# Nutrition, Growth and Health in Tanzanian Infants

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# Nutrition, Growth and Health in Tanzanian Children

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## Abstract

Undernutrition in early life increases children's risk of mortality, morbidity, and impaired growth and development. This thesis analyzes data from two randomized controlled trials in Dar es Salaam, Tanzania. The first trial assessed the effect of daily multivitamin (vitamins B-complex, C and E) supplementation on mortality and morbidity in infants born to HIV-infected mothers. 2387 infants were randomized to multivitamins or placebo at 6 weeks and followed-up for two years. The second trial assessed the effect of zinc and/or multivitamins (vitamins B-complex, C and E) on morbidity in infants born to HIV-uninfected mothers. 2400 infants were randomized to either zinc + multivitamins, zinc only, multivitamins only, or placebo at 6 weeks and were followed for 18 months.

*Chapter 1* assesses the effect of zinc and/or multivitamin supplements in the second trial on longitudinal child growth – defined by change in height-for-age, weight-for-age and weight-for-height z-scores (HAZ, WAZ and WHZ) and stunting, underweight and wasting (<-2 SD 2006 WHO standard for each indicator respectively). We found small, but significant effects of supplements on change in WHZ and WAZ, but did not find a statistically significant effect of zinc and/or multivitamin supplements on stunting, wasting or underweight.

*Chapter 2* assesses the effect of zinc and/or multivitamin supplements in the second trial on early child development (ECD) assessed using the cognitive, motor (fine and gross) and language (receptive and expressive) scales of the Bayley Infant Scales of Development 3rd Edition (BSID-III). We did not find a significant effect of supplements on early childhood development as assessed by the BSID-III.

*Chapter 3* pools the two trials in order to compare mortality, morbidity and growth in HIV-infected, HIV-exposed-but-uninfected (HIV-EU) and HIV unexposed infants. HIV-infected children had the highest rates of mortality, morbidity and growth failure. HIV-EU infants had higher rates of mortality and morbidities than unexposed infants; but lower rates than their HIV-infected peers.

**Conclusions:** Alternative approaches (beyond zinc and/or multivitamin supplementation) to improve growth and ECD in vulnerable populations should be pursued. Child health interventions should target not only HIV-infected but also HIV-EU children, given their increased susceptibility to morbidity and mortality.

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## ACKNOWLEDGEMENTS

# Chapter 1

**The effect of zinc and/or multivitamin supplementation on the growth of Tanzanian children aged 6-84 weeks: a randomized, placebo-controlled, double-blind trial**

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**ABBREVIATIONS**

AI - Adequate Intake

HAZ – height-for-age z-score

IU – international units

MV – multivitamins

Recommended Dietary Allowance (RDA)

WAZ = weight-for-age z-score

WHO – World Health Organisation

WHZ – weight-for-height z-score

Zn – Zinc

Zn + MV – zinc and multivitamins

**CLINICAL TRIAL REGISTRATION**

Clinicaltrials.gov identifier NCT00421668

**KEYWORDS**

Zinc, Multiple Micronutrients, Supplementation, Infancy, Growth

**ABSTRACT**

**Background:** Poor child growth increases the risks of mortality and morbidity. Micronutrient supplements have the potential to improve child growth.

**Objective:** We assessed the effect of daily zinc and/or multivitamin (vitamins C, E & B-complex) supplements on growth among infants in Tanzania.

**Design:** In this randomized, 2x2 factorial, double-blind trial, 2400 infants were randomized to zinc (Zn), multivitamins (MV), zinc and multivitamins (Zn+MV), or placebo at 6 weeks of age and followed for 18 months with monthly growth measurements. Mixed effects models with restricted cubic splines for mean change in anthropometric z-scores were fit for each group. Likelihood ratio tests were used to compare the effect of supplements on growth trajectories. Cox proportional hazards models were used to compare the incidence of stunting, wasting and underweight.

**Results:** Children in all groups experienced growth faltering. At 19 months of age, the prevalence of stunting, wasting and underweight were 19.8%, 6.0% and 10.8% respectively. Change in weight-for-age (WAZ) and weight-for-height (WHZ) z-scores were significantly different across the four groups (p < 0.001 for both). The mean decline in WAZ from baseline until the end of follow-up among the Zn+MV group was significantly less than in the placebo group [mean (SE): -0.36 (0.04) vs -0.50 (0.04), p=0.020], while mean decline in WHZ was significantly greater in the Zn only group relative to placebo [-0.57 (0.07) vs -0.35 (0.07) p=0.021]. Supplements did not have a significant effect on mean change in height-for-age (HAZ), or on rates of stunting, wasting or underweight.

**Conclusion:** Despite small but statistically significant improvements in WAZ in the ZN+MV group, neither daily zinc or multivitamin supplements alone or in combination infants reduced the incidence of underweight, stunting or wasting among Tanzanian infants. Alternative approaches to prevent growth faltering should be pursued.

# INTRODUCTION

Chronic undernutrition, manifested as stunting (height-for-age < -2 SD) currently affects 165 million children under 5 worldwide (1). Children who suffer from undernutrition in early life experience detrimental short and long-term consequences. Undernutrition decreases immune function and increases the risk of infection. Severe wasting in particular (weight-for-height <-3SD of the 2006 WHO standard) - has been shown to increase the risk of mortality 12-fold (2). Among children who survive early childhood, growth faltering can also lead to impaired cognitive development and increased risks of chronic disease, as well pregnancy complications among women (3). Micronutrient deficiencies are particularly prevalent in resource-constrained settings even when caloric intake may be sufficient (1, 4). An estimated 17% of the world's population is at risk of inadequate intake of zinc, an essential mineral that plays an important role in growth, immune function, reproduction and neuro-behavioral development (5). The World Health Organisation (WHO) currently recommends zinc supplementation as part of treatment for diarrheal disease because it can help shorten the duration and severity of disease (6, 7). However, whether routine, preventative zinc supplementation can improve long-term child growth is less clear. Numerous studies have documented a small, but statistically significant effect of zinc supplements on growth in children and adolescents (8-10); however, there has been significant heterogeneity in the impact of zinc supplements on growth based on children's age, study setting, supplement dosage and duration, and underlying zinc status (10). In particular, there are few studies which have initiated high-dose zinc supplementation (multiple times the RDA) during early infancy, and longitudinally assessed whether there is an added benefit of zinc supplementation beyond what infants receive through breastmilk (10).

