prevalence, incidence, and persistence of psychiatric and substance use disorders among mothers living with HIV*. J Acquir Immune Defic Syndr, 2014. **65**(5): p. 526-34.

109. Mellins, C.A., et al., *Longitudinal study of mental health and psychosocial predictors of medical treatment adherence in mothers living with HIV disease*. AIDS Patient Care STDS, 2003. **17**(8): p. 407-16.
110. Webster, K.D., et al., *Caregiver socioemotional health as a determinant of child well-being in school-aged and adolescent Ugandan children with and without perinatal HIV exposure*. Trop Med Int Health, 2019. **24**(5): p. 608-619.
111. Sandman, C.A., et al., *Exposure to prenatal psychobiological stress exerts programming influences on the mother and her fetus*. Neuroendocrinology, 2012. **95**(1): p. 7-21.
112. Schwabe, L., V.D. Bohbot, and O.T. Wolf, *Prenatal stress changes learning strategies in adulthood*. Hippocampus, 2012. **22**(11): p. 2136-43.
113. Coe, C.L. and G.R. Lubach, *Prenatal origins of individual variation in behavior and immunity*. Neurosci Biobehav Rev, 2005. **29**(1): p. 39-49.
114. Frodl, T. and V. O'Keane, *How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans*. Neurobiol Dis, 2013. **52**: p. 24-37.
115. Goff, B., et al., *Reduced nucleus accumbens reactivity and adolescent depression following early-life stress*. Neuroscience, 2013. **249**: p. 129-38.
116. Pechtel, P. and D.A. Pizzagalli, *Effects of early life stress on cognitive and affective function: an integrated review of human literature*. Psychopharmacology (Berl), 2011. **214**(1): p. 55-70.
117. Edmiston, E.E., et al., *Corticostriatal-limbic gray matter morphology in adolescents with self-reported exposure to childhood maltreatment*. Arch Pediatr Adolesc Med, 2011. **165**(12): p. 1069-77.
118. Hanson, J.L., et al., *Early stress is associated with alterations in the orbitofrontal cortex: a tensor-based morphometry investigation of brain structure and behavioral risk*. J Neurosci, 2010. **30**(22): p. 7466-72.
119. Thomaes, K., et al., *Reduced anterior cingulate and orbitofrontal volumes in child abuse-related complex PTSD*. J Clin Psychiatry, 2010. **71**(12): p. 1636-44.
120. Gatt, J.M., et al., *Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety*. Mol Psychiatry, 2009. **14**(7): p. 681-95.
121. Cohen, R.A., et al., *Early life stress and morphometry of the adult anterior cingulate cortex and caudate nuclei*. Biol Psychiatry, 2006. **59**(10): p. 975-82.
122. Gerritsen, L., et al., *BDNF Val66Met genotype modulates the effect of childhood adversity on subgenual anterior cingulate cortex volume in healthy subjects*. Mol Psychiatry, 2012. **17**(6): p. 597-603.
123. Hart, H. and K. Rubia, *Neuroimaging of child abuse: a critical review*. Front Hum Neurosci, 2012. **6**: p. 52.

124. Mueller, S.C., et al., *Early-life stress is associated with impairment in cognitive control in adolescence: an fMRI study*. *Neuropsychologia*, 2010. **48**(10): p. 3037-44.
125. Eisenberger, N.I., et al., *Neural pathways link social support to attenuated neuroendocrine stress responses*. *Neuroimage*, 2007. **35**(4): p. 1601-12.
126. Engert, V., et al., *Investigating the association between early life parental care and stress responsivity in adulthood*. *Dev Neuropsychol*, 2010. **35**(5): p. 570-81.
127. Kim, P., et al., *Perceived quality of maternal care in childhood and structure and function of mothers' brain*. *Dev Sci*, 2010. **13**(4): p. 662-73.
128. Taylor, S.E., et al., *Neural bases of moderation of cortisol stress responses by psychosocial resources*. *J Pers Soc Psychol*, 2008. **95**(1): p. 197-211.
129. Rao, H., et al., *Early parental care is important for hippocampal maturation: evidence from brain morphology in humans*. *Neuroimage*, 2010. **49**(1): p. 1144-50.
130. Elkington, K.S., et al., *Predicting Arrest in a Sample of Youth Perinatally Exposed to HIV: The Intersection of HIV and Key Contextual Factors*. *AIDS Behav*, 2018. **22**(10): p. 3234-3243.
131. Malee, K., *Neuropsychological outcomes among children and adolescents with HIV infection. Oral presentation at in 2nd Annual NeuroAIDS Conference and Research Workshop*. 2016: 2nd Annual NeuroAIDS Conference and Research Workshop, University of Ibadan, Ibadan, Nigeria.
132. Aurpibul, L., et al., *Metabolic syndrome, biochemical markers, and body composition in youth living with perinatal HIV infection on antiretroviral treatment*. *PLoS One*, 2020. **15**(3): p. e0230707.
133. Fourman, L.T., et al., *Association of In Utero HIV Exposure With Obesity and Reactive Airway Disease in HIV-Negative Adolescents and Young Adults*. *J Acquir Immune Defic Syndr*, 2020. **83**(2): p. 126-134.
134. Geffner, M.E., et al., *Changes in insulin sensitivity over time and associated factors in HIV-infected adolescents*. *AIDS*, 2018. **32**(5): p. 613-622.
135. Jao, J., et al. *Lower insulin sensitivity early in life with in utero HIV/ART exposure in Botswana. in CROI*. 2021. Virtual.
136. Blazquez, D., et al., *Lipid and glucose alterations in perinatally-acquired HIV-infected adolescents and young adults*. *BMC Infect Dis*, 2015. **15**: p. 119.
137. Claudio, C.C., et al., *Nutritional status and metabolic disorders in HIV-exposed uninfected prepubertal children*. *Nutrition*, 2013. **29**(7-8): p. 1020-3.
138. Alvarez, P., et al., *Immune activation despite preserved CD4 T cells in perinatally HIV-infected children and adolescents*. *PLoS One*, 2017. **12**(12): p. e0190332.
139. Dirajlal-Fargo, S., et al., *HIV-exposed-uninfected infants have increased inflammation and monocyte activation*. *AIDS*, 2019. **33**(5): p. 845-853.

