Effects of HIV exposure, TB and HIV treatment, and isoniazid preventive therapy on prognosis and growth trajectories of children

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Abstract

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Introduction: Expanded access to antiretroviral therapy (ART) and prevention of mother-to-child transmission of HIV services (PMTCT) have enabled the vast majority of pregnant women living with HIV (WLWH) to give birth to HIV-exposed uninfected (HEU) children. Evidence suggests that birth weight and birth length are lower in HEU than in HIV unexposed uninfected (HUU) children, but there is conflicting evidence on growth after birth. HEU children have a high risk of *Mycobacterium tuberculosis* (Mtb) infection and TB disease, with an increased risk of exposure to known (mothers living with HIV) and unknown sources of infection. Latent TB infection (LTBI) can be effectively treated with isoniazid preventive therapies (IPT). Recently completed randomized controlled trials have examined IPT's efficacy and effectiveness in preventing TB infection and disease in pregnant mothers and infants, but the long-term effects of IPT exposure *in utero* or during infancy on infant growth are unknown. Despite ART and PMTCT, some children still get infected with HIV. Children living with HIV (CHIV) have a high mortality rate, particularly if diagnosed late. TB is one of the most common infections in CHIV. Treating both TB and HIV is vital to decreasing TB-related mortality in CHIV. However, TB-HIV co-treatment poses management challenges and potential effects on childhood growth. The goals of the study are to evaluate the effects of HIV exposure, TB-HIV treatment, and IPT on the prognosis and growth trajectories of children. The project had four aims: **Aim 1:** To determine the effect of HIV and ART exposure *in-utero* on growth and development of HEU infants. **Aim 2.** To determine the effect of maternal pregnancy versus postpartum maternal IPT on growth faltering of HEU infants. **Aim 3:** To evaluate the impact of infant IPT on the rate of growth of HEU infants. **Aim 4:** To determine the effect of TB-HIV co-treatment on the prognosis (viral load suppression, immune reconstitution, and growth).

Methods: This dissertation used four data sources to address the aims of the study. To address Aim #1, we used data from a prospective birth cohort (MiTIPS) of women with and without HIV infection, and their infants in Western Kenya. Infants were enrolled at 6 weeks of age and followed until 24 months of age. We used multivariable linear mixed-effects models to compare growth rates (weight-for-age z-score [WAZ] and height-for-age z-score [HAZ]) and multivariable linear regression to compare overall development z-score between HEU and HIV-unexposed uninfected (HUU) children. To address Aim #2, we used a post-hoc analysis of data from TB APPRISE, a multicenter, double-blind, placebo-controlled trial, which randomized women with HIV to 28-weeks of IPT starting in pregnancy (pregnancy-IPT) or postpartum week 12 (postpartum-IPT). Infants were followed from birth to 48 weeks of age. We used overall and sex-stratified multivariable Cox proportional hazards regression to compare time to infant growth faltering between arms to 12 weeks and 48 weeks postpartum. To address Aim #3, we used data from The infant TB Infection Prevention Study (iTIPS) trial, a non-blinded RCT of IPT versus no IPT among HIV-exposed infants in Kenya. Infants were enrolled at 6 months. Interventions were given for 12 months and observational follow-up continued until 24 months of age. We used intent-to-treat linear mixed-effects models to compare growth rates (weight-for-age z-score [WAZ] and height-for-age z-score [HAZ]) between trial arms. To address Aim #4, we used longitudinal data from the PUSH trial among ART-naïve 0-12 years of age hospitalized CHIV (NCT02063880) in Kenya. TB-ART treated and ART-only groups were compared at 6-months post-ART for viral suppression (<40 c/ml), CD4% change, and growth using generalized linear models, linear regression, and linear mixed-effects models, respectively.

Results: Aim #1: Among 355 infants, 184 were HEU children, 51.3% (182/355) were female, 3.9% (14/355) were low birthweight, and 8.5% (26/307) were preterm. Median maternal age was 25.0 (interquartile range [IQR] 22.0-29.0 years); mothers of HEU children were older and had higher income; all mothers of HEU children received ART in pregnancy, of whom 67.9% (125/184) started ART pre-pregnancy and 87.3% (158/181) received 3TC/FTC, TDF, EFV regimens. In longitudinal linear analyses, HEU children did not differ significantly from HUU in growth or development (p>0.05 for all). Higher maternal education was associated with significantly better growth and development in all children in the first 24 months of age: WAZ (β=0.18 [95% CI:0.01, 0.34]), HAZ (β=0.26 [95% CI: 0.04, 0.48], and overall development (β=0.24 [95% CI: 0.02, 0.46]). Breastfeeding was associated with significantly better HAZ (β=0.42 [95% CI: 0.19, 0.66]) and development (β=0.31 [95% CI: 0.08, 0.53]) in all children.

Aim #2: Among 898 HIV-exposed uninfected (HEU) infants, 447 (49.8%) were females. Infants in the pregnancy-IPT arm had a 1.47-fold higher risk of becoming underweight by 12 weeks of age (aHR 1.47 [95% CI: 1.06, 2.03]) than infants in the postpartum-IPT arm; increased risk persisted to 48 weeks postpartum (aHR 1.34 [95% CI: 1.01, 1.78]). Maternal IPT timing was not associated with stunting or wasting. In sex-stratified analyses, male infants in the pregnancy-IPT arm experienced an increased risk of lower birthweight (LBW) (aRR 2.04 [95% CI: 1.16, 3.68), preterm birth (aRR 1.81 [95% CI: 1.04, 3.21]) and becoming underweight by 12 weeks (aHR 2.02 [95% CI: 1.29, 3.18]) and 48 weeks (aHR 1.82 [95% CI: 1.23, 2.69]). Maternal IPT timing did not influence growth in female infants. Aim #3: Among 298 infants, 150 were randomized to IPT between 6-10 weeks of age, 47.6% were females, median birthweight was 3.4 kg (inter-quartile range [IQR] 3.0-3.7), and 98.3% were breastfed. During 12-month IPT treatment and 12-month post-RCT follow-up, WAZ and HAZ declined significantly with more HAZ decline in male infants. There were no growth differences between trial arms, including in sex-stratified analyses. In longitudinal linear analysis, mean WAZ (β=0.04 [95% CI:-0.14, 0.22]), HAZ (β=0.14 [95% CI:-0.06, 0.34]), and WHZ [β=-0.07 [95% CI:-0.26, 0.11]) z-scores were similar between arms as were WAZ and HAZ growth trajectories. Infants in the IPT arm had a higher monthly WHZ increase (β to 24 months 0.02 [95% CI:0.01, 0.04]) than in the no-IPT arm. Aim #4: Among 152 CHIV, 40.8 % (62) were TB-ART treated. At baseline pre-ART, median age was 2.0 years, VL was 5.6 $\log_{10}$ c/ml, CD4% was 14%, and 40.4% had HAZ<2; CD4% and growth measures were significantly lower, and VL significantly higher in the TB-ART group. At 6 months post-ART, 37.2% had viral suppression and the median (IQR) CD4% increased by 7.2% (2.0%-11.6%) CD4% with no difference between TB-ART and ART-only groups. The TB-ART group had significantly lower WAZ and HAZ throughout follow-up (WAZ -0.81 [95% CI: -1.23, -0.38, p<0.001], HAZ -0.15 [95% CI: -0.29, -0.01, p=0.030]). The TB-ART group had greater rate of WAZ increase in analyses unadjusted and adjusted for baseline WAZ (unadj 0.62 [95% CI: 0.18, 1.07, p=0.006] or adj 0.58 [95% CI: 0.12, 1.03, p=0.013]).

Conclusion: HEU children had a similar growth trajectory and development compared with HUU children. Breastfeeding and maternal education improved weight, height, and overall development of children, suggesting that these factors are more influential than HIV or ART exposure on infant growth in this cohort with universal maternal ART coverage for HEU. Maternal IPT during pregnancy was associated with an increased risk of LBW, preterm birth, and becoming underweight among HEU infants, specifically male infants. In contrast to the impact of maternal pregnancy-IPT on infant growth, infant IPT administration among HEU infants did not significantly impact growth outcomes in the first two years of life. TB-HIV co-treatment did not affect viral suppression and CD4 reconstitution post-ART among young hospitalized children initiating ART, suggesting tolerability and lack of interference of TB treatment on ART levels. TB-ART-treated CHIV had more rapid growth reconstitution, despite which growth deficits persisted, suggesting the need for growth monitoring and management in co-treated CHIV. The findings inform strategies to optimize TB-HIV co-treatment in CHIV, provide additional evidence regarding maternal and infant IPT and safety in infancy, and inform clinical care for CHIV and HEU children.

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Introduction

As a result of the expansion of access to antiretroviral therapy (ART) for prevention of mother-to-child HIV transmission (PMTCT), an increasing proportion of pregnant women living with HIV (WLHIV) are able to give birth to HIV-exposed uninfected (HEU) children (1,2). HIV exposure in-utero is associated with preterm birth, low birth weight (LBW) and length, small for gestational age (SGA), and stillbirth (3,4). In some prior studies, HEU children exposed to a short-course of antiretrovirals *in utero* for PMTCT had slower growth than HUU children (5,6). Similarly, ART-exposed children were significantly smaller and shorter at 24 months of age than those exposed to zidovudine (ZDV) monotherapy during pregnancy (7). This evidence suggests that exposure to both HIV and ART *in utero* may affect infants' growth. However, the evidence regarding growth deficits in HEU children has been inconsistent - with some studies finding no differences between HEU and HUU children (3,8). Most studies to date were from the time period before Option B+ (lifelong ART for PMTCT rather than short-course monotherapy) was adopted and when fewer WLHIV were on ART pre-pregnancy.

There is also evidence that HEU children have a high risk of morbidity and mortality (9–13). HEU children are at high risk of Mtb infection (14), with a high risk of exposure from known (HIV-infected mothers) and unknown sources of infection (15–17). While the timing of progression from latent Mtb infection to progress to TB disease can be variable, and children present with TB disease between the ages of 1-4 years (18). Isoniazid preventive therapy (IPT) effectively treats LTBI. TB prevention among WLWH in TB-endemic areas is a high priority (19). As a result, the World Health Organization (WHO) recommends TB preventive therapy, such as IPT for people living with HIV (PHIV), including pregnant women (20). WHO guidelines also recommend IPT for children under 5 with a known TB exposure (21). However, recent studies estimate that most TB transmission to children occurs without a known contact (22). HEU children have a higher risk of Mtb infection than HUU children (14). IPT has side effects that can potentially compromise caloric intake, including loss of appetite, nausea, vomiting, upset stomach, and fever, and these side effects may affect infant growth (23). Studies that compare TB-exposed to unexposed children to ascertain the effect of IPT on growth would be susceptible to many confounding factors. To date, the effects of administering IPT during infancy on the growth of children are not well characterized. An RCT that evaluated the impact of IPT on the incidence of Mtb infection (24) in HEU infants was recently conducted (24). This RCT offers a unique opportunity to assess infant IPT impact on growth with a rigorous randomized trial design.

A few observational studies in pregnant women demonstrated that IPT does not negatively impact pregnancy outcomes (25,26), despite reports of embryocidal effects in animal studies (27). Trial-based data have been lacking until recently (28). In a recently completed trial, although initiation of IPT during pregnancy appeared as safe as deferred IPT in terms of adverse maternal outcomes, it was associated with a higher incidence of composite adverse pregnancy outcomes (such as stillbirth, spontaneous abortion, low birth weight, and preterm delivery or congenital anomalies in the infant) (28,29). It is still unclear if IPT exposure *in utero*, in conjunction with additional exposure to HIV and ART, affects long-term growth over the first year of life.

HIV infection still occurs among children even with expanded PMTCT and ART (30). Compared to HUU children, CHIV have an 8-fold increased risk of TB (31). Africa contributes approximately 78.0% of the worldwide pediatric TB deaths in CHIV (32). TB-HIV co-treatment, while essential to address both infections, can pose several management challenges, including high pill burden, drug toxicity, drug-drug interactions, and immune reconstitution-inflammatory syndrome (33,34). These may lead to poorer viral suppression, immune reconstitution, or growth.

In this dissertation, we conducted a series of analyses to assess the impact of HIV exposure, maternal IPT, and infant IPT on growth among infants. We assessed other cofactors of growth and development among HEU and HUU infants. Among CHIV, we assessed the impact of TB-ART co-treatment on clinical and growth outcomes.

Chapter 1: Effect of *in utero* HIV and antiretroviral therapy exposure on growth during infancy

Abstract:

Background: Exposure to HIV and antiretroviral therapy (ART) *in utero* may influence infant growth and development. Most available evidence predates the adoption of Option B+ (women with HIV are immediately offered ART, regardless of their CD4 count, in order to prevent vertical transmission). We compared growth and development in HIV-exposed uninfected (HEU) to HIV-unexposed (HUU) infants in a recent cohort.

Methods: This analysis used data from a prospective birth cohort (MiTIPS) of women with and without HIV infection, and their infants in Western Kenya. Women were enrolled during pregnancy and followed up until 24 months postpartum. We used multivariable linear mixed-effects models to compare growth rates (weight-for-age z-score [WAZ] and height-for-age z-score [HAZ]) and multivariable linear regression to compare overall development (assessed with caregiver-reported early development instruments [CREDI]) between HEU and HUU children.

Results: Among 355 infants, 184 (51.8%) were HEU infants, 3.9% (14/355) were low birthweight, and 8.5% (26/307) were preterm. Median maternal age (interquartile range [IQR]) was 25.0 (22.0-29.0) years; mothers of HEU children were older and had higher incomes. During pregnancy all mothers of HEU children received ART; 67.9% (125/184) started ART pre-pregnancy and 87.3% (158/181) received 3TC/FTC,TDF,EFV. longitudinal linear analyses, HEU children did not differ significantly from HUU in growth or development (p>0.05 for all). In the combined HEU/HUU children cohort, adjusted for potential confounders, higher maternal education was associated with significantly better growth and development: WAZ (β=0.18 [95% CI:0.01, 0.34]), HAZ (β=0.26 [95% CI: 0.04, 0.48], and development (β=0.24 [95% CI: 0.02, 0.46]). Breastfeeding was associated with significantly better HAZ (β=0.42 [95% CI: 0.19, 0.66]) and development (β=0.31 [95% CI: 0.08, 0.53])

Conclusion: HEU children had a similar growth trajectory and development to HUU children. Breastfeeding and maternal education improved weight, height, and overall development of children irrespective of maternal HIV status.

Background

Expanded access to combined antiretroviral therapy (ART) and prevention of mother-to-child transmission of HIV services (PMTCT) have enabled the vast majority of pregnant women living with HIV (WLWH) to give birth to HIV-uninfected children (1,2). The population of HIV-exposed uninfected (HEU) children is growing (35). HIV exposure and antiretroviral therapy (ART) may have independent effects on infant growth (3,4,7,36). There is also evidence that HEU children have a higher risk of morbidity (37,38) and mortality (9–13).

HIV exposure in-utero is associated with preterm birth, low birth weight (LBW) and length, small for gestational age (SGA), and stillbirth (3,4). Studies comparing HEU and HUU children in the era of short-course antiretrovirals for PMTCT observed that HEU children had slower growth than HUU children (5,6). More recent data suggest that even with combination ART in pregnancy, growth deficits persist in HEU children (39–41). In a longitudinal study in Botswana, ART-exposed children were significantly smaller and shorter at 24 months of age than those exposed to zidovudine (ZDV) monotherapy during pregnancy (7). Together, these studies suggest that exposure to both HIV and ART *in utero* may affect infants' growth. However, evidence regarding growth deficits in HEU children has been conflicting – with some studies noting no differences between HEU and HUU children (3,8). Similarly, while some studies showed poor language development in HEU children (42), others found no differences in neurodevelopment between HEU and HUU children (43). Further, most existing studies used data from before the adoption of Option B+, a recommendation that all WLHIV initiate lifelong ART. We used a recent longitudinal study to compare the growth and overall development of HEU and HUU children and identify predictors of growth and development.

Methods

Study design:

We used data from Impact of maternal HIV on *Mycobacterium tuberculosis* Infection among Peripartum Women and their Infants (MiTIPS), an observational, prospective study of parallel longitudinal cohorts of pregnant women and their infants in Western Kenya from April 2016 to March 2021. Details of the study have been previously summarized (44). Participants in the study included 200 WLWH and 200 HIV-uninfected pregnant women seeking antenatal care services at three public hospitals in Nyanza Province, Kenya, as well as their infants. HEU children were compared with children born to HIV-uninfected mothers (HUU). Women who had TB in the past year or at enrollment, and women who lived outside of the catchment area or plan to move there were excluded from the parent study.

Infant growth characterization

Mothers were enrolled during pregnancy, and mother-infant pairs were followed up to 24 months postpartum. Infants were enrolled at 6 weeks of age and their birth weight and length were obtained from maternal report and review of maternal child health (MCH) booklet. Study team members were trained by CDC-Kenya mentors on growth measurement. Weight and length of children were measured at 6 weeks and 6, 12, 18, and 24 months of age. Growth was measured twice at each visit, and we used the average value rounded to the nearest 0.1 kg and 0.1 cm for weight and length, respectively.

Weight-for-age z-score [WAZ], weight-for-length z-score [WLZ], and length-for-age z-score [LAZ]) were defined using WHO child growth standards (45). Growth faltering was defined as underweight WAZ <-2, wasting WLZ <-2, and stunting LAZ <-2 (45).

The short caregiver-reported early development instruments (CREDI) – age-specific 20 questions – were used to measure Early Childhood Development (ECD). We used the 24-29 month short CREDI questionnaire. Due to COVID-19, study visits, which were aligned with routine child health of PMTCT follow-up visits were delayed. Data from children who attended study visits from 24-35 months were included for early childhood development analysis. We generated age and sex-specific z-score standards for development.

Maternal age, family income, educational status, marital status, body mass index (BMI), infant sex, and time-varying breastfeeding were assessed as relevant covariates.

Data analyses

Mean and standard deviation (SD) were used to describe normally distributed continuous variables; median and interquartile range (IQR) were used to describe skewed distributions; and, frequency and percentage were used to describe categorical variables.

Multivariable linear mixed-effects models (LMEMs) with autoregression correlation structure, random intercept for subjects, and random slope for follow-up time were used to compare growth (WAZ, WHZ, HAZ) between HEU and HUU children from enrollment to 24 months of age, and multivariable linear regression was used to compare development of children at 24-35 months of age.

Approach to missing data

There were missing data in this study: overall WAZ (3.3%), HAZ (14.5%), and WHZ (14.5%). Multiple imputations by chained equations (MICE), including a series of regression models for each missing variable conditional upon other specified variables, were used to manage missing data. Baseline infant and maternal characteristics (infant's age and sex, breastfeeding, maternal age, education, employment, income, BMI, marital status, and HIV infection) were used in the MICE analyses. We imputed the data 25 times. Pooled parameter estimates and their standard errors were calculated according to Rubin's rules to account for the between- and within-imputation variance (46). We employed one single imputation model to obtain imputed values for outcomes (WAZ, HAZ, and WHZ) missed at each visit during follow-up. In the imputation models, we specified the appropriate distributions for each of the variables in the model. In the presence of missing data, the multiple imputation approach should yield unbiased estimates assuming data are missing at random (MAR).

In all analyses, we used 95% confidence intervals (95% CI) and a p-value <0.05 to determine the statistical significance of associations. R version 3.5.1 and STATA version 16 statistical software were used in the analyses.

Results

Maternal and infant baseline characteristics

Overall, 355 children, 184 HEU and 171 HUU children were included in this analysis. The mean (SD) age of mothers was 26.0 (5.4) years; mean (SD) BMI was 24.05 (4.2); and, median (IQR) monthly income in KSH was 10000 (5000-20000). Overall, 81.4% (289/355) of mothers were currently married, 44.2% (157/355) completed primary school or less, and 46.5% (165/355) were unemployed. Among mothers of HEU children, 47.3% (87/184) used isoniazid preventive therapy (IPT) during pregnancy, 67.9% (125/184) were on ART pre-pregnancy and 87.3% (158/181) received 3TC/FTC,TDF,EFV regimens.

Median infant (IQR) birthweight was 3.2 kg (3.0-3.6); 4.0% (14/353) had LBW; and, 8.5% (26/307) were preterm. Of all infants, 51.4% (182/354) were females, and 96.1% (324/337) of infants were breastfed at enrollment. The frequency of LBW, preterm, female births, and breastfeeding did not differ significantly between HEU and HUU infants (Table 1).

Effect of HIV-ART exposure on growth – WAZ, HAZ, and WHZ

In univariate analyses, HEU children did not differ from HUU children in WAZ, HAZ, or WHZ. Adjusted for mother’s age, BMI, educational level, marital status, income and infant sex and breastfeeding, HEU children had no statistically significant differences in WAZ (β = -0.08 [95% CI: -0.25, 0.09]), HAZ (β = 0.14

[95% CI: -0.09, 0.36]), and WHZ (β = -0.18 [95% CI: -0.42, 0.06]) when compared to HUU children (Table 2). Findings were similar across sex of infants (interaction p-value>0.05). There was no difference in the rate of the monthly change in WAZ, HAZ, and WHZ between HEU and HUU children in the first 24 months, p-value >0.05 (Figure 1).

Cofactors for growth in overall cohort (combined HEU and HUU)

In univariate and multivariate analyses, maternal BMI, educational level, infant sex, and breastfeeding were each independently associated with WAZ and HAZ. In multivariate analyses, for every 1 kg/m2 increase in maternal BMI, infants WAZ increased by 0.04 (β = 0.04 [95% CI: 0.03, 0.06]) z score and HAZ increased by 0.03 (β = 0.03 [95% CI: 0.01, 0.06]) z-score. Infants whose mothers attained secondary school or above had 0.18 WAZ (β = 0.18 [95% CI: 0.01, 0.34]) and 0.26 HAZ (β = 0.26 [95% CI: 0.04, 0.48]) scores higher than infants born from mothers who completed primary school or less. Female infants had 0.24 WAZ (β = 0.24 [95% CI: 0.08, 0.39]) and 0.45 HAZ (β = 0.45 [95% CI: 0.24, 0.66]) scores higher than male infants. Infants who were breastfeeding had higher HAZ (β = 0.42 [95% CI: 0.19, 0.66]) than infants who were not breastfeeding (Table 2). Infants whose monthly household income was 10,000 to <20,000 Kenyan shillings (KSH) had higher WAZ (β = 0.34 [95% CI: 0.11, 0.58]) than infants whose household income was less than 5,000 KSH.

Effect of maternal HIV-ART exposure on overall development z-score

HEU and HUU children had a similar distribution of overall development z-scores (Figure 2). In univariate analyses, HEU and HUU children did not differ in development z-scores. Adjusted for all pre-specified factors, HEU children had similar overall development z-score compared to HUU children, (β = -0.04 [95% CI: -0.27, 0.18]) (Table 3).

Cofactors of development in the combined cohort (HEU and HUU)

In univariate analyses, maternal BMI and breastfeeding at 24 months were associated with the overall developmental score. In multivariate analyses, maternal BMI, educational level, infant’s sex, and breastfeeding were independently associated with overall development score. For every 1 kg/m^2 increase in maternal BMI, infants’ overall development decreased by 0.03 (β = -0.03 [95% CI: -0.05, 0.01]) z score. Infants whose mothers attained secondary school or above had a 0.24 higher overall developmental z-score (β = 0.24 [95% CI: 0.02, 0.46]) than infants born from mothers who completed primary school or less. Infants who were breastfeeding at 24 months had a 0.31 higher overall development z-score (β = 0.31 [95% CI: 0.08, 0.53]) than infants who were not breastfeeding (Table 3).

Growth and development in stratified analyses among HEU children with mothers starting ART before versus during pregnancy and by ART regimen

Adjusted for maternal age, BMI, educational status, marital status, income, sex of children, breastfeeding, ART regimen, pregnancy IPT, and viral load, there was no association between the timing of ART initiation and growth – WAZ (β=-0.04 [95% CI:-0.38, 0.31]), HAZ (β=-0.05 [95% CI: -0.45, 0.35]), and WHZ (β=-0.02 [95% CI:-0.38, 0.34]) – among HEU children, p-value >0.005 (Figure 3). Similarly, adjusted for the same factors, maternal ART regimen had no significant association with growth among HEU children, p-value >0.05 (supplemental Table 1).

ART timing and ART regimen were not also associated with overall development score among HEU children (supplemental Table 2).

Discussion

In this longitudinal prospective cohort study, we found that HEU children had similar growth trajectories and development to HUU children. Maternal BMI, maternal education, and breastfeeding were associated with a significant impact on growth and development in the combined cohort of HEU and HUU children.

