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Decreased growth among antiretroviral drug and HIV exposed uninfected versus unexposed children in Malawi and Uganda

Jim Aizire[1,2], **Alla Sikorskii**[3,4], **Lillian Wambuzi Ogwang**[2], **Rachel Kawalazira**[5], **Alex Mutebe**[2], **Itziar Familiar-Lopez**[4], **MacPherson Mallewa**[6], **Taha Taha**[1], **Michael J. Boivin**[4,7,8], **Mary Glenn Fowler**[9], ***PROMISE-NEURODEV* study team**

[1]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health; [2]Makerere University – Johns Hopkins University (MU-JHU), Kampala, Uganda; [3]Department of Statistics & Probability, Michigan State University; [4]Department of Psychiatry, Michigan State University; [5]College of Medicine – Johns Hopkins University Research Project, Blantyre, Malawi; [6]Malawi College of Medicine, University of Malawi, Blantyre, Malawi; [7]Department of Psychiatry, University of Michigan; [8]Department of Neurology & Ophthalmology, Michigan State University; [9]Department of Pathology, Johns Hopkins University School of Medicine

Abstract

Objective: To compare growth among antiretroviral drug and maternal-HIV exposed uninfected (AHEU) versus age-and-sex-matched HIV unexposed uninfected (HUU) children.

Design: Prospective cohort of AHEU children identified from the PROMISE trial (NCT01061151: clinicaltrials.gov registry) and age-and-sex-matched HUU controls from child-wellness clinics, enrolled (09/2013–10/2014) in Malawi and Uganda.

Methods: Weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ), and head-circumference-for-age (HCAZ) z-scores were derived at 12 and 24 months-of-age. Wilcoxon Rank-Sum and Fischer's exact tests were used for unadjusted exposure group comparisons. Generalized-Estimating-Equations models estimated adjusted relative risks (aRR) for poor growth outcomes.

Results: Overall, 471 (50.5%) AHEU and 462 (49.5%) HUU children were assessed. Ugandan AHEU compared to HUU children had significantly lower mean LAZ ($p<0.001$) and WAZ ($p<0.001$) at 12 and 24 months-of-age and HCAZ ($p=0.016$) at 24 months, with similar but not significant differences among Malawian AHEU and HUU children. The risk of stunting (more than two standard deviations below the WHO-population LAZ median) was increased among AHEU versus HUU children: aRR=2.13 (95% confidence interval (CI): 1.36, 3.33), p=0.001 at 12-months, and aRR=1.67 (95% CI: 1.16, 2.41), p=0.006 at 24-months-of-age in Uganda; and aRR=1.32 (95% CI: 1.10, 1.66), p=0.018, at 24-months-of-age in Malawi. The risk of HCAZ

Corresponding Author: Dr. Jim Aizire, MBCHB, MHS, PhD, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe St., E7132B, Telephone: 410-502-8988, jaizire1@jhu.edu.

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below WHO median was increased among AHEU versus HUU children at 24-months-of-age, aRR = 1.35 (95% CI: 1.02, 1.79), p=0.038 in Uganda; and aRR=1.35 (95% CI: 0.91, 2.02), p=0.139 in Malawi.

Conclusions: Perinatal exposures to maternal-HIV and antiretroviral drugs were associated with lower LAZ (including stunting), WAZ and HCAZ at 24 months-of-age compared HUU children.

Keywords

Growth; HIV Exposed Uninfected children; antiretroviral drug exposures

INTRODUCTION

The efficacy of maternal and infant antiretroviral (ARV) drugs for prevention of mother-to-child transmission of HIV-1 (PMTCT), including prolonged-breastfeeding populations, is well established.[1–3] As this knowledge became available in recent years, the recommended infant feeding and ARV drug strategies for PMTCT evolved rapidly. Currently, the World Health Organization (WHO) recommends breastfeeding for at least 12 months for both HIV-infected and uninfected women to decrease early childhood morbidity/mortality; with use of combination-antiretroviral therapy (cART) for all HIV-infected pregnant and breastfeeding women.[4] Sub-Saharan Africa, a region with more than 90% of the global PMTCT need, and where rapid scale-up of universal cART and breastfeeding programs are underway, will be home to most of the estimated 1.5 million ARV and HIV exposed uninfected (AHEU) children born annually by 2020.[5] Many AHEU children will be exposed to complex ARV drug regimens both antenatally and during lactation through breastmilk. Longer term consequences of prolonged perinatal HIV and ARV drug exposures are currently unknown regarding growth.

Studies among African HIV-exposed uninfected (HEU) children, conducted prior to the ARV drug era, were inconclusive as to whether HIV exposure was associated with poorer growth outcomes compared to HIV unexposed uninfected (HUU) children. Differing results were largely attributed to variations across studies with regard to key cofactors of child-growth such as breastfeeding, and/or maternal health in the absence of cART.[6] More recent observational studies conducted in the context of ARV drug use for PMTCT suggest that growth deficits reported among AHEU versus HUU children are more common and persist through/beyond the first year-of-life.[7–10] However, these more recent studies also had varying limitations that might preclude generalizability to other settings in Sub-Saharan Africa with endemic stunting,[11] and where prolonged breastfeeding is traditionally the norm, with cumulative HIV and ARV drug exposures as long as 27–33 months (9 months *in-utero* plus 18–24 months of extended breastfeeding exposures).[4]

The goal of this analysis was to determine if AHEU children had poorer physical growth compared to HUU children in settings with high background levels of childhood growth faltering due to nutritional deficiencies and infectious diseases, associated with increased morbidity and mortality.[12–14] We tested differences in growth outcomes at 12 and 24 months-of-age among age-and-sex-matched breastfed AHEU and HUU children with

normal birthweights, and similar socio-economic backgrounds, in Malawi and Uganda, both countries with high levels of endemic child stunting.[11]

METHODS

Study Population:

In 2013–2014, a prospective cohort of AHEU (cases) and age-and sex-matched HUU children (controls) and their mothers was enrolled into the Neurodevelopmental and Growth (ND&G) study at two international research sites in Blantyre, Malawi and Kampala, Uganda. Study design and procedures were described previously.[15] Briefly, 240 AHEU and 240 HUU children were targeted at each site. Eligible AHEU children were co-enrolled from the Promoting Maternal and Infant Survival Everywhere (PROMISE) study. Healthy HUU controls were identified from child-wellness/immunization clinics from the same medical facilities from which PROMISE trial participants were sourced.