Recently, public health practitioners have also begun to emphasize the role of multiple micronutrients as opposed to single-nutrient interventions to improve child health (4). Because

multiple deficiencies often co-exist in the same individuals and communities, multiple micronutrient supplements are a particularly attractive and relatively inexpensive intervention (1, 11, 12). Research on which micronutrients, at which doses, and in which settings is essential to formulate policies on multiple micronutrient interventions. WHO currently supports home fortification of food with micronutrient powders containing at least iron, vitamin A and zinc to improve iron status and reduce anaemia among infants and children 6–23 months of age (13); however, the evidence base to support the routine provision of other micronutrients remains incomplete. Our group has demonstrated that the daily provision of vitamins B-complex, C and E to pregnant women can improve birthweight (14) and post-natal infant weight gain(15). The current study aims to assess whether daily provision of these micronutrients directly to infants born to HIV-negative mothers can have a long-term impact on child growth, and furthermore, whether multivitamins have a synergistic effect when combined with zinc supplements.

## SUBJECTS AND METHODS

Subjects in this study were part of a 2x2 factorial, randomized, double-blind, placebo-controlled trial designed to assess whether daily administration of zinc and/or multivitamins to infants born to HIV-negative mothers from 6 weeks of age for a period of 18 months reduced the risk of diarrheal disease and respiratory infection morbidity, compared to placebo (16). A secondary endpoint of this study was to assess whether these supplements improved child growth. Pregnant women <34 weeks gestation who planned to stay in Dar es Salaam for at least two years were informed about the study and consented prenatally. In addition, mothers were also recruited from the labor ward from district hospitals in Dar es Salaam. Inclusion criteria included singleton, live births of HIV-negative mothers. All mothers in the study were confirmed to be HIV-negative 1-2 weeks after delivery. HIV-status was assessed using 2 sequential enzyme-linked immonosorbent assays (ELISAs) that

used the Murex HIV antigen/antibody (Abbot Murex, Dartford, Kent, U.K) followed by the Enzygnost anti-HIV-1/2 Plus (Dade Behring, Marburg, Germany). Discordant results between two ELISAs were resolved by a Western blot assay. Low-birth weight and premature infants were included in the study since they might be particularly likely to benefit from supplements; however infants of multiple births and infants with congenital abnormalities or other medical conditions that would interfere with the study proceedings were excluded.

At 5-7 weeks of age, 2400 infants were randomized to receive one of four treatment regimens: zinc only (Zn), multivitamins only (MV), Zn+MV, or placebo. A biostatistician in Boston prepared a randomization list from 1 to 2400 that used blocks of 20 and was stratified by study clinic. Capsules were packaged in a blister pack of 15 each and numbered boxes containing 6 blister packs were prepared containing the corresponding treatments. Each eligible infant was assigned the next numbered box of capsules at his/her respective site. All study physicians, nurses and participants were blinded to treatment group. The supplements were provided as opaque capsules containing an orange-flavored powder manufactured by Nutriset (Malaunay, France). Mothers were instructed to push the capsule through the back of the blister pack, open the capsule and empty the contents into a clean, plastic cup, mix with 5ml of sterile water and administer the solution to the child orally. All four regimens were tested to ensure they were indistinguishable in appearance, smell and taste. For infants receiving zinc, each capsule contained 5mg of zinc. For infants receiving multivitamin supplements, each capsule contained 60 mg of vitamin C, 8 mg of vitamin E, 0.5 mg of thiamine, 0.6 mg of riboflavin, 4 mg of niacin, 0.6 mg of vitamin B6, 130 μg of folate and 1 μg of vitamin B12. From the time of randomization until 6 months of age, infants received 1 capsule per day, representing between 150-600% of the Recommended Dietary Allowance (RDA) or Adequate Intake (AI) for the micronutrients for infants 0-6 months. From seven months of age until the end

of follow-up, infants received two capsules per day representing 150-400% of the RDA or AI for their age group.

Mothers were instructed to return to the clinic monthly for follow-up visits. All mothers and children received the standard of perinatal and child health care based on the guidelines of the Ministry of Health and Social Welfare in Tanzania. Mothers received prenatal care including anthropometric assessment, iron-folic acid supplementation and intermittent presumptive treatment for malaria. Standard of care for children included monthly growth monitoring, immunizations, routine treatment for illnesses and periodic vitamin A supplementation (100,000 IU at 9 months and 200,000 IU at 15 months). Children who were diagnosed with anemia were treated with iron supplementation.

At each monthly follow-up visit, trained study nurses assessed regimen compliance by counting the number of unconsumed capsules and measured child anthropometry using standard techniques (17). Weight was measured on a digital infant balance scale with 10-g precision (Tanita) and length with 1-mm precision using a rigid length board with an adjustable foot piece.

**ETHICS**

Ethical approval was granted by the Harvard T.H. Chan School of Public Health Human Subjects Committee, the Muhimbili University of Health and Allied Science Committee of Research and Publications, the Tanzanian Institute of Medical Research and the Tanzanian Food and Drug Authority. A Data Safety Monitoring Board also met twice annually over the course of the study. All mothers provided written informed consent to enroll themselves and their infants in the study. The trial was registered at Clinicaltrials.gov with identifier NCT00421668.

## STATISTICS

Power calculations to determine the study sample size were based on the primary outcomes of the parent trial (i.e., clinical symptoms of diarrhea and lower respiratory infection) (16). Post-hoc power calculations for our current growth analysis were conducted for mean change in HAZ without taking splines into account. Based on the power calculation approach described by Basagaña and Spiegelman (18), assuming the coefficient of the change in HAZ and time interaction is 0.01, we have greater than 99% power to detect a linearly divergent difference in growth trajectory in change of HAZ between treatment groups.

