Growth Faltering and Developmental Delay in HIV-Exposed Uninfected Ugandan Infants: A Prospective Cohort Study

Reshma Sirajee[1], Andrea L. Conroy[2]; Sophie Namasopo[3]; Robert O. Opoka[4], Stephanie Lavoie[5], Sarah Forgie[1], Bukola Oladunni Salami[9,10], Michael T. Hawkes*[1,6,7,8,9].

[1]Department of Pediatrics, University of Alberta, Edmonton, Canada; [2]Ryan White Center for Pediatric Infectious Diseases and Global Health, Indiana University School of Medicine, Indianapolis, USA; [3]Department of Paediatrics, Jinja Regional Referral Hospital, Jinja, Uganda; [4]Department of Paediatrics and Child Health, Mulago Hospital and Makerere University, Kampala, Uganda; [5]National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada, [6]Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Canada; [7]School of Public Health, University of Alberta, Edmonton, Canada; [8]Distinguished Researcher, Stollery Science Lab; Member, [9]Women and Children's Health Research Institute; [10]Faculty of Nursing, University of Alberta, Edmonton, Canada;

*Corresponding author: Michael T. Hawkes, Department of Pediatrics, University of Alberta, 3-588D; Edmonton Clinic Health Academy, 11405 87 Ave NW, Edmonton, Alberta, T6G 1C9, Canada, Fax: 1-888-790-1176, Tel: 1-780-248-5540, Email: mthawkes@ualberta.ca

Abstract

Background: HIV exposed but uninfected (HEU) infants are at increased risk of impaired early linear growth and cognitive development. We examined associations between pre and postnatal

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growth and subsequent neurodevelopment in Ugandan HEUs, hypothesizing that early insults may explain alterations in both somatic growth and brain development.

Methods: We prospectively followed a cohort of HEUs from birth to 18 months of age, and measured length/height, weight, head and arm circumference longitudinally. The Malawi Development Assessment Tool (MDAT, 12 and 18 months) and the Color Object Association Test (COAT, 18 months) were used for developmental assessments.

Results: Among 170 HEUs, the prevalence of low birth weight (LBW) and failure to thrive (FTT) was 7.6% and 37%, respectively. HEUs had MDAT scores that were similar to the reference population. The mean (SD) score on the COAT was 5.5 (3.1) compared to 6.9 (5.3) in developmentally normal children. Developmental ability at 18 months of age showed strong cross-sectional correlation with weight- (ρ=0.36, p<0.0001), length/height- (ρ=0.41, p<0.0001), head circumference- (ρ=0.26, p=0.0011), and middle upper arm circumference (MUAC)-for-age (ρ=0.34, p=0.0014). There was a statistically significant correlation between birth weight and MDAT z-score at 18 months (ρ=0.20, p=0.010). FTT was associated with lower MDAT z-score (median -0.13 (IQR -0.75 to +0.14) *versus* +0.14 (IQR -0.44 to +0.63), p=0.042).

Conclusion: Growth faltering in HEUs was associated with lower attainment of developmental milestones at 18 months of age. Our findings point to a simple screening method for identifying HEUs at risk for developmental intervention.

Introduction

In addition to the 1.7 million children and adolescents living with HIV, there are 14.8 million HIV exposed but uninfected (HEU) infants globally, 90% of whom reside in sub-Saharan Africa.[1,2] Thanks to successful interventions such as maternal combination antiretroviral therapy (ART), vertical transmission (VT) of HIV has been reduced to <1% in resource-rich areas and <2.9% in low- and middle-income countries (LMICs).[3-6] HEU newborns significantly outnumber infected infants worldwide and represent nearly 30% of the newborn population in some HIV endemic nations.[7]

Although HEUs do not exhibit severe immunodeficiency and opportunistic infections like HIV infected infants, HEU health outcomes differ from those of HIV-unexposed uninfected infants (HUUs). In LMICs, mortality among HEUs in the first 2 years of life is 2 to 3 times higher than HUUs.[8] HEU survivors have increased risk of impaired early linear growth,[9] psychomotor and cognitive development,[2,10] hearing loss, expressive language expression,[9] and diarrheal disease.[11] Growth faltering and neurodevelopmental delay in HEUs may be due to pre- and post-natal factors, including socioeconomic variables, ART exposure,[8] subtle immune deficits, systemic inflammation, and exposure to infections.[12]

Early infant development is increasingly recognized as a determinant of health and productivity in adulthood and is listed among the United Nations Sustainable Development Goals.[13-16] HEUs are among the estimated 219 million (39%) children younger than 5 years (under-5s) in LMICs at risk of not reaching their developmental potential.[13] The prenatal period and the first 2 years of life are the most sensitive times during which growth faltering is associated with later cognition,

executive function, and school attainment.[17-19] Identifying HEUs before they complete this sensitive period of somatic and brain growth may be crucial to foster optimal child development.

As both growth and neurodevelopment issues of HEU are recognized, our objective was to determine early growth challenges and indicators that can determine neurodevelopmental concerns later in infancy. Uganda is among the top 5 countries in the world with respect to total number of HEU children and represents an ideal context for the study of growth and development of HEUs in a LMIC.[1] We explored associations between prenatal and postnatal growth and subsequent development at 18 months of age.

Methods

Study design

This was a prospective cohort study of HEUs, conducted between March 2016 to December 2018, examining the relationship between growth faltering and neurodevelopment.

Study setting and participants

The prevalence of HIV in Uganda is 0.5% among children.[20] Our study was conducted at two facilities with labor and delivery services: Jinja Regional Referral Hospital (total catchment population 507,700) and Kambuga District Hospital (total catchment population 35,873). Both centers are characterized by high obstetrical volumes, high HIV rates, and numerous mothers with unknown HIV status presenting in labor. The National Program for prevention of vertical transmission in Uganda implemented "Option B+" in 2012[21] for the mother (lifelong ART initiated as soon as HIV detected in pregnancy), nevirapine from birth to 6 weeks of life for the infant, and co-trimoxazole prophylaxis from 6 weeks of age until HIV infection is excluded.[22] HIV-seropositive mothers of any age and their newborn infants were eligible for inclusion in the study. Exclusion criteria were: negative maternal HIV testing at delivery; vertical infection (one or more positive HIV PCR tests in infant); infant death before 18 months of age; and loss to follow-up at 18 months of age.

Study procedures

Consenting mothers with positive or unknown HIV status presenting in labor were tested with point-of-care rapid HIV serologic tests. The HIV testing was conducted in accordance with the Uganda National Policy Guidelines for HIV counselling and testing.[23] Specimens collected were

tested using rapid diagnostic tests in series. The test kits were validated by the national health reference library. The first test was done with Determine™ HIV–1/2 Ag/Ab Combo (Alere, USA). If positive, the result was confirmed by a second test, the HIV 1/2 STAT PAK® Assay (Chembio, USA). In case of a discordant result, a "tie-breaker" test was performed using the Uni-Gold™ Recombigen® HIV-1/2 (Trinity Biotech, Ireland).

Mothers testing positive were managed according to national guidelines.[22] Using standardized case report forms, demographic and clinical information was collected at birth. Follow-up clinic visits occurred at 6 weeks, 12 months and 18 months of age. Neurodevelopment assessments were conducted at 12 and 18 months of age.

Determination of HIV infection status

At each study visit, we collected a venipuncture blood sample and applied three aliquots of 10-20 µL to a filter paper (Whatman® FTA® DMPK-C Cards for Dried Blood Spot, GE Healthcare Life Sciences, USA). The filter paper was dried at room temperature and stored for subsequent shipping to the National Laboratory for HIV Reference Services (NLHRS) in Canada for HIV-1 DNA testing and analysis. For each dried blood spot (DBS) card, a whole spot (~75 µl) was cut and lysed in 2mL of NucliSENS® easyMag® Lysis buffer (bioMérieux, France). The samples were then placed on a shaker for 1 hour at room temperature. The lysed samples were extracted for total nucleic acid using the Generic 2.0.1 protocol on the NucliSENS® easyMag® platform. Extracted elutions were tested for HIV-1 using the NLHRS-molecular algorithm, which includes in-house PCRs targeting DQ-alpha and pol (integrase). Sample quality was first assessed by the in-house DQ-alpha PCR (Taq PCR Core Kit, QIAGEN, Germany). RNA from positive DQ-

alpha samples were synthesized into complementary DNA (cDNA) using the Superscript IV VILO Master Mix (ThermoFisher Scientific, USA) kit. The detection of HIV-1 in the DBS samples was assessed by using an in-house nested pol (integrase) PCR (Taq PCR Core Kit, QIAGEN, Germany). A positive band of 175 bp using a QIAxcel instrument (QIAGEN, Germany) defined HIV-1 infection.