Mother-child pairs were eligible if the case was 6–12 months-of-age (later changed to 6–18 months-of-age), and the appropriate control was age (+/−4 months) and sex-matched; birthweight of two or more kilograms; documented confirmation of AHEU child's HIV-uninfected and mother's HIV-infected statuses; maternal HIV-uninfected status for the HUU child; no known serious/chronic clinical condition; and written informed consent for study participation. Mother-child pairs were excluded if the mother declined home-visits; or resided outside the study catchment area. The study was funded by the US National Institute of Child Health and Human Development (HD 073296), and regulatory oversight was provided by Institutional Review Boards in Malawi and Uganda, and the Johns Hopkins Medical Institute.

The PROMISE (1077BF version) clinical trial (number NCT01061151: clinicaltrials.gov registry) described previously,[1] was an open-label trial that enrolled HIV-infected pregnant women between 2011 and 2016 across several international sites. Three sequential randomizations were conducted. The antepartum (1077BA) randomization of HIV-infected pregnant women from 14 weeks gestation through labor onset compared the safety and efficacy of maternal zidovudine alone and maternal cART regimens. The postpartum (1077BP) randomization of eligible women and their HIV-uninfected infants within 1-week post-delivery compared maternal cART and infant daily nevirapine. Women identified very late during pregnancy were registered to Late Presenter Registration (1077BL) for 1077BP eligibility assessment. Mother-child pairs from 1077BA or 1077BL who did not meet 1077BP eligibility criteria were maintained on standard-of-care observational study follow-up. Standardized procedures were used to institute temporary/permanent study drug holds/switches.

ND&G Study Procedures:

Demographic, socio-economic and clinical data were collected at enrolment; and medical history including child-nutrition/breastfeeding and hospitalizations, physical exam and anthropometric measurements (weight, length and head-circumference) were updated at 24, 36, 48 and 54 months-of-age study follow-up visits. Maternal (confirmed HIV-infection,

viral load, CD4 and ARV use) and infant data (birthweight, and laboratory confirmation of infant HIV-free status at 12 and 24 months-of-age) were abstracted from the PROMISE trial records. Infant date-of-birth, birthweight and immunization history for the control-group children were obtained from the standard-of-care immunization/growth card. HIV rapid tests were used according to country-specific standard-of-care algorithms to screen control-group mothers prior to enrolment, and control-group children at 24-months-of-age. Research experienced staff used standardized procedures including calibrated weight-scales, stadiometer boards and tape-measures to assess child weight, length/height and head-circumference, respectively.

Statistical Methods:

Data were entered and validated using 'FileMaker12/Server14', and analyzed using Stata version 14.2 (Stata, College Station TX, USA). In our published child neurodevelopmental outcomes paper, we described analyses based on the PROMISE antepartum and postpartum randomizations and a comparison group of HUU children.[15] For this paper, the 1077BA randomization was the basis for antepartum exposure determination, while postpartum exposures were based on the 1077BP randomization or ARV drug exposures during standard-of-care observational follow-up for mother-child pairs deemed ineligible for 1077BP randomization. This analysis reports the impact of maternal-HIV and ARV exposures regardless of randomization arm or ARV drug type/combinations on growth of AHEU children compared to age-sex-matched HUU children. This analysis was stratified by country to account for potentially important geographical differences in food-security, and background levels of childhood growth restrictions.[11]

Anthropometric data were used to calculate age-and sex-based Z scores for length-for-age (LAZ); weight-for-length (WLZ); weight-for-age (WAZ) and head-circumference-for-age (HCAZ) using WHO Growth standards (2006).[16] Stunting, wasting and being underweight were defined as LAZ, WLZ and WAZ more than two standard deviations below the medians of the respective WHO references. For the HCAZ scores, indicators of being below the median of the WHO population standard were analyzed.

Wilcoxon Rank Sum and Fischer's exact tests were used to compare continuous and categorical variables, respectively between AHEU and HUU groups using two-tailed tests. To address differences in anthropometric measures in the longitudinal context (12 and 24 months), Generalized Estimating Equation (GEE) models with robust variance estimators were used. To adjust for potential confounding bias, the GEE models included breastfeeding status, maternal age, electricity/gas use and tap-water use as covariates. These variables were defined *apriori* based on subject matter expertise and literature-review. However, income stability was not included as a covariate because of substantial missing data (>10%). Time was entered as a class variable in interaction with exposure group to model potentially varying differences between groups as time progressed. The identity-link was specified for the analysis of Z-scores. The adjusted means for each group at each time point were output from the model, and differences between exposure groups were expressed as β coefficients with 95% Confidence Intervals (CI)). Log-link was used in the analysis of repeated

measures of binary anthropometric indicators, and group differences were summarized using relative risks (RRs) with 95% CIs.

Given the available sample sizes by country, the following differences in proportions of binary outcomes were detectable at each time-point with power of 0.8 or greater in two-tailed tests at a 0.05 level of significance: stunting difference of 14% (assuming 40% HUU rate); underweight difference of 10% (assuming 10% HUU rate), wasting difference of 7% (assuming 5% HUU rate), and 9% difference in proportion with HCAZ below the WHO median (assuming 20% HUU rate).