Descriptive statistics were used to summarize baseline maternal, child and household characteristics. For categorical variables, frequencies in absolute numbers and percentages were assessed and compared using chi-squared tests. For continuous variables mean and standard deviations were derived and compared using ANOVA. Regimen compliance was defined as the average percentage of capsules a child consumed over the number of capsules he/she should have consumed between visits. For our analysis, we calculated age- and sex-specific z-scores for three anthropometric indexes: weight-for-height (WAZ), height-for-age (HAZ) and weight-for-age (WAZ) using the 2006 WHO growth standards (19). In accordance with WHO recommendations, we set all extreme HAZ (<-6 or >6), WHZ (<-5 or >5), and WAZ (<-6 or >5) values to missing as recommended (20). Measurement error in individual children's growth trajectories was also identified by regressing height, weight, HAZ and WAZ independently against age for each child. The greatest and lowest deciles of residual values were individually assessed, and extreme outliers were set to missing. WHZ was set to missing for any visit where HAZ or WAZ were set to missing. Dichotomous variables of wasting, stunting and underweight were created for children who fell below -2 of the relevant z-score.

For the primary analysis, mixed effects models with restricted cubic splines for change in mean anthropometric z-score over time were created for each group using the intent-to-treat principle (21). Change in z-scores were calculated by subtracting a child's baseline z-score from the appropriate anthropometric z-score at each subsequent follow-up visit. For the mixed effects model with restrict cubic splines, knots were placed at 3, 6, 9, 12, 15 and 18 months of age with two additional knots at 6 and 10 weeks in order to improve model fit by accounting for rapid growth in early infancy. In order to assess whether there was a significant interaction effect of combining supplements, we conducted likelihood ratio tests (LRT) to compare mixed models created using the maximum likelihood method. Full models contained all treatment terms (MV, Zn, and MV+Zn), age variables (age as a continuous variable as well as age spline variables), and interaction terms for treatment variables with age variables. Reduced models did not contain interaction terms for MV+Zn with age variables.

Incidence rates of stunting, wasting and underweight were compared using Cox proportional hazards models with the exact method for ties. The assumption of proportional hazards was assessed by comparing Kaplan Meir plots across treatment groups, and also by testing an additional model that included treatment and age interactions in the Cox model. Chi-squared tests were used to compare the number of children who were lost to follow-up and who completed follow-up across the four treatment groups, and t-tests were used to compare mean age of last visit across each treatment group. In addition, baseline socio-demographic characteristics of infants who completed the study and infants who dropped out were compared using t-tests for continuous variables and chi-squared tests for categorical outcomes. Because adjusting for baseline covariates has the potential to improve precision (22) and because baseline z-scores were somewhat different across the four groups, we repeated the primary analyses to assess whether adjusting for baseline anthropometric status or other baseline covariates changed the findings from the mixed effects

models with cubic splines. We also assessed effect modification by using mixed effects models with restricted cubic splines stratified first by infant sex and separately stratified by birthweight (infants in the lowest quartile of birthweight <3000g vs birthweight ≥3000 g). All analyses were conducted in SAS version 9.3 (SAS Institute, Cary, NC USA).

**RESULTS**

A total of 14,901 pregnant women were screened for study enrollment, of whom 3,815 were HIV-uninfected women who met eligibility criteria and consented to participate in the study. After excluding infants with medical conditions at birth, those with unknown delivery status and/or those who did not return for randomization, 2400 infants were randomized to Zn, MV, Zn + MV, or placebo (**Figure 1.1**). Maternal, child and household characteristics at baseline were comparable across the four groups (**Table 1.1**). Over half of all mothers were housewives with no income, and three-quarters had 7 years of education or fewer. For almost one-third of mothers, this was their first pregnancy. Mean maternal age was significantly different across the four

**Figure 1.1: Study profile of randomized trial of multivitamin and zinc supplementation to infants in Dar es Salaam, Tanzania**
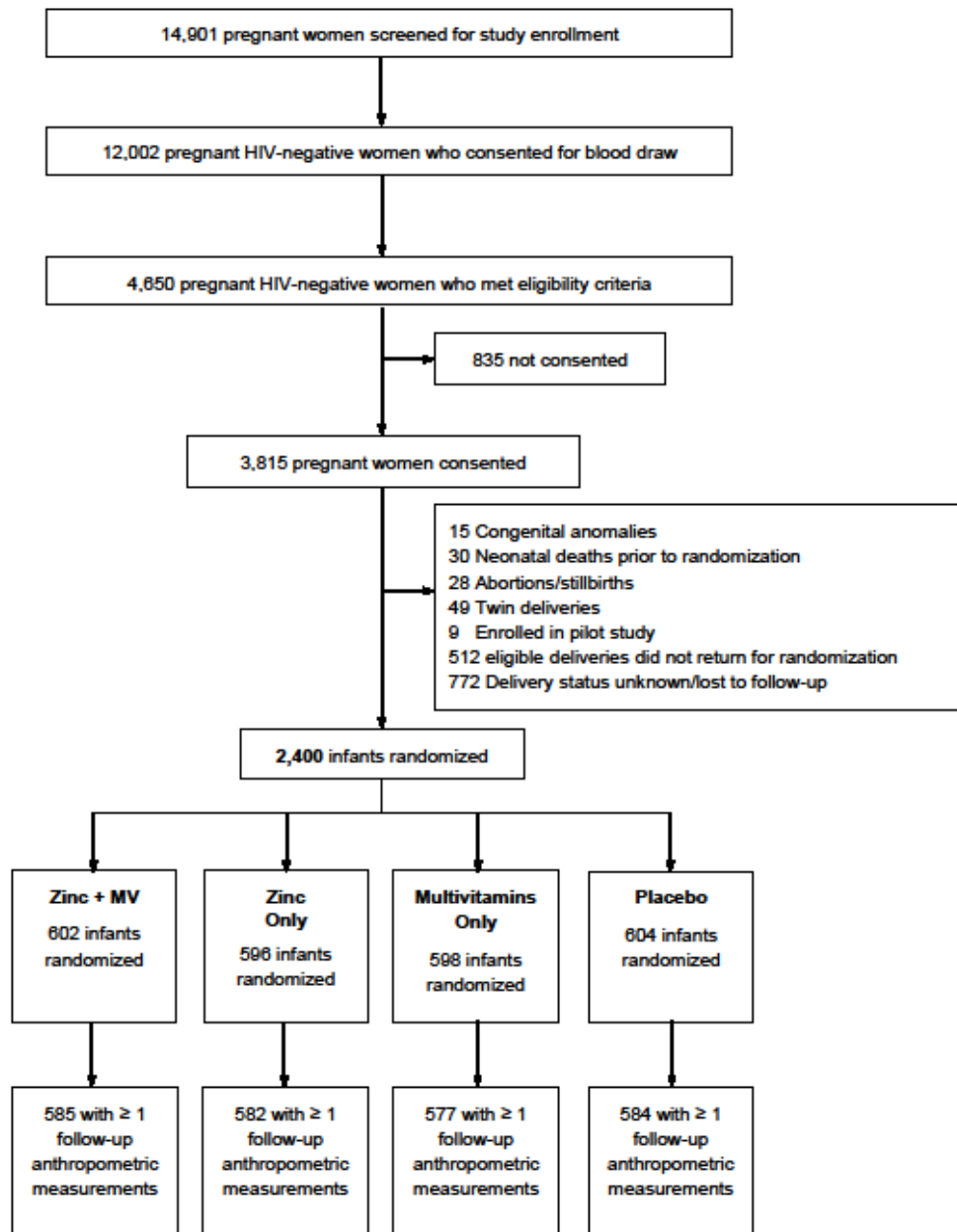
**Table 1.1  Baseline characteristics of mothers and their children enrolled in a trial of zinc and multivitamin supplementation**