140. Wilkinson, J.D., et al., *Cardiac and inflammatory biomarkers in perinatally HIV-infected and HIV-exposed uninfected children*. AIDS, 2018. **32**(10): p. 1267-1277.
141. Angoff, R., R.C. Mosarla, and C.W. Tsao, *Aortic Stiffness: Epidemiology, Risk Factors, and Relevant Biomarkers*. Front Cardiovasc Med, 2021. **8**: p. 709396.
142. Mitchell, G.F., *Aortic stiffness, pressure and flow pulsatility, and target organ damage*. J Appl Physiol (1985), 2018. **125**(6): p. 1871-1880.
143. Alvarez-Bueno, C., et al., *Arterial Stiffness and Cognition Among Adults: A Systematic Review and Meta-Analysis of Observational and Longitudinal Studies*. J Am Heart Assoc, 2020. **9**(5): p. e014621.
144. Mitchell, G.F., et al., *Arterial stiffness, pressure and flow pulsatility and brain structure and function: the Age, Gene/Environment Susceptibility--Reykjavik study*. Brain, 2011. **134**(Pt 11): p. 3398-407.
145. Dirajlal-Fargo, S., et al., *Insulin Resistance and Markers of Inflammation in HIV-infected Ugandan Children in the CHAPAS-3 Trial*. Pediatr Infect Dis J, 2017. **36**(8): p. 761-767.
146. Innes, S., et al., *High Prevalence of Dyslipidemia and Insulin Resistance in HIV-infected Prepubertal African Children on Antiretroviral Therapy*. Pediatr Infect Dis J, 2016. **35**(1): p. e1-7.
147. Jao, J., et al., *A comparison of metabolic outcomes between obese HIV-exposed uninfected youth from the PHACS SMARTT Study and HIV-unexposed youth from the NHANES Study in the U.S.* J Acquir Immune Defic Syndr, 2019.
148. Jao, J., et al., *Distinct cord blood C-peptide, adipokine, and lipidomic signatures by in utero HIV exposure*. Pediatr Res, 2021.
149. Chirinos, J.A., et al., *Large-Artery Stiffness in Health and Disease: JACC State-of-the-Art Review*. J Am Coll Cardiol, 2019. **74**(9): p. 1237-1263.
150. Safar, M.E., et al., *Pulse pressure, arterial stiffness, and end-organ damage*. Curr Hypertens Rep, 2012. **14**(4): p. 339-44.
151. Mattace-Raso, F.U., et al., *Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study*. Circulation, 2006. **113**(5): p. 657-63.
152. Saito, M., et al., *Possible link between large artery stiffness and coronary flow velocity reserve*. Heart, 2008. **94**(6): p. e20.
153. Karagodin, I., et al., *Aortic stiffening precedes onset of heart failure with preserved ejection fraction in patients with asymptomatic diastolic dysfunction*. BMC Cardiovasc Disord, 2017. **17**(1): p. 62.
154. Ohyama, Y., et al., *Association of Aortic Stiffness With Left Ventricular Remodeling and Reduced Left Ventricular Function Measured by Magnetic Resonance Imaging: The Multi-Ethnic Study of Atherosclerosis*. Circ Cardiovasc Imaging, 2016. **9**(7).

155. Chung, G.E., et al., *Clinical significance of increased arterial stiffness associated with atrial fibrillation, according to Framingham risk score*. Sci Rep, 2021. **11**(1): p. 4955.
156. Cremer, A., et al., *Increased arterial stiffness is an independent predictor of atrial fibrillation in hypertensive patients*. J Hypertens, 2015. **33**(10): p. 2150-5.
157. Dernellis, J. and M. Panaretou, *Aortic stiffness is an independent predictor of progression to hypertension in nonhypertensive subjects*. Hypertension, 2005. **45**(3): p. 426-31.
158. Wilson, J. and A.J.S. Webb, *Systolic Blood Pressure and Longitudinal Progression of Arterial Stiffness: A Quantitative Meta-Analysis*. J Am Heart Assoc, 2020. **9**(17): p. e017804.
159. Li, S., et al., *Influence of metabolic syndrome on arterial stiffness and its age-related change in young adults: the Bogalusa Heart Study*. Atherosclerosis, 2005. **180**(2): p. 349-54.
160. Wu, C.F., et al., *Therapeutic modification of arterial stiffness: An update and comprehensive review*. World J Cardiol, 2015. **7**(11): p. 742-53.
161. Anyfanti, P., et al., *Association of non-invasive hemodynamics with arterial stiffness in rheumatoid arthritis*. Scand Cardiovasc J, 2018. **52**(4): p. 171-176.
162. Zanolli, L., et al., *Inflammation and Aortic Stiffness: An Individual Participant Data Meta-Analysis in Patients With Inflammatory Bowel Disease*. J Am Heart Assoc, 2017. **6**(10).
163. Vlachopoulos, C., et al., *The effect of TNF- $\alpha$  antagonists on aortic stiffness and wave reflections: a meta-analysis*. Clin Rheumatol, 2018. **37**(2): p. 515-526.
164. Tomiyama, H., et al., *The Contribution of Inflammation to the Development of Hypertension Mediated by Increased Arterial Stiffness*. J Am Heart Assoc, 2017. **6**(7).
165. Badji, A., et al., *Arterial stiffness and white matter integrity in the elderly: A diffusion tensor and magnetization transfer imaging study*. Neuroimage, 2019. **186**: p. 577-585.
166. Maillard, P., et al., *Effects of Arterial Stiffness on Brain Integrity in Young Adults From the Framingham Heart Study*. Stroke, 2016. **47**(4): p. 1030-6.
167. Mohammadi, H., et al., *Cortical thinning is associated with brain pulsatility in older adults: An MRI and NIRS study*. Neurobiol Aging, 2021. **106**: p. 103-118.
168. Hussein, A., et al., *The association between resting-state functional magnetic resonance imaging and aortic pulse-wave velocity in healthy adults*. Hum Brain Mapp, 2020. **41**(8): p. 2121-2135.
169. Urbina, E.M., et al., *Target Organ Abnormalities in Pediatric Hypertension*. J Pediatr, 2018. **202**: p. 14-22.
170. Patel, K., et al., *Aggregate risk of cardiovascular disease among adolescents perinatally infected with the human immunodeficiency virus*. Circulation, 2014. **129**(11): p. 1204-12.
171. Dirajlal-Fargo, S., et al., *Monocyte activation and gut barrier dysfunction in South African youth on antiretroviral therapy and their associations with endothelial dysfunction*. AIDS, 2020. **34**(11): p. 1615-1623.