Clinical definitions

Failure to thrive (FTT)[24] was defined as downward crossing of two or more major percentile lines on the World Health Organization (WHO) weight-for-age growth chart[25] between 6 weeks and 18 months of age. Major percentile were defined as the 5^{th}, 10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}, 95^{th} lines.[24] Because catch-up or catch-down growth can occur with transition from the intrauterine to the extrauterine environment, the birthweight was excluded in the assessment of FTT.

Growth velocity was determined as the change in weight, height, and head circumference from the 12 month visit to the 18 months visit. This change was compared to the WHO standards[25] to obtain the growth velocity z-score.

Stunting was defined as infant length/height-for-age two standard deviations or more below the mean using WHO growth standards.[25] The z-score for length/height-for-age was calculated using the package *zscorer*[26] in the R statistical environment.

Wasting was defined as infant weight-for-length two standard deviations or more below the mean using WHO growth standards.[25] The z-score for weight-for-length was calculated using the package *zscorer*[26] in the R statistical environment.

Neurodevelopmental ability (rank) was assigned based on the standardized score of Malawi Developmental Assessment Tool (MDAT) milestones achieved at 18 months of age (see Statistical Analysis below).

Small for gestational age was defined as weight below the tenth percentile for gestational age at birth, based on Ugandan norms.[27]

Neurodevelopmental assessment

The MDAT is a culturally appropriate, reliable developmental assessment tool for use in African settings including Uganda[28] for children from birth to 6 years of age.[29] This test examines milestones in the domains of gross motor, fine motor, language, and social development through direct observation of the child and questions to the caregiver. Items are scored as pass/fail. Reference norms for this tool are derived from a population of 1,513 normally healthy children (excluding those with malnutrition, significant medical problems, prematurity, or known neurodisability) from birth to 6 years of age, recruited from clinics at rural and urban sites in Malawi.[29] The MDAT testing protocol was modified for this study by selecting age-appropriate milestones for 12 and 18 months old infants (see Supplemental table S5 for complete list of milestones assessed). Three Ugandan study nurses were trained to administer the MDAT in one half-day training session. The nurses, who had tacit linguistic and cultural understanding,

observed infant behavior and administered the MDAT questionnaire in the local languages (Luganda, Lusoga, and Rukiiga) as a verbal interview, allowing them to provide clarification when necessary around the meaning of the questions

The Color Object Association Test (COAT) measures declarative (explicit) memory at 18 to 36 months of age[30] and has been used in Ugandan infants in previous studies.[31,32] This visually paired association memory test directs children to correctly place a familiar toy/object in its color-associated wooden hinged lid box (e.g., toy car in pink box). After a child underwent learning phase, we conducted 4 trials with increasing number of objects to remember per trial. Two points were given for correctly putting toys in the correct color-pair box, 1 point was given for self-correction, and 0 points were given for incorrect responses. The COAT has demonstrated strong reliability and validity (discriminant and convergent) when compared to established memory tools for children across various ages.[30] Reference norms for the COAT were derived from a US cohort of 281 toddlers aged 18-36 months from a multi-ethnic community of mostly lower- and lower middle-class socioeconomic backgrounds.[30]

During neurodevelopmental testing, the examiner evaluated and rated the child's test behavior as follows: (1) overall typical or atypical behavior; (2) compliance (complies, usually complies, rarely complies); (3) interest in surroundings (alert, somewhat disinterested, seriously disinterested); (4) fearfulness (none, somewhat fearful, very fearful); and (5) attention span (attentive, somewhat distractable, very distractable).[33] These were subjective ratings, intended to systematically note the child's style of interacting with his or her environment.[33]

Statistical analysis

In our primary analysis, we used FTT as the predictor variable and neurodevelopmental ability (rank) at 18 months of age as the outcome variable.[24,34] Neurodevelopmental ability was computed using item response theory (IRT), a psychometric model of a trade-off between test taker's ability and the difficulty of the test.[35] The Rasch Model, a 1-parameter logistic model to determine an infant's ability based on their responses to multiple test items was used.[35] A standardized (mean zero, unit standard deviation) score was determined for each infant for each MDAT developmental domain and an overall developmental score. This allowed ranking of individuals by neurodevelopmental ability within the cohort.

Secondary neurodevelopmental outcomes included scores on the MDAT at 12 months of age and the declarative memory, as assessed by the COAT [30] at 18 months of age. In secondary analyses, the associations between neurodevelopmental outcomes and the following growth parameters were assessed: birth weight, weight-for-age, length/height-for-age, weight-for-length/height, growth velocity between 12 and 18 months of age, mid-upper arm circumference, and head circumference. For secondary analyses involving multiple statistical comparisons, the Holm-Bonferroni correction was used to adjust for the family-wise type 1 error rate.

To examine associations between variables, non-parametric methods (Mann–Whitney U test) were used for continuous data. The two-tailed Pearson Chi-Square or Fisher's exact test were used for categorical data, as appropriate. Correlations between continuous variables were assessed using Spearman's rank correlation coefficient (ρ). To compare milestones achieved at two different time points (MDAT at 12 and 18 months of age), we used the McNemar test for

paired nominal data. To determine if pre-natal (birth weight) and post-natal (FTT) growth parameters were independently associated with MDAT score, we used a multivariable linear regression model. We included the MDAT score as the continuous dependent variable and the birth weight (continuous) and FTT (binary) as independent variables. Variable selection for the multivariable model was guided by theoretical considerations (pre- and post-natal growth measurements), rather than statistical model selection. To determine whether growth faltering increased with age, we used a linear mixed effects (LME) model to account for repeated measurements over time using *lme4*.[36] The z-score (weight-for-age, length/height-for-age, or weight-for-length/height) was entered as a continuous dependent variable into the model and age was entered as a fixed effect. We modeled intercepts and slopes for each subject as random effects. P-values were obtained by likelihood ratio tests of the full model including age as fixed effect against the reduced model without age. Data analyses were performed using GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA), and the R statistical environment.[37]

Sample size calculation

A standard sample calculation [38] showed that we would need 139 patients to detect a difference of 0.5 standard deviations (SDs) in the mean MDAT score between children with and without FTT with 80% power, at the α=0.05 level of significance. This sample size calculation assumed that 35% of children in our cohort would have FTT [39] and that a difference of 0.5 SDs ("medium" effect size) [40] would represent a clinically significant difference in outcome.[41]

Ethical approval and consent to participate

This study was reviewed and approved by the Makerere University School of Biomedical Sciences Research Ethics Committee (REC Protocol #SBSREC 295) and the University of Alberta Research Ethics Committee (Study reference Pro00057175). Regulatory approval for the study was obtained by the Uganda National Council of Science and Technology (registration number HS 1985). All participating children had a parent or caregiver that provided written informed consent.

Results

A total of 1239 pregnant HIV positive mothers and their infants were screened and 375 were enrolled (Figure 1). Eight infants subsequently tested positive by DNA PCR for HIV. Three infants died at the age of 7, 8 and 10 weeks before the primary outcome could be assessed (MDAT at 18 months of age). A further 194 children were lost to follow-up at 18 months of age (Figure 1). Younger maternal age, lower gravidity and parity, fewer antenatal clinic visits, and prematurity were factors associated with loss to follow-up (Supplemental Materials, Table S1). The final cohort consisted of 170 HEU infants who completed the MDAT at 18 months of age. The baseline characteristics of the cohort are reported in Table 1, disaggregated by the primary outcome (developmental ability at 18 months of age).

Growth faltering in HEU infants

Weight-for-age, length/height-for-age, weight-for-length/height, MUAC-for-age, and head circumference-for-age are shown in Figure 1. The proportion of HEU infants who were stunted, wasted, and underweight at each follow-up visit are shown in Table S2. Low birth weight (LBW) was correlated with lower weight, MUAC, and head circumference at 18 months of age, suggesting that small newborns tended to remain small in later infancy. However, an increasing proportion of underweight, stunting, and wasting with increasing age was also observed (Figure 1). LME models confirmed that the z-scores increasingly deviated from the mean with increasing age: change in weight-for-age z-score -0.39 per year (95% CI -0.51 to -0.28, $p<0.0001$); length/height-for-age z-score -0.94 per year (95% CI -1.2 to -0.72, $p<0.0001$); weight-for-length/height z-score -0.41 per year (95% CI -0.70 to -0.13, $p=0.0045$). With respect to growth velocity between 12 and 18 months of age, 22%, 52%, and 37% of infants had weight, height

and head circumference velocity less than -2SD, based on WHO growth charts, respectively. Failure to thrive was observed in 44 infants (37%).