| | ZN + MV (n=602) | ZN only (n=596) | MV only (n=598) | Placebo (n=604) | p value[1] |
|---|---|---|---|---|---|
| *Maternal characteristics* | | | | | |
| Age (y) | 26.1 ± 5.0[2] | 26.8 ± 5.1[2] | 26.2 ± 5.0[2] | 26.5 ± 5.0[2] | 0.049 |
| Formal education [*n* (%)] | | | | | |
| None | 9 (1.5) | 8 (1.4) | 10 (1.7) | 9 (1.5) | |
| 1-7 y | 441 (73.4) | 421 (71.1) | 416 (70.2) | 453 (75.3) | 0.538 |
| ≥ 8 y | 151 (25.1) | 163 (27.5) | 167 (28.2) | 140 (23.3) | |
| Employment [*n* (%)] | | | | | |
| Housewife without income | 357 (59.8) | 365 (62.3) | 337 (56.7) | 386 (64.4) | |
| Housewife with income | 201 (33.7) | 175 (29.9) | 212 (35.7) | 176 (29.4) | 0.125 |
| Other | 39 (6.5) | 46 (7.9) | 45 (7.6) | 37 (6.2) | |
| Marital status [*n* (%)] | | | | | |
| Married or living with partner | 542 (90.5) | 534 (90.5) | 542 (91.4) | 537 (90.0) | 0.860 |
| Prior pregnancies [*n* (%)] | | | | | |
| None | 187 (31.2) | 169 (28.6) | 205 (34.5) | 184 (30.6) | |
| 1-4 | 396 (66.1) | 410 (69.3) | 375 (63.1) | 398 (66.2) | 0.378 |
| ≥ 5 | 16 (2.7) | 13 (2.2) | 14 (2.4) | 19 (3.2) | |
| Mid-upper arm circumference (cm) | 26.7 ± 3.2 | 27.1 ± 3.1 | 27.1 ± 3.1 | 27.0 ± 3.1 | 0.090 |
| *Socioeconomic characteristics* | | | | | |
| Daily food expenditure per person in household is < 1000[3] TSh [*n* (%)] | 170 (29.4) | 162 (28.6) | 164 (28.8) | 158 (27.6) | 0.925 |
| Household possessions[4] [*n* (%)] | | | | | |
| None | 180 (30.0) | 192 (32.7) | 173 (29.2) | 171 (28.4) | |
| 1-3 | 339 (56.5) | 308 (52.4) | 330 (55.7) | 358 (59.5) | 0.292 |
| ≥ 4 | 81 (13.5) | 88 (15.0) | 90 (15.2) | 73 (12.1) | |
| *Child characteristics* | | | | | |
| Age at randomization (wk) | 5.9 ± 0.4 | 5.9 ± 0.4 | 5.9 ± 0.4 | 5.9 ± 0.4 | 0.543 |
| Male sex [*n* (%)] | 289 (48.0) | 300 (50.3) | 316 (52.8) | 311 (51.5) | 0.388 |
| Low birth weight, < 2500 g [*n* (%)] | 22 (3.7) | 21 (3.6) | 21 (3.6) | 18 (3.0) | 0.920 |
| Born preterm, <37 wk [*n* (%)][5] | 67 (12.1) | 80 (14.6) | 66 (12.0) | 77 (14.0) | 0.501 |
| Small for Gestational Age[5, 6] | 53 (9.9) | 48 (8.9) | 35 (6.5) | 55 (10.1) | 0.143 |
| Length-for-age Z-score[7] | -0.37 ± 1.23 | -0.33 ± 1.19 | -0.26 ± 1.20 | -0.17 ± 1.31 | 0.028 |
| Weight-for-length Z-score[7] | 0.15 ± 1.31 | 0.16 ± 1.33 | 0.14 ± 1.29 | 0.05 ± 1.28 | 0.477 |
| Weight-for-age Z-score[7] | -0.26 ± 1.02 | -0.23 ± 0.97 | -0.17 ± 0.99 | -0.16 ± 1.05 | 0.247 |

1 p-value for continuous variables from ANOVA procedure and chi-squared for categorical variables
2 Mean ± SD (all such values unless otherwise indicated)
3 At the time of the study, this was roughly equal to USD 0.75 per day
4 From a list that includes sofa; television; radio; refrigerator; and fan
5 n=2157
6 Calculated based on the standards developed by Oken et al. BMC, 2003 (23)
7 Anthropometric z-scores from baseline (age 5-7 weeks)

groups (p=0.049), with the youngest mothers in the Zn + MV group (26.1 ± 5.0 years) and the

oldest in the Zn only group (26.8 ± 5.1 years). Mean age of children at randomization was 5.9 weeks

in all four groups, and approximately half of the children were male. The prevalence of low-birth-

weight was approximately 3%, and 13% of children were born before 37 weeks gestation. Mean

length-for-age z-score at randomization was significantly different across the four groups (p=0.028).