We found no statistically significant difference in WAZ, HAZ, and WHZ in HEU children compared to HUU children. Our findings are consistent with a study from Zambia (8) but differ from other studies in Nigeria (40), Malawi and Uganda (41), and Kenya (39) which found that HEU children had lower WAZ, HAZ, and WHZ. A study in Botswana also observed a higher risk of stunting in HEU than in HUU children (36). Most studies demonstrating growth deficits in HEU children used data collected at or before 2014 (when Option B+ was rolled out). Our study and the study in Zambia that found no difference in growth between HEU and HUU children both used data after 2017. Care or treatment may have improved over time, which could be partly explained by the higher proportion of mothers (67.9%) who started ART pre-pregnancy. In the study from Botswana, 67% of stunting risk was related to LBW (36). We had a low proportion of infants with LBW (4.0%), but HEU children did have a relatively higher LBW prevalence than the HUU. Breastfeeding prevalence was high in both HEU and HUU children in our study, which may have attenuated differences between the groups. After breastfeeding adjustment, a study in Denmark (3) revealed that WAZ scores of HEU and HUU children were the same. In addition, investigators of that study found that the difference in HAZ and WHZ between HEU and HUU children disappeared after the first year when adjusted for breastfeeding. The study in Uganda and Malawi found significant growth deficits in HEU in Uganda, but not in Malawi (41). Breastfeeding prevalence among HEU children was of the main differences between Uganda (49.0% HEU Vs 86.2% HUU) and Malawi (80.0% HEU vs 97.0% HUU) cohorts. It is also possible that our study lacked a sufficient sample size to detect small differences. The study was able to detect a 0.3 or higher Z-score difference (assuming 1 SD) between HEU and HUU children, a real difference below this threshold would have been missed. Other studies have noted differences of 0.28 or less (40).

We found no differences in development among HEU compared to HUU children, consistent with studies in Botswana (43) and South Africa (47). Our study used a short form CREDI questionnaire to study the overall development of 24-35 months of age children. Both the Botswana and South Africa studies used Bayley Scales of Infant and Toddler Development III (BSID III) (43,47,48). The short CREDI questionnaire provides an overall developmental score and does not assess specific domains (49). It is possible that more sensitive assessment tools may have detected differences between HEU and HUU children in our cohort. Studies in Cameroon (50) and a recent meta-analysis found that HEU children had deficits in gross motor function and poorer expressive language, but similar cognitive development, fine motor skills, and receptive language development compared to HUU (42).

We found several sociodemographic predictors for growth and neurodevelopment in the combined cohort. For every 1 kg/m^2 increase in maternal BMI, infant WAZ and HAZ rose by 0.05 and 0.04 z-scores, respectively, while overall development declined by 0.03 z-score. Prior studies have shown a link between increased BMI and increased growth (51) as well as poorer brain development (52). It is unclear how maternal BMI affects the brain development of infants, but systemic inflammation and altered circulating hormones may play a role (53).

We found that infants born to mothers who had completed secondary education or higher had substantial and significantly better growth and developmental score points than infants born to mothers who had only completed primary school. Among the core socioeconomic factors (occupation, income, and education), maternal education has been shown to most strongly influence children's cognitive development in the United States and the UK (54). Education provides women with a broad range of social and human capital, that influences healthy interaction with their children, parenting style, and knowledge of growth and development that could positively influence a child's development (55).

We observed that breastfed infants had better growth and neurodevelopment in the combined cohort. Formula-fed infants gain weight more rapidly than breastfed ones (56), but formula feeding may lead to diarrhea and slowed growth in settings with poor sanitation (57). Our findings are aligned with a meta-analysis demonstrating better cognitive outcomes with breastfeeding (57).

Female infants had 0.24 higher WAZ and 0.45 higher HAZ z-scores, but the same development score, compared to male infants. This is in line with other studies (42). It is unclear why males are more likely to have growth deficits than females, but it may relate to cultural differences in feeding infants or susceptibility to infections.

This study has several strengths, including prospective longitudinal design. Weight and height/length measurements were both collected twice, reducing measurement errors. The study also has limitations, including small sample size and lack of detailed neurocognitive assessment.

Conclusion: This longitudinal prospective cohort study found no significant differences in growth trajectory and development between HEU and HUU children. Breastfeeding and maternal education positively influenced both growth and development of children in the first 24 months of life. Maternal BMI was positively associated with growth but negatively associated with development. Our results suggest that sociodemographic factors play a more influential role in growth and development of infants than HIV or ART exposure in the context of Option B+ implementation and high pre-pregnancy ART usage. However, larger studies are needed to confirm our findings.

Tables

Table 1: Maternal and Infant Demographics and Clinical Characteristics

Characteristics	HEU* (n=184)	HUU[¥] (n=171)	P-value
Maternal characteristics			
Mean age (SD) – years	27.9 (5.4)	24.1 (4.7)	**<0.001**
Maternal BMI	25.3 (4.1)	24.8 (4.2)	0.250
Mother completed primary school or less	89 (48.4)	68 (39.8)	0.126
Currently married mothers – no. (%)	152 (82.6)	137 (80.1)	0.641
Unemployed mothers – no. (%)	77 (41.8)	88 (51.5)	0.088
Monthly income in KSH – no. (%)			
Less than 5,000	38 (45.8)	44 (54.2)	**0.041**
5,000 to < 10,000	41 (50.0)	41 (50.0)	
10,000 to <20,000	53 (65.4)	28 (34.6)	
20,000 or above	51 (47.2)	57 (52.8)	
Maternal ARV started before pregnancy – no. (%)	125 (67.9)	-	-
Current ART regimen – no. (%)			
3TC/FTC,TDF,EFV	158 (87.3)	-	-
3TC/FTC,ZDV/TDF,NVP	16 (8.8)	-	-
AZT + 3TC + EFV	3 (1.7)	-	-
TDF/AZT + 3TC + LPV/r	4 (2.2)	-	-
IPT during pregnancy – no. (%)	87 (47.3)	-	-
Viral load (>40 HIV copies /ml) – no. (%)	53 (31.4)		
Infant characteristics			
Female infants – no. (%)	99 (53.8)	83 (48.5)	0.376
Breastfeeding at enrollment – no. (%)	168/175 (96.0)	156/162 (96.3)	0.999
Mean birth weight in kg (SD)	3.3 (0.6)	3.3 (0.5)	0.851
Low birth weight (<2.5 kg)[¶] – no./total no. (%)	11/184 (5.9)	3/169 (1.8)	0.080
Mean birth length in cm (SD)	49.3 (3.2)	49.6 (3.2)	0.595
Preterm birth (<37 gestational weeks) – no./total no. (%)	19/184 (10.3)	7/123 (5.7)	0.222

*HEU – HIV-exposed uninfected, and [¥]HUU – HIV-uninfected unexposed. [δ]Body mass index is the weight in kilograms divided by the square of height in meters. [¶]Low birth weight is an infant born weighing 5.5 pounds (2.5 kilograms) or less.

Table 2: Comparison of WAZ, HAZ, and WHZ over a 2-years follow-up between HEU and HUU and evaluation of other cofactors

Variable	**WAZ[¶]**			**HAZ[¥]**			**WHZ[α]**		
	cCoefficient* (95% CI)	**aCoefficient[¤] (95% CI)**	**P-value**	**cCoefficient* (95% CI)**	**aCoefficient[¤] (95% CI)**	**P-value**	**cCoefficient* (95% CI)**	**aCoefficient[¤] (95% CI)**	**P-value**
HEU (ref: HUU)[ʂ]	-0.05 (-0.22, 0.12)	-0.08 (-0.25, 0.09)	0.370	0.10 (-0.11, 0.31)	0.14 (-0.09, 0.36)	0.248	-0.17 (-.36, 0.06)	-0.18 (-0.42, 0.06)	0.134
Age of mothers in years	0.00 (-0.01, 0.02)	-0.01 (-0.02, 0.01)	0.531	0.01 (-0.01, 0.03)	0.00 (-0.02, 0.03)	0.804	-0.01 (-0.03, 0.01)	-0.01 (-0.03, 0.01)	0.454
Mean maternal BMI[δ]	**0.05 (0.03, 0.06)**	**0.04 (0.03, 0.06)**	**<0.001**	**0.04 (0.02, 0.07)**	**0.03 (0.01, 0.06)**	**0.008**	**0.03 (0.00, 0.05)**	**0.03 (0.00, 0.06)**	**0.033**
Secondary school or above (ref: primary school or less)	**0.21 (0.05, 0.38)**	**0.18 (0.01, 0.34)**	**0.038**	**0.26 (0.05, 0.48)**	**0.26 (0.04, 0.48)**	**0.022**	0.12 (-0.09, 0.33)	0.04 (-0.20, 0.27)	0.763
Currently married mothers (ref: not married)	-0.13 (-0.35, 0.08)	-0.05 (-0.27, 0.17)	0.683	-0.15 (-0.42, 0.12)	-0.07 (-0.35, 0.22)	0.659	-0.04 (-0.32, 0.23)	0.03 (-0.26, 0.33)	0.827
Income in KSH									
Less than 5,000	Referent	Referent		Referent	Referent		Referent	Referent	
5,000 to < 10,000	0.01 (-0.22, 0.25)	-0.05 (-0.28, 0.18)	0.669	-0.08 (-0.38, 0.23)	-0.17 (-0.47, 0.13)	0.273	0.05 (-0.25, 0.35)	0.05 (-0.26, 0.35)	0.765
10,000 to <20,000	**0.37 (0.13, 0.61)**	**0.34 (0.11, 0.58)**	**0.004**	0.16 (-0.14, 0.47)	0.07 (-0.24, 0.38)	0.662	**0.33 (0.02, 0.64)**	**0.35 (0.03, 0.68)**	**0.034**
20,000 or above	**0.26 (0.04, 0.49)**	0.12 (-0.11, 0.35)	0.299	0.15 (-0.14, 0.43)	-0.03 (-0.33, 0.27)	0.838	**0.29 (0.00, 0.58)**	0.24 (-0.07, 0.56)	0.130
Female infants	**0.46 (0.26, 0.67)**	**0.24 (0.08, 0.39)**	**0.004**	**0.46 (0.26, 0.67)**	**0.45 (0.24, 0.66)**	**<0.001**	-0.01 (-0.22, 0.21)	-0.03 (-0.24, 0.18)	0.771
Breastfeeding**	**0.42 (0.19, 0.65)**	0.11 (-0.03, 0.25)	0.137	**0.42 (0.19, 0.65)**	**0.42 (0.19, 0.66)**	**<0.001**	0.04 (-0.20, 0.28)	0.00 (-0.25, 0.025)	0.995

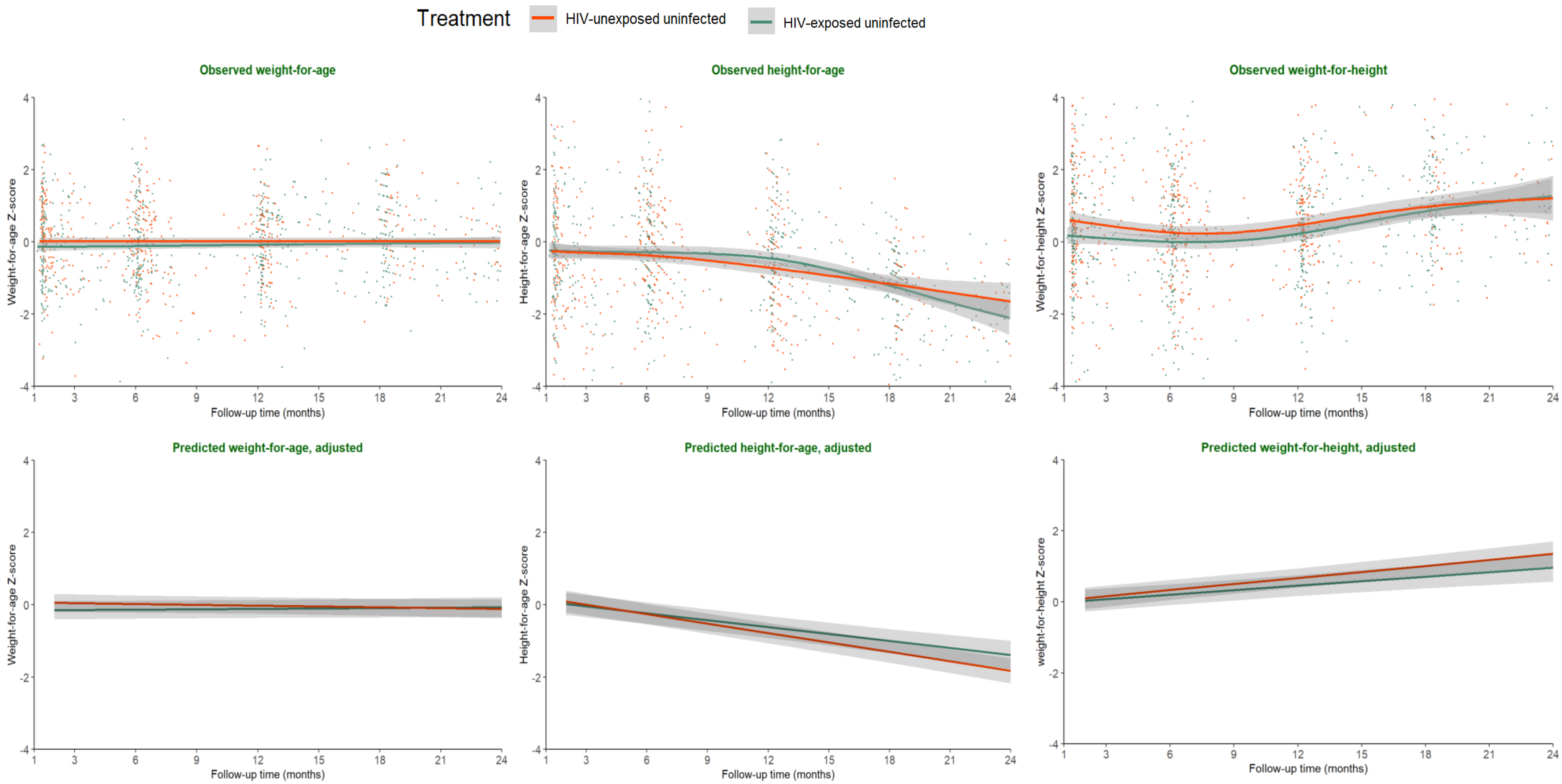
[¶]WAZ – weight-for-age. [¥]HAZ – height-for-age. [α]WHZ – weight-for-height z-score. [ʂ]HEU – HIV-exposed uninfected, and HUU – HIV-uninfected unexposed. [δ]Body mass index is the weight in kilograms divided by the square of height in meters. * cCoefficient – crude (unadjusted) coefficient. [¤]aCoefficient – coefficients adjusted for other cofactors (HIV exposure status, maternal age, education, marital status, household income, infant's sex, and breastfeeding). **Breastfeeding – was a time-varying factor

Table 3: Comparison of HEU to HUU and effect of other cofactors on overall development z-score assessed at 24-35 months

Variable	**Overall development z-score**		
	cCoefficient* (95% CI)	**aCoefficient[¤] (95% CI)**	**P-value**
HEU children (ref: HUU children)*	-0.08 (-0.29, 0.12)	-0.04 (-0.27, 0.18)	0.721
Age of mothers in years	0.00 (-0.02, 0.02)	0.01 (-0.27, 0.18)	0.679
Mean maternal BMI[δ]	**-0.02 (-0.04, 0.00)**	**-0.03 (-0.05, -0.01)**	**0.017**
Secondary school or above (ref: primary school or less)	**0.25 (0.04, 0.46)**	**0.24 (0.02, 0.46)**	**0.031**
Currently married mothers (ref: not married)	-0.04 (-0.30, 0.21)	-0.05 (-0.34, 0.23)	0.707
Income in KSH			
Less than 5,000	Referent	Referent	
5,000 to < 10,000	0.07 (-0.24, 0.37)	0.11 (-0.20, 0.41)	0.495
10,000 to <20,000	0.03 (-0.29, 0.34)	-0.03 (-0.36, 0.29)	0.844
20,000 or above	0.24 (-0.03, 0.51)	0.17 (-0.11, 0.45)	0.239
Female infants	0.03 (-0.18, 0.23)	0.05 (-0.16, 0.26)	0.633
Breastfeeding at 24 months	-0.05 (-0.51, 0.40)	**0.31 (0.08, 0.53)**	**0.008**
Birth weight (kg)	0.05 (-0.14, 0.24)	0.08 (-0.11, 0.27)	0.831

*HEU – HIV-exposed uninfected, and HUU – HIV-uninfected unexposed. [δ]Body mass index is the weight in kilograms divided by the square of height in meters.
* cCoefficient – crude (unadjusted) coefficient. [¤]aCoefficient – coefficients adjusted for other cofactors (HIV exposure status, maternal age, education, marital status, household income, infant's sex, and breastfeeding)

Figures


Observed weight-for-age

Observed height-for-age

Observed weight-for-height

Predicted weight-for-age, adjusted

Predicted height-for-age, adjusted

Predicted weight-for-height, adjusted

Figure 1: Scatter plots of change in observed and adjusted WAZ, HAZ, and WHZ over time by HIV exposure

(Curves on the top are scattered plots of change in observed WAZ, HAZ, and WHZ over time by HIV exposure and the Lowess curves represent a change in mean growth from birth to the 24 months of age. The bottom curves show the change in adjusted WAZ, HAZ, and WHZ.)

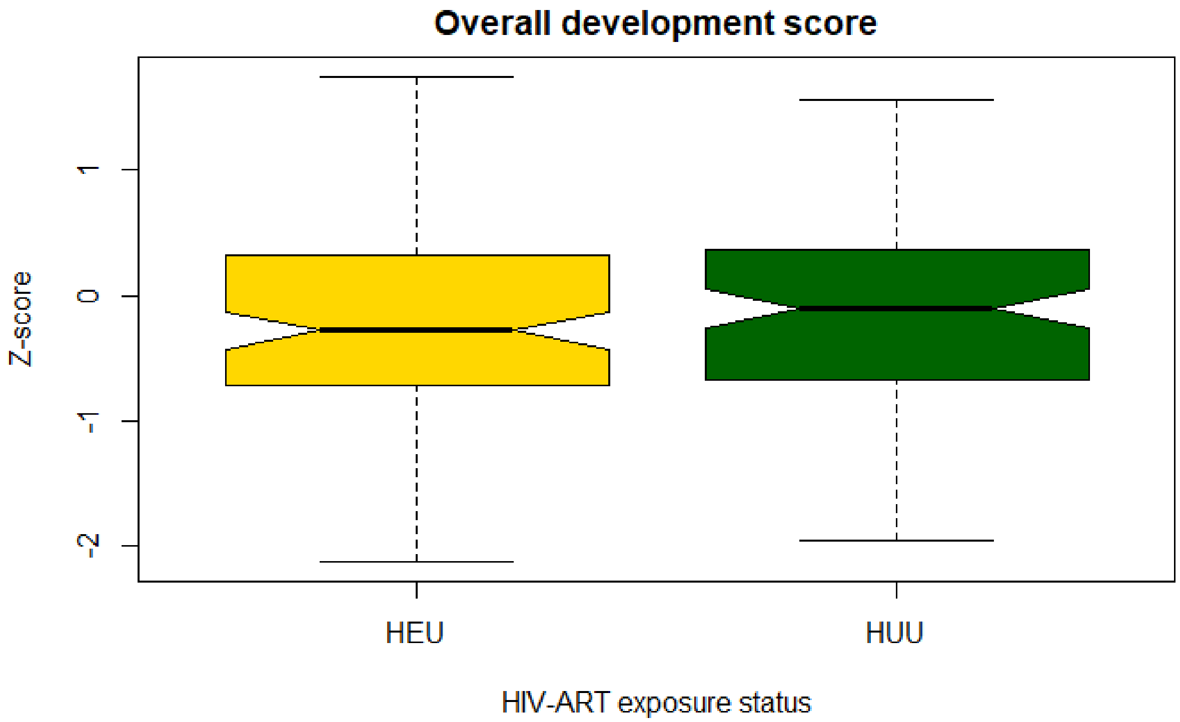


Figure 2: Distribution of overall development z score measured by CREDI between HIV-exposed uninfected (HEU) and HIV-unexposed uninfected (HUU) children at 24-35 months of age.

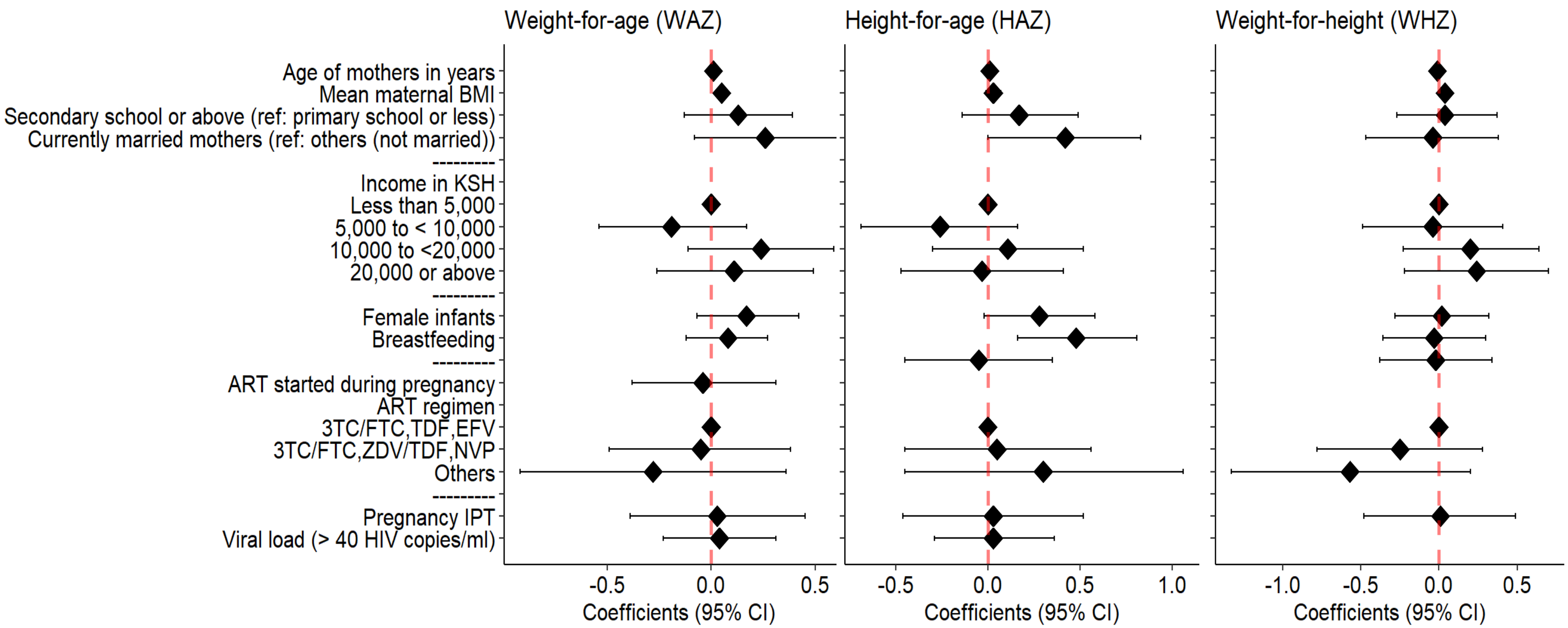


Figure 3: Coefficients (95% CI) for all cofactors among HIV exposed uninfected children

Supplemental Tables

Supplemental Table 1: Evaluation of cofactors of WAZ, and HAZ during a 2-years follow-up among HEU children