Neurodevelopmental outcomes in HEU infants

At 12 and 18 months of age, 109 and 170 HEU infants, respectively, underwent MDAT testing (Figure 2). The proportion of HEU infants who had achieved the selected MDAT milestones was similar or superior to the reference range for normal infants, with the exception of two social milestones at 18 months of age (Figure 2 and Supplemental Table S3). Girls had higher language achievement than boys at 18 months of age ($p=0.0065$, significant after Holm Bonferroni correction for multiple comparisons). Of note, achievement in the four developmental domains were significantly correlated with each other, suggesting that infants strong in one domain tended to be strong in all domains, as opposed to isolated and independent developmental strengths (Supplemental Tables S4 and S5). Furthermore, the MDAT social domain was correlated with the COAT score (Supplemental Table S6). Comparing the MDAT at 12 and 18 months of age, achievement of overlapping milestones in fine motor, language, and social domains increased between 12 to 18 months, as expected ($p<0.05$ for all comparisons, Supplemental Table S7). Test behavior (typical/atypical, interest in surroundings, compliance, distractibility, fearfulness) showed statistically significant associations with MDAT ability (Supplemental Figure S2). The mean (SD) score on the COAT (declarative memory) at 18 months of age was 5.5 (3.1) compared to 6.9 (5.3) in a reference population in the USA.[30] The COAT score was correlated with the MDAT social domain ($\rho=0.21$, $p=0.042$, Table S6).

Growth faltering is associated with lower neurodevelopmental attainment

Cross-sectional correlations between neurodevelopmental scores at 12 and 18 months of age and anthropometric parameters measured at the time of the assessment and are shown in Table 2. Weight-for-age, length/height-for-age, and MUAC-for-age z-scores were statistically significantly associated with the MDAT scores at 12 and 18 months of age (Table 2). Stunting at 18 months of age was strongly associated with lower MDAT score (median -0.19 (IQR -0.89 to -0.19) *versus* +0.38 (IQR -0.18 to +0.75), $p<0.0001$). Similarly, wasting was associated with lower MDAT score (median -0.94 (IQR -1.5 to -0.42) *versus* +0.025 (IQR -0.45 to +0.62), $p=0.0074$). In addition, the head circumference-for-age z-score was correlated with the MDAT score at 18 months of age. The weight-for-length/height z-scores were correlated with the COAT score at 18 months of age (Table 2).

Birth weight (reflecting intrauterine growth), as well as height velocity between 12 to 18 months of age (reflecting postnatal growth) were predictive of developmental scores at 18 months of age. There was a statistically significant correlation between birth weight and lower MDAT score at 18 months ($\rho=0.20$, $p=0.010$, Supplemental Figure S3). Infants with LBW (<2500 g) had lower MDAT scores than children with normal birth weight (median -0.69 (IQR -1.1 to -0.18) *versus* +0.025 (IQR -0.44 to +0.62), $p=0.0065$, Supplemental Figure S3). On the other hand, preterm infants (<37 weeks gestational age at birth) had similar MDAT scores compared to term infants ($p=0.92$). Infants who were small for gestational age had similar MDAT scores compared to infants who were average or large for gestational age ($p=0.12$). The height velocity between 12 and 18 months of age was also correlated with the MDAT z-score at 18 months ($\rho=0.22$, $p=0.026$).

With respect to the primary outcome, FTT was associated with a lower MDAT score (median -0.13 (IQR -0.75 to +0.14) *versus* +0.14 (IQR -0.44 to +0.63, p=0.042, Figure 3). Infants with FTT had a relative risk of 1.5 (95%CI 1.04-2.1, p=0.035) of below-average neurodevelopment attainment compared to infants without FTT. In a multivariable linear regression model, both birth weight (p=0.026) and FTT (p=0.030) were statistically significant independent predictors of MDAT score at 18 months of age.

Discussion

Here we show that growth faltering is common in HEUs and predicts lower neurodevelopmental achievement at 18 months of age. Both LBW, reflecting intrauterine growth restriction, and FTT, reflecting postnatal growth faltering, were independently associated with subsequent lower neurodevelopmental scores.

Our cohort of HIV positive mothers and HEUs was a relatively uniform group of mother-infant pairs, typical of a resource-limited, HIV-endemic setting. Overall, 80% of the mothers received the same publicly available ART regimen (tenofovir disoproxil/lamivudine/efavirenz), 99% of infants were breastfed at 6 weeks of age with 94% weaned by 18 months of age, and all infants were prescribed nevirapine syrup for 6 weeks and co-trimoxazole from week 6 until HIV infection was excluded. The rate of vertical transmission was 8/375 (2.1%) and the child mortality was 3/375 (0.8%) during the follow-up period. This is similar to another study in African mothers with HIV, in which vertical transmission was 3.6% and mortality was 2.2% among HEUs in the first 2 years of life.[42]

The proportion of LBW among HEUs in our study (8%) was similar to that previously reported (8-18%).[43-45] HEUs have a higher prevalence of LBW than HUUs,[9,46] suggesting that growth faltering begins at the fetal stage.[47,48] With respect to postnatal growth faltering, stunting [9,39,43,46], wasting [43,46], underweight [46], and microcephaly [46,49,50] are more prevalent in HEUs than HUUs. In a recent study, HEUs, relative to HUUs, had a higher risk of stunting at 24 months of age (adjusted odds ratio 1.32 and 1.67 for Malawian and Ugandan infants, respectively).[51] In our study, HEUs had high prevalence of stunting, which increased with age (32%, 43% and 58% at 6

weeks, 12 months, and 18 months of age, respectively) (Table S2), a finding that has been documented in three previous longitudinal cohorts in LMICs.[9,39,43] The prevalence of wasting (5-13%) and underweight (7-15%) in our study were similar to other LMIC cohorts.[9,39,43] Likewise, the prevalence of microcephaly in our study was similar to another study in Zimbabwe.[49]

HEUs (relative to HUUs) have subtle neurocognitive deficits that vary between studies and neurodevelopment domains.[52-54] Most studies have found that HEUs are delayed in receptive and expressive language, although cognitive delays may only be detectable at 30-42 months of age.[39,43,47,50,52,53,55-58] Delays in school performance, lower IQ, language, and fine motor development are apparent in HEUs 2-12 years of age.[47,59,60] In several studies, HEUs did not show any significant motor and social delays relative to HUUs.[52,53,55,56] In a recent prospective cohort study from Uganda and Malawi, ante-partum and post-partum exposure to HIV and ART did not result in greater developmental risks for the HEU children through age 60 months, relative to HUUs.[61] Our findings in young infants are consistent with previous studies showing that subtle language and cognitive delays may only appear at later age; nonetheless, by ranking the cohort by developmental ability, we were able to discern associations between growth parameters and neurodevelopment.

In our study, LBW, stunting, wasting, lower height velocity between 12 and 18 months of age, and FTT were associated with lower neurodevelopmental scores at 18 months of age. Other studies have established that stunting is a marker of chronic malnutrition and a strong predictor of poor neurodevelopment in HEUs.[9,52,62,63] Findings from our study confirm the association between stunting and MDAT scores at 12 and 18 months and may suggest that chronic

malnutrition was a cause of both poor linear growth and lower developmental achievement. The COAT score was associated with lower weight-for-length/height but was not associated with stunting. This may suggest that infant memory is less susceptible to effects of chronic undernutrition than other developmental domains. Lastly, some studies showed that microcephaly was linked to poorer school performance and cognitive and memory deficits in school aged HEU children.[63,64] Likewise, in our study, head circumference at 18 months of age was correlated with MDAT scores at 18 months of age ($p=0.001$). Our study is noteworthy for finding multiple associations between prenatal and postnatal growth with cognitive ability in infancy. Taken together, these results suggest that early insults in HEU infants affect both somatic growth and brain development.

Several mechanisms may explain the association between growth faltering and neurodevelopmental delay in some HEUs. HEUs are exposed in utero to HIV virions and proteins, co-infections such as cytomegalovirus, maternal medications, low-grade systemic inflammation, mitochondrial or immunological perturbations, or dysregulation of bone metabolism or mineralization.[31,47,51,65-67] Fetal exposure to chronic inflammation results in both abnormal growth trajectory,[68] structural brain abnormalities, including low hippocampal volumes,[69] and developmental delay.[70] Postnatally, HEUs have more frequent infections,[67] may not be breastfed as long as HUUs,[71,72] and are exposed to prophylactic medications (NVP and CTX).[73] Although molecular details are not fully elucidated, subtle immune deficits, repeated infections, and elevated levels circulating inflammatory biomarkers[70,74] may lead to growth hormone resistance and stunting.[74] Socioeconomic determinants such as the home environment, maternal education level, and household income would likely influence both growth and

development.[47] Households affected by HIV are often food insecure,[75] which may lead to protein-energy malnutrition with deleterious effect on child growth and cognitive development.[76]

Growth disturbances early in infancy predicted subsequent developmental ability, suggesting a screening mechanism to identify susceptible infants for developmental interventions. Strategies for low-income settings that can be used to mitigate the developmental problems include cognitive stimulation with storytelling, singing, and playing with household objects.[77] Platforms for development services can be through home visits, clinic attendance, community-based group sessions, community health workers, and broadcast media such as radio or television.[13,78] Our findings suggest that a subgroup of HEUs at risk of developmental delay can be identified using simple methods such as monitoring early growth (LBW and FTT). This may allow us to introduce early childhood development interventions during the critical first 1000 days of life[79] and track neurodevelopmental trajectory using performance measures such as the MDAT.[29]