Infants in the MV+Zn group had the lowest mean HAZ at baseline (-0.37 ± 1.23), while infants in

the placebo group had the highest (-0.17 ± 1.31).

Median regimen compliance among children was 96% (25th and 75th percentiles: 91% and 99%.) of

the allocated regimen based on pill counts at clinic visits. Children in all supplement groups

experienced significant growth faltering during follow-up. At 19 months of age, the prevalence of

stunting, wasting and underweight were 19.8%, 6.0% and 10.8% respectively. In the likelihood ratio

tests comparing mixed effects models with and without interaction terms for Zn+MV and time

spline variables, the models for change in WAZ (p<0.001) and WHZ (p<0.001) were significantly

improved by including interaction terms for Zn+MV, indicating a significant interaction on growth

outcomes by combining the two supplements (**Figure 1.2**).

**Figure 1.2: Change in height-for-age, weight-for-age and weight-for-height z-scores from 6-84 weeks in four treatment arms**
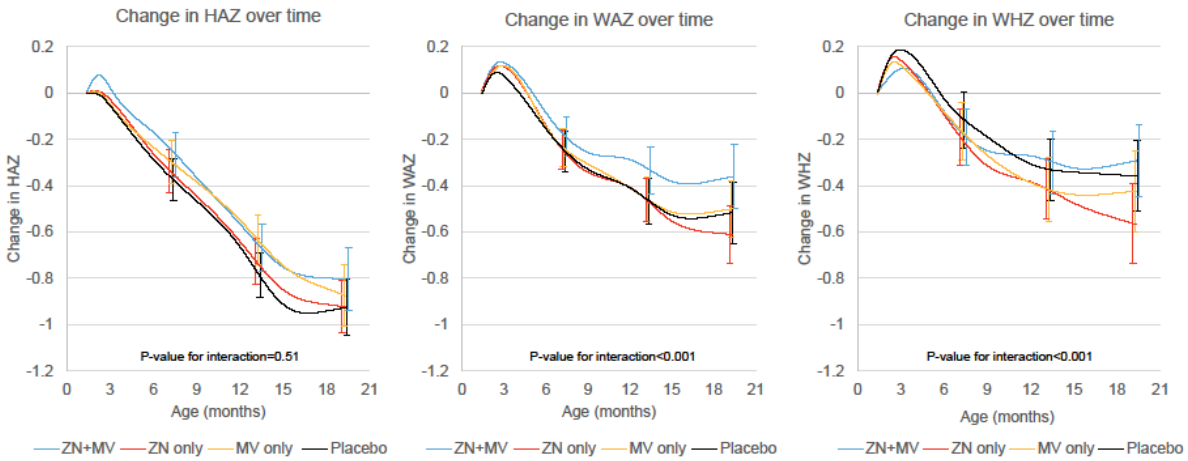


Curves were creating using mixed effects models with restricted cubic splines with knots at 6 & 10 weeks, and 3, 6, 9 12 15 & 18 months of age. N=2336 for HAZ analysis, N=2,347 for WAZ analysis and N=2,332 for WHZ analysis. Bars represent 95% confidence intervals for each of the four treatment groups at 6, 12 and 18 months of follow-up. P-values for interaction were derived from likelihood ratio tests comparing mixed effects models with restricted cubic splines. The full model contained an MV+ZN interaction term as well as interaction terms between MV+ZN and time and all time spline variables. The reduced model did not contain interaction terms for MV+ZN and time or time spline variables.

There was no significant improvement when Zn+MV interaction terms were added to the model for change in HAZ (p=0.505). Pairwise comparisons of mean change in WAZ from baseline to the end of follow-up indicated that the Zn+MV group experienced a significantly smaller decline in WAZ relative to the placebo group [mean change (SE): -0.36 (0.04) vs -0.50 (0.04), p-value for difference: 0.020] (**Table 1.2**). Pairwise comparisons for mean change in WHZ revealed a significantly greater decline in WHZ in the Zn only group relative to placebo [-0.57 (0.07) vs -0.35 (0.07) p=0.021]. The incidence rates of stunting, wasting and underweight were not significantly different in the Zn+MV, Zn only or MV only groups compared to the placebo group (**Table 1.3**). In the analyses adjusted for baseline anthropometry, we found that the decline in WAZ of the ZN+MV group remained significantly smaller than the decline in the placebo group

**Table 1.2: Mean change in height-for-age, weight-for-age and weight-for-height z-score over 18 months of follow-up across the four treatment groups[1]**

|  | ZN + MV (n=602) | Zn only (n=596) | MV only (n=598) | Placebo (n=604) |
|---|---|---|---|---|
| Mean change in HAZ (SE) | -0.79 (0.05) | -0.93 (0.05) | -0.88 (0.05) | -0.93 (0.05) |
| p-value[2] | 0.058 | 0.956 | 0.478 | ref |
| Mean change in WAZ (SE) | -0.36 (0.04) | -0.61 (0.04) | -0.52 (0.04) | -0.50 (0.04) |
| p-value[2] | 0.020 | 0.063 | 0.792 | ref |
| Mean change in WHZ (SE) | -0.31 (0.07) | -0.57 (0.07) | -0.42 (0.07) | -0.35 (0.07) |
| p-value[2] | 0.655 | 0.021 | 0.473 | ref |

1. From mixed effects model with restricted cubic splines
2. P-value from comparison of mean change in z-score relative to the mean change in z-score in the placebo group

