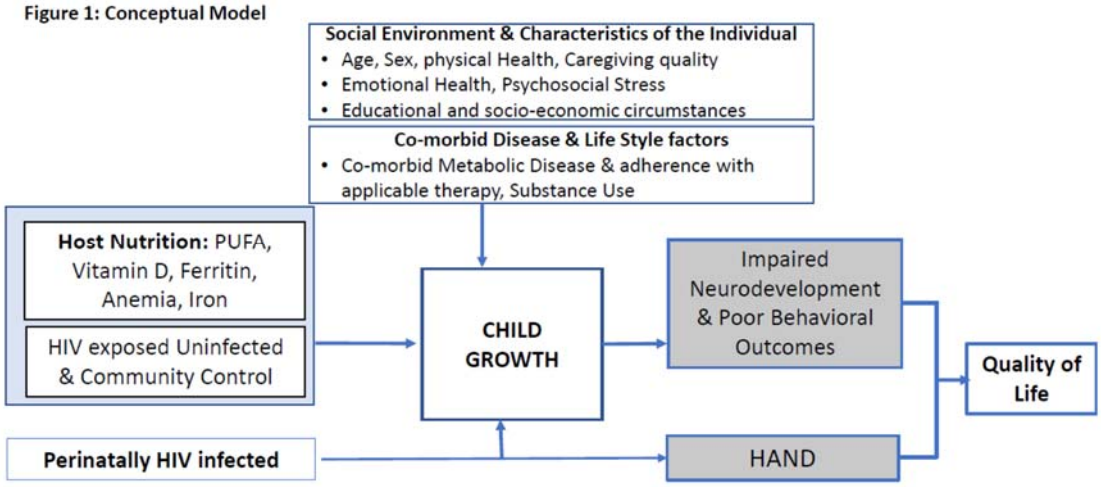
RESEARCH PLAN: *The Morbidity Contribution of Micronutrient and Polyunsaturated Fatty Acid Deficiencies to Growth faltering and Neurocognitive Disorder in Perinatally HIV-exposed and Unexposed Ugandan Children.*

1.1 SIGNIFICANCE: HAART has positively transformed pediatric HIV but evidence of typical development is equivocal. Increasing access to highly active antiretroviral therapy (HAART) for persons living with HIV/AIDS (PLWHA) in low and middle income countries (LMIC) has resulted in large reductions in number of perinatally HIV (PHIV) infected children,[1-4] and earlier time of HAART initiation for current PHIV compared to their pre-HAART era peers.[5] At same time, at least 1.5 million children per year are perinatally HIV exposed but uninfected (HEU). [6] Majority of HEU are exposed to HAART during highly sensitive developmental windows - including the first one thousand days of life, with poorly understood long-term consequences.[6] Perinatal HIV exposure and punctuated/chronic HAART exposure for children of female PLWHA will persist for the foreseeable future and large numbers of these HIV-affected children are expected to survive into adulthood.[1, 3] The evidence that these vulnerable children are developing/thriving in the long-term, particularly with respect to neurodevelopment and quality of life (QOL), is limited.[1, 2, 7-9]

1.2 Impaired growth and neurodevelopment is common in LMIC youth; HIV-infection/exposure magnifies this risk. That all children survive and thrive with appropriate developmental outcomes is a goal of UNAIDS and several NIH centers.[10-12] Successful growth, neurocognitive development and function is a cornerstone of thriving across the lifecourse and the most important determinant of adult productivity.[1, 7, 13, 14] Unfortunately, high rates of child morbidity and mortality is typical in Uganda where an estimated 30% of children are stunted, 15.7% of children exhibit impaired or delayed cognitive development, 26.3% exhibit impaired/delayed socioemotional development and as much as 36.8% are delayed/impaired in either or both developmental dimensions.[13] Against this background of high stunting and neurodevelopmental impairment, HIV-infection and exposure represents an additional set of determinants that may act additively or synergistically with risk factors applicable to HIV unexposed uninfected (HUU) to further impair growth and neurocognitive function in HIV-exposed/infected children. HIV-associated neurocognitive disorder (HAND) - i.e. spectrum of neurocognitive dysfunction observed in PLWHA still affects between 25% to 60%.[15] HAART has successfully reduced the most severe form of HAND, i.e. HIV-associated dementia (HAD), but the prevalence of mild and asymptomatic cognitive deficits has increased in PLWHA.[16]

Figure 1: Conceptual Model



The dearth of robust comparative growth and neurodevelopmental information for school-aged, adolescent and older perinatally HIV-affected relative to HUU community controls is widely recognized.[2] With few exceptions,[17-21] studies in the pre-HAART era found diminished motor, memory, and verbal functions in PHIV compared to HIV-negative controls.[20, 22-24] The vast majority of children in these early studies were ≤5 years old. This knowledge gap is compounded by limited specific studies of the survival experience of vulnerable PHIV, HEU and HUU and partly reflects the relative recency of multi-decade survival of PHIV infected children from LMIC.[22, 25] Our team has enrolled and followed for 12 months 305 6-10 years old Ugandan children with and without perinatal HIV infection/exposure and their caregivers as part of recently completed field work. We are now enrolling another 300 Ugandan adolescents and their caregivers as part of an ongoing NIH supported R21 research program. The goal of the investigations for the AAP supported post-doctoral fellow will investigate the role of nutrition in long-term child growth and neurocognitive function as illustrated in figure 1.