RESULTS

Study profile:

Overall, 944 mother-child pairs were enrolled between August 2013 and December 2014 (Figure 1). Of these, 934 children had at least one complete growth measure by 24-months-of-age. Out of 934 mother-child pairs, there were 472 (50.5%) AHEU and 462 (49.5%) HUU. We accrued 779 (83.4%) of these mother-child pairs within the 12-months-of-age visit window. Of these 54 (6.9%) missed or were lost to follow-up by 24-months-of-age visit, non-differentially by exposure group. The rest were enrolled during the 24-months-of-age visit window.

Maternal HIV and ART exposures:

All AHEU and HUU children included in these analyses were HIV-uninfected through 24-months-of-age. Of the 472 AHEU children, 255 (54.0%), and 213 (45.1%) had cART and non-cART antepartum exposures, respectively, while four (0.9%) were late-presenters and had no antepartum ARV exposures. Ascertainment of postpartum ARV exposures was based on the postpartum randomization status for 419 (88.8%) infants, while 53 (11.3%) children deemed ineligible for randomization, exposure was based on documented standard-of-care ART exposures during observational follow-up. Overall, 238 (50.4%) children including 2 later-presenters had cumulative (antepartum and postpartum) cART exposures only; 100 (21.2%) including 2 late-presenters had cumulative exposures to non-cART regimens only; and 134 (28.4%) had mixed exposures (antepartum-cART followed by postpartum non-cART or vice-versa). Measures of maternal viral load, CD4 and CD8 are summarized in table 1.

Baseline characteristics:

Demographic, socio-economic status (SES), and clinical mother-child characteristics at baseline (or otherwise stated) are summarized in Table 1. There was a tendency towards lower SES among AHEU compared to HUU households based on reported electricity-use ($p<0.001$) in Malawi or tap-water use ($p\leq 0.015$) at both sites. Compared to HUU controls, AHEU children had lower birthweights in Malawi ($p=0.014$) and Uganda ($p<0.001$). The prevalence of breastfeeding was high at both sites, for both HEU and HUU children but also consistently higher among HUU compared to the AHEU children ($p\leq 0.008$). Exposed-group children versus HUU controls were more likely to have had a prior hospitalization reported at baseline, in Uganda, $p=0.024$, but not in Malawi, $p=0.061$.

Mean anthropometric measures at 12 and 24 months-of-age study visits:

The unadjusted mean LAZ and WAZ scores at 12- and 24-months-of-age visits were consistently below the median of the respective age-sex WHO population references for both AHEU and HUU children, but this was not true for WLZ or HCAZ mean scores (Table 2).

In Uganda, at both 12- and 24-months-of-age visits, AHEU versus HUU children had significantly lower mean LAZ ($p<0.001$), and lower mean WAZ ($p<0.001$), respectively. In addition, AHEU children had lower mean HCAZ at 24-months-of-age ($p=0.016$). However, differences in mean HCAZ among AHEU versus HUU children at 12-months-of-age, and mean WLZ at both visits, were not significant. The prevalence of stunting was high, and significantly higher among AHEU versus HUU children: 29.8% versus 13.3%, $p<0.001$ at 12-months-of-age, and 32.3% versus 18.2%, $p=0.001$ at 24-months-of-age.

In Malawi, similar trends of lower mean LAZ, WLZ, WAZ or HCAZ scores and higher stunting were observed among AHEU versus HUU children, although the differences were not significant.

Longitudinal analysis of growth outcomes with adjustment for other risk factors:

The adjusted means of LAZ, WAZ, WHZ and HCAZ from the longitudinal GEE model with the identity-link are depicted by site in Figure 2. The results of the comparisons of the adjusted means are similar to the unadjusted means described above.

The results from the longitudinal GEE model with the log link for binary outcomes of stunting, underweight, wasting, and head circumference below the WHO median for AHEU children compared to HUU controls are summarized in Table 3.

Ugandan AHEU children compared to HUU controls had a higher risk of stunting: adjusted RR (aRR) = 2.13 (95% CI: 1.36, 3.33), $p=0.001$, at 12-months-of-age, and aRR=1.67 (95% CI: 1.16, 2.41), $p=0.006$, at 24-months-of-age. Similarly, AHEU children had an increased risk of HCAZ below the WHO median: aRR=1.35 (95% CI: 1.02, 1.79), $p=0.038$.

Malawian AHEU children compared to HUU controls at 24-months-of-age had a higher risk of stunting aRR=1.32 (95% CI: 1.10, 1.66), $p=0.018$, while the observed increased risk of HCAZ below the WHO median was not significant, aRR= 1.35 (95% CI: 0.91, 2.02), $p=0.139$.

DISCUSSION

In this prospective study at two African sites in Malawi and Uganda, HIV-uninfected children with prolonged perinatal exposures to maternal-HIV and ARV drugs had significantly lower mean-LAZ and lower mean-WAZ at 12- and 24-months-of-age, respectively, and lower mean-HCAZ at 24-months-of-age when compared with age and sex-matched HUU controls. Stunting at 24-months-of-age was common among AHEU children in Malawi (50%) and Uganda (30%). Both Malawian and Ugandan AHEU children had significantly increased risk of stunting at 12- and 24-months-of-age, as well as increased risk

of HCAZ below the WHO population median at 24-months-of-age, relative to HUU controls.