**Table 1.3: Cox proportional hazards models for incidence of stunting, wasting and underweight from 6-84 weeks in the four treatment groups**

|  | Zn + MV | Zn only | MV only | Placebo |
|---|---|---|---|---|
| *Stunting* |  |  |  |  |
| No. of events/No. of child-years | 173/460 | 176/478 | 169/470 | 164/459 |
| HR (95% CI)[1] | 0.84 (0.67, 1.04) | 0.96 (0.77, 1.18) | 0.93 (0.75, 1.15) | Ref |
| p-value | 0.102 | 0.687 | 0.486 |  |
| *Wasting* |  |  |  |  |
| No. of events/No. of child-years | 89/514 | 118/523 | 110/505 | 99/513 |
| HR (95% CI)[2] | 0.92 (0.69, 1.22) | 1.19 (0.91, 1.55) | 1.11 (0.85, 1.46) |  |
| p-value | 0.546 | 0.203 | 0.458 | Ref |
| *Underweight* |  |  |  |  |
| No. of events/No. of child-years | 107/530 | 131/542 | 116/516 | 99/541 |
| HR (95% CI)[3] | 0.97 (0.74, 1.27) | 1.23 (0.95, 1.60) | 1.29 (0.99, 1.69) |  |
| p-value | 0.818 | 0.116 | 0.060 | Ref |

1. Adjusted for baseline HAZ
2. Adjusted for baseline WHZ
3. Adjusted for baseline WAZ

[mean change (SE): -0.42 (0.04) vs -0.55 (0.04), p=0.026). The decline in mean WHZ in the zinc only group also tended to be steeper than the decline in the placebo group [mean change (SE): -0.47 (0.06) vs -0.33 (0.06), p=0.089]. Adding additional baseline covariates (maternal height, parity, infant sex and household asset score) resulted in similar findings as controlling for baseline z-scores only. Because we did not see a significant interaction between the zinc and multivitamin supplements on change in HAZ, we collapsed the four treatment groups so that we could conduct two separate comparisons of infants who received zinc (Zn+) versus those who did not receive zinc (Zn-), and

infants who received multivitamins (MV+) against those who did not receive multivitamins (MV-). In comparing Zn+ vs Zn- infants, we did not see a significant difference in mean change of HAZ, WAZ or WHZ **(Figure 1.3)**. Children who received multivitamins experienced a small but statistically significant reduction in decline in HAZ and WAZ compared to infants who did not receive multivitamins **(Figure 1.4)**. The mean (SE) decline in HAZ over the 18 months of follow-up among infants who received multivitamins was -0.82 (0.03) compared to -0.93 (0.03) among infants who did not received multivitamins (p-value for difference=0.012). In WAZ, the infants who received multivitamins experienced a decline of -0.46 (0.03) versus -0.56 (0.03) in infants who did not receive multivitamins (p-value for difference=0.004). In Cox proportional hazards models, however, there was no significant difference in rates of stunting, wasting or underweight when comparing Zn+ vs Zn- groups, nor in the comparison of MV+ vs MV- groups **(Tables 1.4 & 1.5).** In analyses assessing loss-to-follow-up, rates of completion were similar across all four treatment groups. No baseline socio-demographic characteristics among early drop-outs were significantly different than the characteristics of those who remained in the study until completion with the exception of gestational age. Children with shorter gestational ages were less likely to complete the study; however, this was not significantly different across treatment groups.

In analyses assessing effect modification of the supplements by birthweight, we found that among infants in the lowest quartile of birthweight (<3000g), those who received multivitamins

**Figure 1.3: Change in height-for-age, weight-for-age and weight-for-height z-scores from 6-84 weeks by zinc treatment group**



Curves were creating using mixed effects models with restricted cubic splines with knots at 6 & 10 weeks, and 3, 6, 9 12 15 & 18 months of age. N=2336 for HAZ analysis, N=2,347 for WAZ analysis and N=2,332 for WHZ analysis. Bars represent 95% confidence intervals for each of the four treatment groups at 6, 12 and 18 months of follow-up. P-values for treatment effect were derived from likelihood ratio tests comparing mixed effects models with restricted cubic splines. The full model contained interaction terms between ZN+ and time and all time spline variables, the reduced model had no time & treatment interactions.

**Figure 1.4: Change in height-for-age, weight-for-age and weight-for-height z-scores from 6-84 weeks by zinc treatment group**



Curves were creating using mixed effects models with restricted cubic splines with knots at 6 & 10 weeks, and 3, 6, 9 12 15 & 18 months of age. N=2336 for HAZ analysis, N=2,347 for WAZ analysis and N=2,332 for WHZ analysis. Bars represent 95% confidence intervals for each of the four treatment groups at 6, 12 and 18 months of follow-up. P-values for treatment effect were derived from likelihood ratio tests comparing mixed effects models with restricted cubic splines. The full model contained interaction terms between MV+ and time and all time spline variables, the reduced model had no time & treatment interactions.

**Table 1.4: Cox proportional hazards models for incidence of stunting, wasting and underweight based on zinc supplementation**

|  | Zn+ | Zn- | P-value |
|---|---|---|---|
| *Stunting* |  |  |  |
| No. of events/No. of child-years | 349/938 | 333/929 |  |
| HR (95% CI)[1] | 0.93 (0.80, 1.08) | Ref | 0.341 |
| *Wasting* |  |  |  |
| No. of events/No. of child-years | 207/1037 | 209/1018 |  |
| HR (95% CI)[2] | 1.00 (0.83, 1.21) | Ref | 0.997 |
| *Underweight* |  |  |  |
| No. of events/No. of child-years | 238/1072 | 215/1057 |  |
| HR (95% CI)[3] | 0.96 (0.80, 1.16) | Ref | 0.702 |

1. Adjusted for baseline HAZ
2. Adjusted for baseline WHZ
3. Adjusted for baseline WAZ

**Table 1.5: Cox proportional hazards models for incidence of stunting, wasting and underweight based on multivitamins supplementation**

|  | MV+ | MV- | P-value |
|---|---|---|---|
| *Stunting* |  |  |  |
| No. of events/No. of child-years | 342/930 | 340/937 |  |
| HR (95% CI)[1] | 0.90 (0.77, 1.05) | Ref | 0.168 |
| *Wasting* |  |  |  |
| No. of events/No. of child-years | 199/1019 | 217/1036 |  |
| HR (95% CI)[2] | 0.93 (0.76,1.12) | Ref | 0.429 |
| *Underweight* |  |  |  |
| No. of events/No. of child-years | 223/1046 | 230/108 |  |
| HR (95% CI)[3] | 0.99 (0.83, 1.20) | Ref | 0.948 |