Our study has several strengths and limitations. Our prospective cohort design allowed us to demonstrate predictive value of growth parameters but was subject to significant loss to follow up. Loss to follow-up is common in early infant programs for prevention of vertical HIV transmission in sub-Saharan Africa, ranging from 19% to 89% of mother-infant pairs in published studies.[80] We had a loss to follow-up rate of 194/375 (51%) by 18 months of age, despite our best attempts to ensure that mothers returned to the clinic (e.g., transportation reimbursement, telephone reminders). We found that frequent causes of loss to follow-up were: inability to contact the mother (e.g., wrong telephone number provided); mother had moved out of the community; or attendance at a different follow-up clinic. Anecdotally, stigma associated

with the HIV diagnosis prevented many mothers from openly seeking follow-up care for their infants. This study also did not include a group of healthy controls (HUU) or HIV positive infants to compare the growth and neurodevelopment assessments; however, relative growth faltering and neurodevelopmental delay has been well documented in previous studies. We used the culturally validated tool MDAT which was specifically developed and validated in an African population along with the COAT memory tool. Other studies have employed a wide range of psychometric tests, which limits the comparability of findings between studies.

In summary, we have demonstrated associations between growth faltering (both prenatal and postnatal) and neurodevelopmental ability at 18 months of age. These findings suggest common mechanisms, with onset in utero but persisting after birth, that affect both somatic and brain growth. Future directions of this research include examining biomarkers of inflammation, growth hormone axis, and neuronal injury, in an attempt to shed light on the mechanism affecting impaired growth and development in some HEUs. By demonstrating the association between LBW, FTT, and subsequent developmental ability, our findings point to a simple screening method that could be used to identify children at risk for developmental intervention.

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Table 1: Baseline characteristics of 170 HIV positive mothers and their HIV exposed, uninfected infants, disaggregated by primary outcome (neurodevelopmental score at 18 months of age)

Characteristics	Entire Cohort (N=170)	Below average MDAT 18 (N=81)	Above average MDAT 18 (N=89)	P-value
Maternal, n (%)				
Age [yr], median (IQR)	28 (24-33)	28 (24-34)	29 (24-33)	0.77
Gravidity	4 (2-5)	4 (2-5)	4 (2-5)	0.99
Parity	4 (2-4)	4 (2-4)	3 (2-4)	0.75
Number of Antenatal Clinic Visits	4 (3-5)	4 (3-5)	4 (3-5)	0.81
STI during pregnancy	30 (17)	14 (17)	16 (18)	0.91
CD4+ T-lymphocyte count ($\times 10^6$/L)	550 (400-700)	520 (400-650)	560 (390-820)	0.40
HIV Drug Regimen during pregnancy				0.60
TDF/3TC/EFV	134 (79)	66 (81)	68 (76)	
AZT/3TC/NVP	17 (10)	8 (9.9)	9 (10)	
Other[1]	19 (11)	7 (8.6)	12 (13)	
WHO Clinical Stage at Delivery				0.37
Stage 1	166 (97)	80 (99)	86 (97)	
Stage 2	3 (1.8)	0 (0)	3 (3.4)	
Stage 3	1 (0.60)	1 (1.2)	0 (0)	
Infant, n (%)				
Sex[2]				0.84
Male	79 (47)	39 (48)	40 (45)	
Female	90 (53)	42 (52)	48 (55)	
Gestational age [weeks], median (IQR)	40 (38-41)	40 (38-41)	39 (38-41)	0.67
Premature	18 (11)	8 (9.9)	10 (11)	0.89
Low Birth Weight (<2500g)	13 (7.6)	10 (12)	3 (3.4)	0.034
Mode of Delivery				0.37
Spontaneous Vaginal Delivery	136 (80)	62 (77)	74 (84)	
Caesarean Section	32 (19)	18 (22)	14 (16)	

Breastfed within 1 hour	132 (78)	64 (79)	68 (76)	0.96
APGAR Score at 1 min				0.63
>8	103 (61)	49 (60)	54 (61)	
≤8	58 (34)	27 (33)	31 (35)	
APGAR Score at 5 min				0.32
>8	152 (89)	72 (89)	80 (90)	
≤8	9 (5.3)	4 (4.9)	5 (5.6)	
Suctioned	32 (19)	17 (21)	15 (17)	0.49
Bag-mask ventilation	3 (1.8)	2 (2.5)	1 (1.1)	0.51
Infant feeding option at birth				0.96
Exclusive Breast Feeding	167 (98)	80 (99)	87 (98)	
Replacement Feeding	2 (1.2)	1 (1)	1 (1.1)	
Exclusive breast feeding				
6 weeks	150 (99)	72 (89)	78 (88)	0.99
12 months	2 (1.9)	0	2 (2.2)	0.50
18 months	0	0	0	- [3]
Weaned (no longer breast feeding)				
6 weeks	0	0	0	- [3]
12 months	56 (33)	25 (31)	31 (35)	0.70
18 months	149 (88)	74 (91)	75 (84)	0.24
Trimethoprim-sulfamethoxazole prophylaxis				
6 weeks	112 (66)	56 (69)	56 (63)	0.49
12 months	82 (48)	40 (49)	42 (47)	0.90
18 months	61 (39)	29 (36)	32 (36)	>0.99

Numbers are n (%) unless otherwise indicated

Abbreviations: MDAT 18, Malawi Developmental Assessment Tool at 18 months of age; WHO, World Health Organization; STI, sexually transmitted infection

[1]Other ART regimens included: TDF/3TC/NVP (n=3), AZT/3TC/EFV (n=3), ABC/3TC/ATZ (n=1), ABC/3TC/LPV/r (n=1), and unknown (n=11).

[2]Data on sex was missing for one patient.

[3]No cases.

Table 2: Cross-sectional correlation between growth and developmental assessments

	MDAT (12 months)	MDAT (18 months)	COAT (18 months)
Weight-for-age z-score	ρ=-0.038 p=0.012	ρ=0.36 **p<0.0001***	ρ=0.14 p=0.18
Weight-for-length/height z-score	ρ=-0.12 p=0.69	ρ=0.086 p=0.29	ρ=0.32 **p=0.002***
Length/height-for-age z-score	ρ=0.22 **p=0.00015***	ρ=0.41 **p<0.0001***	ρ= -0.23 p=0.024
Head circumference-for-age z-score	ρ=0.17 p=0.19	ρ=0.26 **p=0.0011***	ρ=-0.094 p=0.36
MUAC-for-age z-score	ρ=0.46 **p=0.0019***	ρ=0.34 **p=0.0014***	ρ=-0.072 p=0.68

*statistically significant using the Holm Bonferroni correction for multiple comparisons
Abbreviations: MDAT Malawi Developmental Assessment Tool; COAT Colour Object Association Test

Figure legends

Figure 1. Growth of 170 Ugandan HEU infants. Growth parameters of girls and boys at 6 weeks, 12 and 18 months of age plotted on World Health Organization growth charts, including length/height-for-age (**A** and **B**), weight-for-age (**C** and **D**), weight-for-length/height (**E** and **F**), head circumference-for-age (**G** and **H**) and Mid-Upper Arm Circumference (MUAC)-for-age (**I** and **J**). Lines indicate major percentiles (5th, 10th, 25th, 50th, 75th, 90th, 95th). Points indicate individual measurements on HEU infants. Two boys had their measurements taken at 5.6 and 6.1 months of age (**B, D, F** and **H**).

Figure 2. Attainment of milestones at 12 and 18 months of age among 170 HEUs. The proportion of HEUs who had achieved age-appropriate Malawi Developmental Assessment Tool (MDAT) milestones is plotted and compared to a normative population.[29] The tool assesses four developmental domains: gross motor (**A** and **B**), fine motor (**C** and **D**), language (**E** and **F**) and social (**G** and **H**). The MDAT was assessed at 12 and 18 months of age.

Figure 3. Association between failure to thrive (FTT) and neurodevelopment among HEUs. Infants with FTT from 6 weeks to 18 months of age had median MDAT standardized ability score of -0.13 (IQR -0.75 to +0.14) compared to infants without FTT, median +0.14 (IQR -0.44 to +0.63, p=0.042).