This is the first study to demonstrate growth deficits among AHEU compared to HUU children through 24 months-of-age in settings where restricted childhood growth is endemic. Two other recent prospective cohort studies conducted in the context of current WHO guidance of universal maternal-ART and breastfeeding between 2013 and 2017, concurrent to our study calendar-period, reported similar growth faltering trends among AHEU versus HUU children through 12- and 18-months-of-age, respectively.[9,10] The high prevalence of stunting among HUU children in this study (40.4% and 42.7% in Malawi, and 13.3% and 18.2% in Uganda, at 12- and 24-months-of-age respectively), and the Nigerian cohort (30.9% at 18-months-of-age), relative to the South African cohort (4.0% at 12-months-of-age) is a reflection of the background stunting rates.[11] Additionally, longer breastfeeding duration through 12-months-of-age was observed in this study (majority (80%) in Malawi and about half in Uganda), comparable to the Nigerian cohort (64.0%), relative to the median duration of about 4 months in the South African cohort.

Earlier studies had considerable limitations. A Botswana study by Powis *et al* based on abstracted data from two completed trials conducted between 2001–2003 and 2006–2008, reported lower mean LAZ and WAZ scores from birth through 24-months-of-age among AHEU children with antepartum exposures to cART versus ZDV-monotherapy; while levels of stunting and wasting reported at 24-months-of-age were similar across the two groups.[7] However, there was no HUU comparison, the two trials were non-concurrent (3-year lag-period with a potential for secular-trends bias), and breastfeeding cessation was encouraged as early as 6-months-of-age. Previously, two cross-sectional designs, a hospital-based survey in Uganda (2010–2011) during the *'WHO option-A or B' era,* and a population-based survey in Botswana (2013–2014) during the *'WHO option B-plus'* era also reported inferior length-for-age among AHEU versus HUU children through 6–12 months, and through 12–24-months-of-age, respectively.[8,17] However, both studies had limitations in ascertaining ARV exposure type/duration. Also, reliance on standard-of-care infant HIV-testing algorithms with wide-intervals (HIV-NAT at 6 weeks-of-age and HIV-antibody test at 18-months-of-age) during a period of sustained breastmilk maternal-HIV transmission risk may have misclassified infant AHEU status. In contrast, a South African study by Patel *et al* reported no differences in weight-for-age for AHEU and HUU children through 24-months-of-age although ARV drug exposures were limited to perinatal single-dose nevirapine.[18] Other studies that assessed growth among HEU children were conducted before ARV-based PMTCT strategies were used in these settings, and reported mixed findings.[6]

In this large cohort, mean infant birth-weights were lower among AHEU versus HUU children; and relative-risk estimates of growth deficits through 24-months-of-age were similar in separate analyses that modeled cumulative (antepartum plus postpartum) exposures compared to antepartum exposure only models (results not shown). Although not conclusive, these findings are suggestive of *in-utero* onset childhood growth-perturbations. Fetal growth restriction is associated with early childhood growth impairment, and about 20% of childhood stunting is attributed to small-for-gestation-age.[8,10,12] However, the lack of linear-growth anthropometric measurements at birth and gestational-age among HUU

children in our study, precluded inclusion of birth anthropometric measures in these analyses, and therefore not able to perform mediation analyses to ascertain the proportion of the observed relative-risk of early childhood stunting attributed to fetal growth restriction. Furthermore, the tendency towards significant linear but not ponderal growth deficits among AHEU compared to HUU controls, at 12- and 24-months-of-age, is suggestive of long-term perturbations earlier during infancy or *in-utero*, since stunting is a biomarker of chronic growth impairment, while underweight and wasting reflect acute or recent nutritional deficits, respectively.[16] We also observed differences across sites. The relative-risk of stunting comparing AHEU and HUU children was higher among Ugandan compared to Malawian children, a heterogeneity-of-effects partly explained by the lower baseline-normal LAZ scores among Malawian HUU compared to Ugandan HUU children, $p<0.001$. The observed overall site differences in stunting among healthy HUU children at baseline are consistent with the population-level estimates of stunting: 50% in Malawi and 30% in Uganda; and the relative food-security in the respective countries.[11] In addition, the negative impact of the devastating 2014/2015 Malawian floods on child-nutrition across both AHEU and HUU groups potentially attenuated group differences in stunting risk estimates at the Malawi site. Flood-exposed populations are associated with chronic malnutrition and increased levels of stunting.[19]

Limitations of this analysis include inability to tease out the independent effects of maternal-HIV and prophylactic-ART exposures on childhood growth. Suggested biological mechanisms for ARV-drug-induced effects on child growth include mitochondrial perturbations that could cause end-organ toxicity; hematopoietic stem-cell nuclear DNA toxicity; and dysregulation of bone metabolism and/or mineralization, in the developing fetus and the young infant.[20,21] Maternal-HIV exposures have been associated with immunological perturbations such as immune activation, decreased number and function of T-cells, and deranged humoral immune responses.[22,23]Also, the impact of disaggregated ART exposures (cART versus non-cART) or specific ART drug types on child growth outcomes, were not available for these analyses pending primary PROMISE trial data analyses being done across all 14 PROMISE trial sites by the PROMISE team. Another limitation pertains to potential residual confounding due to unmeasured external or individual-level factors such as immunizations or endemic infections like measles, diarrhea, pneumonia, malaria or tropical enteropathy – an acquired syndrome characterized by altered gut immunity and diminished capacity to absorb nutrients, associated with poor sanitary conditions.[24] Despite these limitations, the study has important strengths. The data are robust with use of standardized instruments for growth measurements, performed by trained staff at both sites, as well as the longitudinal aspects to the growth data through 12–24 months-of-age and accurate ongoing data on breastfeeding duration, social-economic and clinical data collected throughout the ND&G study. In addition, the abstracted data for AHEU children are from a well monitored multi-country NIH clinical trial. To mitigate heterogeneity on external (environmental or SES) factors, AHEU and HUU participants were sourced from the same public medical facilities, and matched on sex and age, both known strong predictors of child-growth. At analysis, we adjusted for breastfeeding status, and household factors (tap-water and electricity/gas use), both surrogates of SES and

environmental – water hygiene and sanitation (WASH) factors, which are recognized key-determinants of early-childhood growth in resource-constrained settings.[12,24]