1.3 Optimal nutrition is protective and may mitigate growth faltering and neurocognitive impairment/HAND. Substantial evidence exists for the beneficial effects of vitamins B-complex, C, and E for immunity and that sufficiency in these micronutrients alleviates high level of inflammation, improves several aspects of immune function and reduces morbidity. [26-31]. Vitamin D's role in optimal growth and skeletal health is well defined.[32-35] Likewise, vitamin D sufficiency has been linked with proper immune function and a range of cardiometabolic health benefits.[36-41] In addition, an emerging body of work has linked vitamin D sufficiency to lower prevalence of a range of mental health problems including depressed mood,[42-44] anxiety,[45] atypicality and externalizing behavioral disorders.[42] More recently, vitamin D's role in brain health and neurocognitive development has been theorized. [46-48] Epidemiologic data also confirms a high prevalence of vitamin D insufficiency in broad segments of children and adults with[49-60] and without[61-65] HIV. However, there remains

substantial gaps in our understanding of its relevance for cognitive function and quality of life (QOL) among children/adolescents with and without chronic HIV infection.

Polyunsaturated fatty acids (PUFA), including omega-3 and omega-6, have salutary impacts on immune function, heart health and the central nervous system through regulation of membrane fluidity, intra-cellular signaling and gene expression and down modulation of inflammatory responses.[66-71] In addition to salutary impacts on cardio-metabolic functions,[72-77] certain PUFA (e.g. arachidonic acid,) have structural roles in the brain and combine with the docosahexaenoic acid (an omega-3 PUFA) as essential co-factors for normal brain development and function.[70] Optimal intakes of omega-3 PUFAs are essential for optimal visual, neural, and behavioral development among infants[78]. Beyond infancy, optimal omega-3 PUFA intake has been associated with improvements in attention, learning, and behavioral disorders throughout the life course.[79-89]

High or low ferritin - a biomarker of bio-available iron[90] - anemia and other facets of impaired hematologic status (IHS) are common and often persistent in African children/adolescents[91-94] and in PLWHA in spite of HAART treatment.[95-100] The dynamic interactions between HIV and iron create a positive feedback loop that increases the risk of iron overload, onset and persistence of non-iron deficiency anemia and may partly mediate HIV persistence and rebound.[90, 99, 101] Persistent immune activation typical in HIV infection contributes to anemia of chronic disease.[102] Anemia, regardless of etiology, predicts sub-optimal immune recovery[103] and mortality[104] in persons living with HIV/AIDS. High serum ferritin has been associated with sub-optimal immune recovery and mortality in PLWHA[101, 102, 105, 106] and represents anemia of chronic disease typified by limited bio-availability of iron. Low serum ferritin on the other hand results from impaired gastrointestinal nutrient absorption and is associated with folate deficiency, iron deficiency anemia[104, 107-109], fatigue and depressed mood.[110] Hence low ferritin, regardless of HIV status, is expected to impair QOL and cognitive performance. Understanding the possible roles of IHS could be an important strategy for slowing HIV disease progression[111] and improving functional outcomes in nutritionally depleted children and adolescents.

2.0 Specific Research Project: We will work with postdoctoral fellow to quantify the modifiable role of key nutritional variables on sub-optimal long-term growth and impaired neurodevelopment/HAND in this vulnerable population. In this cohort (n=500 caregiver child pairs enrolled to date), HIV-infection/exposure presents a heightened risk of both growth faltering and impaired neurocognitive development as noted in conceptual Model (**Figure 1**). [14, 46, 112-115] These risk factors which may act in additive or synergistic manner to impair growth and neurocognitive development, in addition to the interplay of other environmental, demographic, medical, and lifestyle factors.[116, 117]

- **Specific Aim #1:** To quantify PUFA, vitamin D and vitamin B-12 related differences in growth trajectory over 12 months in Ugandan children 6 – 10 years old.
- **Specific Aim #2:** To determine PUFA, Vitamin D and vitamin B-12 related differences in change in neurocognitive function and neurocognitive disorders over 12 months in Ugandan children 6-10 years.