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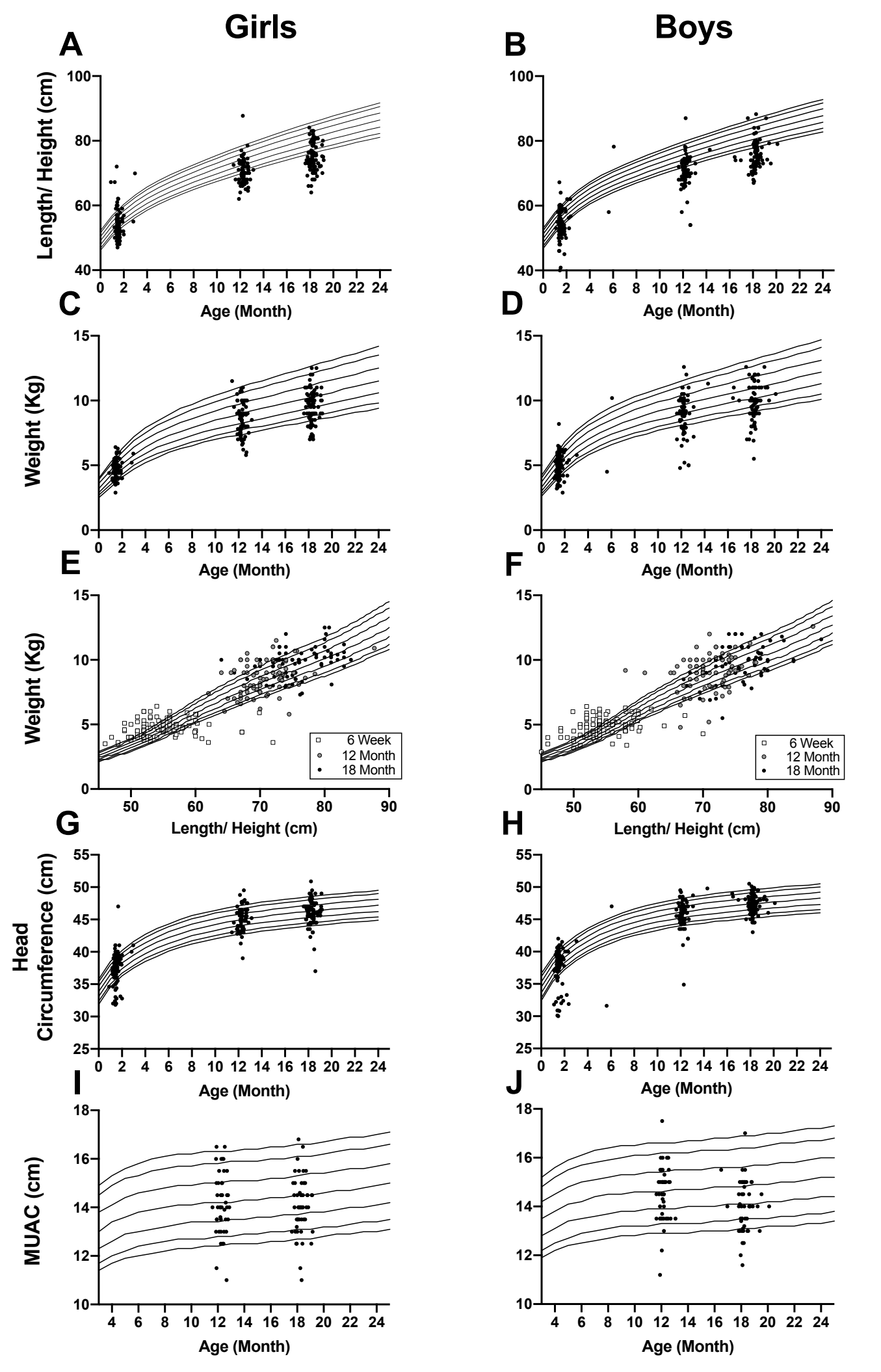
Girls
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A
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Length/ Height (cm)
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Length/ Height (cm)
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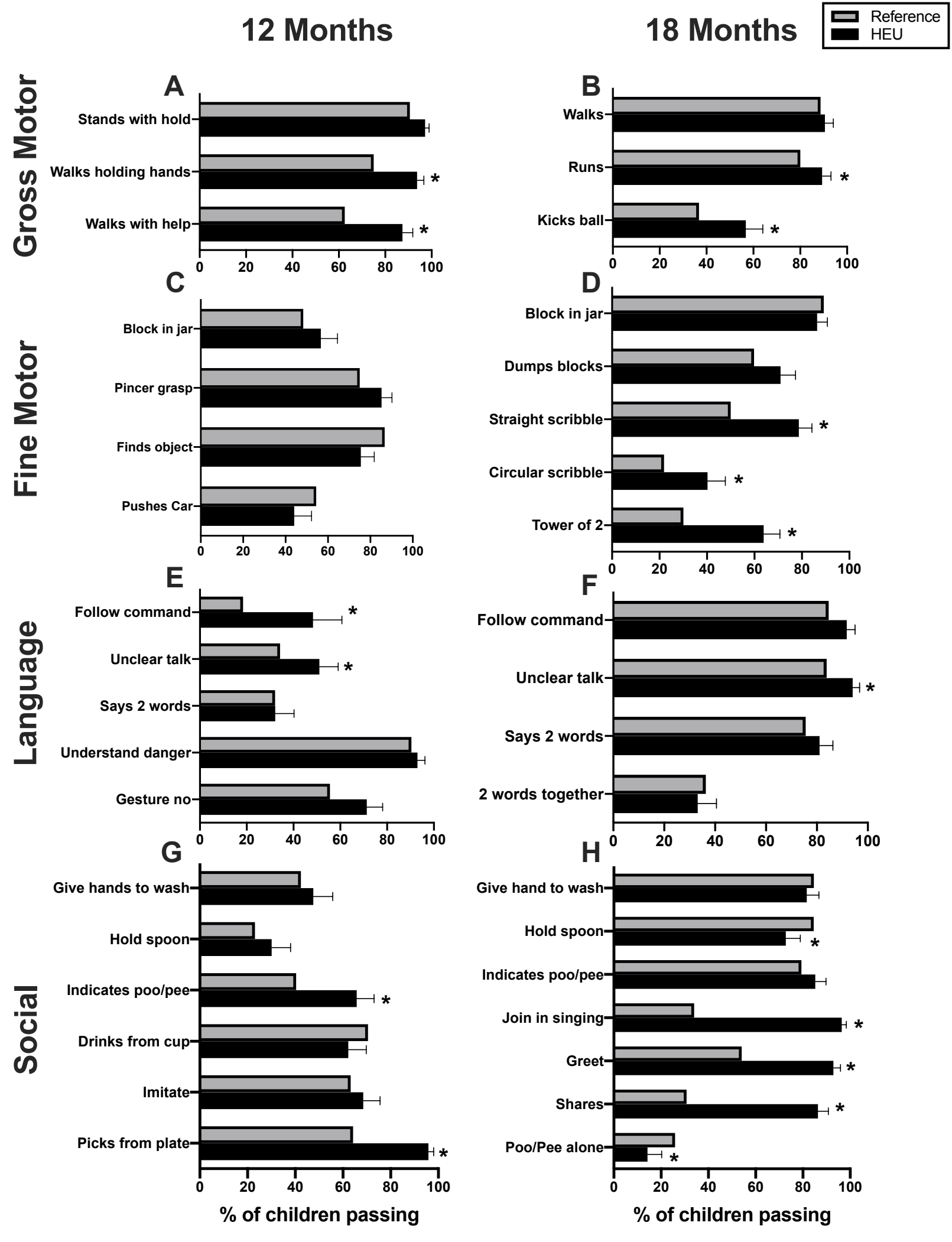
12 Month
18 Month