The overall high prevalence of stunting and increased risk among AHEU compared to HUU children underscores stunting as an urgent public health priority in Malawi and Uganda; and breastfed AHEU children remain a vulnerable subgroup, who should be prioritized for careful growth monitoring with early intervention of children falling off their growth trajectory to achieve the global PMTCT goal of optimal pediatric HIV-free survival. Decreased LAZ, WAZ and HCAZ are measurable and modifiable indicators of underlying childhood ill-health which early interventions can impact to decrease childhood morbidity and mortality. Stunting and underweight are associated with childhood infectious disease severity and malnutrition, and both contribute up to 14% of attributed child deaths.[12–14] In addition, early childhood growth-faltering even with catch-up later in childhood has been associated with non-communicable diseases later in adulthood.[25] Poor child development among school-going-age children including cognitive and school-learning outcomes, have been attributed to early-childhood stunting and decreased HCAZ.[26,27] Head-circumference through 24 months-of-age is correlated with brain size, and lower HCAZ scores among AHEU children may be a reflection of impaired brain function.[28] We previously demonstrated that HAZ was significantly predictive of Mullens based cognitive ability scores at 24-months-of-age in this cohort of children born at term.[15] Similarly, pre-term birth, a correlate of early childhood HAZ or stunting, was predictive of cognitive developmental delays based on the Bayley scales (BSID-III) assessments in a South Africa study.[29] There were no differences in cognitive development between AHEU and HUU children in this study cohort,[15] as well as a recent Botswana study.[30]

Our findings are likely generalizable to other breastfeeding populations in sub Saharan Africa with endemic child undernutrition and high infectious disease burden. Anthropometric monitoring is critical for adequate child health care in these settings. These data are crucial additions to previous reports suggesting the critical period of maternal-HIV and ARV drug exposures is likely *in-utero.* Reassuringly, prolonged postpartum exposures did not appear to further increase the risk of growth deficits. Further research is needed to better understand the role of maternal-HIV milieu and/or ART drug exposures during this critical period of fetal growth; and whether growth deficits among AHEU children persist throughout childhood.

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J.A contributed to study conceptualization, protocol development and coordination (trainings, SOPs, IRB, and monthly conference calls), statistical analysis and interpretation of findings, and completed the manuscript first draft; A.S was the lead protocol clinical methodologist and biostatistician, verified data integrity, analysis and interpretation of findings; L.W.O, R.K and M.M provided clinical care oversight at the respective research sites; coordinated participant scheduling for clinical assessments; supervised site-level data quality assurance and management; A.M and F.I.L supervised and coordinated the data management at both sites and worked with J.A and A.S on data integrity and quality assurance; T.T provided overall scientific leadership of the Malawi site as site PI, participated in data analysis plans, interpretation of principal findings; M.J.B and M.G.F, both co-protocol chairs of the ND&G study, and M.G.F as the protocol chair of the PROMISE trial, participated in all phases of study conceptualization, study design, proposal writing for funding, study implementation of protocols, data analysis and interpretation of findings, results dissemination. All authors participated in the manuscript writing and approved the final draft for publication.

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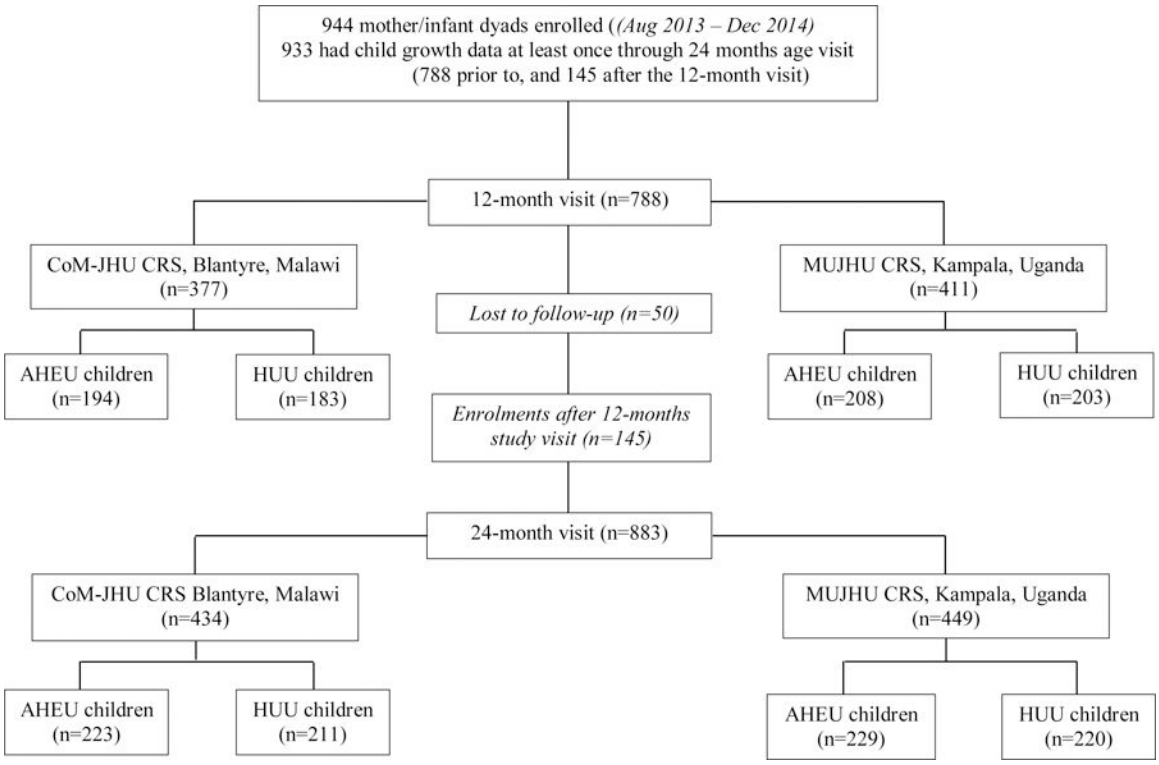

Figure 1.