Variable		WAZ[¶]			HAZ[¥]			WHZ[α]	
	cCoefficient* (95% CI)	aCoefficient[¤] (95% CI)	P-value	cCoefficient* (95% CI)	aCoefficient[¤] (95% CI)	P-value	cCoefficient* (95% CI)	aCoefficient[¤] (95% CI)	P-value
Age of mothers in years	0.01 (-0.01, 0.03)	0.01 (-0.02, 0.03)	0.501	0.02 (-0.01, 0.04)	0.01 (-0.02, 0.04)	0.415	0.00 (-0.02, 0.03)	-0.01 (-0.04, 0.02)	0.647
Mean maternal BMI[δ]	**0.05 (0.03, 0.08)**	**0.05 (0.02, 0.08)**	**0.001**	0.18 (-0.09, 0.46)	0.03 (-0.01, 0.07)	0.096	0.04 (0.01, 0.07)	0.04 (0.00, 0.08)	0.027
Secondary school or above (ref: primary school or less)	0.15 (-0.08, 0.37)	0.13 (-0.13, 0.39)	0.330	0.18 (-0.09, 0.46)	0.17 (-0.14, 0.49)	0.280	0.11 (-0.17, 0.40)	0.04 (-0.27, 0.37)	0.773
Currently married mothers (ref: others (not married))	0.12 (-018, 0.42)	0.26 (-0.08, 0.61)	0.129	0.18 (-0.18, 0.55)	**0.42 (0.00, 0.83)**	**0.048**	0.02 (-0.37, 0.40)	-0.04 (-0.47, 0.38)	0.844
Income in KSH									
Less than 5,000	Referent	Referent		Referent	Referent		Referent	Referent	
5,000 to < 10,000	-0.04 (-0.39, 0.31)	-0.19 (-0.54, 0.17)	0.306	-0.23 (-0.66, 0.20)	-0.26 (-0.69, 0.16)	0.223	0.10 (-0.34, 054)	-0.04 (-0.49, 0.41)	0.854
10,000 to <20,000	0.27 (-0.05, 0.60)	0.24 (-0.11. 0.59)	0.183	0.09 (-0.30, 0.48)	0.11 (-0.30, 0.52)	0.609	0.22 (-0.20, 0.63)	0.20 (-0.23, 0.64)	0.359
20,000 or above	0.12 (-0.21, 0.45)	0.11 (-0.26, 0.49)	0.547	-0.01 (-0.41, 0.39)	-0.03 (-0.47, 0.41)	0.900	0.26 (-0.15, 0.68)	0.24 (-0.22, 0.70)	0.318
Female infants	0.21 (-0.02, 0.44)	0.17 (-0.07, 0.42)	0.172	0.38 (0.10, 0.65)	0.28 (-0.02, 0.58)	0.069	0.02 (-0.29, 0.32)	0.02 (-0.28, 0.32)	0.989
Breastfeeding	0.02 (-0.16, 0.21)	0.08 (-0.12, 0.27)	0.444	0.52 (0.21, 0.84)	**0.48 (0.16, 0.81)**	**0.004**	-0.09 (-0.42, 0.23)	-0.03 (-0.36, 0.30)	0.856
ART started during pregnancy	-0.08 (-0.33, 0.16)	-0.04 (-0.38, 0.31)	0.840	-0.16 (-0.45, 0.13)	-0.05 (-0.45, 0.35)	0.807	0.02 (-0.28, 0.32)	-0.02 (-0.38, 0.34)	0.910
ART regimen									
3TC/FTC,TDF,EFV	Referent	Referent		Referent	Referent		Referent	Referent	
3TC/FTC,ZDV/TDF,NVP	0.06 (-0.35, 0.47)	-0.05 (-0.49, 0.38)	0.818	0.12 (-0.38, 0.61)	0.05 (-0.45, 0.56)	0.837	-0.06 (-0.56, 0.44)	-0.25 (-0.78, 0.28)	0.357
Others	-0.07 (-0.68, 0.53)	-0.28 (-0.92, 0.36)	0.398	0.55 (-0.19,1.29)	0.30 (-0.45, 1.06)	0.429	-0.55 (-1.29, 0.18)	-0.57 (-1.33, 0.20)	0.150
Pregnancy IPT	-0.87 (-0.41, 0.23)	0.03 (-0.39, 0.45)	0.878	-0.12 (-0.50, 0.26)	0.03 (-0.46, 0.52)	0.897	-0.04 (-0.43, 0.36)	0.01 (-0.48, 0.49)	0.972
Viral load (≥ 40 HIV copies/ml)	-0.13 (-0.38, 0.13)	0.04 (-0.23, 0.31)	0.768	-0.04 (-0.34, 0.26)	0.03 (-0.29, 0.36)	0.833			

[¶]WAZ – weight-for-age. [¥]HAZ – height-for-age. [α]WHZ – weight-for-height z-score. *HEU – HIV-exposed uninfected, and HUU – HIV-uninfected unexposed.
[δ]Body mass index is the weight in kilograms divided by the square of height in meters. * cCoefficient – crude (unadjusted) coefficient. [¤]aCoefficient – coefficients adjusted for other cofactors (HIV exposure status, maternal age, education, marital status, household income, infant's sex, and breastfeeding)

Supplemental Table 2. Evaluation of cofactors of WAZ, and HAZ among HUU children

Variable	WAZ[¶]			HAZ[¥]			WHZ[ɑ]		
	cCoefficient* (95% CI)	**aCoefficient[¤] (95% CI)**	**P-value**	**cCoefficient* (95% CI)**	**aCoefficient[¤] (95% CI)**	**P-value**	**cCoefficient* (95% CI)**	**aCoefficient[¤] (95% CI)**	**P-value**
Age of mothers in years	-0.01 (-0.03, 0.02)	**-0.03 (-0.06, -0.00)**	**0.04**	0.00 (-0.03, 0.04)	-0.02 (-0.06, 0.02)	0.390	-0.001 (-0.04, 0.02)	-0.02 (-0.06, 0.02)	0.347
Mean maternal BMI[δ]	**0.04 (0.01, 0.07)**	**0.04 (0.01, 0.07)**	**0.003**	**0.05 (0.01, 0.09)**	**0.04 (0.01, 0.08)**	**0.020**	0.01 (-0.02, 0.05)	0.02 (-0.02, 0.05)	0.385
Secondary school or above (ref: primary or less)	**0.28 (0.04, 0.52)**	**0.26 (0.02, 0.49)**	**0.036**	**0.37 (0.005, 0.70)**	**0.39 (0.06, 0.73)**	**0.021**	0.11 (-0.21, 0.43)	0.00 (-0.33, 0.34)	0.996
Currently married mothers (ref: others (not married))	-0.38 (-0.68, 0.09)	**-0.37 (-0.68, -0.06)**	**0.020**	**-0.47 (-0.85, -0.08)**	**-0.50 (-0.92, -0.07)**	**0.022**	-0.11 (-0.50, 0.27)	0.00 (-0.43, 0.42)	0.984
Income in KSH									
Less than 5,000	Referent	Referent		Referent	Referent		Referent	Referent	
5,000 to < 10,000	0.06 (-0.26, 0.39)	0.07 (-0.25, 0.38)	0.685	0.05 (-0.38, 0.49)	0.00 (-0.43, 0.43)	0.997	0.03 (-0.39, 0.45)	0.08 (-0.35, 0.51)	0.713
10,000 to <20,000	**0.51 (0.14, 0.87)**	**0.48 (0.12, 0.83)**	**0.009**	0.17 (-0.33, 0.67)	0.03 (-0.46, 0.53)	0.905	**0.61 (0.11, 0.11)**	**0.67 (0.16, 0.117)**	**0.010**
20,000 or above	**0.38 (0.08, 0.68)**	0.19 (-0.11, 0.50)	0.219	0.29 (-0.12, 0.68)	-0.03 (-0.46, 0.38)	0.859	0.33 (-0.06, 0.71)	0.34 (-0.09, 0.76)	0.123
Breastfeeding	**0.25 (0.04, 0.46)**	**0.24 (0.03, 0.46)**	**0.025**	**0.46 (0.11, 0.81)**	**0.44 (0.09, 0.79)**	**0.015**	0.07 (-0.30, 0.44)	0.07 (-0.31, 0.44)	0.730
Female infants	**0.29 (0.06, 0.52)**	0.20 (-0.20, 0.42)	0.076	**0.56 (0.25, 0.87)**	**0.47 (0.17, 0.78)**	**0.003**	-0.02 (-0.32, 0.28)	-0.11 (-041, 0.20)	0.496

[¶] WAZ – weight-for-age. [¥] HAZ – height-for-age. [ɑ] WHZ – weight-for-height z-score. [δ] Body mass index is the weight in kilograms divided by the square of height in meters. * cCoefficient – crude (unadjusted) coefficient. [¤] aCoefficient – coefficients adjusted for other cofactors (HIV exposure status, maternal age, education, marital status, household income, infant's sex, and breastfeeding)

Suppleme*n*tal Table 3: Effect cofactors on overall development z-score assessed by CREDI questionnaire at 24-35 months among 24-35 months of age HEU children

Variable	**Overall development z-score**		
	cCoefficient* (95% CI)	**aCoefficient[¤] (95% CI)**	**P-value**
Age of mothers in years	0.00 (-0.03, 0.02)	0.00 (-0.02, 0.03)	0.746
Mean maternal BMI[δ]	-0.02 (-0.06, 0.01)	-0.02 (-0.06, 0.01)	0.211
Secondary school or above (ref: primary school or less)	**0.34 (0.05, 0.63)**	**0.44 (0.13, 0.76)**	**0.006**
Female infants	-0.03 (-0.32, 0.26)	0.02 (-0.29, 0.32)	0.918
Birth weight (kg)	0.12 (-0.13, 0.38)	0.08 (-0.18, 0.35)	0.516
Breastfeeding at 24 months	0.34 (0.04, 0.64)	**0.34 (0.01, 0.66)**	**0.041**
ART started during pregnancy	-0.13 (-0.44, 0.18)	-0.23 (-0.58, 0.12)	0.199
ART regimen			
3TC/FTC,TDF,EFV	Referent	Referent	
3TC/FTC,ZDV/TDF,NVP	-0.03 (-0.57, 0.50)	-0.10 (-0.63, 0.43)	0.711
Others	-0.18 (-0.86, 0.49)	-0.47 (-1.19, 0.25)	0.195
Viral load (≥ 40 HIV copies/ml)	-0.01 (-0.33, 0.32)	0.01 (-0.33, 0.34)	0.973

[δ] Body mass index is the weight in kilograms divided by the square of height in meters. * cCoefficient – crude (unadjusted) coefficient. [¤] aCoefficient – coefficients adjusted for other cofactors (HIV exposure status, maternal age, education, ART timing, ART regimen, viral load, infant's sex, and breastfeeding)

Supplemental Table *4*: Effect cofactors on overall development z-score assessed by CREDI questionnaire among 24-35 months of age H*U*U children

Variable	**Overall development z-score**		
	cCoefficient* (95% CI)	**aCoefficient[¤] (95% CI)**	**P-value**
Age of mothers in years	0.01 (-0.02, 0.05)	0.03 (-0.01, 0.06)	0.140
Mean maternal BMI[δ]	-0.02 (-0.05, 0.01)	-0.03 (-0.06, 0.06)	0.097
Secondary school or above (ref: primary school or less)	0.13 (-0.18, 0.44)	0.16 (-0.16, 0.49)	0.320
Female infants	0.09 (-0.20, 0.39)	0.14 (-0.17, 0.45)	0.365
Birth weight (kg)	-0.08 *-0.37, 0.22)	-0.03 (-0.33, 0.26)	0.817
Breastfeeding at 24 months	0.29 (-0.05, 0.63)	0.32 (-0.04, 0.68)	0.080

[δ] Body mass index is the weight in kilograms divided by the square of height in meters. * cCoefficient – crude (unadjusted) coefficient. [¤] aCoefficient – coefficients adjusted for other cofactors (HIV exposure status, maternal age, education, infant’s sex, and breastfeeding)

Supplemental material: Short for CREDI questionnaire.

This questionnaire is used to derive the overall development score, but not the specific domains of development (motor, language, cognition, social-emotional, and mental health). We used the short form CREDI for 24-29 months of age but it could also be applied to children 30-35 months of age.

Caregiver Reported Early Development Instruments (CREDI)
Short Form: 24-29 MONTHS

Assessor Instructions:

- Administer the set of questions that corresponds with the child’s age band.
- Remember to show caregivers the corresponding full-page illustration for those items that include an image.
- Before administering items, SAY (to caregivers):

Now I am going to ask you about the types of things your child is currently able to do. Please answer "yes" or "no" to these questions. If you are unsure, you can also answer by saying “don’t know.” Please keep in mind that children learn and grow at different rates, so it is fine if your child can't yet do these things. Some of these skills children only achieve at older ages. If there is any question you feel uncomfortable answering, please let me know and we can move to the next question.

Item #	Item	Image	Response		
E1	If you show the child an object he/she knows well (e.g., a cup or animal), can he/she consistently name it?		Yes	No	DK
E2	Can the child say ten or more separate words (e.g., names like "Mama" or objects like "ball")?		Yes	No	DK
E3	Can the child sing a short song or repeat parts of a rhyme from memory by him/herself?		Yes	No	DK
E4	Can the child jump with both feet leaving the ground?		Yes	No	DK
E5	Can the child speak using sentences of three or more words that go together (e.g., "I want water" or "The house is big")?		Yes	No	DK
E6	Can the child correctly ask questions using any of the words "what," "which," "where," or "who"?		Yes	No	DK
E7	Can the child correctly use any of the words "I," "you," "she," or "he" (e.g., "I go to store," or "He eats rice")?		Yes	No	DK
E8	Does the child ask about familiar people other than parents when they are not there (e.g., "Where is the neighbor?")?		Yes	No	DK
E9	Can the child count up to five objects (e.g., fingers, people)?		Yes	No	DK
E10	Can the child identify at least one color (e.g., red, blue, yellow)?		Yes	No	DK
E11	Does the child often kick, bite, or hit other children or adults?		Yes	No	DK
E12	If you show the child two objects or people of different size, can he/she tell you which one is the big one and which is the small one?		Yes	No	DK

E13	Does the child become extremely withdrawn or shy in new situations?		Yes	No	DK
E14	If you point to an object, can the child correctly use the words "on," "in," or "under" to describe where it is (e.g., "The cup is on the table" instead of "The cup is in the table.").		Yes	No	DK
E15	Does the child ask "why" questions (e.g., "Why are you tall?")?		Yes	No	DK
E16	If you ask the child to give you three objects (e.g., stones, beans), does the child give you the correct amount?		Yes	No	DK
E17	Can the child explain in words what common objects like a cup or chair are used for?		Yes	No	DK
E18	Can the child dress him/herself (e.g., put on his/her pants and shirt without help)?		Yes	No	DK
E19	Can the child say what others like or dislike (e.g., "Mama doesn't like fruit," "Papa likes football")?		Yes	No	DK
E20	Can the child talk about things that have happened in the past using correct language (e.g., "Yesterday I played with my friend" or "Last week she went to the market")?		Yes	No	DK

Chapter 2: Effect of pregnancy versus postpartum maternal isoniazid preventive therapy on infant growth in HIV-exposed uninfected infants

Abstract

Background: IPT initiation during pregnancy was associated with increased incidence of adverse pregnancy outcomes in the TB APPRISE trial. Effects of *in utero* IPT exposure on infant growth are unknown.

Methods: This post-hoc analysis used data from TB APPRISE, a multicenter, double-blind, placebo-controlled trial, which randomized women to 28-week IPT starting in pregnancy (pregnancy-IPT) or postpartum week 12 (postpartum-IPT). We used overall and sex-stratified multivariable Cox proportional hazards regression to compare time to infant growth faltering between arms to 12 weeks and 48 weeks postpartum.

Results: Among 898 HIV-exposed uninfected (HEU) infants, 447 (49.8%) were females. Infants in pregnancy-IPT had a 1.47-fold higher risk of becoming underweight by 12 weeks (aHR 1.47 [95% CI: 1.06, 2.03]) than infants in the postpartum-IPT; increased risk persisted to 48 weeks postpartum (aHR 1.34 [95% CI: 1.01, 1.78]). Maternal IPT timing was not associated with stunting or wasting. In sex-stratified analyses, male infants in the pregnancy-IPT arm experienced an increased risk of lower birthweight (LBW) (aRR 2.04 [95% CI: 1.16, 3.68), preterm birth (aRR 1.81 [95% CI: 1.04, 3.21]) and becoming underweight by 12 weeks (aHR 2.02 [95% CI: 1.29, 3.18]) and 48 weeks (aHR 1.82 [95% CI: 1.23, 2.69]). Maternal IPT timing did not influence growth in female infants.

Conclusion: Maternal IPT during pregnancy was associated with an increased risk of LBW, preterm birth, and becoming underweight among HEU infants, particularly male infants. These data add to prior

TB APPRISE data, suggesting that IPT during pregnancy impacts infant growth, which could inform management, and warrants further examination of mechanisms.

Background:

Prevention of tuberculosis (TB) among women living with HIV (WLWH) is a high priority in TB endemic areas (19). Children born to WLWH who are HIV-exposed but uninfected (HEU) have a higher risk of TB exposure, infection, and TB-related morbidity and mortality compared to HIV-unexposed uninfected (HUU) children (58). Thus, the World Health Organization (WHO) recommends TB preventive therapy such as isoniazid preventive therapy (IPT), which reduces the risk of progression from TB infection to TB disease (59,60), for PLWH, including during pregnancy (20).

Observational studies on pregnant women, primarily secondary analyses, did not reveal associations of pregnancy IPT with adverse pregnancy outcomes (25,26). Until recently, safety data regarding IPT in pregnancy rigorously assessed in a trial have been lacking. The TB APPRISE trial evaluated the safety of the immediate (pregnancy) IPT) vs. deferred (postpartum) IPT in WLWH on antiretroviral therapy (ART) (29). In this study, although pregnancy-IPT was as safe as postpartum-IPT with regards to adverse maternal outcomes, pregnancy-IPT was associated with an increased incidence of composite adverse pregnancy outcomes (stillbirth or spontaneous abortion, low birth weight (LBW), preterm delivery, or infant congenital anomalies) (29).

HEU children are exposed to HIV and ART in-utero, both of which may increase the risk of preterm birth, LBW and low birth length, small for gestational age (SGA), stillbirth (3–5,7), and growth compromise (61). The potential effect of *in utero* IPT exposure on long-term growth in HEU infants is not known. The TB APPRISE study provides an opportunity to compare the effect of maternal pregnancy-IPT versus postpartum-IPT on the growth of HEU infants in the first year of life. We compared randomized arms in the TB APPRISE trial for differences in infant growth outcomes and evaluated cofactors of growth among HEU infants. Additionally, we evaluated whether infant sex-modified maternal IPT effect on birth outcomes and infant growth.

Methods

Parent trial design and intervention*:* This post-hoc analysis utilized data from the TB APPRISE trial – a randomized, double-blind, placebo-controlled, multicenter study to evaluate pregnancy vs. postpartum IPT with respect to maternal safety. The trial, as reported in detail previously (29), was conducted in eight countries at 13 different sites with high TB prevalence (>60 cases per 100,000). Participants were randomized to receive a 28-week course of IPT (300 mg daily) either during pregnancy (pregnancy-IPT) or at postpartum week 12 (postpartum-IPT). The pregnancy-IPT arm received isoniazid daily for 28 weeks (initiated between 14- and 34-weeks gestation, immediately after enrollment), then switched to placebo until the 40th week postpartum. The postpartum-IPT arm initiated placebo immediately after trial entry during pregnancy until the 12th week postpartum and then switched to isoniazid daily until the 40th week postpartum. All participants received vitamin B6 and a prenatal multivitamin from week 0 to week 40 postpartum. The randomization was stratified by the gestational age at trial entry (≥14 weeks to <24 weeks or ≥24 weeks to ≤34 weeks) and was balanced between arms at each site.

Participants and study period*:* Pregnant WLWH, 14 to 34 weeks of gestation, weighing >35 kg, with >750 absolute neutrophil count cells/mm^3, >7.5 gm/dL hemoglobin, and >50,000 platelets count/mm^3 were eligible. Participants were required to have enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and total bilirubin) at or below 1.25 times the upper limit of the normal range within 30 days prior to study entry. Women with active TB, recent TB exposure, TB treatment for more than 30 days in the previous year, or peripheral neuropathy of grade 1 or higher were excluded. The original study included 956 participants, 477 randomized to pregnancy-IPT and 479 to postpartum-IPT arm. Participants were enrolled between August 2014 and April 2016.

This analysis was restricted to HEU infants born to mothers participating in the RCT. Exclusion criteria for this analysis included lack of infant information (withdrawal from the study before birth, still birth, or lack of any growth measurement), HIV infection of the infant, and twin births.

Infant growth characterization: Mother-infant pairs were followed up to 48 weeks postpartum. Weight and length of infants were measured at birth, 4th, 8th, 12th, 24th, 36th, 44th, and 48th weeks postpartum to the nearest 0.1 kg and 0.1 cm. There was a two-week extension period for mothers who did not attend their last visit. Missing values at the scheduled last visit were replaced by measurements within two weeks after the end of the study.

Low birth weight (LBW) was defined as less than 2.5 kg regardless of gestational age. Birth before completion of 37 weeks of pregnancy was regarded as preterm. Small for gestational age (SGA) was defined by weight less than the 10th percentile for gestational age using INTERGROWTH standards (62).

Weight-for-age z-score [WAZ], weight-for-length z-score [WLZ], and length-for-age z-score [LAZ]) were defined using WHO child growth standards (45). Growth faltering was less than 2 Z-scores; with underweight defined as WAZ <-2, wasting WHZ <-2, and stunting LAZ <-2.

Cofactors of growth faltering: Cofactors of growth faltering assessed in the analyses included: maternal characteristics at enrollment, including body mass index (BMI), age, ART regimen, viral non-suppression (viral load $\geq$ 40 copies/ml), CD4 count (cells/mm^3), education, household food insecurity (experiencing lack of resources to get food, or going to sleep hungry in the past 30 days, and/ or passing whole day and night hungry), and infant sex.

Statistical analysis:

Means and standard deviations (SDs) were used to describe normally distributed continuous variables, medians and interquartile ranges (IQRs) to describe skewed distributions, and frequencies and percentages to describe categorical variables. Baseline maternal and infant characteristics were compared between pregnancy-IPT and postpartum-IPT randomization groups using two-sided t-tests (Mann-Whitney U tests if assumptions were not met) for continuous variables and Pearson χ^2 tests (Fisher's exact tests if assumptions were not met) for categorical variables.

Because randomization was carried out on pregnant women in the primary study and some women had an abortion, were stillbirth, or were withdrawn from the study, the randomization groups were compared in modified intent-to-treat analyses adjusted for predetermined potential confounding variables.

Adverse birth outcomes: Effects of pregnancy-IPT on LBW and preterm birth were examined in the primary trial publication; however, sex-stratified analyses of these outcomes were not conducted. We examined the effects of pregnancy-IPT on birth outcomes (LBW, preterm birth, and SGA) using generalized linear models with a Poisson family and a log link (to estimate relative risks) in overall and sex-stratified analyses. Multivariable generalized linear models were fitted to control potential confounders.

Growth faltering during infancy: Mothers in the postpartum-IPT arm initiated IPT at 12 weeks after delivery, therefore data was censored at 12-week postpartum to examine the effect of pregnancy-IPT compared to no IPT during pregnancy and early postpartum. In addition, to compare the longer-term effects of pregnancy-IPT on growth faltering, randomized arms were compared up to 48 weeks after birth. Growth faltering was compared between randomized groups using Cox proportional hazards regression models and generalized estimated equations (GEE).

We used Kaplan-Meier survival analysis to compare, unadjusted, time to the first event of growth faltering (underweight, wasting, or stunting) to 12 weeks postpartum and 48 weeks postpartum in overall and sex-stratified analyses. Univariate and multivariable Cox proportional hazards regression models and models including interaction terms between randomization arm and infants' sex were fit to compare the risk of experiencing the first episode of growth faltering between the randomized groups and any effect modification by infant sex. For these analyses, time zero was the randomization date, and no failure (no growth faltering) was assumed prior to birth. Infants lost to follow-up or who died prior to failure were censored at their last visit date. Growth data from visits following growth faltering were censored. We used multivariable Cox proportional hazards regression models to identify cofactors (maternal BMI, age, ART regimen, viral non-suppression, CD4 count (cells/mm3), education, and household food insecurity and infant sex) of growth faltering. As fewer than 5% of at-risk participants remained in the study after 60 post-randomization weeks, the values at 60th week and after were censored.

Univariate and multivariable generalized estimated equations (GEE) were fit with a Poisson family and a log link (to estimate relative risk) and exchangeable correlation structure to compare risks of growth faltering (underweight, wasting, and stunting) at any time up until 12 weeks postpartum and up to 48 weeks, as well as testing for interaction by infants' sex and analyses stratified by sex. Infants who experienced growth faltering anytime were not censored in this analysis. The multivariate GEE model was used to identify cofactors of growth faltering in HEU infants. We used R version 4.1.0 for analyses.

Results

Maternal and infant baseline characteristics

In this study, 898 infants were included: 448 in the pregnancy-IPT and 450 in the postpartum-IPT arm (Figure 1).

The median age of mothers at enrollment was 29 years (IQR 24-33), and median BMI was 26.2 (IQR 23.3-29.7). All mothers were on ART and the majority received either efavirenz-tenofovir-lamivudine (EFV, TDF, 3TC, 57.3% (480/850)) or efavirenz-tenofovir-emtricitabine (EFV, TDF, FTC, 26.2% (222/850)). Almost half (49.2%) of mothers had a CD4 count above 500 cells/mm3, and 37.6% (316/850) of mothers had a viral load of >40 HIV RNA copies/ml. Almost half (447/898) of the infants were female – 228 in the pregnancy-IPT arm and 219 in the postpartum-IPT arm. Baseline maternal and infant demographic and clinical characteristics were similar between randomized arms (Table 1).

Maternal and infant TB status throughout the study period

As reported in the primary analysis, six mothers (0.4%) and one infant (0.1%) developed TB, and 241 mothers (32.4%) and 41 (5.8%) infants tested positive on a QuantiFERON-TB Gold In-Tube (QGIT) test. There was no significant difference in TB disease or infection incidence in mothers or infants between arms.