12 Months
18 Months
Reference
HEU
Gross Motor
Fine Motor
Language
Social
A
Stands with hold
Walks holding hands
Walks with help
B
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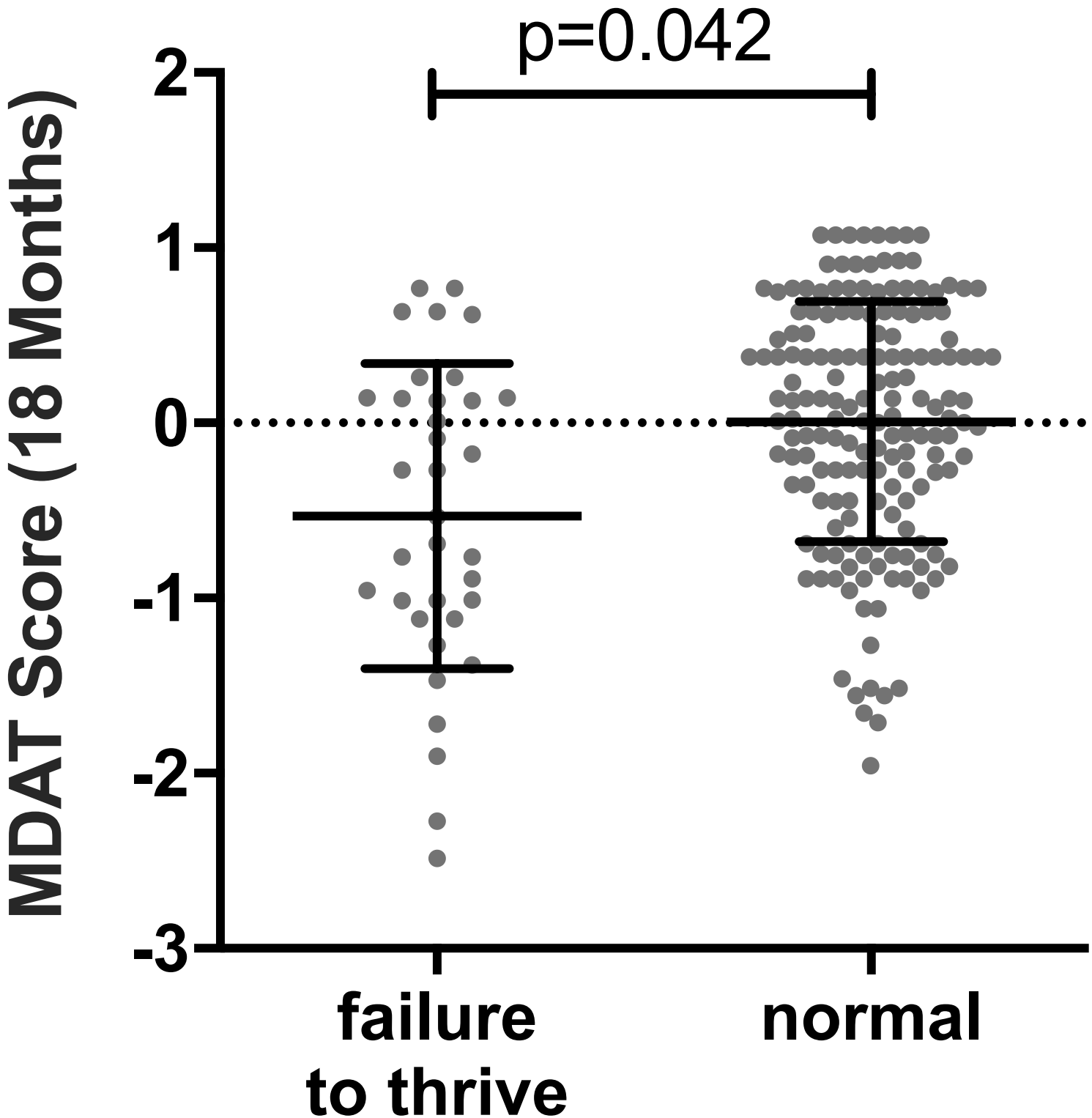
Runs
Kicks ball
C
Block in jar
Pincer grasp
Finds object
Pushes Car
D
Block in jar
Dumps blocks
Straight scribble
Circular scribble
Tower of 2
E
Follow command
Unclear talk
Says 2 words
Understand danger
Gesture no
F
Follow command
Unclear talk
Says 2 words
2 words together
G
Give hands to wash
Hold spoon
Indicates poo/pee
Drinks from cup
Imitate
Picks from plate
H
Give hand to wash
Hold spoon
Indicates poo/pee
Join in singing
Greet
Shares
Poo/Pee alone
0 20 40 60 80 100
% of children passing
% of children passing


p=0.042
MDAT Score (18 Months)
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1
0
-1
-2
-3
failure to thrive
normal

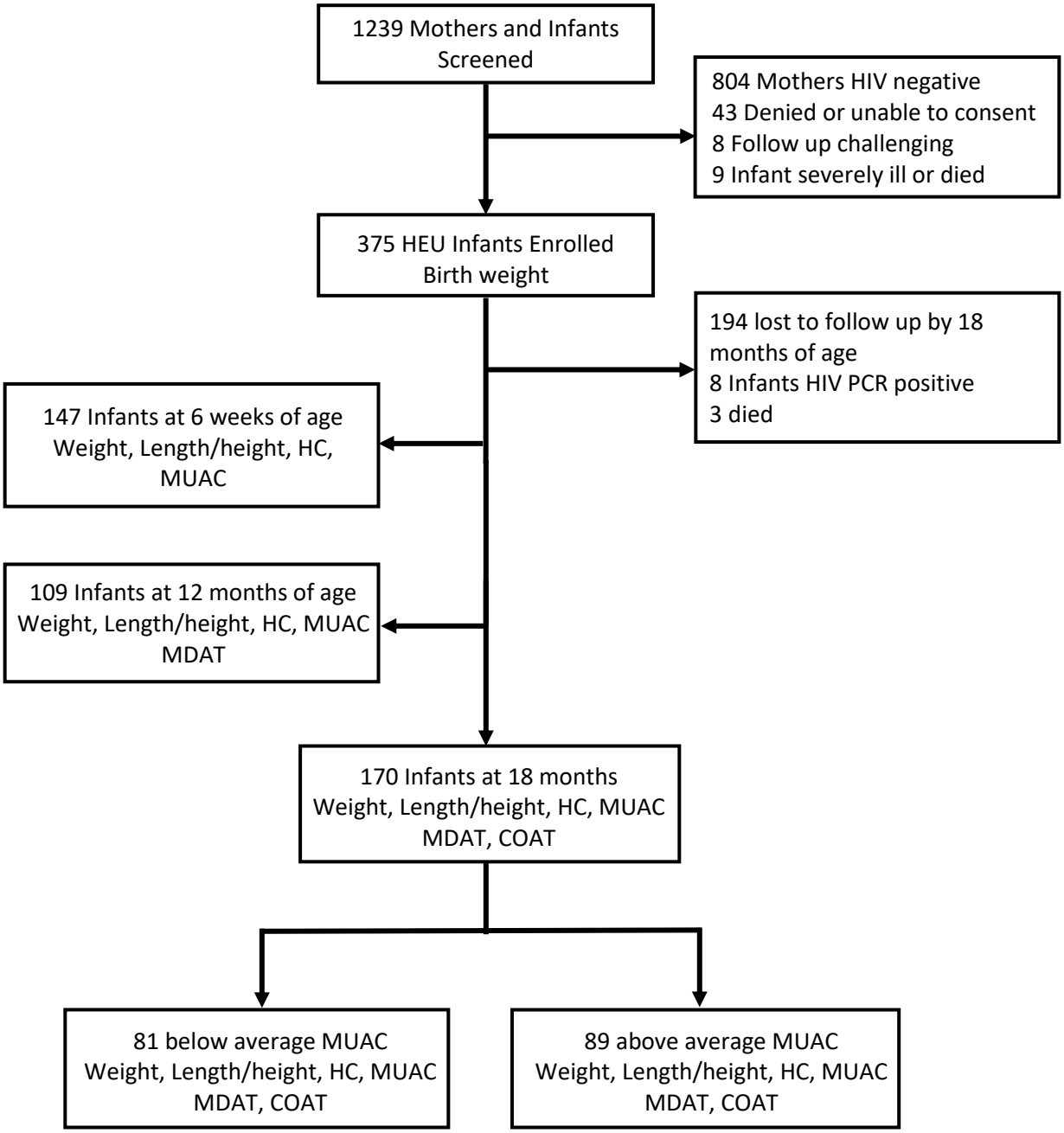

Figure S1. Trial flow diagram. 1241 mothers-infant pairs at the Jinja Regional Referral Hospital or Kambuga Hospital were screened and 170 were included. Anthropomorphic data (weight, length/height/height, head circumference (HC), and mid-upper arm circumference (MUAC)) were recorded at 6 weeks, 12 months and 18 months of age. Neurodevelopment assessment was performed using the Malawi Development Assessment Tool (MDAT) at 12 and 18 months; and the Color Object Association Test (COAT) at 18 months. The cohort with complete growth and neurodevelopment data (n=170) was dichotomized based on the average MDAT score at 18 months of age.