172. Mahtab, S., et al., *Endothelial Dysfunction in South African Youth Living With Perinatally Acquired Human Immunodeficiency Virus on Antiretroviral Therapy*. Clin Infect Dis, 2020. **71**(10): p. e672-e679.
173. Miller, T.L., et al., *Biomarkers of vascular dysfunction in children infected with human immunodeficiency virus-1*. J Acquir Immune Defic Syndr, 2010. **55**(2): p. 182-8.
174. O'Rourke, M.F. and M.E. Safar, *Relationship between aortic stiffening and microvascular disease in brain and kidney: cause and logic of therapy*. Hypertension, 2005. **46**(1): p. 200-4.
175. Van Essen, D.C., et al., *The Human Connectome Project: a data acquisition perspective*. Neuroimage, 2012. **62**(4): p. 2222-31.
176. Wulfert, E., et al., *Delay of gratification: impulsive choices and problem behaviors in early and late adolescence*. J Pers, 2002. **70**(4): p. 533-52.
177. Koffarnus, M.N. and W.K. Bickel, *A 5-trial adjusting delay discounting task: accurate discount rates in less than one minute*. Exp Clin Psychopharmacol, 2014. **22**(3): p. 222-8.
178. Basgoze, Z., et al., *Valence-based Word-Face Stroop task reveals differential emotional interference in patients with major depression*. Psychiatry Res, 2015. **229**(3): p. 960-7.
179. Estle, S.J., et al., *Differential effects of amount on temporal and probability discounting of gains and losses*. Mem Cognit, 2006. **34**(4): p. 914-28.
180. Green, L., et al., *Do adjusting-amount and adjusting-delay procedures produce equivalent estimates of subjective value in pigeons?* J Exp Anal Behav, 2007. **87**(3): p. 337-47.
181. Myerson, J., L. Green, and M. Warusawitharana, *Area under the curve as a measure of discounting*. J Exp Anal Behav, 2001. **76**(2): p. 235-43.
182. Carter, C.S., et al., *CNTRICS final task selection: social cognitive and affective neuroscience-based measures*. Schizophr Bull, 2009. **35**(1): p. 153-62.
183. National Institutes of Health and Northwestern University. *NIH Toolbox*. 2017.
184. Barch, D.M., et al., *Function in the human connectome: task-fMRI and individual differences in behavior*. Neuroimage, 2013. **80**: p. 169-89.
185. Gioia, G.A., Isquith, P.K., Guy, S.C., Kenworthy, L., *BRIEF: Behavior Rating Inventory of Executive Function*. Child Neuropsychol, 2000. **6**: p. 235-238.
186. Lovstad, M., et al., *Behavior Rating Inventory of Executive Function Adult Version in Patients with Neurological and Neuropsychiatric Conditions: Symptom Levels and Relationship to Emotional Distress*. J Int Neuropsychol Soc, 2016. **22**(6): p. 682-94.
187. Gross, J.J. and O.P. John, *Individual differences in two emotion regulation processes: implications for affect, relationships, and well-being*. J Pers Soc Psychol, 2003. **85**(2): p. 348-62.
188. Gullone, E. and J. Taffe, *The Emotion Regulation Questionnaire for Children and Adolescents (ERQ-CA): a psychometric evaluation*. Psychol Assess, 2012. **24**(2): p. 409-17.

189. Barch, D.M., et al., *Demographic and mental health assessments in the adolescent brain and cognitive development study: Updates and age-related trajectories*. Dev Cogn Neurosci, 2021. **52**: p. 101031.
190. Bunford, N., et al., *The Difficulties in Emotion Regulation Scale-Parent Report: A Psychometric Investigation Examining Adolescents With and Without ADHD*. Assessment, 2020. **27**(5): p. 921-940.
191. Achenbach, T.M., *The Child Behavior Checklist and Related Instruments*, in *The use of psychological testing for treatment planning and outcomes assessment*, E.J. Maan, Editor. 1999, Lawrence Erlbaum Associates Publishers. p. 429-466.
192. Achenbach, T.M., et al., *Multicultural assessment of child and adolescent psychopathology with ASEBA and SDQ instruments: research findings, applications, and future directions*. J Child Psychol Psychiatry, 2008. **49**(3): p. 251-75.
193. Nakamura, B.J., Ebesutani, C., Bernstein, A. Chorpita, B.F., *A Psychometric Analysis fo the Child Behavior Checklist DSM-Oriented Scales*. J Psychopathol Behav Assess, 2009. **31**: p. 178-189.
194. Achenbach, T.M., Rescorla, L.A., *Manual for the ASEBA School-Age Forms & Profiles*. 2001, Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.
195. Rescorla, L.A., Achenbach, T.M., *The Achenbach System of Empirically Based Assessment (ASEBA) for Ages 18 to 90 Years*, in *The use of psychological testing for treatment planning and outcomes assessmsent: Instruments for adults*, M.E. Maruish, Editor. 2004, Lawrence Erlbaum Associates Publishers. p. 115-152.
196. Atlas, J., *Test review of the Children's Depression Inventory 2nd Edition*, in *The nineteenth mental measures yearbook*, K.F.G. J.F. Carlson, & R.A. Spies, Editor. 2014, Buros Institute's Test Reviews Online.
197. Kroenke, K., R.L. Spitzer, and J.B. Williams, *The PHQ-9: validity of a brief depression severity measure*. J Gen Intern Med, 2001. **16**(9): p. 606-13.
198. Spitzer, R.L., K. Kroenke, and J.B. Williams, *Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire*. JAMA, 1999. **282**(18): p. 1737-44.
199. Behrens, B., et al., *The Screen for Child Anxiety Related Emotional Disorders (SCARED): Informant Discrepancy, Measurement Invariance, and Test-Retest Reliability*. Child Psychiatry Hum Dev, 2019. **50**(3): p. 473-482.
200. Birmaher, B., et al., *Psychometric properties of the Screen for Child Anxiety Related Emotional Disorders (SCARED): a replication study*. J Am Acad Child Adolesc Psychiatry, 1999. **38**(10): p. 1230-6.
201. Birmaher, B., et al., *The Screen for Child Anxiety Related Emotional Disorders (SCARED): scale construction and psychometric characteristics*. J Am Acad Child Adolesc Psychiatry, 1997. **36**(4): p. 545-53.