Table 1: Nutritional Indices vary substantially by perinatal HIV status among early School-aged Ugandan children with and without Perinatal HIV infection/exposure

	PHIV	HEU	HUU	
Nutrient	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	P-value
Vitamin D (ng/mL)	21.75 (6.7)	21.24 (9.1)	20.48 (7.40)	0.504
Total n3	2.97 (1.29)	2.80 (1.06)	2.92 (1.21)	0.635
Omega-3 Index	3.38 (1.58)	3.15 (1.23)	3.29 (1.49)	0.600
Total HUFA	16.10 (3.36)	14.40 (2.94)	14.82 (2.85)	0.001
T/T ratio	0.009 (0.01)	0.007(0.00)	0.007 (0.00)	0.003
Palmitelaidic acid	0.08 (0.05)	0.07 (0.04)	0.07 (0.04)	0.080
Stearic acid	11.82 (2.07)	10.42 (1.99)	10.72 (2.05)	<0.001
Linoleic acid	35.40 (5.42)	37.74 (5.08)	37.13 (5.34)	0.014
Linoelaidic acid	0.010 (0.01)	0.005 (0.01)	0.01 (0.01)	0.026
GLA	0.07 (0.06)	0.05 (0.04)	0.06 (0.05)	0.029
Arachidic acid	0.22 (0.06)	0.18 (0.07)	0.19 (0.07)	0.011
DGLA	0.73 (0.28)	0.45 (0.19)	0.49 (0.18)	<0.001
Mead acid	0.11 (0.07)	0.07 (0.04)	0.07 (0.04)	<0.001
Arachidonic acid	12.50 (2.40)	11.31 (2.23)	11.57 (2.09)	0.002
Nervonic acid	0.75 (0.24)	0.80 (0.25)	0.85 (0.25)	0.020

PHIV = Perinatally HIV infected, HEU = Perinatally HIV Exposed Uninfected. HUU = HIV unexposed Uninfected. GLA = gamma linolenic acid. HUFA = highly unsaturated fatty acid. DGLA =Dihomo-γ-linolenic acid. T/T ratio = triene/tetraene ratio

Nutritional Biomarkers. Serum assessments of hemoglobin, vitamin-D, ferritin and PUFA is made at baseline using standard methodology for each. In brief, hemoglobin is measured as part of complete blood count and used to define anemia per WHO age-sex thresholds as follows: <11.5 g/dl if ≤11 years, <12 g/dl if male or female 12 – 14 years or non-pregnant female aged >15 years and >13.0 g/dl if male ≥15 years old.[118] Vitamin-D will be measured as 25-hydroxyvitaminD [25(OH)D] by high performance liquid chromatography tandem mass spectrometry.[119] Categories based on the concentration (in nmol/L) of 25(OH)D are defined as follows: highly sufficient [>70), sufficient [50–70), insufficient (25–50) and deficient [<=25). These maybe collapsed based on distribution to create sufficient, insufficient vs low 25(OH)D level.[120] Serum ferritin will be measured using enzyme-linked immunosorbent assays (ELISA).[121, 122] Per prior precedent, low ferritin was defined as <30ng/L.[121] High ferritin was defined as >200 ng/L for males and >150 ng/L for females.[121] PUFA levels will be measured in serum using gas chromatography as in our previous studies[123, 124].

Outcome Measures: We have two main outcome measures for this study – growth, neurocognitive function and quality of life (QOL). Growth measures include: height for age, weight for age and body-mass-index for age. Cognitive measures include: executive function and socio-emotional adjustment measured using the behaviour

rated inventory of executive function and behaviour assessment system for children. QOL is measured using the Pediatric Quality of Life Inventory (PedsQL) questionnaire. All outcomes are multi-dimensionally assessed in both caregivers and children. Locally relevant relateive measures in Ugandan children/adolescents will be derived by internally standardizing raw scores for each respondent to derive a z-score. Z-scores correspond to the deviation in respondents score above or below the mean score for adolescents of same age and sex specific in the sample as follows: $X_i = (X_{raw} - X_{(sample\ age,sex\ mean)}] / SD_{(age,\ sex)}$. Scores warranting clinical vigilance are defined as scores ≥1.5 standard deviations in the direction of risk relative sample age/sex mean score per principles firmly established in prior work[125] and applied in context of our cognition studies in Ugandan children/adolescents.[8]

3.0 STATEMENT BY MENTORING TEAM AND ENVISONED MENTORING PLAN
To provide trainee with scientific and logistic tools needed to move forward in this exciting area of research, this team has assembled a mentoring team that includes myself-Dr. Jenifer Fenton as primary mentor, Dr. Amara Ezeamama as MSU co-mentor, Dr. Ezekiel Mupere as and Dr. Sarah Zalwango as Ugandan co-mentors. Each contributor's expertise and how they articulate with and complement each other in mentoring the AAP post-doctoral fellow is further described in mentoring plan.