Study flow diagram. Enrolment and study evaluations at the study clinics in Malawi and Uganda, August 2013 – April 2016

Legend: AHEU, Antiretroviral and HIV exposed uninfected children; HUU, HIV Unexposed Uninfected children; CoM-JHU CRS (College of Medicine-Johns Hopkins University Clinical Research Site); MU-JHU CRS (Makerere University – Johns Hopkins University Clinical Research Site).

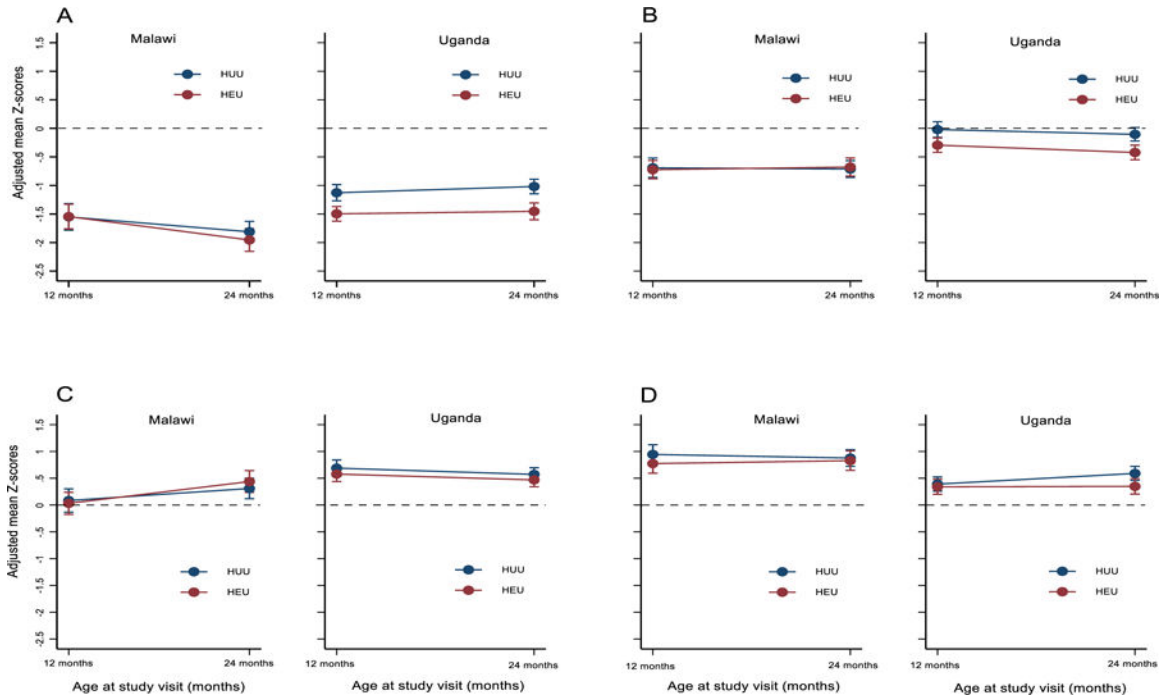

Figure 2.

Adjusted means of Z-scores and 95% CIs for weight-for-age (A), length-for-age (B), weight-for-height (C), and head circumference-for-age (D) of AHEU and HUU children at 12 and 24 months by site

Legend: Depicted data points are Z scores with 95% confidence intervals (CIs), calculated by use of adjusted Generalized Estimation Equations (GEE) linear regression models. All models adjusted for measured potential confounders defined *aprior* and these included breastfeeding statuses; maternal-age; electricity/gas use and tap-water use. HEU=HIV-exposed uninfected. HUU=HIV-unexposed uninfected. AHEU, Antiretroviral and HIV exposed uninfected children; HUU, HIV Unexposed Uninfected children; CoM-JHU CRS (College of Medicine-Johns Hopkins University Clinical Research Site); MU-JHU CRS (Makerere University – Johns Hopkins University Clinical Research Site).

Table 1.

Baseline maternal/child characteristics by exposure (maternal HIV and prophylactic antiretroviral drugs) status, and by site [Ω]