202. Spitzer, R.L., et al., *A brief measure for assessing generalized anxiety disorder: the GAD-7*. Arch Intern Med, 2006. **166**(10): p. 1092-7.
203. Kroenke, K., et al., *Anxiety disorders in primary care: prevalence, impairment, comorbidity, and detection*. Ann Intern Med, 2007. **146**(5): p. 317-25.
204. Plummer, F., et al., *Screening for anxiety disorders with the GAD-7 and GAD-2: a systematic review and diagnostic metaanalysis*. Gen Hosp Psychiatry, 2016. **39**: p. 24-31.
205. Aidala, A., et al., *Development and validation of the Client Diagnostic Questionnaire (CDQ): a mental health screening tool for use in HIV/AIDS service settings*. Psychology, Health & Medicine, 2004. **9**(3): p. 362-380.
206. Buysse, D.J., et al., *The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research*. Psychiatry Res, 1989. **28**(2): p. 193-213.
207. Koita, K., et al., *Development and implementation of a pediatric adverse childhood experiences (ACEs) and other determinants of health questionnaire in the pediatric medical home: A pilot study*. PLoS One, 2018. **13**(12): p. e0208088.
208. Gortmaker, S.L., et al., *Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1*. N Engl J Med, 2001. **345**(21): p. 1522-8.
209. Howland, L.C., et al., *Effects of negative life events on immune suppression in children and youth infected with human immunodeficiency virus type 1*. Pediatrics, 2000. **106**(3): p. 540-6.
210. Storm, D.S., et al., *Protease inhibitor combination therapy, severity of illness, and quality of life among children with perinatally acquired HIV-1 infection*. Pediatrics, 2005. **115**(2): p. e173-82.
211. Jefferies, P., L. McGarrigle, and M. Ungar, *The CYRM-R: A Rasch-Validated Revision of the Child and Youth Resilience Measure*. J Evid Based Soc Work (2019), 2018: p. 1-23.
212. Fung, S.F., *Validity of the Brief Resilience Scale and Brief Resilient Coping Scale in a Chinese Sample*. Int J Environ Res Public Health, 2020. **17**(4).
213. Resilience Research Centre. *CYRM and ARM user manual*. 2018; Available from: <http://www.resilienceresearch.org/>
214. Goodman, R., H. Meltzer, and V. Bailey, *The Strengths and Difficulties Questionnaire: a pilot study on the validity of the self-report version*. Eur Child Adolesc Psychiatry, 1998. **7**(3): p. 125-30.
215. Eisenberg, N., *Emotion, regulation, and moral development*. Annu Rev Psychol, 2000. **51**: p. 665-97.
216. Zucker, R.A., et al., *Assessment of culture and environment in the Adolescent Brain and Cognitive Development Study: Rationale, description of measures, and early data*. Dev Cogn Neurosci, 2018. **32**: p. 107-120.
217. Petersen, A.C., et al., *A self-report measure of pubertal status: Reliability, validity, and initial norms*. J Youth Adolesc, 1988. **17**(2): p. 117-33.

218. Nielson, D.M., et al., *Detecting and harmonizing scanner differences in the ABCD study - annual release 1.0*. bioRxiv, 2018.
219. Knutson, B., et al., *Anticipation of increasing monetary reward selectively recruits nucleus accumbens*. Journal of Neuroscience, 2001. **21**(16): p. art. no.-RC159.
220. Botdorf, M., et al., *Adolescent risk-taking is predicted by individual differences in cognitive control over emotional, but not non-emotional, response conflict*. Cognition & Emotion, 2017. **31**(5): p. 972-979.
221. Langenecker, S.A., et al., *Cognitive control neuroimaging measures differentiate between those with and without future recurrence of depression*. Neuroimage-Clinical, 2018. **20**: p. 1001-1009.
222. Owen, A.M., et al., *N-back working memory paradigm: A meta-analysis of normative functional neuroimaging studies*. Hum Brain Mapp, 2005. **25**(1): p. 46-59.
223. Glasser, M.F., et al., *The Human Connectome Project's neuroimaging approach*. Nat Neurosci, 2016. **19**(9): p. 1175-87.
224. Delgado, M.R., et al., *Tracking the hemodynamic responses to reward and punishment in the striatum*. J Neurophysiol, 2000. **84**(6): p. 3072-7.
225. Hariri, A.R., et al., *The amygdala response to emotional stimuli: a comparison of faces and scenes*. Neuroimage, 2002. **17**(1): p. 317-23.
226. Drobyshevsky, A., S.B. Baumann, and W. Schneider, *A rapid fMRI task battery for mapping of visual, motor, cognitive, and emotional function*. Neuroimage, 2006. **31**(2): p. 732-44.
227. Hagler, D.J., Jr., et al., *Image processing and analysis methods for the Adolescent Brain Cognitive Development Study*. Neuroimage, 2019. **202**: p. 116091.
228. Kriska, A.M., et al., *Development of questionnaire to examine relationship of physical activity and diabetes in Pima Indians*. Diabetes Care, 1990. **13**(4): p. 401-11.
229. Wallace, T.M., J.C. Levy, and D.R. Matthews, *Use and abuse of HOMA modeling*. Diabetes Care, 2004. **27**(6): p. 1487-95.
230. Lagrand, W.K., et al., *C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon?* Circulation, 1999. **100**(1): p. 96-102.
231. Lau, B., et al., *C-reactive protein is a marker for human immunodeficiency virus disease progression*. Arch Intern Med, 2006. **166**(1): p. 64-70.
232. Burdo, T.H., et al., *Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy*. J Infect Dis, 2011. **204**(1): p. 154-63.
233. Burdo, T.H., et al., *Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients*. J Infect Dis, 2011. **204**(8): p. 1227-36.