Effect of pregnancy-IPT on adverse birth outcomes – underweight, preterm, and SGA

Overall, 8.6% of infants were premature, 10.5% were LBW, and 18.8% were SGA at birth. Adjusted for relevant cofactors, infants in the pregnancy-IPT arm had a 1.60-fold higher risk of LBW (aRR 1.60 [95% CI: 1.07, 2.41]) than infants in the postpartum-IPT arm (Table 2). There was no significant difference in risk of being preterm (aRR 1.31 [95% CI: 0.87, 1.97]) or SGA (aRR 0.97 [95% CI: 0.71, 1.32]) between randomization arms.

In sex-stratified analyses adjusted for all cofactors, male infants in the pregnancy-IPT arm had a 2.04-fold higher risk of LBW (aRR 2.04 [95% CI: 1.16, 3.68) and 1.81-fold increased risk of preterm birth (aRR 1.81 [95% CI: 1.04, 3.21]) than those in the postpartum-IPT arm. Pregnancy-IPT was not associated with LBW or preterm delivery among female infants or SGA among male and female infants (Table 2).

Risk of experiencing growth faltering during infancy

The overall risk of being underweight during the 48-week follow-up period was 22.8 per 100 person-years (95% CI: 19.4, 26.0), risk of stunting was 40.1 per 100 person-years (95% CI: 35.9, 44.1), and risk of wasting was 32.8 per 100 person-years (95% CI: 28.9, 36.5).

In univariate analysis, male infants in the pregnancy-IPT had a significantly higher cumulative probability of being underweight (30.5 per 100 person-years [95% CI: 22.02, 38.1] vs 19.7 per 100 person-years [95% CI: 13.5, 25.5]), p=0.041) than male infants in the postpartum-IPT arm (Figure 2), but the same cumulative probability of stunting and wasting. Pregnancy-IPT has no statistically significant effect on the cumulative probability of being underweight, stunting, and wasting in females, p-value > 0.05.

In multivariable Cox regression models, pregnancy-IPT was associated with infant underweight analyses to 12 weeks and 48 weeks postpartum. Infants in the pregnancy-IPT arm experienced a 1.47-fold higher risk of becoming underweight in the first 12 weeks (aHR 1.47 [95% CI: 1.06, 2.03]) and a 1.34-fold higher risk of becoming underweight in the first 48 weeks (aHR 1.34 [95% CI: 1.01, 1.78]) than infants in the postpartum-IPT arm. Maternal IPT timing was not associated with stunting (aHR by 12 weeks 1.12 [95% CI: 0.91, 1.39] and aHR by 48 weeks 1.08 [95% CI: 0.89, 1.30]) or wasting (aHR by 12 weeks 1.09 [95% CI: 0.81, 1.45] and aHR by 48 weeks 1.02 [95% CI: 0.79, 1.32]) (Table 3).

Infant sex significantly modified the effect of pregnancy-IPT on underweight in analyses to 12 weeks (p-value=0.037) and 48 weeks (p-value=0.022). Male infants in the pregnancy-IPT arm experienced a

2.02-fold increased risk of becoming underweight in the first 12 weeks (aHR 2.02 [95% CI: 1.29, 3.18) and a 1.82-fold increased risk of becoming underweight in the first 48 weeks (aHR 1.82 [95% CI: 1.23, 2.69]) than male infants in the postpartum-IPT arm (Table 3). Pregnancy-IPT has no statistically significant effect on growth in females, p-value > 0.05.

There was also a statistically significant sex-specific effect of pregnancy-IPT on wasting in male and female infants by 12 weeks (p-value=0.021), but not by 48 weeks (p-value=0.057). Male infants in the pregnancy-IPT arm experienced a 1.61-fold higher risk of becoming wasted in the first 12 weeks (aHR 1.61 [95% CI: 1.04, 2.49), and a 1.43-fold higher risk (nonsignificant) of becoming wasted in the first 48 weeks (aHR 1.40 [95% CI: 0.95, 2.06) than male infants in the postpartum-IPT arm (Table 3).

Among female infants, pregnancy-IPT was not associated with growth faltering – underweight (aHR at 12 weeks 0.97 [95% CI: 0.59, 1.58] and aHR at 48 weeks 0.90 (95% CI: 0.59, 1.38]), stunting (aHR at 12 weeks 0.98 [95% CI: 0.71, 1.35] and aHR at 48 weeks 1.00 [0.75, 1.33), and wasting (aHR at 12 weeks 0.76 [95% CI: 0.51, 1.12] and aHR at 48 weeks 0.79 [95% CI: 0.55, 1.13]).

We also fitted GEE multivariable models to estimate the repeated prevalence of growth faltering during infancy which yielded similar results to Cox regression analyses (Supplemental tables 1).

Cofactors of growth faltering

For every 1 kg/m^2 increase in maternal BMI, infant risk of underweight decreased by 7% (aHR 0.93 [95% CI: 0.90, 0.96]), and wasting risk decreased by 8% (aHR 0.92 [95% CI: 0.89, 0.94]) (Table 4).

For every additional year of maternal age, the risk of wasting in infants increased by 4% (aHR 1.04 [95% CI: 1.01, 1.06]), while the risk of stunting decreased by 2% (aHR 0.98 [95% CI: 0.96, 1.00]). Infants born to mothers who used NVP-regimens experienced a 1.6-fold increased risk of becoming underweight (aHR 1.62 [95% CI: 1.09, 2.41]) and 1.34 increased risk of stunting (aHR 1.34 [95% CI: 1.00, 1.79]) compared to EFV,TDF,3TC/FTC regimens. Infants born from mothers who have secondary education have 21% lower risk of stunting (aHR 0.79 [95% CI: 0.64, 0.98]) but a 45% higher risk of wasting (aHR 1.45 [1.04, 2.03]) (Table 4).

Multivariable GEE models used to assess cofactors of growth faltering yielded similar results as Cox proportional hazard regression models above (Supplemental Table 2).

Discussion

In this post-hoc analysis of a multi-site RCT evaluating maternal IPT in pregnancy versus postpartum, timing of maternal IPT influenced growth outcomes among HEU infants with a significantly higher risk of underweight among infants born to mothers in the pregnancy-IPT arm. There was effect modification of associations of IPT with growth by infant sex, with significantly increased underweight and wasting in males born to mothers in the pregnancy-IPT versus postpartum-IPT arms. Our findings suggest that the timing of maternal IPT may influence birth size as well as postnatal growth in infants and provide valuable data for policymakers and clinicians considering the optimal timing of IPT in pregnant WLWH.

Our data suggest growth-altering effects of *in utero* IPT exposure. Infants born to mothers randomized to pregnancy-IPT had a 1.60-fold higher risk of LBW and of becoming underweight during the first year of life than infants born to mothers randomized to postpartum-IPT. During pregnancy, IPT causes embryocidal effects on rats and rabbits, delays neurodevelopment in zebrafish, and affects postnatal growth, development, and cognitive ability in rats (27,63,64). In vitro studies have demonstrated cytotoxic effects of IPT that disturb the cell cycle in mammalian cells (64). Antenatal IPT may induce poor appetite, nausea, emesis, and hepatic changes in the mother that could, in turn, affect infant growth (59,65). In addition, since isoniazid crosses placenta barrier (27), direct drug effects on the fetus could influence growth.

We found that associations between pregnancy-IPT and growth were modified by infant sex. Male infants in the pregnancy-IPT arm had a significantly higher risk of preterm birth, LBW, and longer-term growth faltering than male infants in the postpartum-IPT arm. In contrast, birth outcomes and growth in female infants were not affected by pregnancy-IPT. *In utero* growth trajectories differ by sex; male fetuses typically grow faster (66) and may not alter their growth trajectory when facing adverse challenges (67–70) Due to the continued growth without adaptations early in pregnancy, male fetuses exposed to adverse intrauterine exposure (maternal health/social/environmental challenges) may have adverse outcomes later in pregnancy (67,68), including growth retardation (71,72), than female infants. Placental gene transcription differences (68,73), prenatal testosterone exposure (74), differences in expression of sex-specific genes (XX-specific versus XY-specific) (67,68,73,74), and maternal glucocorticoids (68) influence sex-related differential adaptation response to intrauterine exposure. These factors may contribute to the observed sex-differential growth associations related to *in utero* IPT. Our findings also suggest that *in utero* IPT continues to affect male growth after birth.

Pregnancy-IPT was not associated with overall or sex-stratified SGA. The fact that male infants in pregnancy-IPT had a 1.81 higher risk of preterm birth while there was no difference in SGA risk than male infants in postpartum-IPT suggests that the mechanism for LBW in male infants may be through preterm birth.

In addition to our primary goal of defining the impact of pregnancy IPT exposure on infant growth, we assessed other cofactors of growth in HEU infants. We found expected associations between maternal BMI and being underweight. Nutritional status during pregnancy affects the fetus's nutrition, potentially altering its growth. We also found that stunting risk decreased by 2% for every additional year of maternal age. This is consistent with a study using data from 18 countries' Demographic Health Surveys, which found that infants of young mothers had lower heights than infants born to older mothers (75). Infants of young mothers may be more prone to intrauterine growth retardation (66). The maternal use of nevirapine regimens was associated with growth faltering; potential mechanisms for this association are unclear.

This study has multiple strengths, including randomized allocation of IPT timing, excellent retention, and sufficient sample size to investigate growth outcomes. The study also has limitations. This post-hoc analysis was designed after the primary RCT was completed. After 12 weeks of age, infants in the deferred arm received postpartum IPT, so there is not a no-IPT comparator after this timepoint. However, in analyses that excluded the time period with exposure to postpartum IPT, growth effects of pregnancy-IPT remained similar.

Conclusion: In this post-hoc analysis, maternal IPT during pregnancy was associated with a significantly increased risk of LBW and risk of becoming underweight among HEU infants. Male infants exposed to pregnancy-IPT had a significant risk of LBW, preterm birth, and longer-term risk of being underweight that persisted over the first year of life. These data add to prior TB APPRISE findings of increased risk of composite adverse birth outcomes associated with IPT during pregnancy, suggesting IPT during pregnancy also impacts the birth size and infant growth specifically among male infants. These data could inform monitoring and management and warrants further examination of potential mechanisms.

Tables

Table 4: Baseline Maternal and Infant Demographics and Clinical Characteristics

Characteristics	Randomized to start IPT during		P-value
	Pregnancy (n=448)	Postpartum (n=450)	
Maternal characteristics at baseline			
Median age (IQR) – years	29 (25-33)	29 (24-33)	0.362
Education achievement of mothers – no. (%)			
Primary school completed or less	106 (45.7)	126 (54.3)	0.319
Secondary school	306 (51.2)	292 (48.8)	
Some college education	36 (52.9)	32 (47.1)	
Median body mass index (IQR)[¥]	26.3 (23.5-30.2)	26.1 (23.1-2.59)	0.306
Median CD4 count (IQR)	493.5 (360-678.3)	497 (356.5-665.5)	0.701
CD4 count (cells/mm^3)			
<200	27 (46.6)	31 (53.4)	0.846
200-500	201 (50.5)	197 (49.5)	
>500	218 (49.5)	222 (50.5)	
ART regimen – no. (%)			
Efavirenz–tenofovir–emtricitabine/lamivudine	373 (50.0)	373 (50.0)	0.388
Efavirenz–zidovudine–lamivudine	3 (27.3)	8 (72.7)	
Nevirapine–zidovudine or tenofovir–emtricitabine or lamivudine	58 (49.2)	60 (50.8)	
Lopinavir or atazanavir–ritonavir with tenofovir or Zidovudine–emtricitabine or lamivudine	12 (63.2)	7 (36.8)	
Efavirenz only	1	0	
Median HIV-1 RNA copies /ml (IQR)	39 (39-107)	39 (39-138)	0.129
Viral load (≥40 HIV copies /ml) – no. (%)	165 (36.9)	173 (38.5)	0.667
Cotrimoxazole use – no. (%)	198 (44.3)	189 (42.1)	0.544
Positive IGRA status – no./total no. (%)	127/442 (28.7)	134/445 (30.1)	0.842
Mean gestation age at enrollment ± sd	26.1 ± 5.3	25.8 ± 5.3	0.419
Infant characteristics at birth			
Female infants – no. (%)	228 (49.1)	219 (51.3)	0.548
Mean birthweight in kilogram (SD)	3.0 (0.6)	3.0 (0.6)	0.606
Low birthweight – no./total no. (%)[¶]	59/425 (13.9)	40/433 (9.2)	**0.043**
Preterm birth – no. (%)	54 (12.1)	42 (9.3)	0.226
Small for gestational age – no./total no. (%)[¤]	79/425 (18.6)	86/433 (19.7)	0.699

[¥] Body mass index is the weight in kilograms divided by the square of height in meters. [¤] Preterm is a baby born before the 37th week of gestation. [§] Small for gestation age is defined as weight less than 10th percentile for gestational age using intergrowth growth standards. [¶] Low birth weight is an infant born weighing 5.5 pounds (2.5 kilograms) or less.

Table 5: Risks of low birth weight, preterm, and small for gestational age overall and stratified by infant sex

Analysis	Model	Isoniazid started during	Low birthweight			Preterm			Small for gestational age		
			cRR[¥] (95% CI)	aRR[¤] (95% CI)	P-value	cRR[¥] (95% CI)	aRR[¤] (95% CI)	P-value	cRR[¥] (95% CI)	aRR[¤] (95% CI)	P-value
Birth	Overall	Pregnancy	**1.50 (1.01, 2.26)**	**1.60 (1.07, 2.41)**	**0.022**	1.29 (0.86, 1.94)	1.31 (0.87, 1.97)	0.197	0.94 (0.69, 1.27)	0.96 (0.71, 1.31)	0.818
		Postpartum	1	1		1	1		1	1	
	Male infants	Pregnancy	**1.87 (1.08, 3.35)**	**2.04 (1.16, 3.68)**	**0.015**	**1.79 (1.04, 3.15)**	**1.81 (1.04, 3.21)**	**0.038**	1.12 (0.74, 1.69)	1.21 (0.79, 1.84)	0.373
		Postpartum	1	1		1	1		1	1	
	Female infants	Pregnancy	1.18 (0.66, 2.13)	1.25 (0.69, 2.27)	0.468	0.87 (0.47, 1.60)	0.94 (0.50, 1.75)	0.854	0.75 (0.47, 1.19)	0.70 (0.43, 1.12)	0.149
		Postpartum	1	1		1	1		1	1	

[¥] cRR – crude relative risk. [¤] aRR – relative risk-adjusted for maternal body mass index (weight in kilograms divided by the square of height in meters), age in years, ART regimen, viral suppression, CD4 count, education, and household food insecurity. [¶] Small for gestation age is defined as less than or equal to 10th percentile weight for gestational age using intergrowth growth standards

Table 6: Risks of growth faltering overall and stratified by infant sex in analyses to 12 and 48 weeks postpartum

			Underweight[ş]			Stunting[¶]			Wasting[β]		
			cHR[¥] (95% CI)	aHR[¤] (95% CI)	P-value	cHR[¥] (95% CI)	aHR[¤] (95% CI)	P-value	aHR[¥] (95% CI)	aHR[¤] (95% CI)	P-value
12-week postpartum	Overall	Pregnancy	1.37 (0.99, 1.89)	**1.47 (1.06, 2.03)**	**0.021**	1.10 (0.89, 1.36)	1.12 (0.91, 1.39)	0.280	1.11 (0.83, 1.47)	1.09 (0.81, 1.45)	0.577
		Postpartum	1	1		1	1		1	1	
	Male infants	Pregnancy	**1.83 (1.18, 2.84)**	**2.02 (1.29, 3.18)**	**0.002**	1.21 (0.91, 1.62)	1.22 (0.91, 1.64)	0.187	1.43 (0.94, 2.19)	**1.61 (1.04, 2.49)**	**0.031**
		Postpartum				1	1		1	1	
	Female infants	Pregnancy	0.97 (0.60, 1.56)	0.97 (0.59, 1.58)	0.889	0.98 (0.72, 1.34)	0.98 (0.71, 1.35)	0.903	0.88 (0.60, 1.29)	0.76 (0.51, 1.12)	0.168
		Postpartum	1	1		1	1		1	1	
48 weeks postpartum	Overall	Pregnancy	1.26 (0.96, 1.67)	**1.34 (1.01, 1.78)**	**0.042**	1.05 (0.86, 1.26)	1.08 (0.89, 1.30)	0.460	1.03 (0.80, 1.33)	1.02 (0.79, 1.32)	0.871
		Postpartum	1	1		1	1		1	1	
	Male infants	Pregnancy	**1.66 (1.13, 2.44)**	**1.82 (1.23, 2.69)**	**0.003**	1.11 (0.85, 1.45)	1.13 (0.86, 1.48)	0.385	1.23 (0.84, 1.79)	1.40 (0.95, 2.06)	0.091
		Postpartum	1	1		1	1		1	1	
	Female infants	Pregnancy	0.92 (0.61, 1.40)	0.90 (0.59, 1.38)	0.630	0.99 (0.75, 1.30)	1.00 (0.75, 1.33)	0.988	0.88 (0.62, 1.25)	0.79 (0.55, 1.13)	0.204
		Postpartum	1	1		1	1		1	1	

[ş]Underweight – defined as weight-for-age (WAZ)<-2). [¶]Wasting – defined as weight-for-length (WLZ)<-2. [β]Stunting – defined as length-for-age (LAZ)<-2. [¥]cHR – crude hazard ratio. [¤]HRa – hazard ratio adjusted for maternal body mass index (weight in kilograms divided by the square of height in meters), age in years, ART regimen, viral suppression, CD4 count, education, and household food insecurity.

Table 7: Cofactors of risk of growth faltering in the overall cohort of HEU infants in analysis to 48 weeks postpartum

Variables	Underweight[s]			Stunting[¶]			Wasting[β]		
	cHR[¥] (95% CI)	aHR[¤] (95% CI)	P-value	cHR[¥] (95% CI)	aHR[¤] (95% CI)	P-value	aHR[¥] (95% CI)	aHR[¤] (95% CI)	P-value
IPT started during pregnancy	1.26 (0.96, 1.67)	**1.34 (1.01, 1.78)**	**0.042**	1.05 (0.86, 1.26)	1.08 (0.89, 1.30)	0.460	1.06 (0.82, 1.38)	1.05 (0.81, 1.37)	0.717
Male infant	1.23 (0.93, 1.63)	1.27 (0.96, 1.69)	0.095	1.14 (0.94, 1.37)	1.14 (0.94, 1.38)	0.197	0.80 (0.61, 1.03)	0.82 (0.63, 1.07)	0.140
Mother's BMI[§]	**0.93 (0.90, 0.96)**	**0.93 (0.90, 0.96)**	**<0.001**	**0.98 (0.96, 1.00)**	0.98 (0.96, 1.00)	0.075	**0.93 (0.90, 0.96)**	**0.92 (0.89, 0.95)**	**<0.001**
Age of a mother in years	1.00 (0.97, 1.02)	1.00 (0.97, 1.03)	0.998	**0.98 (0.96, 1.00)**	**0.98 (0.96, 1.00)**	**0.035**	1.03 (1.01, 1.05)	**1.04 (1.01, 1.06)**	**0.004**
Art regimen									
3TC/FTC,TDF,EFV	1	1		1	1		1	1	
3TC, ZDV, EFV	0.82 (0.20, 3.29)	0.89 (0.22, 3.65)	0.876	2.03 (1.01, 4.10)	1.91 (0.93, 3.91)	0.078	-	--	
3TC/FTC, LVP/ATV, TDF/ZDV	0.96 (0.35, 2.58)	0.82 (0.30, 2.24)	0.701	1.36 (0.75, 2.48)	1.32 (0.72, 2.43)	0.365	1.14 (0.47, 2.77)	1.10 (0.45, 2.70)	0.829
3TC/FTC, ZDV/TDF, NVP	1.36 (0.93, 1.99)	**1.59 (1.06, 2.37)**	**0.024**	1.20 (0.91, 1.57)	**1.34 (1.00, 1.79)**	**0.049**	1.09 (0.75, 1.58)	1.13 (0.77, 1.67)	0.532
Viral load (≥40 HIV copies /ml)	1.07 (0.81, 1.43)	1.11 (0.80, 1.51)	0.550	1.11 (0.92, 1.36)	1.13 (0.91, 1.40)	0.256	0.90 (0.68, 1.17)	0.83 (0.62, 1.12)	0.227
CD4 count (cells/mm^3)									
<200	1	1		1	1		1	1	
200-500	0.71 (0.42, 1.22)	0.65 (0.37, 1.12)	0.118	0.95 (0.64, 1.40)	0.91 (0.61, 1.36)	0.658	0.92 (0.55, 1.54)	0.87 (0.52, 1.47)	0.608
>500	0.80 (0.47, 1.36)	0.75 (0.43, 1.32)	0.320	0.98 (0.66, 1.44)	0.94 (0.62, 1.41)	0.767	0.81 (0.49, 1.36)	0.78 (0.45, 1.34)	0.367
Mother's education									
Primary school completed or less	1	1		1	1		1	1	
Secondary school	0.91 (0.67, 1.25)	0.88 (0.63, 1.22)	0.434	**0.79 (0.64, 0.98)**	0.82 (0.65, 1.02)	0.070	1.49 (1.07, 2.07)	**1.45 (1.04, 2.03)**	**0.030**
Some college education	0.66 (0.34, 1.26)	0.65 (0.33, 1.26)	0.198	0.70 (0.47, 1.05)	0.66 (0.43, 1.01)	0.056	1.26 (0.71, 2.23)	1.30 (0.73, 2.34)	0.373
Food insecure household	0.94 (0.61, 1.45)	0.87 (0.56, 1.36)	0.550	1.10 (0.83, 1.46)	1.04 (0.78, 1.39)	0.796	0.91 (0.61, 1.37)	0.91 (0.60, 1.38)	0.653

[s] Underweight – defined as weight-for-age (WAZ)<-2). [¶] Wasting – defined as weight-for-length (WLZ)<-2. [β] Stunting – defined as length-for-age (LAZ)<-2. [¥] cHR – crude hazard rate. [¤] aHR – hazard ratio adjusted for maternal body mass index (weight in kilograms divided by the square of height in meters), age in years, ART regimen, viral suppression, CD4 count, education, and household food insecurity. [§] Body mass index is the weight in kilograms divided by the square of height in meters.