Table S1: Baseline characteristics of HIV positive mothers and HIV exposed, uninfected infants in Uganda

Characteristics	Entire Cohort (N=375)	Included (N=170)	Lost to follow up (N=194)	HIV infected (N=8)	Fatal outcome (N=3)	P-value[1]
Maternal, n (%)						
Age [yr], median (IQR)	27 (23-31)	28 (24-33)	25 (23-30)	26 (22-27)	21,25,38	0.00018
Gravidity	3 (2-4)	4 (2-5)	3 (2-4)	2 (1-3)	2,9,2	0.00069
Parity	3 (2-4)	4 (2-4)	3 (2-4)	2 (1-2)	2,9,2	0.0056
CD4+ T-lymphocyte count ($\times 10^6$/L)[2]	554 (396-788)	547 (397-696)	548 (395-810)	794[3]	-	0.48
Number of Antenatal Clinic Visits	4 (3-4)	4 (3-5)	4 (3-4)	4 (3-5)	1,2,3	0.014
STI during pregnancy	65 (17)	30 (18)	33 (17)	0	2 (67)	>0.99
HIV Drug Regimen during pregnancy						0.44
TDF/3TC/EFV	305 (81)	134 (79)	166 (86)	5 (63)	0	
AZT/3TC/NVP	31 (8.3)	17 (10)	13 (6.7)	1 (13)	0	
Other[4]	37 (9.9)	19 (11)	15 (7.7)	2 (13)	3	
WHO Clinical Stage at Delivery						0.90
Stage 1	364 (97)	166 (97)	188 (97)	7 (100)	3 (100)	
Stage 2	7 (1.9)	3 (1.8)	4 (2.1)	0	0	
Stage 3	2 (0.80)	1 (0.60)	2 (1.0)	0	0	
Infant, n (%)						
Sex[5]						0.079
Male	197 (53)	79 (47)	112 (58)	4 (50)	2 (66)	
Female	173 (46)	90 (53)	78 (40)	4 (50)	1 (33)	
Gestational age [wk], median (IQR)	39 (38-41)	40 (38-41)	39 (38-41)	39 (36-38)	36,36,37	0.44
Premature	51 (17)	18 (12)	31 (20)	2 (29)	2 (67)	0.047
Low Birth Weight	29 (7.9)	13 (7.8)	16 (8.3)	0	0	>0.99
Mode of Delivery						0.59
Spontaneous Vaginal Delivery	297 (79)	136 (80)	153 (79)	5 (63)	3 (100)	
Caesarean Section	76 (20)	32 (19)	41 (21)	3 (38)	0	
Breastfed within 1 hour	287 (77)	132 (78)	151 (79)	3 (38)	1 (33)	0.59
APGAR Score at 1 min						0.26
>8	222 (62)	103 (61)	117 (59)	2 (33)	1 (100)[6]	
≤8	133 (37)	58 (34)	71 (36)	4 (67)		

APGAR Score at 5 min						0.64
>8	333 (93)	152 (94)	176 (94)	4 (67)	1 (100)[5]	
≤8	21 (6.4)	9 (5.6)	11 (5.9)	2 (33)		
Suctioned	64 (17)	32 (19)	31 (16)	1 (13)	0	0.50
Bag-mask ventilation	6 (1.7)	3 (1.9)	2 (1.1)	1 (13)	0	0.47
Infant Feeding Option						0.55
Exclusive Breast Feeding	365 (98)	167 (98)	188 (98)	7 (100)	3 (100)	
Replacement Feeding	6 (1.6)	2 (1.1)	4 (2.1)	0	0	
Exclusive breast feeding						
6 weeks	273 (73)	150 (99)	117 (98)	3 (75)	3 (100)	0.33
12 months	2 (1.3)	2 (1.2)	0	0	0	-[7]
18 months	0	0	0	0	0	-[7]
Weaned (no longer breast feeding)						
6 weeks	0	0	0	0	0	-[7]
12 months	68 (44)	56 (50)	10 (24)	2 (67)	0	0.015
18 months	172 (93)	149 (88)	21 (95)	2 (100)	0	0.40
Trimethoprim-sulfamethoxazole prophylaxis						
6 weeks	210 (77)	112 (76)	94 (80)	3 (75)	1 (50)	0.68
12 months	122 (83)	82 (95)	37 (90)	3 (100)	0	0.54
18 months	73 (40)	61 (39)	10 (43)	2 (100)	0	0.53

STI sexually transmitted infection; WHO World Health Organization

[1]P-value compares infants included in the analysis (N=170) to those who were excluded (lost to follow-up, HIV infected, and deceased, N=205).

[2]CD4+ T-lymphocyte count was available for 84 (22%) mothers

[3]CD4 count was available for only one of the eight mothers.

[4]other cART regimens included 1. for whole cohort: TDF/3TC/NVP (n=6), AZT/3TC/EFV (n=7), ABC/3TC/ATZ (n=1), ABC/3TC/LPV/r (n=1), and unknown (n=22). 2. For included cohort: TDF/3TC/NVP (n=3), AZT/3TC/EFV (n=3), ABC/3TC/ATZ (n=1), ABC/3TC/LPV/r (n=1), and unknown (n=11). 3. For loss to follow up cohort: TDF/3TC/NVP (n=3), AZT/3TC/EFV (n=4), and unknown (n=8). 4. HIV positive: TDF/2TC/EFV (n=1), and unknown (n=1). 5. For Fatal Outcome cohort: unknown (n=3).

[5]Sex was missing for 5 infants

[6]APGAR score was available for one of the three infants.

[7]No cases found.

Table S2: Anthropomorphic measurements of 170 HIV exposed uninfected infants from week 6 to 18 months of age.

Growth Measures	**6 weeks**	**12 months**	**18 months**
Weight [kg], median (IQR)	4.7 (4.2 to 5.3)	9.0 (7.9 to 9.8)	9.8 (9.0 to 10)
Length/height [cm], median (IQR)	55 (52 to 58)	70 (68 to 73)	75 (72 to 79)
Head Circumference [cm] , median (IQR)	38 (37 to 39)	46 (44 to 47)	47 (46 to 48)
Underweight, n (%)	18 (7.4)	23 (15)	26 (15)
Stunting, n (%)	60 (32)	65 (43)	102 (58)
Wasting, n (%)	26 (13)	10 (6.7)	9 (5.1)
Microcephaly, n (%)	29 (12)	7 (4.7)	9 (4.9)

Table S3: Comparison of Ugandan HEU infants and normative population with respect to MDAT milestones at 12 and 18 months of age

	Correct (N=170)	**Observed (%)**	**Expected (%)[1]**	**P-value**
12 months				
Gross Motor				
Ability to stand if holding on to things	100	98	91	0.016
Walks using both hands of someone	99	97	75	**<0.0001***
Walks with help using some's hand or furniture	92	90	63	**<0.0001***
Fine Motor				
Neat pincer grasp, picks up maize or bean with thumb and one finger	90	88	75	0.0030
Puts blocks into jar in imitation. Puts	60	59	48	0.043
Finds object under piece of cloth	82	80	87	0.084
Pushes a little car along	45	45	54	0.059
Language				
Understands when being cautioned about danger	95	93	90	0.44
Indicates by gesture to say "No."	71	70	56	0.0059
Follows simple commands (1 stage) eg. "give me the cup"	35	35	18	**<0.0001***
Unclear talk/jabber in sentences - pretends to talk but does not actually make sense	51	50	34	**0.00087***
Says 2 words, but words other than mama/dada	35	34	32	0.72
Social				
Drinks form a cup well without spilling	63	62	71	0.067
Is able to indicate, by pointing, that they want something	69	67	63	0.41
Can the child eat by picking posho/kaloo from a plate in morsels that mum has made?	97	95	64	**<0.0001***
Puts hands out to have them washed by mum	47	46	42	0.51
Can hold a spoon and take food by self, but spills some	30	29	23	0.16
Indicates in some way that they need to go for a poo/pee	67	66	40	**<0.0001***
18 months				
Gross Motor				
Walks well	153	92	89	0.34
Runs, but basic running – may fall over at times	151	90	80	**0.0011***
Kicks a ball in any way/tries to kick a ball	96	57	37	**<0.0001***

Fine Motor				
Puts blocks into jar in imitation	145	88	89	0.67
Dumps blocks out of jar purposefully	119	72	60	0.0018
Scribbles on paper (straight scribble)	133	81	50	**<0.0001***
Scribbles on paper (circular scribble)	68	41	22	**<0.0001***
Tower of 2 blocks	107	65	30	**<0.0001***
Language				
Follows simple commands (1 stage) e.g., "give me the cup"	154	92	85	0.015
Unclear talk/jabber in sentences - pretends to talk but does not actually make sense.	158	94	84	**0.00046***
Says 2 words, but words other than mama/dada	135	80	76	0.18
Says 2 words together	55	33	37	0.40
Social				
Puts hands out to have them washed by mum	136	81	85	0.23
Can hold a spoon and take food by self, but spills some	124	74	85	**0.00020***
Indicates in some way that they need to go for a poo/pee	144	86	79	0.050
Wants to join in with singing games	162	97	34	**<0.0001***
Able to greet either by extending hand or verbally	156	93	54	**<0.0001***
Sharing things, including food with others	145	86	31	**<0.0001***
Does a poo or pees by themselves without wetting their pants	24	14	26	**0.00092***

*statistically significant using the Holm Bonferroni correction for multiple comparisons
MDAT Malawi Developmental Assessment Tool
[1]Based on normative population of Malawian infants

Table S4: Correlation between developmental domains of the MDAT (12 months)

	Gross motor	Fine motor	Language	Social
Gross motor				
Fine motor	ρ=0.28 **p=0.0010***			
Language	ρ=0.39 **p<0.0001***	ρ=0.45 **p<0.0001***		
Social	ρ=0.23 **p=0.0075***	ρ=0.17 **p=0.043***	ρ=0.29 **p=0.00054***	