234. Lien, E., et al., *Elevated levels of serum-soluble CD14 in human immunodeficiency virus type 1 (HIV-1) infection: correlation to disease progression and clinical events*. *Blood*, 1998. **92**(6): p. 2084-92.
235. Olson, N.C., et al., *Soluble CD14, Ischemic Stroke, and Coronary Heart Disease Risk in a Prospective Study: The REGARDS Cohort*. *J Am Heart Assoc*, 2020. **9**(6): p. e014241.
236. Hardwick, J.P., et al., *Eicosanoids in metabolic syndrome*. *Adv Pharmacol*, 2013. **66**: p. 157-266.
237. Palmu, J., et al., *Eicosanoid Inflammatory Mediators Are Robustly Associated With Blood Pressure in the General Population*. *J Am Heart Assoc*, 2020. **9**(19): p. e017598.
238. Fischer, R., et al., *Dietary omega-3 fatty acids modulate the eicosanoid profile in man primarily via the CYP-epoxygenase pathway*. *J Lipid Res*, 2014. **55**(6): p. 1150-64.
239. Alpert, K., Kogan, A., Parrish, T., Marcus, D., & Wang, L., *The Northwestern University Neuroimaging Data Archive (NUNDA)*. *Neuroimage*, Under Review.
240. Marcus, D.S., et al., *The open-source neuroimaging research enterprise*. *J Digit Imaging*, 2007. **20 Suppl 1**: p. 130-8.
241. Marcus, D.S., et al., *The Extensible Neuroimaging Archive Toolkit: an informatics platform for managing, exploring, and sharing neuroimaging data*. *Neuroinformatics*, 2007. **5**(1): p. 11-34.
242. Desikan, R.S., et al., *An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest*. *Neuroimage*, 2006. **31**(3): p. 968-980.
243. Destrieux, C., et al., *Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature*. *Neuroimage*, 2010. **53**(1): p. 1-15.
244. Smith, S.M., et al., *Advances in functional and structural MR image analysis and implementation as FSL*. *Neuroimage*, 2004. **23 Suppl 1**: p. S208-19.
245. Cox, R.W., *AFNI: software for analysis and visualization of functional magnetic resonance neuroimages*. *Comput Biomed Res*, 1996. **29**(3): p. 162-73.
246. Csernansky, J.G., et al., *Computational anatomy and neuropsychiatric disease: probabilistic assessment of variation and statistical inference of group difference, hemispheric asymmetry, and time-dependent change*. *Neuroimage*, 2004. **23 Suppl 1**: p. S56-68.
247. McKeown, M.J., et al., *Analysis of fMRI data by blind separation into independent spatial components*. *Hum Brain Mapp*, 1998. **6**(3): p. 160-88.
248. Calhoun, V.D., et al., *The chronnectome: time-varying connectivity networks as the next frontier in fMRI data discovery*. *Neuron*, 2014. **84**(2): p. 262-74.
249. Hutchison, R.M., et al., *Dynamic functional connectivity: promise, issues, and interpretations*. *Neuroimage*, 2013. **80**: p. 360-78.

250. Wu, S.J., et al., *Longitudinal fMRI task reveals neural plasticity in default mode network with disrupted executive-default coupling and selective attention after traumatic brain injury*. Brain Imaging Behav, 2019.
251. Raamana, P.R., et al., *Thickness network features for prognostic applications in dementia*. Neurobiol Aging, 2015. **36 Suppl 1**: p. S91-S102.
252. Raamana, P.R., et al., *Novel ThickNet features for the discrimination of amnesic MCI subtypes*. Neuroimage Clin, 2014. **6**: p. 284-95.
253. Popuri, K., et al., *Development and validation of a novel dementia of Alzheimer's type (DAT) score based on metabolism FDG-PET imaging*. Neuroimage Clin, 2018. **18**: p. 802-813.
254. Han, X., et al., *Reliability of MRI-derived measurements of human cerebral cortical thickness: the effects of field strength, scanner upgrade and manufacturer*. Neuroimage, 2006. **32**(1): p. 180-94.
255. Reuter, M., et al., *Within-subject template estimation for unbiased longitudinal image analysis*. Neuroimage, 2012. **61**(4): p. 1402-18.
256. Christensen, A., et al., *Hippocampal subfield surface deformity in non-semantic primary progressive aphasia*. Alzheimers Dement (Amst), 2015. **1**(1): p. 14-23.
257. Khan, A.R., L. Wang, and M.F. Beg, *Multistructure large deformation diffeomorphic brain registration*. IEEE Trans Biomed Eng, 2013. **60**(2): p. 544-53.
258. Khan, A.R., L. Wang, and M.F. Beg, *FreeSurfer-initiated fully-automated subcortical brain segmentation in MRI using Large Deformation Diffeomorphic Metric Mapping*. Neuroimage, 2008. **41**(3): p. 735-46.
259. Wang, L., et al., *Fully-automated, multi-stage hippocampus mapping in very mild Alzheimer disease*. Hippocampus, 2009. **19**(6): p. 541-548.
260. Beg, M.F., et al., *Computing Large Deformation Metric Mappings via Geodesic Flows of Diffeomorphisms*. International Journal of Computer Vision, 2005. **61**(2): p. 139.
261. Grenander, U. and M.I. Miller, *Computational Anatomy: An Emerging Discipline*. Quarterly of Applied Mathematics, 1998. **LVI**(4): p. 617-694.
262. Beg, M.F., et al., *Comparison of four shape features for detecting hippocampal shape changes in early Alzheimer's*. Stat Methods Med Res, 2012.
263. Wang, L., et al., *Large deformation diffeomorphism and momentum based hippocampal shape discrimination in dementia of the Alzheimer type*. IEEE Trans Med Imaging, 2007. **26**(4): p. 462-70.
264. Csernansky, J.G., et al., *Abnormalities of thalamic volume and shape in schizophrenia*. Am J Psychiatry, 2004. **161**(5): p. 896-902.
265. Harms, M.P., et al., *Thalamic shape abnormalities in individuals with schizophrenia and their nonpsychotic siblings*. J Neurosci, 2007. **27**(50): p. 13835-42.