Figures

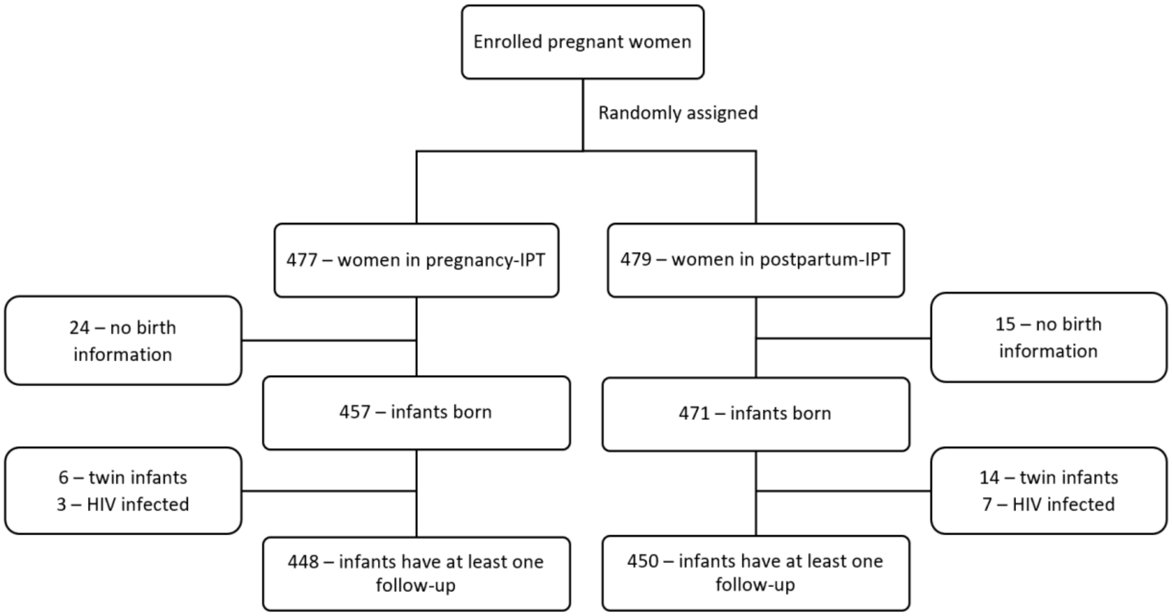


Figure 4: Enrollment, randomization, post-hoc exclusion criteria, and analysis

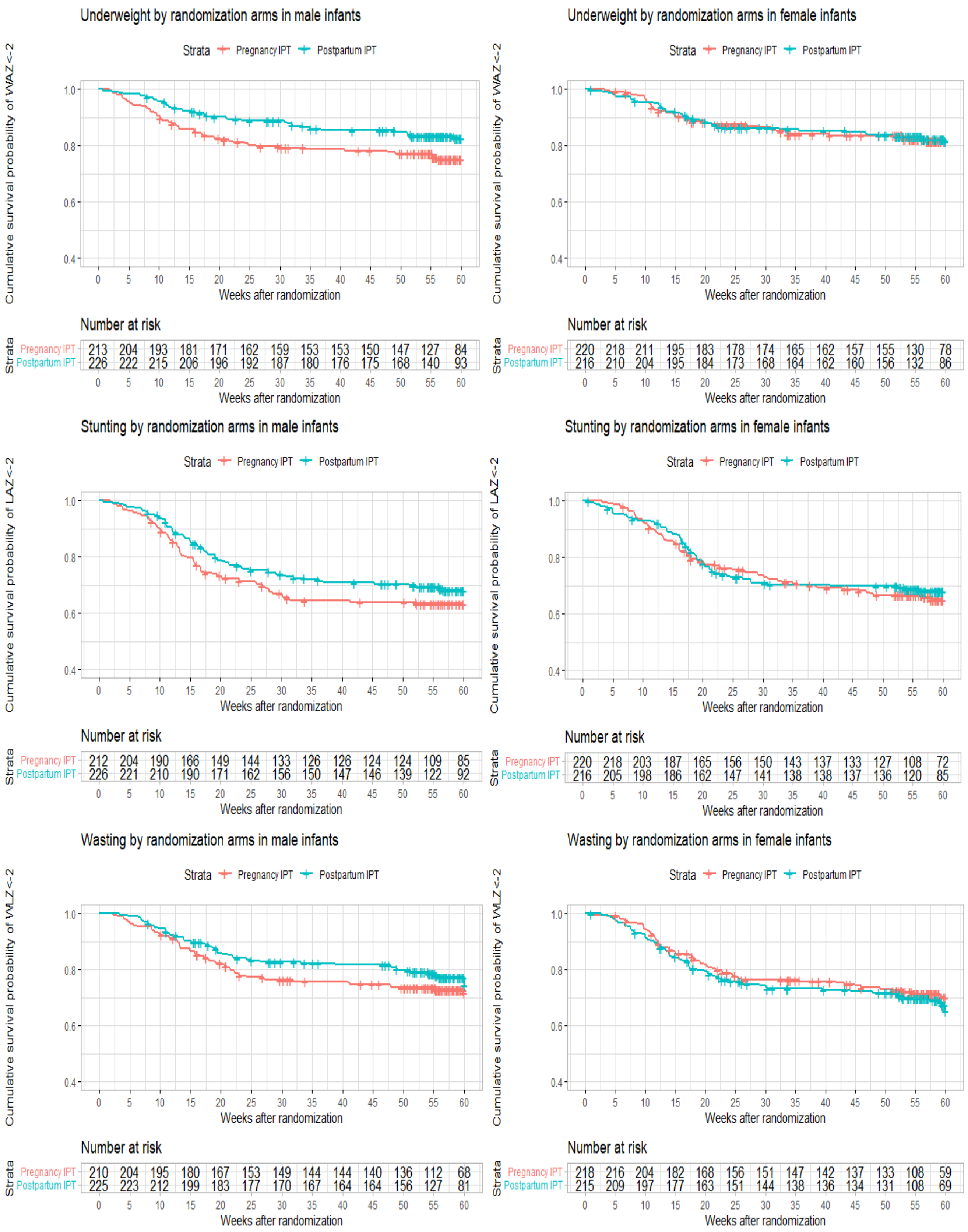


Figure 2: Comparison of sex-stratified cumulative survival probability of underweight, and stunting by randomized arms.

Underweight (defined as weight-for-age (WAZ)<-2), wasting (defined as weight-for-length (WLZ)<-2),

Supplemental tables

Supplemental Table 1: Risk of underweight, stunting, and wasting overall and stratified by infant sex in analysis to 12 and 48 weeks postpartum using GEE models with recurrent outcomes

Analysis	Model	Isoniazid started during	Underweight[§]			Stunting[¶]			Wasting[β]		
			cRR[¥] (95% CI)	aRR[¤] (95% CI)	P-value	cRR[¥] (95% CI)	aRR[¤] (95% CI)	P-value	cRR[¥] (95% CI)	aRR[¤] (95% CI)	P-value
12-week postpartum	Overall	Pregnancy	**1.38 (1.00, 1.91)**	**1.47 (1.07, 2.02)**	**0.017**	1.14 (0.95, 1.38)	1.18 (0.98, 1.42)	0.080	1.08 (0.82, 1.44)	1.06 (0.81, 1.39)	0.648
		Postpartum	1	1		1	1		1	1	
	Male infants	Pregnancy	**1.68 (1.11, 2.56)**	**1.78 (1.18, 2.69)**	**0.006**	1.27 (1.00, 1.63)	1.27 (0.99, 1.63)	0.058	1.42 (0.95, 2.13)	**1.63 (1.10, 2.42)**	**0.015**
		Postpartum	1	1		1	1		1	1	
	Female infants	Pregnancy	1.04 (0.63, 1.72)	1.09 (0.66, 1.81)	0.729	1.01 (0.76, 1.33)	1.02 (0.76, 1.36)	0.915	0.91 (0.62, 1.33)	**0.82 (0.56, 1.20)**	**0.306**
		Postpartum	1	1		1	1		1	1	
48 weeks postpartum	Overall	Pregnancy	1.28 (0.97, 1.70)	**1.36 (1.04, 1.78)**	**0.027**	1.12 (0.94, 1.34)	1.16 (0.97, 1.39)	0.103	0.98 (0.75, 1.29)	0.98 (0.75, 1.27)	0.856
		Postpartum	1	1		1	1		1	1	
	Male infants	Pregnancy	**1.59 (1.09, 2.32)**	**1.69 (1.19, 2.41)**	**0.004**	1.23 (0.97, 1.56)	1.25 (0.98, 1.59)	0.070	1. 34 (0.89, 2.02)	1.48 (1.00, 2.21)	0.052
		Postpartum	1	1		1	1		1	1	
	Female infants	Pregnancy	0.98 (0.64, 1.50)	1.01 (0.67, 1.52)	0.968	1.00 (0.76, 1.30)	1.01 (0.77, 1.32)	0.948	0.75 (0.52, 1.08)	**0.68 (0.47, 0.99)**	**0.042**
		Postpartum	1	1		1	1		1	1	

[§] Underweight – defined as weight-for-age (WAZ)<-2). [¶] Wasting – defined as weight-for-length (WLZ)<-2. [β] Stunting – defined as length-for-age (LAZ)<-2. [¥] cRR – crude relative risk. [¤] aRR – relative risk adjusted for maternal body mass index (weight in kilograms divided by the square of height in meters), age in years, ART regimen, viral suppression, CD4 count, education, and household food insecurity.

Supplemental Table 2: Cofactors of growth faltering (becoming underweight, wasted, or stunted) during follow-up in the overall cohort of HEU infants in analysis to 48 weeks postpartum

Variables	Underweight[ş]			Stunting[¶]			Wasting		
	cRR[¥] (95% CI)	aRR[¤] (95% CI)	P-value	cRR[¥] (95% CI)	aRR[¤] (95% CI)	P-value	cRR[¥] (95% CI)	aRR[¤] (95% CI)	P-value
IPT started during pregnancy	1.28 (0.97, 1.70)	**1.36 (1.04, 1.78)**	**0.027**	1.12 (0.94, 1.34)	1.16 (0.97, 1.39)	0.103	0.98 (0.75, 1.29)	0.98 (0.75, 1.27)	0.856
Male infant	1.37 (1.03, 1.82)	**1.40 (1.07, 1.85)**	**0.016**	1.23 (1.03, 1.48)	**1.23 (1.03, 1.48)**	**0.022**	0.81 (0.61, 1.06)	0.83 (0.63, 1.09)	0.179
Mother's BMI[§]	0.91 (0.89, 0.94)	**0.91 (0.88, 0.94)**	**<0.001**	0.98 (0.96, 1.00)	**0.98 (0.96, 1.00)**	**0.041**	0.93 (0.90, 0.96)	**0.92 (0.89, 1.09)**	**<0.001**
Mother's age in years	1.00 (0.97, 1.02)	1.00 (0.98, 1.03)	0.771	0.99 (0.97, 1.00)	0.99 (0.97, 1.00)	0.152	1.03 (1.00, 1.05)	**1.03 (1.01, 1.06)**	**0.001**
ART regimen									
3TC/FTC,TDF,EFV	1	1		1	1				
3TC, ZDV, EFV	0.94 (0.25, 3.48)	0.99 (0.28, 3.49)	0.985	1.47 (0.75, 2.88)	1.44 (0.73, 2.84)	0.287	-	-	
3TC/FTC, LVP/ATV, TDF/ZDV	0.76 (0.25, 2.32)	0.65 (0.22, 1.90)	0.432	1.43 (0.85, 2.42)	1.34 (0.79, 2.26)	0.278	0.84 (0.30, 2.31)	0.78 (0.29, 2.15)	0.635
3TC/FTC, ZDV/TDF, NVP	1.35 (0.92, 1.96)	**1.66 (1.14, 2.43)**	**0.009**	1.34 (1.05, 1.71)	**1.48 (1.15, 1.91)**	**0.002**	1.15 (0.79, 1.68)	1.14 (0.78, 1.69)	0.499
Viral load (≥40 HIV copies /ml)	1.26 (0.95, 1.67)	1.27 (0.95, 1.71)	0.109	1.09 (0.91, 1.31)	1.18 (0.97, 1.44)	0.089	0.94 (0.70, 1.24)	0.94 (0.70, 1.27)	0.691
CD4 count (cells/mm^3)									
<200	1	1		1	1		1	1	
200-500	0.68 (0.41, 1.14)	0.64 (0.39, 1.06)	0.081	1.12 (0.97, 1.30)	0.94 (0.64, 1.37)	0.734	1.19 (0.65, 2.19)	1.22 (0.66, 2.23)	0.526
>500	0.72 (0.43, 1.19)	0.72 (0.44, 1.21)	0.215	1.00 (1.00, 1.00)	1.10 (0.75, 1.62)	0.622	1.16 (0.63, 2.13)	1.25 (0.68, 2.32)	0.475
Mother's education									
Primary school completed or less	1	1		1	1	1	1	1	
Secondary school	0.91 (0.66, 1.25)	0.89 (0.65, 1.22)	0.473	0.78 (0.64, 0.94)	**0.80 (0.65, 0.97)**	**0.027**	1.46 (1.03, 2.06)	**1.44 (1.02, 2.04)**	**0.041**
Some college education	0.63 (0.33, 1.23)	0.65 (0.34, 1.24)	0.187	0.81 (0.56, 1.17)	0.80 (0.55, 1.16)	0.241	1.10 (0.59, 2.04)	1.17 (0.63, 2.18)	0.626
Food insecure household	0.82 (0.52, 1.30)	0.77 (0.49, 1.20)	0.254	0.99 (0.75, 1.30)	0.94 (0.72, 1.24)	0.671	0.84 (0.54, 1.30)	0.83 (0.53, 1.30)	0.412

[ş]Underweight – defined as weight-for-age (WAZ)<-2). [¶]Wasting – defined as weight-for-length (WLZ)<-2. [β]Stunting – defined as length-for-age (LAZ)<-2.[¥]cRR – crude relative risk. [¤]aRR – relative risk adjusted for maternal body mass index (weight in kilograms divided by the square of height in meters), age in years, ART regimen, viral suppression, CD4 count, education, and household food insecurity. [§]Body mass index is the weight in kilograms divided by the square of height in meters.

Chapter 3: Isoniazid preventive therapy during infancy has no adverse effect on growth among HIV-exposed uninfected children: secondary analysis of data from a randomized controlled trial

Abstract

Background: Isoniazid preventive therapy (IPT) decreases risk of tuberculosis (TB) disease. It is challenging to evaluate the growth impact of infant IPT in observational studies because of confounding by indication - children who received IPT may differ from those who did not. In a recent randomized trial (RCT) we assessed IPT effects on infant growth without a known TB exposure.

Methods: The infant TB Infection Prevention Study (iTIPS) trial was a non-blinded RCT among HIV-exposed uninfected (HEU) infants in Kenya. Inclusion criteria for the parent RCT were age 6-10 weeks, birthweight >2.5 kg, and gestation >37 weeks. Infants in the IPT arm received 10 mg/kg isoniazid daily for 12 months while the control trial received no intervention; post-trial observational follow-up continued through 24 months of age. We used intent-to-treat linear mixed-effects models to compare growth rates (weight-for-age z-score [WAZ] and height-for-age z-score [HAZ]) between trial arms.

Results: Among 298 infants, 150 were randomized to IPT, 47.6% were females, median birthweight was 3.4 Kg (inter-quartile range [IQR] 3.0-3.7), and 98.3% were breastfed. During 12-month intervention period and 12-month post-RCT follow-up, WAZ and HAZ declined significantly in all children with more HAZ decline in male infants. There were no growth differences between trial arms, including in sex-stratified analyses. In longitudinal linear analysis, mean WAZ (β=0.04 [95% CI:-0.14, 0.22]), HAZ (β=0.14 [95% CI:-0.06, 0.34]), and WHZ [β=-0.07 [95% CI:-0.26, 0.11]) z-scores were similar between arms as were

WAZ and HAZ growth trajectories. Infants in the IPT arm had higher monthly WHZ increase (β to 24 months 0.02 [95% CI:0.01, 0.04]) than the no-IPT arm.

Conclusion: IPT administered to HEU infants did not significantly impact growth outcomes in the first two years of life.

Background

Infection with *Mycobacterium tuberculosis* (Mtb) before the age of 2 can progresses to severe TB disease, with rapid progression occurring within the first year of Mtb infection (76–80). Without treatment, children with Mtb infection, which is also called latent TB infection (LTBI), have about a 19% risk of developing TB within two years (76–79,81,82). HIV-exposed uninfected (HEU) children have a higher risk of TB and Mtb infection than HIV-unexposed uninfected (HUU) children (14). Africa accounted for 30.5% of the global pediatric TB burden in 2018 (83) and 37.0% of TB-related deaths among children under 15 years of age. Therefore, approaches to prevent and treat TB infection in TB endemic areas among HEU children are a priority.

Isoniazid preventive treatment (IPT) decreases progression of LTBI to TB disease (84–86). The World Health Organization (WHO) recommends IPT in children under 5 with known TB exposure and children with HIV (CHIV), older than one year of age (21). Recent studies suggest that household exposure accounts for less than 30% of Mtb transmission to children, with most Mtb transmission occurring without a known contact (22). This suggests that WHO recommendations may miss many children at risk for TB. Because progression from Mtb infection to TB disease is rapid during infancy, IPT administration, regardless of contact history, may be warranted in some higher-risk groups of infants. We conducted a randomized trial (RCT) (NCT02613169) to examine the impact of IPT on the incidence of Mtb infection in HEU infants (24). IPT was associated with a non-significant trend for decreased Mtb infection (predominantly assessed by Tuberculin skin tests (TST) during the first year of life (87), which was not sustained up to 24 months of age (88).

While IPT has clear benefits in decreasing risk of TB disease, it has side effects that can potentially compromise caloric intake, including loss of appetite, nausea, vomiting, and upset stomach (23). These, in turn, could influence infant growth. To date, the effects of IPT during infancy on the growth of children have not been well characterized. In observational studies, assessing the effect of IPT on growth in infants has been a challenge because of confounding by indication: children exposed to TB or children with HIV regardless of exposure, who are eligible for IPT, may have different characteristics that influence growth. The infant TB Infection Prevention Study (iTIPS) provided a unique opportunity to compare growth in HEU infants receiving IPT versus those not receiving IPT in a randomized controlled trial, unconfounded by known TB exposure or other related characteristics. Therefore, we leveraged the RCT design of the parent study to rigorously examine the effect of IPT on infants' growth over the first two years of life.

Methods

Parent trial design and intervention

This secondary analysis used data from the iTIPS trial, a non-blinded RCT among HIV-exposed infants in Western Kenya. As reported in detail previously (24), randomization was stratified by site and generated by the study statistician using a computer-generated random block size. Infants 6-10 weeks of age in the IPT arm initiated isoniazid 10 mg/kg once daily for 12 months. The control arm received no intervention. Standardized weight-based isoniazid dosing (by weight band using 100 mg scored tablets) was used, corresponding to Kenya and WHO recommendations (24). Pyridoxine was provided to children randomized to isoniazid to decrease peripheral neuropathy risk. To ensure full dose usage and ease of infant administration, caregivers were advised to pulverize isoniazid and pyridoxine and mix them with small quantities of breastmilk, clean water, or liquid cotrimoxazole. The intervention ended after 12 months post-randomization (~14 months of age); post-trial observational follow-up continued until 24 months of age for both arms.

Participants and study period

Overall, 300 HIV-exposed infants were enrolled from Prevention of Mother to Child HIV Transmission (PMTCT) clinics in Western Kenya. Inclusion criteria for the parent study were 6-10 weeks of age, birth weight >2.5 Kg, and not premature (>37 weeks of gestation). Exclusion criteria included known household TB exposure, including mothers with a history of TB in the past year, and infants enrolled in other TB prevention programs or vaccine studies. The parent trial and this analysis excluded two infants diagnosed with HIV. Follow-up visits were conducted at 10 and 14 weeks of age, and 6, 9, 12, and ~14 (12 months post-randomization) months of age. Observational follow-up continued through 24 months of age. The trial lasted from August 2016 to July 2019, but the observational follow-up continued until September 2020.

Ethical approvals

The University of Nairobi/Kenyatta National Hospital Ethics and Research Committee (P571/08/2015), Jaramogi Oginga Odinga Teaching and Referral Hospital Ethical Review Committee, University of Washington Institutional Review Board (STUDY00000761), and Kenya Pharmacy and Poisons Board approved the study. Caregivers gave informed consent. An external and independent Data and Safety Monitoring Board monitored adverse events in the parent trial.

Infant growth characterization

Infants were enrolled at 6-10 weeks of age and their birth weight and length were based on a review of maternal child health cards and maternal reports. Weight and length of children were measured at 6, 10, 14 weeks, 6, 12, ~14 (12 months post enrolment), and 24 months of age. A CDC-Kenya team trained data collectors for two days on growth monitoring prior to trial initiation. Infants were measured twice at each visit, and the average weight and length were rounded to the nearest 0.1 kg and 0.1 cm, respectively.

Weight-for-age z-score [WAZ], weight-for-length z-score [WLZ], and length-for-age z-score [LAZ]) were defined using WHO child growth standards (45). Growth faltering was defined as underweight WAZ <-2, wasting WLZ <-2, and stunting LAZ <-2 (45).

Maternal and infant information

Baseline maternal and infant characteristics were collected using standardized questionnaires and medical record abstraction administered by trained study staff. Maternal characteristics, including age in years, educational achievement of mothers, current marital status, employment status, household income, mode of delivery, smoking, alcohol and drug use during pregnancy, timing of the mother's HIV diagnosis, use of antiretroviral therapy (ART), TB diagnosis and, any maternal IPT used during pregnancy were collected. The following baseline infant characteristics were collected: age in weeks, sex assigned at birth, current breastfeeding status, presence of BCG scar, birth weight, current cotrimoxazole use, and ART use for prevention of maternal-to-child transmission of HIV.

Statistical analyses

Means and standard deviations (SD) were used to describe normally distributed continuous variables, medians and interquartile ranges (IQR) were used to describe skewed distributions, and frequency and percentage to describe categorical variables. We compared baseline maternal and infant characteristics between IPT and no-IPT arms using a two-sided t-test (the Mann-Whitney U test if assumptions were not met) for continuous variables and the Pearson χ^2 test (Fisher's exact test if assumptions were not met) for categorical variables.

The primary analysis was an intent-to-treat (ITT) analysis, comparing the growth (WAZ, HAZ, and WHZ) of infants in IPT and no-IPT arms. Linear regression was used to compare the WAZ, HAZ, and WHZ of children measured at ~14 months of age (12 months post-randomization – the end of the trial) and 24 months of age (extended observational follow-up) between the arms. We also fitted linear mixed-effects models (LMEMs) with autoregression correlation structure, random intercept for subjects, and random slope for follow-up time, to compare growth (WAZ, WHZ, HAZ) trajectories between IPT vs no-IPT arms from enrollment to ~14 and 24 months of age.

Linear regression was used to determine factors associated with WAZ, HAZ, and WHZ of children measured at ~14 and 24 months of age and LMEMs to determine factors associated with WAZ, HAZ, and WHZ of children from enrollment to ~14 and 24 months of age.

Approach to data missingness

There were missing data in this study: overall WAZ (9.2%), HAZ (9.7%), and WHZ (9.7%). Multiple imputations by chained equations (MICE), which ran a series of regression models for each missing variable conditional upon other specified variables, were used to manage missing data. Baseline infant and maternal characteristics (infant's age, sex, BCG scar, current cotrimoxazole use, baseline WAZ, HAZ, WHZ, and maternal age, education, employment, and marital status) were used in the MICE analyses. We imputed the data 25 times. Pooled parameter estimates and their standard errors were calculated according to Rubin's rules to account for the between- and within-imputation variance (46). We employed one single imputation model to obtain imputed values for outcomes (WAZ, HAZ, and WHZ) missed at each visit during follow-up. In the imputation models, we specified the appropriate distributions for each of the variables in the model. In the presence of missing data, the multiple imputation approach should yield unbiased estimates assuming data are missing at random (MAR) (46).

Results

Maternal and infant baseline characteristics

Among 298 HEU infants enrolled in the parent trial, 150 were randomized to IPT and 148 to the control no-IPT arm and all were included in this secondary analysis. Baseline maternal and infant characteristics were previously reported in the primary RCT publication and were uniformly distributed between the randomization arms (Table 1) (24). The mean (SD) age of mothers was 28 (±5) years and median (IQR) monthly income in 1000 KSH was 10 (6 – 15). Most (86.6% [258/298]) were currently married, 53.3% (159/298) completed primary school or less, and 49.7% (148/298) were unemployed. Few (14.4% [43/298]) mothers delivered via cesarean section. Only one mother smoked, 9 mothers drank alcohol and 10 used drugs during pregnancy. All participating mothers had HIV, 23.8% (71/298) of whom were diagnosed with HIV during pregnancy, 73.2% (218/298) started ART before the pregnancy, 51.3% (153/298) had taken IPT during pregnancy, and 10.4% (31/298) had ever been diagnosed with TB.

The median age of the infants at enrollment was 6.3 weeks (6.0-6.6 weeks), and the median birthweight was 3.4 Kg (IQR 3-3.7). All infants were of normal birth weight, due to eligibility criteria which excluded low birth weight infants. Of all infants, 47.6% (142/298) were females, 92.6% (276/298) had BCG scars, 90.0% (268/298) were on cotrimoxazole, and almost all (295/298) received ART for PMTCT after birth. Most (98.3% [293/298]) infants were breastfed. Infant mean WAZ (SD) was 0.2 (1.0), mean HAZ was -0.25 (1.2), and mean WHZ was 0.7 (1.6) at enrollment. And, as reported in primary analyses, only 3.7% of infants in this study experienced gastroenteritis.

Changes in growth by ~14 and 24 months of age

There was a decline in growth z-scores, with average WAZ value of all infants decreasing by 0.33 (β = -0.33 [95% CI: -0.48, -0.19]) and 0.58 (β = -0.58 [95% CI: - 0.75, - 0.42]) z-scores by ~14 and 24 months of age, respectively. Similarly, the average HAZ of infants participating in this study decreased by 0.50 (β = - 0.50 [95% CI: -0.66, -0.34]) and 1.74 z-scores (β = - 1.74 [95% CI: - 1.99, - 1.52]) z-scores by ~14 and 24 months of age, respectively. WHZ decreased significantly by 0.40 (β = -0.40[95% CI: -0.65, -0.16]) by ~14 months of age but the change was not significant (β=0.27 [95% CI: -0.02, 0.56]) by 24 months of age. There were no significant differences between the IPT vs. no-IPT arms in WAZ, HAZ, and WHZ at ~14 months and 24 months. Infants in the IPT arm had similar mean WAZ (β = 0.22 [95% CI: -0.07, 0.50]), HAZ (β = 0.08 [95% CI: -0.31, 0.47]), and WHZ (β = 0.21 [95% CI: -0.19, 0.60]) at 24 months of age compared with children in the non-IPT arm. The observed mean change in growth is shown in Figure 1. At 24 months of age, 5.8% (13/225) were underweight, 47.9% (104/217) stunted, and 1.4% (3/215) wasted and did not differ significantly by arm.