Characteristics	Malawi site (N=456)			Uganda site (N=477)		
	Exposed (AHEU) (N= 231)	Controls (HUU) (N=225)	*P*-value	Exposed (AHEU) (N=240)	Controls (HUU) (N=237)	*P*-value
Maternal characteristics						
Maternal age (years), median [IQR]	28.0 [25.0–31.0]	23.0 [20.0–28.0]	**<0.001**	27.0 [24.0–30.0]	25.0 [22.0–30.0]	0.053[a]
Maternal care (primary care giver), n (%)						
12-month visit	176 (99.8%)	167 (99.4%)	0.488	206 (99.5%)	202 (99.8%)	1.000[b]
24-month visit	209 (99.5%)	200 (99.5%)	1.000	217 (96.4%)	210 (95.9%)	0.809[b]
Household income stability score, n (%)						
Relatively-stable	46 (22.1%)	49 (22.7%)	0.973	47 (22.1%)	87 (48.3%)	**<0.001**[b]
Fairly-stable	42 (20.2%)	45 (20.8%)		54 (25.3%)	49 (27.2%)	
Unstable	120 (57.7%)	122 (56.5%)		112 (52.6%)	44 (24.4%)	
Electricity or gas in household, n (%)	93 (42.3%)	130 (59.4%)	**<0.001**	98 (41.0%)	118 (50.2%)	0.053[b]
Refrigerator in household, n (%)	26 (11.8%)	36 (16.4%)	0.173	36 (15.1%)	52 (22.1%)	0.058[b]
Water source for household use, n (%)						
Tap-water (in house)	33 (15.4%)	35 (15.9%)	**0.015**	74 (31.4%)	51 (21.7%)	**0.013**[b]
Tap-water (communal)	124 (58.0%)	151 (68.6%)		105 (44.5%)	135 (57.4%)	
Borehole and other sources	57 (26.6%)	34 (15.5%)		57 (24.1%)	49 (20.9%)	
Maternal viral load, copies/ml, median [IQR]						
12-month visit	302 [39–8359]	--		1022 [19–16591]	--	
24-month visit	213 [39–4663]	--		2230 [19–18391]	--	
Maternal CD4 cells count, median [IQR]						
12-month visit	571 [445–757]	--		705 [509–1008]	--	

Characteristics	Malawi site (N=456)			Uganda site (N=477)		
	Exposed (AHEU) (N= 231)	**Controls (HUU) (N=225)**	***P*-value**	**Exposed (AHEU) (N=240)**	**Controls (HUU) (N=237)**	***P*-value**
24-month visit	604 [470–766]	--		636 [480–892]	--	
Maternal CD8 cells count, median [IQR]						
12-month visit	852 [640–1102]	--		875 [685–1110]	--	
24-month visit	797 [627–1061]	--		830 [654–1111]	--	
Infant characteristics						
Females, n (%)	111 (48.0%)	108 (48.0%)	1.000	117 (48.8%)	115 (48.5%)	1.000[b]
Age (months), median [IQR]						
12-month visit	14.4 [13.1–16.3]	14.7 [13.7–15.9]	0.775	13.1 [12.0–16.3]	12.7 [12.0–15.7]	0.777[a]
24-month visit	24.1 [24.0–25.0]	24.1 [24.0–25.7]	0.751	24.1 [24.0–25.0]	24.1 [24.0–25.2]	0.986[a]
Birthweight (kilograms), median [IQR]	3.0 [2.7–3.3]	3.0 [2.8–3.3]	**0.014**	3.0 [2.8–3.4]	3.3 [3.0–3.6]	**<0.001**[a]
Breastfeeding status (yes), n (%)						
12-month visit	151 (79.9%)	173 (96.7%)	**<0.001**	102 (49.0%)	175 (86.2%)	**<0.001**[b]
24-month visit	14 (6.7%)	60 (29.3%)	**<0.001**	15 (6.6%)	32 (14.6%)	**0.008**[b]
Hospitalization before baseline (yes), n (%)	8 (3.6%)	2 (0.9%)	0.061	53 (22.1%)	33 (13.9%)	**0.024**[b]

Key: AHEU, Antiretroviral and HIV Exposed Uninfected; HUU, HIV Unexposed Uninfected; IQR, Interquartile range;

[Ω] The median (IQR) maternal viral load (copies/ml), CD4 and CD8 cells count at 12 and 24-month study visits are presented for AHEU group mothers.

[a] *p*-value from Wilcoxon rank-sum test;

[b] p-value from Fisher's exact test.

Highlighted p-values are statistically significant. Missing data: maternal-age (2.7%); care-giver (3.9%); maternal wellbeing (9.9%); Household income (12.4%); electricity-use (2.1%); refrigerator-use (1.9%); water-source (3.0%); infant gender (0.0%); infant-age (0.0%); birthweight (0.2%); breastfeeding (0.8%); prior hospitalizations (0.3%). Household-income-stability index score was based on a composite of five binary-questions (yes/no) of the standardized *AFASS* questionnaire: *"Is mother currently working?", "Is mother the primary bread-winner?", "Is pooled income sufficient?", "Is pooled income sufficient for 12-months?", "Is there enough to spend on infant nutrition and transport to healthcare?".* Equal weights (yes=1; no=0) were applied to each question and based on the total score, household-income was considered relatively-stable (5, 4 or 3); fairly-stable (2) or unstable (1 or 0).

Table 2.

Mean Anthropometric Z-scores at 12 and 24 months-of-age visits by exposure group and by site

Category	Mean anthropometric z-score (95% Confidence Interval), *p*-value					
	Malawi			Uganda		
	Exposed (AHEU)	Controls (HUU)	*P*-value	Exposed (AHEU)	Controls (HUU)	*P*-value
12 months	***(n= 189)***	***(n=179)***		***(n=208)***	***(n=203)***	
Length-for-age	−1.51 (−1.71, −1.31)	−1.62 (−1.84, −1.40)	0.476	−1.43 (−1.57, −1.29)	−1.01 (−1.14, −0.87)	<**0.0001**
Weight-for-length	0.04 (0.14, 0.22)	0.17 (0.04, 0.37)	0.372	0.56 (0.42, 0.71)	0.71 (0.58, 0.84)	0.136
Weight-for-age	−0.69 (−0.84, −0.54)	−0.66 (−0.83, −0.50)	0.830	−0.27 (−0.41, −0.13)	0.04 (−0.07, 0.17)	<**0.0001**
Head circumference	0.81 (0.64, 0.98)	0.85 (0.68, 1.01)	0.783	0.36 (0.22, 0.49)	0.46 (0.34, 0.57)	0.289
24 months	***(n= 210)***	***(n=221)***		***(n=229)***	***(n=220)***	
Length-for-age	−1.96 (−2.12, −1.80)	−1.90 (−2.10, −1.73)	0.568	−1.56 (−1.70, −1.42)	−1.10 (−1.22, −0.98)	<**0.0001**
Weight-for-length	0.39 (0.23, 0.55)	0.36 (0.18, 0.53)	0.787	0.45 (0.33, 0.57)	0.54, (0.43, 0.66)	0.272
Weight-for-age	−0.74 (−0.88, −0.61)	−0.73 (−0.87, −0.59)	0.890	−0.48 (−0.60, −0.36)	−0.17 (−0.28, −0.05)	<**0.0001**
Head circumference	0.96 (0.81, 1.11)	0.84 (0.69, 0.99)	0.255	0.30 (0.16, 0.44)	0.53 (0.40, 0.66)	**0.016**