266. Mamah, D., et al., *Basal ganglia shape abnormalities in the unaffected siblings of schizophrenia patients*. Biol Psychiatry, 2008. **64**(2): p. 111-20.
267. Mamah, D., et al., *Structural analysis of the basal ganglia in schizophrenia*. Schizophr Res, 2007. **89**(1-3): p. 59-71.
268. Smith, M.J., et al., *Thalamic morphology in schizophrenia and schizoaffective disorder*. J Psychiatr Res, 2011. **45**(3): p. 378-85.
269. Wang, L., et al., *Validity of large-deformation high dimensional brain mapping of the basal ganglia in adults with Tourette syndrome*. Psychiatry Res, 2007. **154**(2): p. 181-90.
270. Williams, A.C., et al., *A pilot study of basal ganglia and thalamus structure by high dimensional mapping in children with Tourette syndrome [v1; ref status: approved 1, <http://f1000r.es/1yu>]*. F1000Research 2013. **2**(207).
271. Dager, S.R., et al., *Shape mapping of the hippocampus in young children with autism spectrum disorder*. AJNR Am J Neuroradiol, 2007. **28**(4): p. 672-7.
272. Geller, B., et al., *Effects of age, sex, and independent life events on amygdala and nucleus accumbens volumes in child bipolar I disorder*. Biol Psychiatry, 2009. **65**(5): p. 432-7.
273. Gordon, E.M., et al., *Generation and Evaluation of a Cortical Area Parcellation from Resting-State Correlations*. Cereb Cortex, 2016. **26**(1): p. 288-303.
274. Bullmore, E.T. and O. Sporns, *Complex brain networks: Graph theoretical analysis of structural and functional systems*. Nature Reviews Neuroscience, 2009. **10**(3): p. 186-198.
275. Guimera, R. and L.A. Amaral, *Cartography of complex networks: modules and universal roles*. J Stat Mech, 2005. **2005**(P02001): p. nihpa35573.
276. Newman, M.E. and M. Girvan, *Finding and evaluating community structure in networks*. Phys Rev E Stat Nonlin Soft Matter Phys, 2004. **69**(2 Pt 2): p. 026113.
277. Rubinov, M. and O. Sporns, *Complex network measures of brain connectivity: uses and interpretations*. Neuroimage, 2010. **52**(3): p. 1059-69.
278. Smith, S.M., et al., *Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data*. Neuroimage, 2006. **31**(4): p. 1487-505.
279. Mori, S., et al., *Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template*. Neuroimage, 2008. **40**(2): p. 570-582.
280. Benjamini, Y. and D. Yekutieli, *The Control of the False Discovery Rate in Multiple Testing under Dependency*. The Annals of Statistics, 2001. **29**(4): p. 1165-1188.
281. Vanderweele, T., *Explanation in Causal Inference: Methods for Mediation and Interaction*. 2015: Oxford University Press.
282. VanderWeele, T.J. and P. Ding, *Sensitivity Analysis in Observational Research: Introducing the E-Value*. Ann Intern Med, 2017. **167**(4): p. 268-274.

283. Liu, H. and T. Wu, *Sample Size Calculation and Power Analysis of Time-Averaged Difference*. Journal of Modern Applied Statistical Methods, 2005. **4**(2).
284. Buss, C., et al., *Maternal care modulates the relationship between prenatal risk and hippocampal volume in women but not in men*. J Neurosci, 2007. **27**(10): p. 2592-5.
285. Hayati Rezvan, P., K.J. Lee, and J.A. Simpson, *The rise of multiple imputation: a review of the reporting and implementation of the method in medical research*. BMC Med Res Methodol, 2015. **15**: p. 30.
286. Seaman, S.R. and I.R. White, *Review of inverse probability weighting for dealing with missing data*. Stat Methods Med Res, 2013. **22**(3): p. 278-95.
287. Fortin, J.P., et al., *Harmonization of cortical thickness measurements across scanners and sites*. Neuroimage, 2018. **167**: p. 104-120.
288. Johnson, W.E., C. Li, and A. Rabinovic, *Adjusting batch effects in microarray expression data using empirical Bayes methods*. Biostatistics, 2007. **8**(1): p. 118-27.
289. Sato, T. and Y. Matsuyama, *Marginal structural models as a tool for standardization*. Epidemiology, 2003. **14**(6): p. 680-6.
290. Bouthoorn, S.H., et al., *Maternal educational level and blood pressure, aortic stiffness, cardiovascular structure and functioning in childhood: the generation R study*. Am J Hypertens, 2014. **27**(1): p. 89-98.
291. Huang, C., et al., *Maternal Education Before Childbirth and Cardiovascular Diseases in Offspring During Early Adulthood: A Danish Population-Based Cohort Study*. Can J Cardiol, 2021. **37**(12): p. 1951-1958.
292. Kayeyi, N., I.F. Sandoy, and K. Fylkesnes, *Effects of neighbourhood-level educational attainment on HIV prevalence among young women in Zambia*. BMC Public Health, 2009. **9**: p. 310.
293. Andrade, J.L., et al., *Adverse Childhood Experiences Are Associated with Cardiometabolic Risk among Hispanic American Adolescents*. J Pediatr, 2021. **237**: p. 267-275 e1.
294. Klassen, S.A., et al., *Linking systemic arterial stiffness among adolescents to adverse childhood experiences*. Child Abuse Negl, 2016. **56**: p. 1-10.
295. Young-Wolff, K.C., et al., *Adverse childhood experiences, mental health, substance use, and HIV-related outcomes among persons with HIV*. AIDS Care, 2019. **31**(10): p. 1241-1249.
296. Cruickshank, J.K., et al., *Ethnic Differences in and Childhood Influences on Early Adult Pulse Wave Velocity: The Determinants of Adolescent, Now Young Adult, Social Wellbeing, and Health Longitudinal Study*. Hypertension, 2016. **67**(6): p. 1133-41.
297. Dipietro, L., et al., *Physical Activity and Cardiometabolic Risk Factor Clustering in Young Adults with Obesity*. Med Sci Sports Exerc, 2020. **52**(5): p. 1050-1056.