Effect of IPT on growth – WAZ, HAZ, and WHZ – at all follow-up timepoints

Multivariable models adjusted sex of infants, maternal education, age of mothers, timing of maternal HIV diagnosis, maternal IPT use during pregnancy, viral load, WAZ at baseline. Adjusted for all other factors, IPT use during infancy did not have a statistically significant effect on growth, either during the trial period up to ~14 months of age or through the observational follow-up until 24 months of age (p-value > 0.05). Infants in IPT arm had similar mean WAZ (β to ~14 months of age 0.00 [95% CI: -0.19, 0.19] and β to 24 months of age β=0.04 [95% CI: -0.14, 0.22]), HAZ (β to ~14 months of age 0.16 [95% CI: -0.05, 0.37] and β to 24 months of age β=0.13 [95% CI: -0.06, 0.33]), and WHZ (β to ~14 months of age -0.14 [95% CI: -0.32, 0.05] and β to 24 months of age β=-0.08 [95% CI: -0.26, 0.10]) z-scores compared to infants in no-IPT arm. There was no statistically significant difference in rate of WAZ (β to ~14 months of age 0.00 [95% CI: -0.01, 0.02] and β to 24 months of age 0.01 [95% CI: 0.00, 0.02]), and HAZ (β to ~14 months of age - 0.01 [95% CI: -0.03, 0.01] and β to 24 months of age -0.01 [95% CI: -0.02, 0.00]) change in a month between the randomized groups. However, infants in IPT arm had an increased rate of WHZ (β to ~14 months of age

0.02 [95% CI: 0.00, 0.04] and β to 24 months of age 0.02 [95% CI: 0.01, 0.04]) change in a month than infants in no-IPT arm (Table 2). The adjusted change in growth is also shown in Figure 1.

Factors associated with growth change

Variables fitted in the model to identify factors associated with growth include infant IPT use, sex of infants and age, maternal educational status, timing of maternal HIV diagnosis (before vs during pregnancy), any maternal IPT exposure during pregnancy, and viral load. Adjusted for all other variables, infants whose mothers completed secondary school had 0.26 WAZ (at ~14 months of age β =0.26 [95% CI: 0.00, 0.52]) and HAZ (at 24 months of age β =0.38 [95% CI: 0.00, 0.75]) higher than infants whose mothers completed primary school or less. Male infants had 0.53 HAZ (β = -0.53 [95% CI: -0.83, -0.23]) lower at ~14 months of age than female infants (Supplemental Table 1).

Adjusted for all factors, male infants had significantly lower WAZ (β to ~14 months of age -0.21 [95% CI: -0.04, 0.02] and β to 24 months of age -0.18 [95% CI: -0.36, -0.01]) and lower HAZ (β to ~14 months of age -0.34 [95% CI: -0.55, -0.13] and β to 24 months of age -0.28 [95% CI: -0.48, -0.09]) than female infants (Table 3).

Discussion

In this secondary analysis of a randomized clinical trial, we found that IPT administered to HEU infants during the first year of life without known TB exposure did not significantly impact growth outcomes (WAZ, HAZ, and WHZ) during a 2-year follow-up. Infants in IPT arm had similar mean WAZ, HAZ, and WHZ z-scores compared to infants in the no-IPT arm. Despite the fact that isoniazid can cause side effects that could compromise caloric intake, including nausea, vomiting, and upset stomach (23), only 3.7% of the children in this study experienced gastroenteritis (87). Our data suggest that infant IPT has no impact on growth, adding to safety data for IPT administered to infants (76–80). The risk of exposure (14), progression, morbidity (11,13,37,58), and mortality (9,10,89) for Mtb infection is high among infants and even higher in those who are HEU children and CHIV. Approaches to prevent and treat TB infection in TB endemic areas in young children are important to decrease TB-related morbidity and mortality.

Considering the benefits of IPT in treating LTBI and preventing active TB disease in children (84–86) and people with HIV and without HIV, WHO recommends IPT to children with known TB exposure and children living with HIV (CHIV), older than one year of age (21). However, most TB cases in children occur without a known contact (22) and therefore are not included in the WHO recommendation. The iTIPS trial did not find a significant benefit of IPT to prevent Mtb infection among HEU children (87,88); a post-hoc analysis demonstrated that with the observed ~10% cumulative incidence of Mtb infection (which was lower than anticipated at study design), there may not have been sufficient sample size to demonstrate benefit of IPT to prevent Mtb infection. It is plausible while IPT decreases progression to TB, it may not prevent Mtb infection. The analysis of the parent RCT demonstrated no difference in adverse events between trial arms. Our new findings of comparable growth between arms provide additional evidence of safety applicable to pediatric IPT use in general.

In our study, WAZ and HAZ significantly declined after enrollment in all HEU cohort. The mean HAZ of all infants significantly decreased by 1.74 Z-scores in 24 months. The proportion of stunting increased from 8.4% to 47.9% from baseline to 2 years of age. This implies that a significant number of HEU infants born with normal HAZ in this study became stunted within 24 months of their birth. Stunting often indicates poor growth and development, and the fact that it's irreversible after 1000 days of life makes (90) losing HAZ in the first 730 days a concern for HEU children.

Our study had several strengths, with a strong study design – a randomized trial with over 90% retention. Weight and length of infants were measured twice, and the average values were used for analysis to reduce measurement error. This study also has limitations. Only normal birth weight and term infant were included in the parent trial, so results do not generalize to low-birthweight and preterm babies.

Conclusion: In this secondary analysis of an RCT, IPT administered to healthy HEU infants ages 6-10 weeks over the first year of life without a known TB exposure, did not significantly impact growth outcomes through the first 2 years of life. However, there is universal poor linear growth, especially height in this cohort of children with HIV exposure.

Tables

Table 8: Baseline maternal and infant demographics and clinical characteristics

Characteristics	IPT* (n=150)	No-IPT*(n=148)	P-value
Maternal characteristics			
Mean age (SD) – years	27.6 (5.1)	28.1 (4.9)	0.344
Primary school completed or less – no. (%)	75 (50.0)	84 (56.8)	0.292
Currently married mothers – no. (%)	134 (89.3)	124 (83.8)	0.217
Maternal employment status – no. (%)			
Unemployed	73 (48.7)	75 (50.7)	0.402
Salaried or irregular working hours	17 (11.3)	23 (15.5)	
Self-employed	60 (40.0)	50 (33.8)	
Median income in 1000 KSH (IQR)	10 (6 – 15)	10 (6 – 15)	0.598
Delivered by c-section – no. (%)	17 (11.4)	26 (17.6)	0.179
Tobacco use during pregnancy – no. (%)	1	0	1.000
Alcohol use during pregnancy – no. (%)	3 (2.0)	6 (4.0)	0.494
Drug use during pregnancy – no. (%)	5 (3.4)	5 (3.4)	0.999
Timing of HIV diagnosis – no. (%)			
Before pregnancy	115 (76.6)	110 (74.3)	0.841
While pregnant for this child	34 (22.7)	37 (25.0)	
After delivery of this child	1 (0.7)	1 (0.7)	
Any IPT exposure during pregnancy – no. (%)	77 (51.3)	76 (51.4)	1.00
Ever diagnosed with tuberculosis – no. (%)	15 (10.0)	16 (10.8)	0.967
Infant characteristics			
Mean infant age in weeks (SD)	6.6 (1.0)	6.6 (1.2)	0.882
Female infants – no. (%)	71 (47.3)	71 (48.0)	1.000
Currently breastfeeding – no. (%)	146 (97.3)	147 (99.3)	0.375
Infant has BCG scar – no. (%)	140 (93.3)	136 (92.5)	0.962
Median birth weight in Kg (IQR)*	3.5 (3.0 – 3.6))	3.4 (3.0 – 3.7)	0.786
Taking cotrimoxazole	136 (90.7)	132 (89.2)	0.817
Mean weight-for-age z-score	0.2 (1.0)	0.3 (1.0)	0.503
Underweight at enrollment	3 (2)	2 (1.3)	1.000
Mean height-for-age z-score	-0.2 (1.2)	-0.3 (1.3)	0.176
Stunted at enrollment	9 (6.0)	16 (10.9)	0.148
Mean weight-for-height z-score	0.5 (1.6)	0.9 (1.7)	**0.043**
Wasted at enrollment	10 (6.7)	5 (3.4)	0.290

*IPT – isoniazid preventive therapy.

Table 9: Effect of IPT on Growth – WAZ, HAZ, WHZ – from enrollment to ~14 and 24 months of age – intent-to-treat analysis – longitudinal and cross-sectional linear analyses

Follow-up	Model	Randomization arm	**WAZ at all follow-up timepoints[¶] (95% CI)**		**HAZ[¥] at all follow-up timepoints (95% CI)**		**WHZ[α] at all follow-up timepoints (95% CI)**	
			Coefficient (95% CI)	P-value	Coefficient (95% CI)	p-value	Coefficient (95% CI)	P-value
Longitudinal[§] up to ~14 months of age	Overall	IPT arm (ref: no-IPT)	0.00 (-0.19, 0.19)	0.985	0.16 (-0.05, 0.37)	0.139	-0.14 (-0.32, 0.05)	0.139
	Male	IPT arm (ref: no-IPT)	0.02 (-0.27, 0.31)	0.886	0.22 (-0.07, 0.52)	0.141	-0.18 (-0.45, 0.10)	0.202
	Female	IPT arm (ref: no-IPT)	-0.02 (-0.26, 0.21)	0.852	0.09 (-0.19, 0.37)	0.535	-0.09 (-0.33, 0.14)	0.435
Longitudinal[§] up to ~24 months of age	Overall	IPT arm (ref: no-IPT)	0.04 (-0.14, 0.22)	0.657	0.13 (-0.06, 0.33)	0.189	-0.08 (-0.26, 0.10)	0.399
	Male	IPT arm (ref: no-IPT)	0.07 (-0.20, 0.34)	0.611	0.17 (-0.11, 0.46)	0.231	-0.09 (-0.36, 0.18)	0.521
	Female	IPT arm (ref: no-IPT)	0.01 (-0.21, 0.24)	0.927	0.09 (-0.17, 0.35)	0.500	-0.07 (-0.30, 0.17)	0.578
Cross-sectional[¤] at ~14 months of age	Overall	IPT arm (ref: no-IPT)	-0.01 (-0.28, 0.25)	0.915	0.13 (-0.17, 0.42)	0.392	-0.11 (-0.42, 20)	0.487
	Male	IPT arm (ref: no-IPT)	0.10 (-0.30, 0.50)	0.610	0.32 (-0.09, 0.72)	0.123	-0.07 (-0.53, 0.38)	0.751
	Female	IPT arm (ref: no-IPT)	-0.14 (-0.48, 0.19)	0.401	-0.08 (-0.49, 0.33)	0.697	-0.15 (-0.58, 0.28)	0.488
Cross-sectional[¤] at 24 months of age	Overall	IPT arm (ref: no-IPT)	0.19 (-0.08, 0.45)	0.171	0.00 (-0.35, 0.35)	0.995	0.25 (-0.13, 0.62)	0.200
	Male	IPT arm (ref: no-IPT)	0.22 (-0.18, 0.62)	0.275	-0.11 (-0.60, 0.39)	0.676	0.40 (-0.14, 0.94)	0.148
	Female	IPT arm (ref: no-IPT)	0.15 (-0.20, 0.50)	0.396	0.12 (-0.37, 0.60)	0.636	0.08 (-0.43, 0.59)	0.745

[¶]WAZ – weight-for-age. [¥]HAZ – height-for-age. [α]WHZ – weight-for-height z-score. *IPT – Isoniazid preventive therapy. [§]Longitudinal – analysis included all data from 6th weeks to 14/24 months of age. [¤]Cross-sectional – analysis is based on one-time data at 14/24 months of age.

Table 10: Factors associated with growth at ~14 and 24-month age – longitudinal

Follow-up	Variable	WAZ[¶] at all follow-up timepoints			HAZ[¥] at all follow-up timepoints			WHZ[¤] at all follow-up timepoints		
		cCoefficient	**aCoefficient***	P-value	**cCoefficient**	**aCoefficient***	P-value	**cCoefficient**	**aCoefficient***	P-value
Up to ~14 months of age	Infant in IPT-arm[§]	-0.00 (-0.19, 0.19)	-0.04 (-0.22, 0.14)	0.675	0.17 (-0.05, 0.38)	0.15 (-0.06, 0.36)	0.166	-0.14 (-0.33, 0.04)	-0.18 (-0.37, 0.00)	0.054
	Male infants	-0.11 (-0.29, 0.08)	**-0.21 (-0.40, -0.02)**	**0.027**	-0.35 (-0.56, -0.14)	**-0.34 (-0.55, -0.13)**	**0.002**	0.13 (-0.06, 0.31)	0.07 (-0.12, 0.25)	0.490
	Secondary school or above	0.13 (-0.06, 0.32)	0.12 (-0.07, 0.30)	0.210	0.07 (-0.15, 0.28)	-0.03 (-0.18, 0.24)	0.771	0.13 (-0.05, 0.32)	0.14 (-0.05, 0.32)	0.141
	Age of mothers	-0.01 (-0.03, 0.01)	-0.00 (-0.02, 0.01)	0.646	-0.02, -0.04, 0.00)	-0.02 (-0.04, 0.00)	0.120	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)	0.397
	Tested HIV positive during pregnancy	0.08 (-0.15, 0.30)	0.06 (-0.17, 0.29)	0.605	0.09 (-0.16, 0.34)	-0.03 (-0.30, 0.23)	0.808	0.03 (-0.19, 0.25)	0.11 (-0.12, 0.34)	0.348
	Pregnancy-IPT	0.21 (0.02, 0.39)	0.17 (-0.01, 0.36)	0.062	0.12 (-0.10, 0.33)	0.14 (-0.07, 0.35)	0.182	0.14 (-0.04, 0.33)	0.13 (-0.05, 0.32)	0.166
	Suppressed viral load (<40 copies/ml)	0.08 (-0.17, 0.33)	0.04 (-0.19, 0.28)	0.706	-0.01 (-0.28, 0.26)	0.05 (-0.22, 0.32)	0.730	0.10 (-0.14, 0.33)	0.02 (-0.21, 0.26)	0.846
	WAZ at birth	0.27 (0.18, 0.36)	0.27(0.20, 0.38)	<0.001				0.17 (0.09, 0.26)	**0.17 (0.08, 0.26)**	**<0.001**
Up to 24 months of age	Infant in IPT-arm	0.04 (-0.14, 0.22)	-0.01 (-0.18, 0.17)	0.945	0.14 (-0.06, 0.34)	0.13 (-0.07, 0.33)	0.215	-0.08 (-0.26, 0.10)	-0.13 (-0.31, 0.05)	0.143
	Male infants	-0.08 (-0.26, 0.10)	-0.18 (-0.36, -0.01)	0.041	-0.29 (-0.49, 0.10)	**-0.28 (-0.48, -0.09)**	**0.005**	0.11 (-0.07, 0.29)	0.04 (-0.14, 0.22)	0.693
	Secondary school or above	0.06 (-0.02, 0.33)	0.15 (-0.02, 0.32)	0.090	0.11 (-0.09, 0.31)	0.09 (-0.11, 0.29)	0.399	0.13 (-0.05, 0.31)	0.13 (-0.05, 0.31)	0.153
	Age of mothers	-0.00 (-0.02, 0.01)	0.00 (-0.02, 0.02)	0.931	-0.02 (-0.04, 0.00)	-0.02 (-0.04, 0.00)	0.090	0.01 (-0.01, 0.03)	0.01 (-0.00, 0.03)	0.134
	Tested HIV positive during pregnancy	0.06 (-0.15, 0.27)	0.08 (-0.14, 0.30)	0.486	0.05 (-0.18, 0.28)	-0.04 (-0.29, 0.21)	0.751	0.06 (-0.16, 0.27)	0.14 (-0.08, 0.36)	0.223
	Pregnancy-IPT	0.15 (-0.03, 0.32)	0.12 (-0.06, 0.29)	0.188	0.08 (-0.12, 0.27)	0.11 (-0.09, 0.31)	0.275	0.14 (-0.04, 0.32)	0.12 (-0.06, 0.30)	0.199
	Suppressed viral load (<40 copies/ml)	0.00 (-0.23, 0.23)	-0.04 (-0.26, 0.18)	0.740	-0.05 (-0.31, 0.20)	0.01 (-0.26, 0.24)	0.946	0.05 (-0.18, 0.28)	-0.01 (-0.24, 0.21)	0.903
	WAZ at birth	0.24 (0.16, 0.33)	**0.26 (0.18, 0.35)**	**<0.001**				0.17 (0.08, 0.26)	**0.17 (0.08, 0.26)**	**<0.001**

[¶]WAZ – weight-for-age. [¥]HAZ – height-for-age. [¤]WHZ – weight-for-height z-score. [§]IPT – Isoniazid preventive therapy. [¤]cCoefficient - crude coefficient. *aCoefficient – adjusted coefficient for all other variables (infant IPT-arm, sex of infants (ref: female infants), maternal education (ref: primary or less), age of mothers, timing of maternal HIV diagnosis (ref: tested positive before pregnancy), any maternal IPT exposure during pregnancy (ref: no use of IPT during pregnancy), viral load (ref: >=40 copies/ml), and WAZ at baseline.

Figures

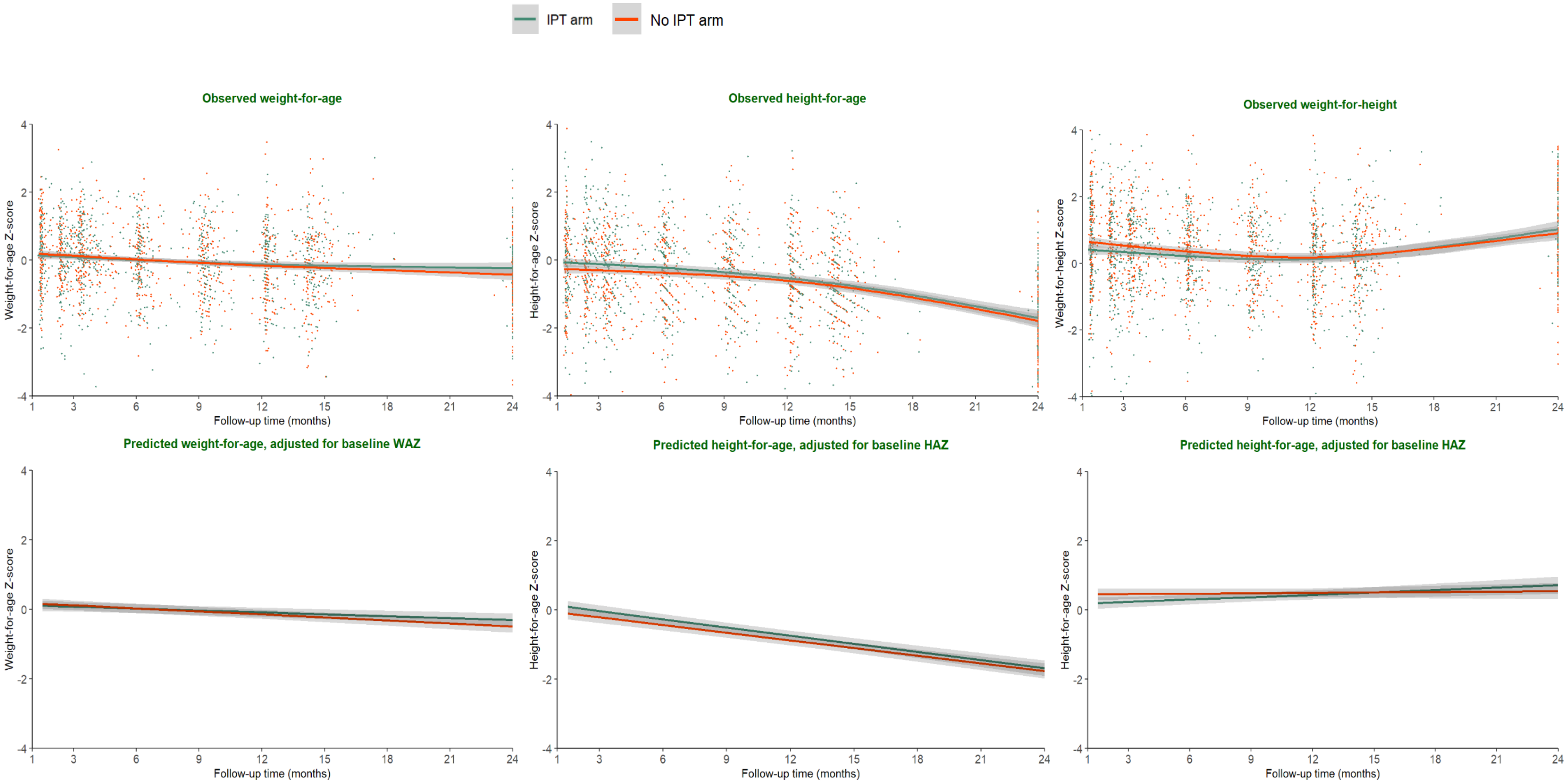


Figure 5: Scatter plots of change in WAZ, HAZ, and WHZ, over time by the randomized arm

(The Lowess curves represent a change in mean growth from randomization to 24 months of age. Bottom curves are adjusted change in WAZ, HAZ, and WHZ.)

Supplemental table

Supplemental Table 1: Factors associated with growth at ~14 and 24-month age

Follow-up	Variable		**WAZ[¶]**			**HAZ[¥]**			**WHZ[α]**	
		cCoefficient (95% CI)[¤]	**aCoefficient* (95% CI)**	P-value	**cCoefficient (95% CI)**	**aCoefficient* (95% CI)**	P-value	**cCoefficient (95% CI)**	**aCoefficient* (95% CI)**	P-value
One point: ~14 months of age	Infant in IPT-arm[§]	-0.01 (-0.29, 0.25)	-0.05 (-0.32, 0.21)	0.686	0.13 (-0.17, 0.42)	0.06 (-0.23, 0.36)	0.678	-0.11 (-0.42, 0.20)	-0.12 (-0.44, 0.20)	0.449
	Male infants	-0.15 (-0.42, 0.11)	-0.25 (-0.52, 0.02)	0.071	-0.52 (-0.81, -0.23)	**-0.53 (-0.83, -0.23)**	**0.001**	0.11 (-0.21,0.42)	0.04 (-0.29, 0.36)	0.830
	Secondary school or above	0.21 (-0.06, 0.47)	0.22 (-0.05, 0.48)	0.108	0.05 (-0.24, 0.35)	0.01 (-0.28, 0.31)	0.943	0.25 (-0.07, 0.57)	0.29 (-0.02, 0.61)	0.071
	Age of mothers	0.00 (-0.03, 0.02)	-0.00 (-0.03, 0.02)	0.794	-0.02 (-0.05, 0.00)	-0.02 (-0.06, 0.01)	0.107	0.01 (-0.2, 0.04)	0.01 (-0.02, 0.61)	0.521
	Tested HIV positive during pregnancy	0.03 (-0.28, 0.34)	0.04 (-0.30, 0.38)	0.822	-0.25 (-0.60, 0.10)	-0.31 (-0.69, 0.06)	0.102	0.21 (-0.17, 0.58)	0.25 (-0.16, 0.66)	0.233
	Pregnancy-IPT	0.27 (0.01, 0.53)	0.24 (-0.02, 0.51)	0.075	-0.01 (-0.30, 0.29)	0.01 (-0.28, 0.31)	0.923	0.37 (0.06, 0.69)	**0.35 (0.03, 0.67)**	**0.033**
	Suppressed viral load (<40 copies/ml)	0.08 (-0.27, 0.42)	0.04 (-0.30, 0.38)	0.827	-0.26 (-0.64, 0.12)	-0.19 (-0.57, 0.19)	0.322	0.27 (-0.14, 0.69)	0.19 (-0.22, 0.60)	0.359
	WAZ at birth	0.24 (0.12, 0.37)	**0.26 (0.13, 0.39)**	**<0.001**				0.24 (0.09, 0.39)	0.24 (0.08, 0.39)	0.003
One point: 24 months of age	Infant in IPT-arm	0.19 (-0.08, 0.46)	0.10 (-0.17, 0.37)	0.468	-0.00 (-0.35, 0.35)	0.01 (-0.36, 0.38)	0.952	0.25 (-0.13, 0.63)	0.12 (-0.25, 0.50)	0.512
	Male infants	0.01 (-0.26, 0.27)	-0.09 (-0.36, 0.18)	0.493	-0.02 (-0.37, 0.34)	0.00 (-0.37, 0.37)	0.999	0.00 (-0.38, 0.37)	-0.11 (-0.49, 0.28)	0.726
	Secondary school or above	0.25 (-0.00, 0.51)	**0.26 (0.00, 0.52)**	**0.050**	0.31 (-0.06, 0.67)	**0.38 (0.00, 0.75)**	**0.048**	0.10 (-0.27, 0.48)	0.07 (-0.31, 0.44)	0.726
	Age of mothers	0.01 (-0.02, 0.04)	0.02 (-0.01, 0.05)	0.173	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.02)	0.334	0.03 (-0.01, 0.07)	**0.04 (0.01, 0.08)**	**0.023**
	Tested HIV positive during pregnancy	0.01 (-0.33, 0.35)	0.13 (-0.23, 0.50)	0.473	-0.14 (-0.58, 0.29)	-0.08 (-0.56, 0.40)	0.745	0.17 (-0.29, 0.63)	0.28 (-0.21, 0.77)	0.260
	Pregnancy-IPT	-0.06 (-0.33, 0.21)	-0.06 (-0.34, 0.21)	0.639	-0.13 (-0.48, 0.22)	-0.07 (-0.43, 0.30)	0.709	0.10 (-0.29, 0.49)	0.05 (-0.33, 0.44)	0.787
	Suppressed viral load (<40 copies/ml)	-0.26 (-0.60, 0.07)	-0.31 (-0.65, 0.03)	0.070	-0.26 (-0.70, 0.18)	-0.27 (-0.71, 0.17)	0.231	-0.17 (-0.63, 0.29)	-0.22 (-0.68, 0.25)	0.357
	WAZ at birth	0.16 (0.03, 0.29)	**0.18 (0.05, 0.31)**	**0.008**				0.14 (-0.04, 0.32)	0.15 (-0.03, 0.34)	0.101

[¶] WAZ – weight-for-age. [¥] HAZ – height-for-age. [α] WHZ – weight-for-height z-score. [§] IPT – Isoniazid preventive therapy. [¤] cCoefficient - crude coefficient.