*statistically significant using the Holm Bonferroni correction for multiple comparisons
MDAT Malawi Developmental Assessment Tool

Table S5: Correlation between developmental domains of the MDAT (18 months)

	Gross motor	Fine motor	Language	Social
Gross motor				
Fine motor	ρ=0.43 **p<0.0001***			
Language	ρ=0.34 **p<0.0001***	ρ=0.25 **p=0.0012***		
Social	ρ=0.069 p=0.38	ρ=0.18 **p=0.022***	ρ=0.19 p=0.016	

*statistically significant using the Holm Bonferroni correction for multiple comparisons
MDAT Malawi Developmental Assessment Tool

Table S6: Correlation between developmental domains of the MDAT (18 months) and the COAT (also conducted at 18 months of age)

MDAT (18 months)	COAT
Gross Motor	ρ=0.053 p=0.61
Fine Motor	ρ=0.12 p=0.23
Language	ρ=0.051 p=0.62
Social	ρ=0.21 p=0.042

MDAT Malawi Developmental Assessment Tool
COAT Color Object Association Test

Table S7: Comparison of MDAT Milestones assessed at both 12 and 18 months of age

Category	Milestone	12 months	18 months	p-value[1]
Fine Motor	Puts blocks into jar in imitation. Puts at least one block into the jar when shown by the examiner	81/141 (57)	147/167 (88)	**<0.0001***
Language	Follows simple commands (1 stage) e.g., "give me the cup"	45/141 (32)	155/170 (91)	**<0.0001***
	Unclear talk/jabber in sentences - pretends to talk but does not actually make sense	72/141 (51)	159/170 (94)	**<0.0001***
	Says 2 words, but words other than mama/dada	45/142 (32)	137/170 (81)	**<0.0001***
Social	Puts hands out to have them washed by mum	68/142 (48)	138/170 (81)	**<0.0001***
	Can hold a spoon and take food by self, but spills some	43/142 (30)	124/170 (73)	**<0.0001***
	Indicates in some way that they need to go for a poo/pee	94/142 (66)	145/170 (85)	**0.0023***

*statistically significant using the Holm Bonferroni correction for multiple comparisons
MDAT Malawi Developmental Assessment Tool
[1]McNemar test for paired nominal data

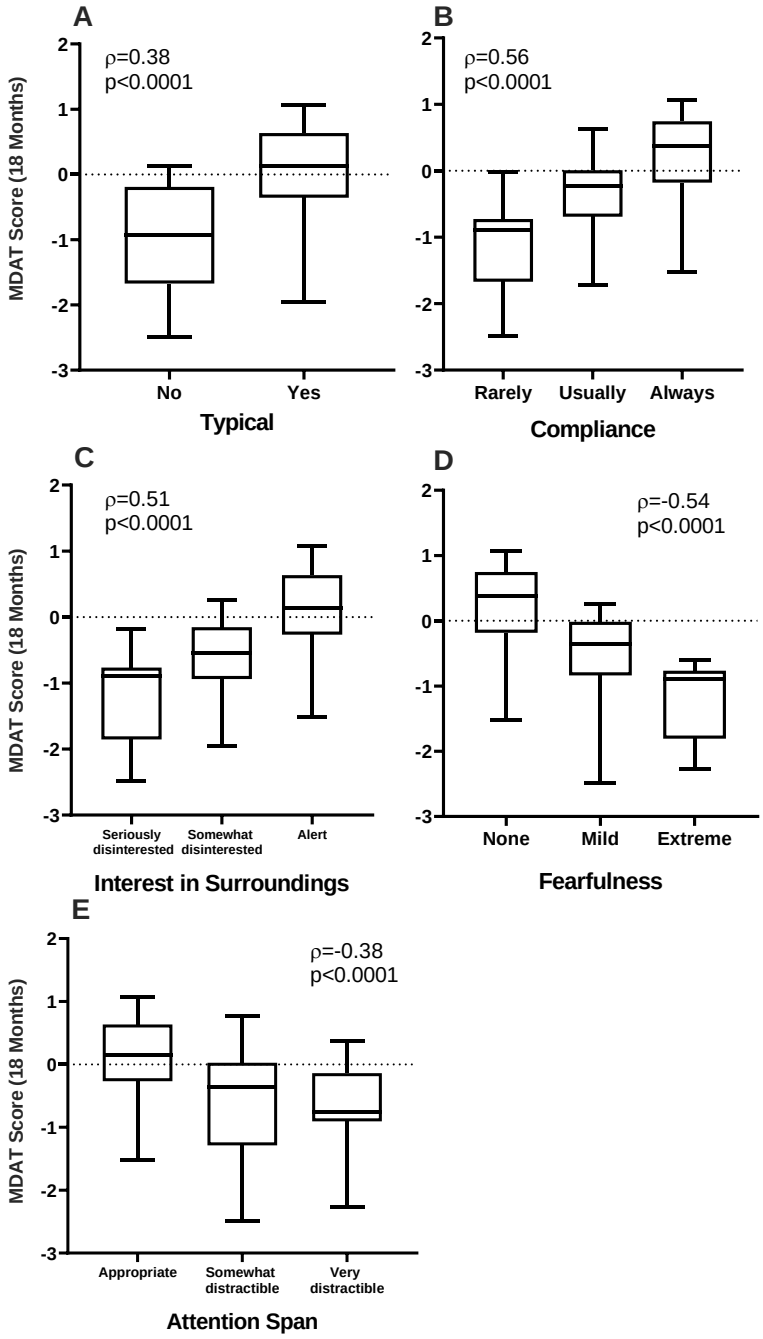

Figure S2. Association between MDAT score at 18 months of age and Test Behaviors. The examiner rated each child according to the following five test behaviors: **(A)** Overall typical vs atypical behavior; **(B)** Compliance; **(C)** Interest in Surroundings; **(D)** Fearfulness; and **(E)** Attention span. There were statistically significant associations between adverse test behaviors and lower MDAT scores.

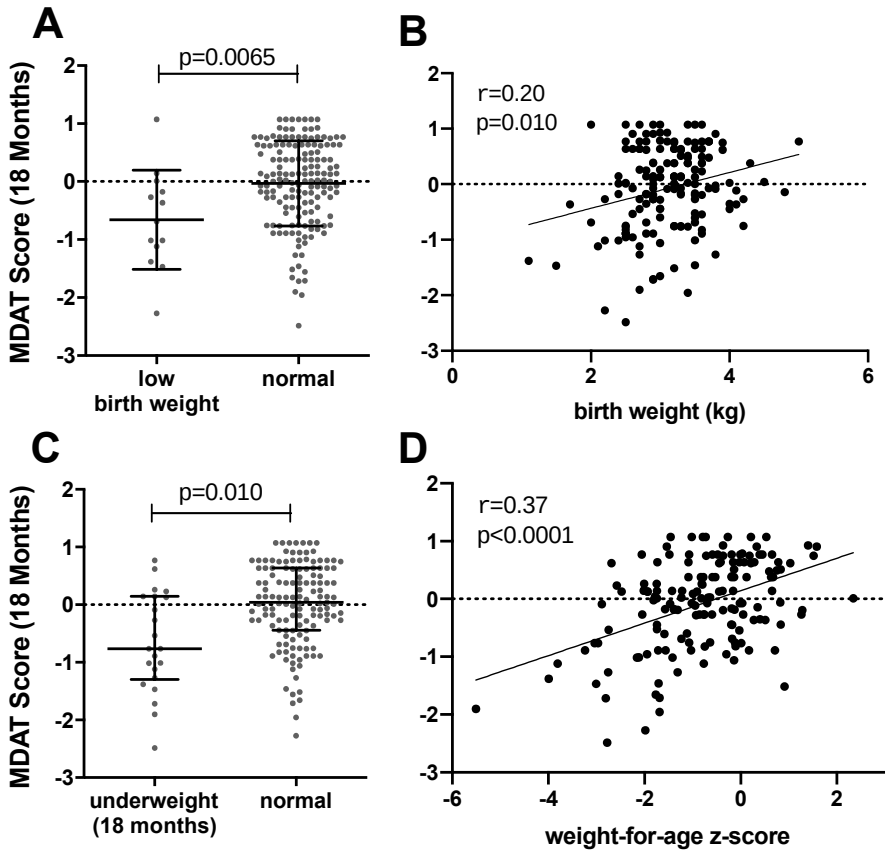

Figure S3. Association between growth and neurodevelopment among HEUs. Lower neurodevelopmental ability (MDAT score at 18 months of age) was associated with low birth weight (**A** and **B**) and underweight at 18 months of age (**C** and **D**).

Supplementary References

1. Gladstone M, Lancaster GA, Umar E, et al. The Malawi Developmental Assessment Tool (MDAT): the creation, validation, and reliability of a tool to assess child development in rural African settings. *PLoS Med.* 2010;7(5):e1000273.