298. Dirajlal-Fargo, S., et al., *Brief Report: Youth Living With Perinatally Acquired HIV Have Lower Physical Activity Levels as They Age Compared With HIV-Exposed Uninfected Youth*. *J Acquir Immune Defic Syndr*, 2021. **87**(1): p. 700-705.
299. Jao, J., et al., *Distinct Lipidomic Signatures in People Living With HIV: Combined Analysis of ACTG 5260s and MACS/WIHS*. *J Clin Endocrinol Metab*, 2022. **107**(1): p. 119-135.
300. Friedman, J., T. Hastie, and R. Tibshirani, *Sparse inverse covariance estimation with the graphical lasso*. *Biostatistics*, 2008. **9**(3): p. 432-41.

**APPENDIX I: SCHEDULE OF EVALUATIONS FOR THE YOUTH PHEU COHORT**

Participants in the Youth Longitudinal Cohort will complete all of the assessments in the table below at Entry and again at the 2-Year (24-Month) Follow-Up. It is recommended that each study visit be completed in two separate sessions occurring on two separate days. The **cognitive/behavioral session** includes cognitive, mental health, and behavioral health assessments, questionnaires for demographic and clinical (e.g., pubertal status; medications) information. The **neuroimaging session** includes training, practice, and MRI scanning. The cognitive/behavioral session should be conducted first except where needed to accommodate scheduling constraints. The two sessions should be approximately 3 weeks apart. Chart abstraction must be completed on or within 3 months after completion of the first session of the study visit. The target date for the 2-Year (24-Month) Follow-Up visit should be the 2-year anniversary of the Entry visit date (+/- approximately 3 months).

In the time between the two study visits, the site research staff will contact the participant once every six months to check-in on how they are doing and update the family’s contact information, if necessary. For telephone contact, each phone call will take about 5 minutes.

In addition to self-report, the caregiver will complete assessments regarding the emotional/behavioral well-being of the participant and report information on behalf of the participant.

<b>Task/Assessment</b>	<b>Entry</b>	<b>Year 2 (Month 24 +/- approximately 3 months)</b>	<b>Comments</b>
Identify and pre-screen for eligibility, obtain informed consent/assent, confirm eligibility, and enroll	X		
<b>Cognition and Mental and Behavioral Assessments</b>			
Computerized Tests (Administer using iPad)			
– Delay Discounting Task	X	X	
– Emotion-Word/Emotion-Face Stroop	X	X	
NIH Toolbox <sup>1</sup> (Administer using iPad)			
– Flanker Inhibitory Control and Attention – List Sorting – Dimensional Change Card Sort – Pattern Comparison – Picture Sequence – Picture Vocabulary	X	X	

– Oral Reading Recognition – Rey-Auditory Verbal Learning Test – Social Support			
<b>Examiner-Administered</b>			
– Cash Choice Task	X	X	
<b>Paper-and-Pencil Questionnaires (Administered as an interview with examiner for youth self-report; self-administered for caregiver-report)</b>			
– BRIEF-2 <sup>1</sup>	X	X	Caregiver-report; include self-report for youth aged 11 years and older only
– ASEBA CBCL <sup>1</sup>	X	X	Caregiver-report; include self-report for youth aged 11 years and older only
– CDI-2	X	X	Self- and caregiver-report
– SCARED	X	X	Self- and caregiver-report
– PEARLS	X	X	Caregiver-report; include self-report for youth aged 12 and older only
– Life Events Checklist	X	X	Self-report only
– Demographics Questionnaire	X	X	Caregiver-report only
– CYRM-R <sup>1</sup>	X	X	Self-report only
– Prosocial Behavior Survey Questions	X	X	Self-report only
– ERQ-CA	X	X	Self-report only
– DERS-P	X	X	Caregiver-report only
– PDS	X	X	Caregiver-report
<b>Online Survey</b>			
– Substance Use	X	X	Self-report
– PDS	X	X	Self-report
<b>Neuroimaging Session</b>			
– T1 – Task fMRI (MID, SST, and EN-back) – DTI – Resting-state fMRI – T2 – Resting-state fMRI	X	X	
<b>Chart Abstraction</b>			

<ul style="list-style-type: none"> <li>– Current medications since last SMARTT visit</li> <li>– Neurologic and psychiatric diagnoses since last SMARTT visit</li> </ul>	X	X	
<b>Existing SMARTT Data to be Utilized</b>			
<ul style="list-style-type: none"> <li>– Demographics, including family resources data</li> <li>– Medical and medications history</li> <li>– Caregiver CDQ</li> <li>– Birth history, including perinatal ARV exposures</li> </ul>	X	X	Data to be obtained for analysis from the DMC at Frontier Science. No chart abstraction/data entry required at the clinical sites.

<sup>1</sup>Do not complete if completed as part of SMARTT within the 3 months prior to the TERBO BRAIN study visit.

**APPENDIX II: SCHEDULE OF EVALUATIONS FOR THE YOUNG ADULT PHEU AND PHIV COHORT**

Participants in the Young Adult Cross-Sectional Cohort will complete all of the assessments in the table below at Entry. It is recommended that the study visit be completed in two separate sessions occurring on two separate days. The **cognitive/behavioral session** includes cognitive, mental health, and behavioral health assessments, questionnaires for demographic and clinical (e.g., medications) information. The **neuroimaging session** includes training, practice, and MRI scanning. The two sessions should be approximately 3 weeks apart. Chart abstraction must be completed on or within 3 months after completion of the first session of the study visit.