*aCoefficient – adjusted coefficient for all other variables (infant IPT-arm, sex of infants, maternal education, age of mothers in years, timing of maternal HIV diagnosis, maternal IPT use during pregnancy, viral load, WAZ at baseline.

Chapter 4: Effect of TB-HIV co-treatment on clinical and growth outcomes among hospitalized children newly initiating antiretroviral therapy

Abstract

Objective: To evaluate the effects of TB-HIV co-treatment on clinical and growth outcomes in children living with HIV (CHIV).

Design: Longitudinal study among Kenyan ART-naïve hospitalized CHIV enrolled in the PUSH trial (NCT02063880).

Methods:

CHIV enrolled in the PUSH trial started ART within 2 weeks after enrollment; all children underwent intensified TB case finding including symptom assessment, tuberculin skin test (TST), chest X-ray (CXR), and mycobacterial culture and Xpert MTB/RIF of respiratory and stool specimens. Anti-TB therapy (ATT) was started based on clinical evaluation and results of TB diagnostic testing. Children were followed for 6 months with serial viral load, CD4%, and growth assessments (weight-for-age [WAZ], height-for-age [HAZ], and weight-for-height [WHZ]). TB-ART treated and ART-only groups were compared at 6-months post-ART for viral suppression (<40 c/ml), CD4% change, and growth using generalized linear models, linear regression, and linear mixed-effects models, respectively.

Result: Among 152 CHIV, 40.8 % (62) were TB-ART treated. At baseline pre-ART, median age was 2.0 years, VL was 5.6 $\log_{10}$ c/ml, CD4% was 14%, and 40.4% had HAZ<2; CD4% and growth measures were significantly lower, and VL significantly higher in the TB-ART group. group. After 6 months on ART, 37.2% of CHIV had viral suppression and the median CD4% increased by 7.2% (IQR 2.0%-11.6%) with no difference between TB-ART and ART-only groups. The TB-ART group had significantly lower WAZ and HAZ over the 6 months of follow-up (WAZ -0.81 [95% CI: -1.23, -0.38], p<0.001; HAZ -0.15 [95% CI: -0.29, -

0.01], p=0.030). The TB-ART group had a greater rate of WAZ increase in analyses unadjusted and adjusted for baseline WAZ (unadj 0.62 [95% CI: 0.18, 1.07, p=0.006] or adj 0.58 [95% CI: 0.12, 1.03, p=0.013]).

Conclusion: TB-HIV co-treatment did not adversely affect early viral suppression and CD4 reconstitution post-ART, suggesting tolerability and lack of interference of TB treatment on ART levels. TB-ART treated CHIV had more rapid growth reconstitution, though growth deficits persisted after 6 months of therapy suggesting the need for intensive growth monitoring and management in this group.

Background

Children living with HIV (CHIV) have a high risk of *Mycobacterium tuberculosis* (Mtb) infection (14) and TB-related morbidity (11,13,37,58) and mortality (9,10,89). Once exposed, CHIV are eight times more likely to develop TB than HIV-unexposed uninfected (HUU) children (31). TB is the leading cause of death (40%) and admission to the hospital (18%) in people living with HIV (34). Africa accounted for 30.5% of the global pediatric TB burden in 2018 (83) and 37.0% of TB-related deaths among children under 15 years of age. Approximately 36.0% of pediatric TB deaths in this region were among CHIV (32), representing 78.0% of TB deaths in CHIV worldwide. Treatment of both TB and HIV is vital to reducing TB-related mortality (84,85) in CHIV. However, TB-HIV co-treatment poses management challenges, including high pill burden, drug toxicity, drug-drug interactions, and immune reconstitution inflammatory syndrome (33,34).

Rifampicin, a component of standard treatment for TB, is a potent inducer of cytochrome P450 isoenzymes (CYP) and p-glycoprotein (PGP) (91–93) – a primary mechanism of drug elimination that reduces plasma concentrations of concomitantly administered protease inhibitors (PI), and non-nucleoside reverse transcriptase inhibitors (NNRTI) (92). Thus, rifampicin may lower plasma ART concentrations and result in inferior virologic and immunological responses (33,91,92). CHIV have a higher incidence of virologic failure than adults due to dosing, bad-tasting formulations, higher likelihood of drug resistance, adherence, social support, and developmental stage (94,95).

TB increases the metabolic rate or resting energy expenditure and impairs substrate handling, facilitating ingested amino acid oxidation instead of protein anabolism (96,97). Thus, TB contributes to malnutrition – mainly wasting and weight loss (96,97). Children typically regain weight after six months of TB treatment (98). Similarly, CHIV have substantially reduced growth (wasting or weight loss) (99) as a consequence of reduced energy intake and increased metabolic rates associated with HIV infection and drug side effects (100). ART restores the growth of CHIV (99,101). TB/HIV co-infection may synergistically exacerbate children's growth deficits (97). While TB-HIV co-treatment improves child survival (84,85), it is unclear how TB-ART treatment influences growth in CHIV receiving ART. Growth trajectories between CHIV with and without TB-HIV co-treatment may differ due to the differential growth effects of TB disease and its' resolution or due to side effects of TB medications. The few studies that have evaluated HIV-TB co-infected children had inconsistent findings (102,103) regarding the impact of co-treatment on viral suppression or immunologic responses in CHIV. A better understanding of viral suppression and the pattern of growth reconstitution during TB-HIV co-treatment can inform treatment approaches and nutritional monitoring and supplementation strategies.

Methods

Study design: This was a retrospective cohort study using data from the Pediatric Urgent Start of Highly Active Antiretroviral Treatment (PUSH) study. PUSH was an unblinded randomized controlled trial of urgent (within 48 hours of enrollment) versus post-stabilization (7 to 14 days after enrollment) ART between 04/ 2013 and 11/ 2015 (NCT02063880). For this secondary analysis, participants were classified based on TB-HIV co-treatment as TB-ART vs. ART-only.

Study population: The PUSH study included ART-naive hospitalized CHIV aged 0-12 years of age. Children were enrolled in four hospitals in Kenya from April 2013 to November 2015 and followed for 6 months. Children with suspected or confirmed central nervous system infections were excluded (104).

The PUSH study obtained ethical approval from Kenyatta National Hospital (KNH)/University of Nairobi (UoN) Ethics Research Committee (ERC), Kenya Pharmacy and Poisons Board (PPB), and the University of Washington (UW) Institutional Review Board. Participants gave written informed consent before participating in the study.

Outcome variables

Viral load suppression: Viral load (VL) was measured at baseline and the 6th month of follow-up. VL suppression was defined as VL of <40 HIV RNA copies/ml at a 6-month follow-up.

CD4% was measured at baseline and 6th month of follow-up. Mean CD4% at 6-months and change in CD4% from baseline were compared.

Growth: Weight and height (length) were collected 9 times (enrollment, when treatment initiated, two weeks, and monthly thereafter), including at baseline. Weight-for-age z-score [WAZ], weight-for-height z-score [WHZ], and height/length-for-age z-score [HAZ]) were defined using WHO child growth standards.

Growth faltering was defined as underweight (WAZ<-2), stunting (HAZ<-2), or wasting (WHZ <-2) (105). WHZ was defined only for children younger than ten years of age.

Exposure variable

TB-ART treatment: TB-HIV treated children are the exposed group and ART-only treated children are the control (comparison) group. The TB_ART treated group received treatment both for TB and HIV. In this study, TB was diagnosed using robust diagnostic methods, including sputum and gastric aspirate Xpert and culture, stool Xpert, urine LAM, chest X-ray, and clinical signs (103,105). Identified TB cases received TB treatment (rifampicin, isoniazid, pyrazinamide, and ethambutol for a 2-month intensive phase and rifampicin and isoniazid for the 4-month continuation phase per Kenyan and WHO guidelines) (103).

Covariates: Potential confounding factors include baseline maternal characteristics such as maternal age in years and educational status (primary or less, secondary or above) and children's baseline characteristics including sex, age (<2, 2-<5, and 5+ years of age), baseline CD4 percent, VL ($\log_{10}$ RNA copies/mL), underweight, stunting, and wasting.

Statistical Analysis*:*

Mean and standard deviation (SD) were used to describe normally distributed continuous variables. Median and interquartile range (IQR) were used to describe skewed distributions, while frequency and percentage were used to describe categorical variables. We compared baseline caregiver and infant characteristics between TB-ART treated and ART-only treated groups, using a two-sided t-test (the Mann-Whitney U test if assumptions were not met) for continuous variables and the Pearson χ^2 test (Fisher's exact test if assumptions were not met) for categorical variables.

We fitted a multivariable generalized mixed linear model (GLM) using Poisson regression with a log link (to estimate relative risk) to compare VL suppression and a multivariable linear regression to compare CD4% at the end of TB treatment between TB-ART treated and ART-only CHIV.

We used the baseline growth data analysis as a visit zero for the longitudinal measurement. We used linear mixed-effects models with autoregression correlation structure, random intercept for subjects, and random slope for follow-up time to compare growth (WAZ, WHZ, HAZ) between TB-ART and ART-only participants. Since TB diagnosis is associated with lower weight or poorer growth, and lower baseline growth may be associated with greater reconstitution, we conducted analyses both with and without adjustment for baseline growth. We fitted the following three mixed models for all growth outcomes - WAZ, WHZ, HAZ: (Model 1) unadjusted model, (Model 2) adjusted for maternal age and education, children's sex, children's age, baseline viral load, and CD4%, and (Model 3) including Model 2 variables and relevant baseline growth marker (WAZ, WHZ, or HAZ) for each outcome. Interaction terms were fitted between TB-ART treatment and follow-up time to examine the rate of change in WAZ and HAZ in Models 2 and 3 for WAZ and HAZ analyses.

Kaplan-Meier survival analysis was used to compare the time from baseline to the first event of increasing WAZ, HAZ, or WHZ by at least 0.5 z-scores from the baseline to the 6 months TB treatment period. Univariate and multivariable Cox proportional hazards regression models were fitted to compare the probability of improving WAZ, HAZ, and WHZ by at least 0.5 z-scores after treatment between the TB-ART treated and ART-only children.

Approach to missing data

Some visits had missing data: for example, WAZ (6%-16% missing per visit), VL (7% baseline, 15% at 6 months), and 6th month CD4% (12%). Multiple imputations by chained equations (MICE), including a series of regression models for each missing variable conditional upon other specified variables were used to manage missing data. In addition to covariates of the current study – TB treatment, child's age, sex, CD4%, ART regimen, baseline WAZ, HAZ, caregiver's age, and education. We imputed the data 25 times. Pooled parameter estimates and their standard errors were calculated according to Rubin's rules to account for the between- and within-imputation variance (107,108). We employed one single imputation model to obtain imputed values for outcomes – WAZ and HAZ missed at each visit during follow-up, baseline WAZ, and baseline viral load. We also ran another imputation model to obtain imputed data for the 6th month CD4% and VL by including only participants who were alive by the end of the study. In the imputation models, we specified the appropriate distributions for each of the variables in the model. In the presence of missing data, the multiple imputation approach should yield unbiased estimates assuming data are missing at random (MAR).

P-values of <.05 cut-offs and 95% confidence intervals were used to determine statistical significance. R version 3.5.1 and STATA version 16 statistical software were used for analyses.

Results

Among the 181 participants in the PUSH RCT, 2 children who had confirmed TB disease but did not start TB treatment and 27 children with no follow-up after baseline (5 lost-to-follo-up and 22 deaths) were excluded. Thus, 152 eligible CHIV children were included in the current analyses: 62 TB-ART co-treated and 90 ART-only. Of these 152 children, 15 died before study completion.

Maternal and children's characteristics

Participants' median (IQR) age was 2.0 (1.1-6.1) years, 53.3% (81/152) were males, 58.6% (89/152) started NNRTI-based ART regimens, 67.1% (102/152) were WHO stage 3 or 4, and median (IQR) baseline CD4% was 14% (IQR 9.0% – 22.3%). Ten percent (15/152) of CHIV's mothers were employed (Table 1).

At baseline, the TB-ART group had higher mean VL (5.7 vs 5.4 $\log_{10}$ c/ml), lower mean CD4% (12.5% vs 15.2%), and a higher proportion of children who were WHO stage 3 or 4 (62.3% vs 51.1%), underweight (72.6% vs 50.0%), or stunted (66.1% vs 55.6%) than the ART-only group.

Effect of TB-HIV co-treatment on change in CD4 percent and VL suppression

Following 6-months of ART, overall, 37.2% (45/121) CHIV had undetectable VL <40 RNA copies/ml, 42.9% (21/49) in TB-ART group and 33.3% (24/72) in ART-only group. Overall, 63.6% (77/121) and 71.1 (86/121) were virally suppressed to <400 c/ml and <1000 c/ml respectively. The median CD4% increase during 6-month follow-up was 7.2% (2.0-11.6); 7.3% (1.6-11.9) in TB-ART group and 7.0% (2.7, 11.6) in ART-group. In univariate models, female sex and higher baseline viral load were associated with significantly lower frequency of viral suppression at 6 months post-ART. In univariate and multivariate models, adjusted for maternal age, education, child sex, age, baseline VL, and CD4%, the TB-ART group had a comparable proportion of VL suppression at 6-months follow-up compared to ART-only group (aRR=1.13 [95% CI: 0.96, 1.33], p = 0.153) (Table 2). Similarly, CD4% increase post-ART did not differ significantly between the TB-ART and ART-only groups (β=-0.84 [95% CI: -3.64, 1.97], p=0.555) (Table 3). Because PI-based ART may particularly interact with TB regimens, we examined the subset of CHIV receiving PI-ART and found no significant differences in viral suppression frequency (50% [14/28] vs 40.0% [18/45]) or change in CD4% (8.34% vs 7.0%) between TB-ART vs ART-only groups in CHIV receiving PI-ART.

In sensitivity analysis, we compared VL outcomes in the TB-ART and ART only groups using varied VL cut-offs ($\geq$ 400 copies/mL vs <400 copies/mL) and also (> 1000 copies/mL vs <1000 copies/mL), and we found the same results as when viral load suppression was defined as <40 copies/mL (Supplemental Table 1).

Growth between pre-ART and 6-months post-ART

Overall, participants' unadjusted average WAZ and HAZ scores increased substantially and significantly by 1.05 (95% CI: 0.81, 1.30) and 0.30 (95% CI: 0.14, 0.45) z-scores, respectively, from baseline to 6-month follow-up. In the TB-ART group, average WAZ score increased significantly by 1.42 (95% CI: 1.04, 1.80), while HAZ did not (0.07 (95% CI: -0.17, 0.31). In the ART-only group, the average WAZ and HAZ scores increased significantly by 0.72 (95% CI: 0.47, 0.98) and 0.44 (95% CI: 0.24, 0.64), respectively.

Effect of TB-HIV co-treatment on change in WAZ

After adjustment for maternal age, education, and children's sex, age, and baseline VL, but not baseline WAZ, the TB-ART group had WAZ scores that were 0.91 less than ART-only group (β=-91 [95% CI: -1.35, -0.47], p<0.001) (Table 3). TB-ART treated children had a significantly more rapid increase in WAZ during the treatment period than the ART-only children. TB-ART treated children gained 0.07 more WAZ scores per month during the treatment period than ART-only treated children (β=0.07 [95% CI: 0.02, 0.13], p=0.006) (Figure 1).

Because the TB-ART group started from lower growth at baseline, their more rapid growth reconstitution could have been due to baseline growth differences. To understand how much of the growth reconstitution differences were due to baseline differences in growth, we adjusted for both confounding variables *and* the baseline pre-ART growth. In these adjusted analyses, there was no statistically significant difference in WAZ between the TB-ART and the ART groups during the treatment period (β=-0.06 [95% CI: -0.28, 0.16], p=0.575) (Table 3). However, the rate of growth recovery remained significantly faster in TB-ART treated children than in ART-only children. After adjustment for baseline WAZ, TB-ART treated children gained 0.08 more WAZ scores per month during the treatment period than ART-only children (β=0.08 [95% CI: 0.03, 0.13], p=0.003) (Figure 1).

Effect of TB-HIV co-treatment on change in HAZ

After adjustment for maternal age, education, child sex, age, and baseline VL, but not baseline HAZ, CHIV receiving TB-ART had a significant lower HAZ during the treatment period (β=-0.56 [95% CI: -1.06, -0.07], p =0.027) (Table 3). The TB-ART group had a statistically significantly slower HAZ increase per month than ART-only children (β=-0.04 [95% CI: -0.08, -0.01]), p =0.026) (Figure 1).

Adjusting for confounding variables *and* baseline pre-ART HAZ, there was a statistically significant association between TB-HIV co-treatment and HAZ during the treatment period. TB-ART treated children had on average 0.21 lower HAZ scores during the study period than ART-only children (β=-0.21 [95% CI: -0.36, -0.06], p=0.003) (Table 3). The TB-ART group had a statistically significantly slower HAZ increase per month than ART-only children (β=-0.05 [95% CI: -0.08, -0.01], p=0.007) (Figure 1).

Effect of TB-HIV co-treatment on change in WHZ

After adjustment for maternal age, education, and children's sex, age, and baseline VL, but not baseline WHZ, the TB-ART group had WHZ scores that were 0.93 less than ART-only group (β=-93 [95% CI: -1.39, -0.47], p<0.001) (Supplemental Table 2). TB-ART treated children had a significantly more rapid increase in WHZ during the treatment period than the ART-only children. TB-ART treated children gained 0.18 more WHZ scores per month during the treatment period than ART-only treated children (β=0.18 [95% CI: 0.11, 0.26], p<0.001).

After adjusting for both confounding variables *and* the baseline pre-ART growth, there was no statistically significant difference in WHZ between the TB-ART and the ART groups during the treatment period (β=-0.08 [95% CI: -0.41, 0.24], p=0.616) (Supplemental Table 2). However, the rate of growth recovery remained significantly faster in TB-ART treated children than in ART-only children. After adjustment for baseline WHZ, TB-ART treated children gained 0.19 more WHZ scores per month during the treatment period than ART-only children (β=0.19 [95% CI: 0.11, 0.27], p<0.001).

The probability of improving WAZ, HAZ, and WHZ by at least 0.5 Z-scores among CHIV who were underweight or stunted at baseline

At baseline, more than half of the children were underweight (64.5%) or stunted (60.8%), with a higher frequency of underweight and stunting in the TB-ART group (Table 1). We examined time to growth recovery during 6-month treatment. Figure 2 shows the cumulative probability of improving WAZ, HAZ, and WHZ by at least 0.5 Z-score among CHIV who were <2 z-scores for the relevant growth parameter at baseline (Supplemental Table 3). Children treated with TB-HIV had no significant difference in their chances of experiencing a minimum of 0.5 improvements in WAZ (aHR=1.21 [95% CI: 0.78, 1.89]) and HAZ (aHR=0.65 [95% CI: 0.38, 1.10]) scores compared to children treated with ART alone.

Discussion

In this study, among hospitalized CHIV newly initiating ART, TB-ART co-treatment did not compromise viral suppression or immune reconstitution during the 6-month follow-up. CHIV receiving TB-HIV co-treatment gained weight more rapidly but still had significantly lower weight and height for age than ART-only children by the end of TB treatment period. TB-ART treated children lagged height reconstitution despite robust weight reconstitution compared to CHIV receiving ART only. Overall, our data suggest good clinical outcomes but persistent growth deficits in TB-ART treated children that will require continued growth monitoring and potential nutritional interventions.

We found that TB-ART co-treatment did not compromise immune reconstitution and viral load suppression compared to ART-only group. There are conflicting data on the impact of TB co-treatment on viral suppression and immune recovery from other studies, some using different VL cut-offs (102,103). One study from South Africa, which used a <50 c/ml cut-off (similar to our study) found no difference in viral suppression between CHIV receiving TB-ART and those on ART-only (102), findings consistent with our results. Although some studies which used a higher VL cut-off (400 c/ml) found that TB-ART co-treatment decreased frequency of viral suppression (103), we failed to find an association in sensitivity analyses using this higher cut-off. Both studies, however, have noted that the impact of TB co-treatment varies by ART regimen, with the limited impact of TB co-treatment on VL suppression among CHIV receiving NNRTI-based regimens, in contrast to CHIV on PI-based regimens which may have inferior virologic and immunologic responses with TB co-treatment (102,103). In our study, CHIV receiving PI-ART had a lower frequency of viral suppression than those receiving NNRTI-based regimens in univariate but not multivariate analyses and decreased CD4 recovery in multivariate analyses. However, in the subset of CHIV receiving PI-based regimens, there was no difference in viral suppression and immune recovery in those receiving TB-ART versus ART, although we had limited statistical power in this sub-group analysis. CHIV in the TB-ART group on LPV/r, received boosted ritonavir to compensate for potential drug-drug interactions which may have attenuated the impact of TB co-treatment on viral suppression or immune reconstitution.

As expected, ART resulted in rapid growth reconstitution in all CHIV, including those receiving TB-ART (109). TB-ART treated children had lower WAZ and HAZ at baseline and despite more rapid growth reconstitution, had residual deficits throughout the treatment period. Children treated for TB and HIV have a double burden of disease that contributes to their weight loss (110). TB-ART treated children gained weight more rapidly than ART-only children, on average about 0.08 WAZ scores more per month than the ART-only group. In children treated for TB, weight gain is an important indicator of prognosis (111). Rapid growth reconstitution is encouraging but residual growth deficits persisted at 6 months, underscoring the importance of continued growth and nutritional monitoring and support.

The TB-ART treated group had more rapid weight reconstitution but lower height reconstitution for their ages than ART-only treated group. This may be due to some persistent metabolic costs of TB and HIV during the first 6 months of treatment (96,97,100). Height deficits might eventually result in stunting, with long-term implications on late childhood and adult obesity and related metabolic disorders (112).

Our study has strengths and limitations. In this study, about 70 % of CHIV patients who were on TB-ART, started ART and TB treatment within 8 days intervals, which provides information for clinicians and policymakers about the effects of ART and TB co-treatment at ART initiation. We were able to assess important HIV outcomes, viral suppression, CD4 reconstitution, and growth parameters during the critical first 6 months post-ART but not longer-term outcomes. This cohort of CHIV who were hospitalized had high mortality, which decreased the sample size and may have led to selection bias in estimating the impact of TB-HIV co-treatment on viral suppression, immune recovery, and growth. Follow-up was limited to 6 months which relatively encompasses sufficient time for viral suppression and completion of TB treatment but does not span the entire period of potential growth recovery. If there was a meaningful difference below what this study was powered to identify, a type II error might have occurred.

Conclusion

TB co-treatment did not affect immune reconstitution and viral load suppression at 6-months post-ART among CHIV newly initiating ART. TB-ART treated children had more rapid weight gain during the follow-up period but still had lower weight and height than ART-only treated children by the end of the 6-month follow-up period. TB-ART treated children had robust weight reconstitution but lagged height reconstitution compared to CHIV receiving ART-only. Despite good clinical outcomes and rapid growth reconstitution, growth deficits are persistent in TB co-treated children, which will require continued growth monitoring and nutritional interventions.