1. Adjusted for baseline HAZ
2. Adjusted for baseline WHZ
3. Adjusted for baseline WAZ

had improved growth outcomes across all three anthropometric measures compared to infants who did not receive multivitamins. Specifically, infants who received multivitamins experienced a mean (SE) decline in HAZ of -0.30 (0.10) compared to a decline of -0.68 (0.10) among infants who did not receive multivitamins (p-value for difference=0.001). There was a similar tendancy in WHZ [-0.35 (0.11) vs. -0.60 (0.12), p-value for difference=0.070]. Of particular note, infants in the lowest quartile of birthweight who received multivitamins actually experienced improvements in WAZ,

with a mean (SE) increase of 0.16 (0.10) compared to a decline among infants in the lowest quartile of birthweight who did not receive multivitamins of -0.19 (0.11) [p-value for difference<0.001]. There was no significant effect of multivitamins in infants born ≥3000g. By contrast, infants who received zinc supplements did not have significantly different declines in WHZ or WAZ in either birthweight category, while infants in the lowest quartile of birthweight who received zinc experienced greater declines in HAZ compared to infants in this birthweight range who did not receive zinc [mean change (SE): -0.61 (0.10) vs. -0.34 (0.11)) p-value for difference=0.020]. There was no significant effect modification of treatment by infant sex.

**DISCUSSION**

In this randomized, 2x2 factorial, clinical trial among infants born to HIV-negative mothers in Dar es Salaam, Tanzania, neither daily zinc nor multivitamin supplements –alone or in combination - had a significant effect on the incidence of stunting, wasting or underweight. There was a small difference in mean change in WAZ and WHZ across treatment groups. Children in the MV + Zn group experienced a significantly smaller decline in WAZ compared to children in the placebo group, a trend that was replicated in change in HAZ. By contrast, children in the Zn only group experienced a significantly greater decline in WHZ, a trend that was also replicated in decline in WAZ compared to the placebo group.

Our findings on the effect of zinc supplementation are somewhat surprising. Several meta-analyses have found a small, but significant, positive effect of zinc supplementation on child growth. In two meta-analyses in 2002 and again in 2009, Brown and colleagues found that zinc supplementation had a positive effect on linear growth in pre-pubertal children (including infants) in both developed and resource-constrained countries (8, 24). In two separate meta-analyses published in 2011, Ramakrishnan et al. (25) and  Imdad and Bhutta (9) narrowed inclusion criteria to focus on the

effect of zinc supplements on growth specifically among children under 5 years of age in low and middle-income countries. While Imdad and Bhutta concluded that a dose of 10 mg zinc/day for 24 weeks led to a net gain in height of 0.37 (±0.25) cm, Ramakrishnan et al found no effect of zinc supplementation on height gain, and only modest increases in WHZ. Most recently, a Cochrane review by Mayo-Wilson et al. found a significant positive effect of zinc supplementation on both height and weight among children from 6 months through 12 years. It is worth noting, however, that the effect on height and weight in the Mayo-Wilson review was driven by studies in children over age 5. In children under 5 years of age, zinc supplementation appeared to reduce both height and weight gain; there was no effect of zinc supplementation on the prevalence of stunting in any age range (10).

Our findings support the argument that if there is an effect of zinc supplementation on growth in infancy and early childhood, it is quite small and may not be beneficial, particularly if it is not balanced with the addition of other micronutrients. It is worth noting that infants in our study initiated zinc supplementation at 6 weeks of age. In early infancy, zinc concentration in breastmilk and infant absorption of zinc are both relatively high often resulting in sufficient zinc intake for estimated requirements (26). In our study, it is likely that zinc supplements provided no additional benefit, and by contrast may have led to a nutrient imbalance by inhibiting absorption of nutrients such as iron and copper (27-29). The statistically significant difference in decline in WAZ among the Zn only group highlights the importance of establishing the appropriate micronutrient dosage and combination in order to minimize potentially harm from supplements.

Our finding that zinc supplements, when given in combination with multivitamin supplements, has a small, but statistically significant beneficial effect on growth outcomes is in line with the literature on multiple micronutrient supplements. Multiple micronutrient supplements have gained significant attention as an inexpensive strategy to combat deficiencies, and potentially improve child growth,

because several micronutrient deficiencies often coexist within the same individual and communities in resource-constrained settings (4, 30). Numerous studies have assessed the effect of multiple micronutrient supplements on anemia and hemoglobin concentration (11, 31-36), but fewer have assessed long-term outcomes including child growth (34, 36). Some studies have demonstrated significant benefits of multiple micronutrient supplements on growth (11, 37, 38); however, micronutrient combinations and supplement dosages vary significantly across studies complicating efforts to compare the evidence.

In two different studies, one in HIV-positive mothers(15) and the other in HIV-negative mothers(14), our group has shown that providing this particular combination of multiple micronutrients (B-complex, vitamins C & E) to pregnant women can improve growth outcomes in their offspring. However, our group recently reported the results of a trial that was similar in design to the current study, where we provided direct daily supplementation to the infants of HIV-infected women in Dar es Salaam with vitamins B, C and E from 6-weeks until 24 months(39). In the study of infants born to HIV-infected mothers, the mothers in both the placebo and intervention groups received daily multivitamin supplements as well, thus potentially reducing the benefit of multivitamin supplementation in their infants. We did not find a significant difference in child growth across treatment arms. In the current study, although we found a statistically significant improvement in WHZ among children in the MV + Zn group, the difference in change in mean z-scores across the treatment groups was quite small, and there was no difference in any of the treatment groups in incidence rates of stunting, wasting or underweight. These findings do not support the use of this particular combination of micronutrient supplements to infants as a strategy to promote growth in the general population.