<b>Task/Assessment</b>	<b>Entry</b>	<b>Comments</b>
Identify and pre-screen for eligibility, obtain informed consent/assent, confirm eligibility, and enroll	X	
<b>Cognition and Mental and Behavioral Assessments</b>		
Computerized Tests (Administer using laptop)		
–Delay Discounting	X	
–Penn Emotion Recognition	X	
NIH Toolbox <sup>1</sup> (Administer using iPad)		
–Flanker Inhibitory Control and Attention –List Sorting –Dimensional Change Card Sort –Pattern Comparison –Picture Sequence –Picture Vocabulary –Oral Reading Recognition –Rey-Auditory Verbal Learning Test –Social Support (Instrumental and Emotional Support included within the Social Relationships subdomain) –Emotional Well-being (Negative Affect, Psychological Well-being, Social Relationships, and Stress and Self-Efficacy)	X	
Paper and Pencil (Self-administered; can be interviewer-administered if participant requires assistance)		
–BRIEF-A	X	
–ASEBA ASR	X	
–PHQ-9	X	
–ERQ	X	

– 7-day alcohol and tobacco use	X	
<b>Online Survey<sup>2</sup></b>		
– GAD-7	X	
– Adult Transition Milestones	X	
– Pittsburgh Sleep Quality Inventory	X	
– Substance Use	X	
– Sexual Risk Behaviors	X	
– ACEs-Revised Questionnaire	X	
– Stressful Life Events	X	
– Brief Resilience Scale (BRS)	X	
– Household and Financial Resources	X	
<b>Clinical Evaluations</b>		
– HIV viral load	X	For YAPHIV with no result from within the 3 months prior
– CD4 T cell count and percent	X	For YAPHIV with no result from within the 3 months prior
<b>Neuroimaging Session</b>		
– T1 – Task fMRI (GAM, EP, and NB-WM) – DTI – Resting-state fMRI – T2	X	
<b>Chart Abstraction</b>		
– Current medications, including ARVs for YAPHIV, since last AMP Up visit – Neurologic and psychiatric diagnoses since last AMP Up visit – For YAPHIV HIV only: HIV viral load, CD4 T cell count and percent, and any changes in CDC class since last AMP Up visit	X	
<b>Existing AMP Up Data to be Utilized</b>		
– Demographics, including household and financial resources data – Medical and medications history – Participant CDQ	X	Data to be obtained for analysis from the DMC at Frontier Science. No chart abstraction/data entry required at the clinical sites.

– Birth history, including perinatal ARV exposures – For YAPHIV only: HIV disease history, including HIV viral load, CD4 T cell count and percent, and changes in CDC class, and historical and current ARV exposures		
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<sup>1</sup>Do not complete the Cognitive battery if it was completed as part of AMP Up within the 3 months prior to the TERBO BRAIN study visit. Complete the designated surveys from the Emotion domain regardless of whether they were completed as part of AMP Up within the 3 months prior to the TERBO BRAIN study visit.

<sup>2</sup>If the online survey cannot be completed in clinic, it may be completed at home or another location outside of the clinic. The research staff will ensure that the participant is able to complete the survey independently and completes it within 2 weeks after the study visit.

**APPENDIX III: ASTRO NESTED SUBSTUDY SCHEDULE OF EVALUATIONS**

Participants in the Youth and Young Adult Cohorts will complete all assessments outlined in the table below at Entry. It is recommended that the study visit be completed on the same day as the cognitive/behavioral session of TERBO BRAIN. Participants should be fasting for 8 hours (except water and medications) prior to blood draw. Accordingly, when ASTRO and TERBO visits are performed on the same day, it is recommended that ASTRO procedures be performed first (while fasting) with a meal provided prior to the start of TERBO procedures. All ASTRO procedures should preferably be completed within 6 months following the final session of the TERBO BRAIN Entry visit.

<b>Task/Assessment</b>	<b>Entry</b>	<b>Comments</b>
Identify and pre-screen for eligibility, obtain informed consent/assent, confirm eligibility	X	
<b>Study Procedures</b>		
Carotid-femoral pulse wave velocity (cfPWV)	X	Using SphygmoCor device
Vital signs and anthropometrics		
– Blood pressure	X	
– Weight	X	
– Height	X	
– Mid-waist circumference	X	
– Hip circumference	X	
Laboratories		
– Plasma <sup>1</sup>	X	
– Serum <sup>1</sup>	X	
– Repository specimens <sup>2</sup>	X	To be stored in the PHACS Repository for yet-to-be-determined future studies
Interviewer-administered questionnaires		
– Caregiver education	X	Participant or caregiver may respond
– IPAQ short-form	X	For young adult participants only
<b>Existing Data from Dr. Urbina</b>		
– cfPWV data from general population controls	X	
<b>Existing SMARTT Data to be Utilized</b>		

– For YPHEU only: Prenatal maternal characteristics (e.g., CD4 T cell count)	X	
<b>Existing AMP Up Data to be Utilized</b>		
– For YAPHIV only: HIV disease history, including HIV viral load and CD4 T cell count	X	
<b>Existing TERBO BRAIN Data to be Utilized</b>		
– Neuroimaging data	X	
– Neurocognitive testing data	X	
– Survey data	X	
– For YAPHIV only: CD4 T cell count and HIV viral load, if obtained	X	
– Data abstracted from AMP and AMP Up	X	

<sup>1</sup>Approximately 16 mL of fasting blood will be drawn. Plasma for glucose, sCD163, sCD14, and eicosanoids. Serum for insulin and lipids. See ASTRO Substudy LPC for specimen processing details.

<sup>2</sup>Approximately 14 mL of fasting blood will be drawn, processed per the ASTRO Substudy LPC and stored in the PHACS Repository for yet-to-be-determined future studies.

#### **APPENDIX IV: TERBO BRAIN/ASTRO PROTOCOL PARTICIPATING SITES**

Participating sites will be selected based on the following criteria:

- Affiliated with a neuroimaging center that utilizes the study-required scanner platforms and has task fMRI capabilities and experience;
- Ability to contribute to accrual based on their SMARTT and/or AMP Up enrollment numbers; and
- Has available staff member who can be trained to administer study assessment measures, including task fMRI and the NIH Toolbox.