3.1 MSU MENTORING TEAM: *Jenifer Fenton, PhD, MPH (Primary), Amara E. Ezeamama, PhD (co-mentor)*; Trainee will gain proficiency in the following competencies from her/his interaction with the MSU based mentoring team over 12 months: 1) systematic evaluation of macro and micronutrient status in children, 2) ethical and scientifically sound methods of establishing and maintaining a research cohort, 3) strategies for enrolling, and tracking large amounts of data from the study participants with the least amount of disruption to both participants and the study enrollment site, 4) reliable assessment of neurocognitive measures via direct and proxy reports, 5) methods of systematically evaluating the quality of caregivers and environmental quality for child rearing which may confound or modify the primary relationships of interest and 6) mentoring on manuscript development and grant strategy to ensure support for future work that will emerge naturally from the activities begun as part of this fellowship.

3.2 MAKERERE UNIVERSITY TEAM: Ezekiel Mupere, MBChB, PhD; Sarah Zalwango, MS, MBBS
Respectively Senior Lecturer and Chair, Department of Pediatrics, Makerere University School of Medicine, Kampala, Uganda & Director of Medical Services, Directorate of Public Health and Environment, Kampala City Council Authority. Dr. Mupere is a pediatric infectious disease specialist and current Department Head/Chair in the Department of Pediatrics, Makerere School of Medicine. Dr. Zalwango brings expertise as a physician with specialization in Pediatrics and expertise in the implementation of clinical epidemiologic studies of HIV, TB and childhood diseases. She is the local principal investigator leading enrolment and recruitment efforts for the study cohort. She has more than ten years of experience in epidemiologic studies of TB/HIV in Kampala, Uganda. Drs Mupere and Zalwango will contribute expertise in clinical management of pediatric HIV/AIDS and will mentor trainee on identifying, measuring and interpreting metabolic complications expected to influence neurologic development, psychosocial adjustment and quality of life in this vulnerable population.

MENTORING PLAN

Proficiency Area	Mentors Involved	Format	Frequency	Duration
Biomarker and Anthropometric methods for assessing growth and nutritional quality	Fenton, Ezeamama	Face to face meetings, e-mail or teleconferences, and response to trainee initiated inquiries	As needed (PRN) and in the context of conference calls to discuss research progress.	PRN
Analysis and interpretation of functional deficits & detangling confounding effects of functional measures by HAART, coincident micronutrient deficits and HIV	Fenton, Ezeamama	Face to face meetings while in the field, e-mail or teleconferences, and response to trainee initiated inquiries	PRN	As needed with multiple weekly interactions
Ethical Conduct of Human Subjects Research	Fenton, Mupere, Ezeamama, Zalwango	Face to face meetings while in the field, e-mail or teleconferences, and response to trainee initiated inquiries	PRN and via weekly conference calls to discuss research progress particularly in early phases of study	~1-2 hours per week particularly in early study phase
Implementation of epidemiologic study in resource constrained setting	Fenton, Ezeamama, Zalwango, Mupere	Face to face meetings while in the field, e-mail or teleconferences, and response to trainee initiated inquiries	PRN and in the context of weekly/bi-weekly conference calls to discuss research progress	~2 – 3 hrs per week
Grant writing and manuscript preparation	Fenton, Ezeamama, Mupere	Face to face meetings, PRN research meetings and e-mails	PRN	~15-20 hours per week
Managing and balancing the demands of conducting high impact science, service and teaching in an academic research career track	Fenton, Ezeamama, Mupere	Experiential learning and response to trainee initiated specific queries	PRN and weekly via designated research meeting time in the Fenton or Ezeamama research group.	~1 hour per month
Research Dissemination through conferences and Networking with other investigators	Fenton, Ezeamama, Mupere, Zalwango	Telephone, e-mails and face to face meetings	PRN and monthly in the context of designated study progress report meetings	~1 – 2 hours per month

DELIVERABLES OVER 1 YEAR OF SUPPORT: 2 peer-reviewed publications (submitted) and developed research proposal in mutually agreed research direction by July 2021. ***Strength of Mentoring Team & Team's Ideal Position to Mentor Future Leaders in African Research and Practice****:* This team has a strong track record in Uganda resulting in solid infrastructure that provides us access to relevant study populations and already collected data to support the post-doctoral trainee. In addition, the team has demonstrated experience evaluating the role of nutritional indices in HIV-related outcomes. Lastly, the investigator team has solid history of productively mentoring students in the implementation of cutting-edge nutritional and epidemiologic research and are ideally situated to facilitate the training of the AAP trainee as part of this fellowship program.

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