Key: AHEU, Antiretroviral and HIV Exposed uninfected; HUU, HIV Unexposed Uninfected; P-value based on a two-sample t-test (with un-equal variances) comparing mean Z-scores (exposed versus control group), respectively; Mean anthropometric Z-score comparisons by combined-antiretroviral therapy (cART) versus non-cART exposed-group children, at 12 and 24-months-of-age were not significant, $p > 0.05$ (not presented); Missing data: 9/788 (1.1%) across sites at 12 months; 3/883 (0.3%) across sites at 24 months, independent of exposure, respectively.

AIDS. Author manuscript; available in PMC 2021 February 01.

Table 3.

Risk and Relative Risk of stunting, underweight, wasting and smaller head circumference at 12 and 24-month-age visits, by exposure and site

Exposure categories	Relative Risk (RR), 95% Confidence Interval (CI)									
	Malawi					Uganda				
	N, Risk (%)	Bivariate	*P*-value	Multivariate	*P*-value	N, Risk (%)	Bivariate	*P*-value	Multivariate	*P-value*
Stunting*(LAZ more than 2 SDs below WHO population median)*										
12 months										
Exposed (AHEU)	69 (36.5%)	0.86 (0.66, 1.11)	0.247	0.99 (0.75, 1.32)	0.982	62 (29.8%)	**2.37 (1.59, 3.51)**	**<0.001**	**2.13 (1.36, 3.33)**	**0.001**
Controls (HUU)	74 (41.4%)	-ref-		-ref-		27 (13.3%)	-ref-		-ref-	
24 months										
Exposed (AHEU)	108 (48.9%)	1.13 (0.92, 1.38)	0.264	**1.32 (1.10, 1.66)**	**0.018**	74 (32.3%)	**1.79 (1.28, 2.52)**	**0.001**	**1.67 (1.16, 2.41)**	**0.006**
Controls (HUU)	90 (42.9%)	-ref-		-ref-		40 (18.2%)	-ref-			
Underweight*(WAZ more than 2 SDs below WHO population median)*										
12 months										
Exposed (AHEU)	16 (8.3%)	0.83 (0.45, 1.54)	0.562	0.91 (0.47, 1.78)	0.793	9 (4.3%)	2.71 (0.78, 2.46)	0.117	1.92 (0.48, 7.63)	0.353
Controls (HUU)	19 (10.4%)	-ref-				3 (1.5%)	-ref-		-ref-	
24 months										
Exposed (AHEU)	21 (9.4%)	0.90 (0.51, 1.57)	0.699	0.98 (0.48, 1.99)	0.948	11 (4.8%)	2.67 (0.85, 8.44)	0.094	2.41 (0.74, 7.83)	0.144
Controls (HUU)	22 (10.4%)	-ref-				4 (1.8%)	-ref-		-ref-	
Wasting*(WHZ more than 2 SDs below WHO population median)*										
12 months										
Exposed (AHEU)	5 (2.6%)	0.45 (0.16, 1.24)	0.122	0.45 (0.14, 1.39)	0.125	2 (1.0%)	1.96 (0.18, 21.63)	0.583	0.48 (0.10, 3.55)	0.459
Controls (HUU)	10 (5.5%)	-ref-				1 (0.5%)	-ref-		-ref-	
24 months										

Exposure categories	Relative Risk (RR), 95% Confidence Interval (CI)									
	Malawi					Uganda				
	N, Risk (%)	Bivariate	*P*-value	Multivariate	*P*-value	N, Risk (%)	Bivariate	*P*-value	Multivariate	*P-value*
Exposed (AHEU)	8 (3.6%)	1.39 (0.48, 4.03)	0.539	1.61 (0.48, 5.47)	0.441	1 (0.4%)	0.96 (0.10, 15.30)	0.977	0.48 (0.10, 3.55)	0.459
Controls (HUU)	5 (2.4%)	-ref-				1 (0.5%)	-ref-		-ref-	
Small heads*(HCAZ score below WHO population median)*										
12 months										
Exposed (AHEU)	45 (23.2%)	1.00 (0.69, 1.45)	0.982	1.19 (0.80, 1.77)	0.381	74 (35.6%)	1.20 (0.92, 1.57)	0.171	1.19 (0.87, 1.63)	0.284
Controls (HUU)	41 (22.4%)	-ref-				62 (30.5%)	-ref-		-ref-	
24 months										
Exposed (AHEU)	50 (22.4%)	1.10 (0.74, 1.50)	0.787	1.35 (0.91, 2.02)	0.139	86 (37.6%)	**1.36 (1.10, 1.78)**	**0.022**	**1.35 (1.02, 1.79)**	**0.038**
Controls (HUU)	46 (21.8%)	-ref-				62 (28.2%)	-ref-		-ref-	

Key: AHEU, Antiretroviral and HIV Exposed Uninfected; HUU, HIV Unexposed Uninfected; Multivariate regression models included breastfeeding status; maternal-age; and additional variables (electricity/gas use and tap water use) with a p-value <0.15 in bivariate analyses assessing potential associations with the outcome, respectively.