Tables

Table 11: Baseline maternal and child demographic and clinical characteristics

Variable	Treatment	
	TB-ART cotreated (n=62)	ART-only (n=90)
Maternal baseline characteristics		
Mean age in years (IQR)	29 (24.0 – 33.0)	29 (24.0-32.0)
Marital status no. (%)		
Living with partner(s)	40 (38.8)	63 (61.2)
Separated, Widow, widower	17 (43.6)	22 (56.4)
Single	4 (44.4)	5 (55.6)
Maternal education – no. (%)		
Primary or less	43 (69.4)	56 (62.2)
Secondary or above	19 (30.1)	34 (37.8)
Maternal employment – no./total no. (%)		
Employed	3 (20)	12 (80)
Unemployed	36 (46.2)	42 (53.9)
Other	23 (38.9)	36 (61.0)
Children's baseline characteristics		
Age – no (%)		
Less than two years of age	28 (38.9)	44 (61.1)
2-<5 years of age	14 (36.8)	24 (63.2)
5+ years of age	20 (47.6)	22 (52.4)
Female sex – no (%)	30 (48.4)	41 (45.6)
ART regimen		
NNRTI-based	32 (52.5)	53 (63.1)
LPV/r-based	29 (47.5)	31 (36.9)
WHO stage 3 or 4 – no (%) *	56 (90.3)	46 (51.1)
Mean CD4% (SD)*	12.5 (9.2)	15.2 (10.1)
Severe immune suppression – no (%)$^{\delta}$	47 (73.4)	77 (67.5)
Mean $\log_{10}$ viral load (IQR)*	5.7 (0.1)	5.4 (0.1)
Underweight– no (%) *	45 (72.6)	45 (50.0)
Mean WAZ (IQR)*	-3.3 (1.7)	-2.2 (1.5)
Stunting (HAZ <-2) – no (%)	41 (66.1)	50 (55.6)
Median HAZ (IQR)	-2.7 (1.8)	-2.3 (1.6)

$^{\delta}$ Severe immune suppression – CD4% (<12 months:<25%, 12–35 months:<20%,>36 months: <15%) or, in absence of CD4%, CD4 cell count (<12 months: <1500 cells/ml, 12–35 months: <750 cells/ml, >36 months <350 cells/ml. *These are baseline characteristics that are unevenly distributed between the exposure groups, p-value <0.05.

Table 12: Cofactors of viral load suppression and CD4% increase in hospitalized CHIV initiating ART

Model	Treatment	Viral load suppressed (<40 copies/mL)		CD4%	
		cRR$^{\sigma}$ (95% CI)	aRR$^{\S\beta}$ (95% CI)	cCoefficient$^{¤}$	aCoefficient$^{\S\beta}$
Overall	TB-ART co-treated (ref: ART-only)	1.25 (0.79, 1.99)	1.29 (0.79, 2.11)	-2.31 (-5.59, 0.97)	-0.83 (-3.68, 2.01)
	Maternal age in years	1.01 (0.98, 1.05)	1.01 (0.97, 1.05)	-0.12 (-0.38, 0.14)	-0.14 (-0.37, 0.09)
	Maternal education: secondary or above (ref: Primary or less)	0.73 (0.43, 1.24)	0.90 (0.52, 1.56)	-3.86 (-7.18, -0.54)**	-0.23 (-3.40, 2.94)
	Female children	0.61 (0.36, 1.01)	0.63 (0.37, 1.07)	4.24(1.10, 7.38)**	2.44 (-0.34, 5.24)
	Age of children				
	<2 years of age	1	1	1	1
	2-<5 years of age	1.92 (1.01, 3.63)*	0.86 (0.25, 2.90)	-2.03 (-5.98, 1.91)	-1.51 (-6.66, 3.64)
	5+ years of age	2.17 (1.20, 3.91)*	0.74 (0.20, 2.72)	-2.82 (-6.65, 1.00)	-0.87 (-7.26, 5.52)
	Baseline $\log_{10}$ viral load	0.90 (0.85, 0.96)**	0.92 (0.85, 0.98)		
	Baseline CD4 percent			0.55 (0.43, 0.67)***	-1.02 (-6.48, 4.44)
	ART regimen: LPV/r-based (ref: NNRTI-based)	0.53 (0.31, 0.92)*	0.50 (0.16, 1.52)	1.43 (-2.13, 4.99)	0.56 (0.41, 0.70)**
PI regimen	TB-ART co-treated (ref: ART-only)	1.09 (0.64, 1.88)	1.08 (0.63, 1.87)	0.50 (-4.82, 5.82)	0.27 (-4.98, 5.27)

$^{\sigma}$ cRR – crude relative risk. §aRR – adjusted relative risk. $^{¤}$cCoefficient - crude coefficient. §aCoefficient – adjusted coefficient. $^{\beta}$ Multivariable models adjusted for all other variables (TB treatment, maternal age, education, baseline viral load/CD4 percent, children sex, and age). *P-value <0.05. **P-value<0.01. ***P-value <0.001.

Table 3:Cofactors of WAZ and HAZ reconstitution in hospitalized CHIV initiating ART

	WAZ[¶]			HAZ[¥]		
	Model 1 cCoefficient[¤]	**Model 2 aCoefficient[§]**	**Model 3 aCoefficient[β]**	**Model 1 cCoefficient[¤]**	**Model 2 aCoefficient[§]**	**Model 3 aCoefficient[β]**
TB-ART co-treated	-0.89 (-1.31, -0.46)***	-0.91 (-1.35, -0.47)***	-0.06 (-0.28, 0.16)	-0.59 (-1.08, -0.10)*	-0.56 (-1.06, -0.07)*	-0.21 (-0.36, -0.06)**
Maternal age in years	-0.00 (-0.04, 0.03)	-0.01 (-0.05, 0.03)	0.00 (-0.02, 0.01)	0.01 (-0.03, 0.05)	-0.01 (-0.05, 0.04)	0.00 (-0.01, 0.01)
Maternal education: secondary or above (ref: Primary or less)	-0.04 (-0.48, 0.40)	0.00 (-0.45, 0.46)	0.02 (-0.19, 0.23)	0.11 (-0.60, 0.38)	-0.17 (-0.68, 0.34)	0.04 (-0.11, 0.20)
Female children(ref: male)	0.23 (-0.21, 0.67)	0.20 (-0.22, 0.62)	0.22 (0.03, 0.42)*	0.19 (-0.30, 0.68)	0.18 (-0.30, 0.66)	0.00 (-0.15, 0.14)
Age of children						
<2 years of age	1	1	1	1		1
2-<5 years of age	0.46 (-0.08, 0.99)	0.36 (-0.16, 0.88)	0.31 (0.08, 0.54)*	0.10 (-0.59, 0.61)	-0.07 (-0.66, 0.53)	-0.06 (-0.24, 0.11)
5+ years of age	0.34 (-0.19, 0.87)*	0.36 (-0.17, 0.89)	0.17 (-0.08, 0.41)	0.49 (-0.09, 1.07)	0.48 (-0.12, 1.08)	0.05 (-0.13, 0.23)
Baseline $\log_{10}$ viral load	-0.29 (-0.55, -0.04)	-0.04 (-0.14, 0.05)	-0.02 (-0.06, 0.02)	-0.44 (-0.72, -0.15)*	-0.11 (-0.22, -0.01)*	-0.03 (-0.06, 0.01)
Baseline WAZ/HAZ	0.69 (0.62, 0.76)8***	δ	0.71 (0.65, 0.77)***	0.84 (0.80, 0.89)***		0.84 (0.80, 0.88)***

[¶] WAZ – weight-for-age. [¥] HAZ – height-for-age. [¤] cCoefficient - crude coefficient. [§] aCoefficient – adjusted coefficient for all other variables (TB treatment, maternal age, education, baseline viral load, CD4 percent, children sex, age). [§] aCoefficient – adjusted coefficient including baseline WAZ for WAZ modes/ baseline HAZ for HAZ models. *P-value <0.05. **P-value<0.01. ***P-value <0.001. [δ] Variable not adjusted

Figures

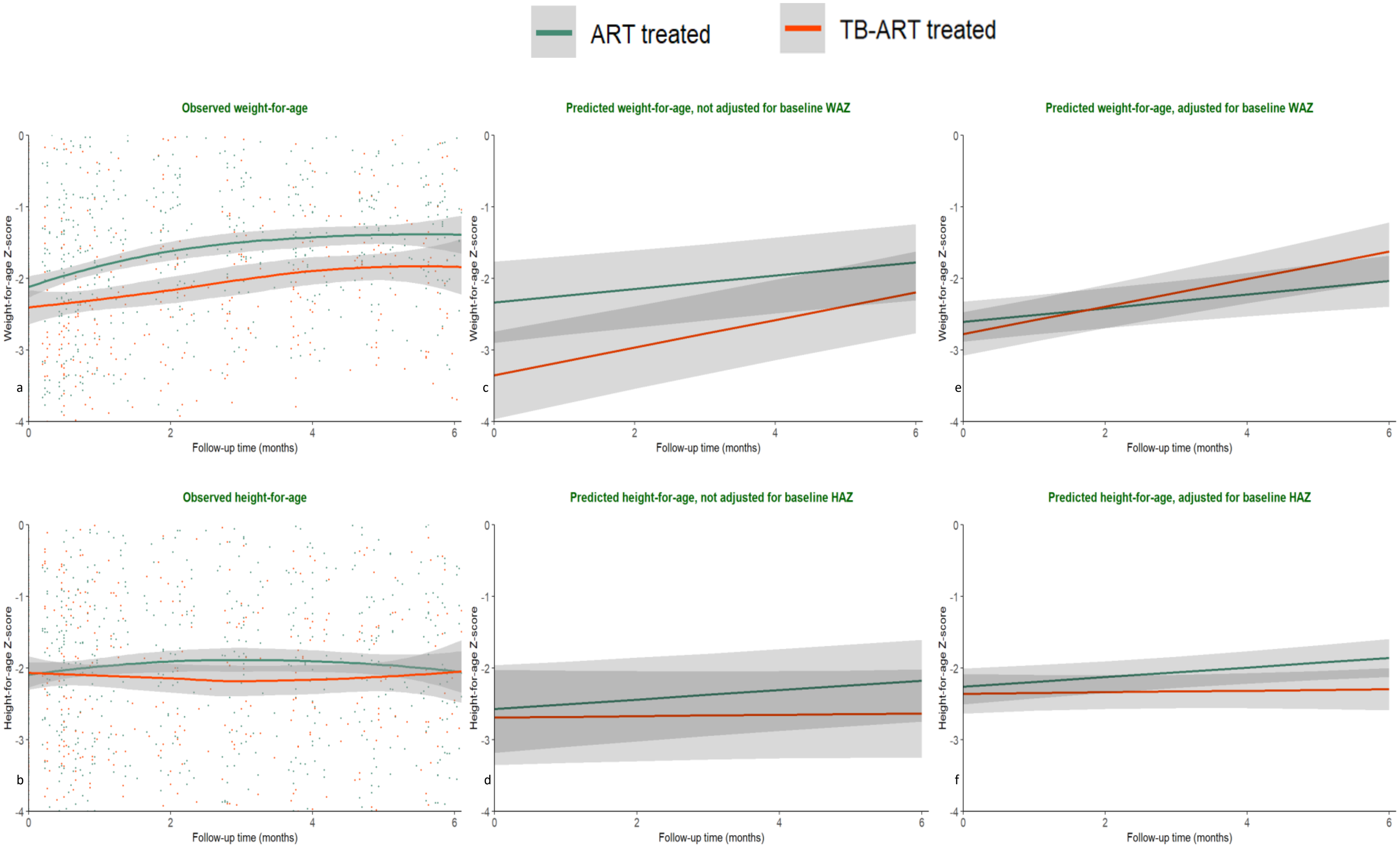


Figure 6: The left two curves are scatter plot of change in WAZ and HAZ over time by TB treatment

(The Lowess curves represent growth reconstitution following TB and /or ART treatment. The middle and right curves show adjusted change in WAZ and HAZ without and with basleine growth adjusted, respectively.)

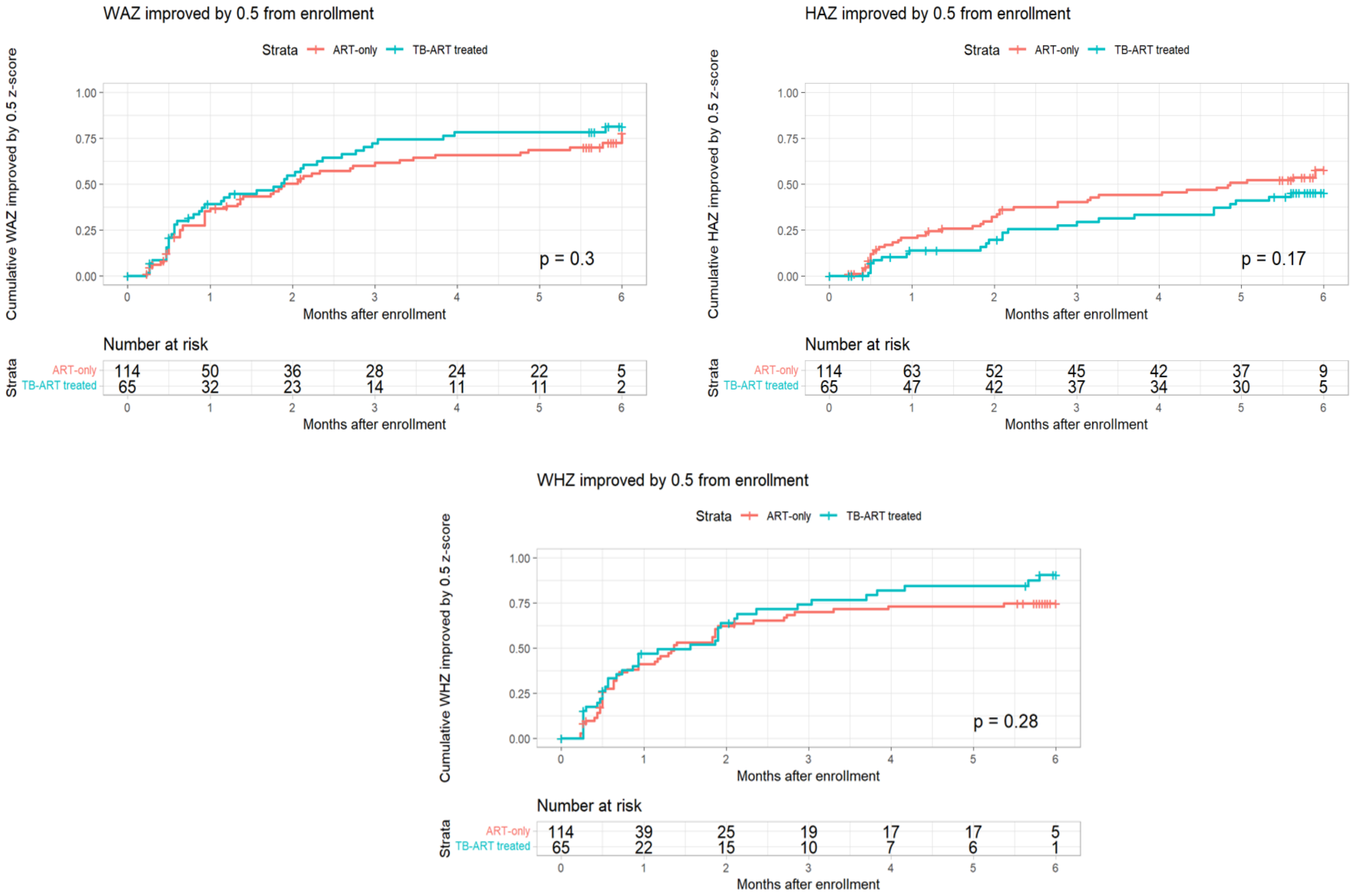


Figure 7: The figure shows cumulative probability of weight-for-age (WAZ), weight-for-length (WLZ), and length-for-age (LAZ) improved by at least 0.5 by TB-treatment

Supplemental tables

Supplemental Table 1: Association of cofactors and viral load suppression defined as <400 copies/mL and <1000 copies/mL

Model	Treatment	**Viral load suppressed (<1000 copies/mL)**		**Viral load suppressed (<400 copies/mL)**	
		cRR$^{\sigma}$ (95% CI)	**aRR$^{\S\beta}$ (95% CI)**	**cRR$^{\sigma}$ (95% CI)**	**aRR$^{\S\beta}$ (95% CI)**
Overall	TB-ART co-treated (ref: ART-only)	0.99 (0.77, 1.28)	1.10 (0.85, 1.43)	0.92 (0.68, 1.24)	1.02 (0.76, 1.37)
	Maternal age in years	1.01 (0.99, 1.03)	1.01 (0.99, 1.03)	1.02 (0.99, 1.03)	1.01 (0.99, 1.04)
	Maternal education: secondary or above (ref: Primary or less)	1.08 (0.84, 1.39)	1.21 (0.93, 1.57)	0.98 (0.73, 1.33)	1.15 (0.86, 1.56)
	Female children	0.92 (0.71, 1.20)	0.99 (0.76, 1.29)	0.88 (0.65, 1.18)	0.98 (0.72, 1.31)
	Age of children				
	<2 years of age	1	1	1	1
	2-<5 years of age	1.79 (1.30, 2.48)***	1.51 (0.86, 2.66)	2.25 (1.54, 3.30)	1.74 (0.95, 3.20)
	5+ years of age	1.67 (1.21, 2.30)**	1.23 (0.66, 2.30)	1.89 (1.27, 2.81)	1.27 (0.65, 2.50)
	Baseline $\log_{10}$ viral load	0.98 (0.93, 1.03)	1.00 (0.94, 1.05)	0.95 (0.91, 1.01)	0.98 (0.93, 1.05)
	Baseline CD4 percent				
	ART regimen: LPV/r-based (ref: NNRTI-based)	0.62 (0.46, 0.84)**	0.74 (0.46, 1.19)	0.59 (0.42, 0.84)**	0.74 (0.46, 1.19)
PI regimen	TB-ART co-treated (ref: ART-only)	1.09 (0.64, 1.88)	1.08 (0.63, 1.87)	1.09 (0.64, 1.88)	1.08 (0.63, 1.87)

$^{\sigma}$ cRR – crude relative risk. §aRR – adjusted relative risk. $^{\beta}$ Multivariable models adjusted for all other variables (TB treatment, maternal age, education, baseline viral load/CD4 percent, children sex, and age). *P-value <0.05. **P-value<0.01. ***P-value <0.001.

Supplemental Table 2: Cofactors of WHZ reconstitution in hospitalized CHIV initiating ART

		WHZ[¶]	
	Model 1 **cCoefficient[¤]**	**Model 2** **aCoefficient[§]**	**Model 3** **aCoefficient[β]**
TB-ART co-treated	-0.90 (-1.34, -0.46)***	-0.93 (-1.39, -0.47)***	-0.08 (-0.41, 0.24)
Maternal age in years	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.01)	-0.02 (-0.05, 0.00)
Maternal education: secondary or above (ref: Primary or less)	0.20 (-0.29, 0.70)	0.23 (-0.25, 0.72)	0.08 (-0.22, 0.39)*
Female children(ref: male)	0.01 (-0.46, 0.47)	0.09 (-0.36, 0.54)	0.19 (-0.09, 0.48)
Age of children			
<2 years of age	1	1	1
2-<5 years of age	0.54 (-0.01, 1.10)	0.61 (0.07, 1.15)*	0.60 (0.27, 0.94)*
5+ years of age	0.21 (-0.35, 0.78)	0.44 (-0.13, 1.01)	0.30 (-0.09, 0.69)
Log 10 of baseline viral load	-0.01 (-0.12, 0.09)	0.03 (-0.08, 0.14)	0.00 (-0.06, 0.06)
Baseline WAZ/HAZ	0.55 (0.48, 0.62)		0.55 (0.47, 0.62)***

Supplemental Table 3:Cofactors of WAZ and HAZ improved by at least 0.5

	WAZ improved by at least 0.5			HAZ improved by at least 0.5			WHZ improved by at least 0.5		
Treatment	cHR[σ] (95% CI)	aHR[§] (95% CI)	P-value	cHR[σ] (95% CI)	aHR[§] (95% CI)	P-value	cHR[σ] (95% CI)	aHR[§] (95% CI)	P-value
TB-ART co-treated (ref: ART-only)	1.23 (0.83, 1.83)	1.21 (0.78, 1.89)	0.398	0.71 (0.43, 1.17)	0.65 (0.38, 1.10)	0.102	1.26 (0.82, 1.92)	1.32 (0.81, 2.22)	0.266
Maternal age in years	1.00 (0.97, 1.03)	1.00 (0.96, 1.03)	0.799	1.01 (0.97, 1.05)	1.01 (0.97, 1.06)	0.592	0.99 (0.96, 1.03)	0.99 (0.95, 1.03)	0.603
Maternal education: secondary or above (ref: Primary or less)	0.78 (0.51, 1.19)	0.83 (0.52, 1.31)	0.418	1.84 (1.14, 2.99)	1.58 (0.95, 2.64)	0.078	0.91 (0.58, 1.43)	1.04 (0.64, 1.70)	0.866
Female children (ref: male)	1.10 (0.74, 1.63)	1.11 (0.73, 1.71)	0.626	0.61 (0.38, 1.00)	0.62 (0.37, 1.04)	0.071	1.13 (0.74, 1.72)	1.04 (0.64, 1.70)	0.486
Age of children									
<2 years of age	1	1	1	1	1	1	1	1	
2-<5 years of age	1.57 (0.98, 2.53)	1.59 (0.95, 2.64)	0.076	0.71 (0.39, 1.28)	0.81 (0.44, 1.50)	0.505	1.79 (1.12, 2.86)	1.92 (1.16, 3.16)	0.011
5+ years of age	1.46 (0.90, 2.38)	1.45 (0.83, 2.52)	0.192	0.58 (0.32, 1.06)	0.59 (0.31, 1.14)	0.119	1.11 (0.62, 1.98)	1.13 (0.58, 2.18)	0.722
Baseline $\log_{10}$ viral load	0.95 (0.88, 1.04)	0.96 (0.88, 1.06)	0.419	1.03 (0.92, 1.15)	1.03 (0.92, 1.16)	0.583	0.98 (0.90, 1.07)	0.97 (0.88, 1.07)	0.646

[σ] cHR– crude hazard ratio. [§]aHR – adjusted hazard ratio - adjusted for all other variables (TB treatment, maternal age, education, children sex, age, and baseline viral load).

Overall summary

This dissertation contributes to the understanding of the effects of administering IPT during pregnancy and infancy, the effects of HIV and ART exposure *in utero* on growth and development of HEU children, and the effect of TB-HIV cotreatment on viral suppression and immunological and growth reconstitution. We found that growth trajectories and development of HEU children were similar to those of HUU children. Maternal BMI, maternal education, and breastfeeding had a greater effect on growth and development of HEU children than HIV exposure in the setting of universal maternal ART. Maternal pregnancy-IPT influenced growth outcomes among HEU infants with an increased risk of low birth weight, preterm birth, and underweight among males. In contrast, infant IPT administration among HEU infants during the first year of life did not significantly impact growth outcomes during a 2-year follow-up. Among CHIV, TB-ART treated CHIV showed less height reconstitution despite robust weight gain compared to CHIV receiving only ART. TB-ART co-treated children (who had lower baseline WAZ) had persistent growth deficits after 6 months of treatment compared to ART-treated children, despite more rapid growth reconstitution.

The results of Chapter 1 did not confirm some prior studies that HEU children have growth and development deficits compared to HUU children. These studies used data collected before or around the time of adoption of option B+ PMTCT regimens and had a lower prevalence of breastfeeding, and relatively larger sample size than our study. We used recent data from a cohort with universal ART use in pregnancy with almost 70% of mothers on ART pre-pregnancy and almost universal breastfeeding. Lack of growth and developmental difference between HEU and HUU children in our study may be due to earlier viral suppression and breastfeeding. Despite a relatively small sample size, we found a substantial and significant impact of maternal education and breastfeeding on infant growth and development, suggesting that these factors may have more impact than HIV or ART exposure in the current context.

Although WHO recommends IPT for WLHIV during pregnancy, findings in Chapter 2 indicate that pregnancy-IPT has an adverse impact on birth weight, preterm birth, and growth, specifically among male infants. In the parent trial, there was no difference in maternal and infant TB infection and disease whether IPT was initiated during pregnancy or postpartum, suggesting that administering postpartum IPT could have the same impact on preventing LTBI and TB disease without the adverse risking adverse birth outcome. Moreover, Chapter 3 demonstrates that infant IPT had no effect on infant growth and poses no significant adverse effects, suggesting that IPT could be used to prevent and treat TB infection during infancy if large studies indicated that it could be effective.

In Chapter 4, we found that TB co-treatment did not have any effect on immune reconstitution and viral load suppression after six months of ART regardless of the ART regimen. TB-HIV treated children had great growth deficits at baseline which persisted throughout the 6-months follow-up period despite rapid growth recovery. TB-ART co-treated children had a slower height gain at 6 months post-ART treatment. Despite good clinical outcomes and rapid growth recovery, growth deficits persist in TB co-treated children, requiring continued growth monitoring and nutritional interventions.

In conclusion, we found that maternal education and breastfeeding influenced the growth and development of children more than *in utero* HIV and ART exposure. Postpartum maternal or infant IPT could be a safer alternative than IPT during pregnancy for treating and preventing TB infection. Due to the double burden of increased metabolic rate, TB-ART cotreatment is associated with persistent growth deficits that require continued monitoring after 6 months of treatment.

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