It is worth noting, however, that the prevalence of low birthweight (<2500g) in our current study was very low. In our analyses of effect modification, we found that multivitamins were particularly

beneficial for infants with a birthweights below 3000g, but there was no effect of multivitamins in infants born above 3000g. These findings indicate that the minimal effect of supplementation in the current study may be due to the low prevalence of low birthweight at baseline. Further research on multivitamin supplementation specifically targeting infants with poor nutritional status at baseline may provide insight into the potential mechanisms and effectiveness of this intervention.

The limitations of our study are similar to those of other large-scale randomized controlled trials in Sub-Saharan Africa. Our study experienced loss-to-follow-up over the 18 months of follow-up; however, our analyses indicated that this loss was non-differential across treatment groups, and thus was unlikely to cause bias in our findings on the effect of treatment on growth. In addition, although our findings may not be generalizable to rural communities where baseline micronutrient status and the prevalence of other risk factors for poor growth may differ, our study is likely generalizable to peri-urban settings in Sub-Saharan Africa.

To our knowledge, this is the largest randomized controlled trial of zinc and multivitamin supplements among African infants to date. Our 2x2 factorial design and extended follow-up enabled a rigorous assessment of the effect of both nutrient interventions alone, and in combination, on early childhood growth. In particular, the initiation of supplements at a very young age (6 weeks), the provision of dosages at multiple times the RDA, and the long duration of supplementation enhanced our ability to assess the impact of the provision of these micronutrients on child growth. Although our group previously reported improvements in morbidity among the children in the current study who received zinc supplements(16), our current findings do not support the use of daily zinc and/or multivitamins (vitamins B, C and E) to improve early childhood growth. Alternative approaches, including interventions that improve infant and young child feeding practices, increase nutrient density and prevent infection would likely have a greater impact on long-term growth outcomes (1, 40).

## AUTHOR CONTRIBUTIONS

CD, WWF & KPM designed research (project conception, development of overall research plan, and study oversight). KPM, RK, RK & SA conducted research (hands-on conduct of the experiments and data collection); LML, CMM, RK and MW analyzed data and performed statistical analyses. LML wrote the paper and LML & CD had primary responsibility for final content. All authors read and approved the final manuscript.

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# Chapter 2

## The Effect of Daily Zinc and/or Multivitamin Supplements on Early Childhood Development in Tanzania: Results from a Randomized Controlled Trial

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**ABBREVIATIONS**

AI - Adequate Intake

BSID-III Bayley Scales of Infant and Toddler Development 3rd Edition

ECD – Early Childhood Development

IU – international units

MV – multivitamins

RDA - Recommended Dietary Allowance

WHO – World Health Organisation

Zn – Zinc

Zn + MV – zinc and multivitamin

## ABSTRACT

Impaired childhood development has lifelong consequences for educational attainment and wage-earning potential. Micronutrient supplements have the potential to improve development. The objective of this study was to determine the effect of daily zinc and/or multivitamin (vitamins C, E & B-complex) supplements on development among Tanzanian infants. In this randomized, 2x2 factorial, double-blind trial, 2400 infants were randomized to zinc (Zn), multivitamins (MV), zinc and multivitamins (Zn+MV), or placebo at 6 weeks of age. At approximately 15 months, a sub-sample of 247 children underwent developmental assessment using the cognitive, language (receptive and expressive) and motor (fine and gross) scales of the Bayley Scales of Infant and Toddler Development 3rd Edition (BSID-III). Mean BSID-III scores were compared using two-sided t-tests and multivariate linear regression models adjusted for child's sex, post-conceptual age and test administrator. Logistic regressions were used to assess odds of a low developmental scores. We did not detect a significant difference in mean BSID-III scores in any of the five domains in univariate or multivariate models comparing each of the four treatment groups. We also did not detect a significant difference in mean BSID-III scores when comparing children who received zinc supplements versus those who did not, or in comparisons of children who received multivitamin supplements versus those who did not. There was no significant difference in odds of a low BSID-III score in any of the 5 domains in treatment arms either. Because neither daily zinc nor multi-vitamin (vitamins B-complex, C and E) supplementation led to improvements in any of the developmental domains assessed using the BSID-III, we recommend pursuing alternative interventions to promote early childhood development in vulnerable populations.

## INTRODUCTION

Each year, an estimated 200 million children under age 5 years fail to fulfill their developmental potential (Grantham-McGregor et al., 2007). Poverty, malnutrition, exposure to pollutants, poor health and unstimulating home environments interact to adversely affect their cognitive, motor and social-emotional development. The first few years of life are particularly important for brain development because modest detrimental effects on developmental processes can have life-long effects on the brain's structure and capacity (Shonkoff and Phillips, 2000). Several studies from developed and developing countries indicate that cognitive and social-emotional development in the first few years of life are strong predictors of school progress (Grantham-McGregor et al., 2007, Stith et al., 2003, Gorman and Pollitt, 1996, Liddell and Rae, 2001, Currie and Thomas, 2012, Feinstein, 2003). Children who suffer from developmental delays are likely to become less productive adults due to both fewer years of schooling and reduced learning per year of school (Grantham-McGregor et al., 2007). The economic costs for the individuals, communities and countries are staggering when one considers that, globally, each year of additional schooling is associated with an average increase in wages of 9.7% (Psacharopoulos and Patrinos*, 2004).

Micronutrient deficiencies are among the most widespread risk factors for poor child development, despite the fact that they are readily preventable (Bhutta et al., 2008). Several researchers have documented the long-lasting and detrimental effect of iron and iodine deficiencies on child development (Falkingham et al., 2010, Grantham-McGregor and Ani, 2001, Stoltzfus, 2003, Zimmermann et al., 2006, Gordon et al., 2009). More recently, interest in the role of additional micronutrients in early childhood development has grown (Black, 2003, Walker et al., 2007); however, the benefits of supplementation of different nutrients and nutrient combinations has yet to be clearly established.

Zinc is an essential mineral required for the synthesis of over 100 enzymes involved in major metabolic pathways including DNA and protein synthesis, and cell division (Hotz and Brown, 2004, Gibson, 2006). Zinc deficiency may affect various aspects of cognitive development including attention, activity, neuropsychological behavior and motor development, likely by interfering with neurogenesis, neuronal migration synaptogenesis and neurotransmission (Bhatnagar, 2001, Sandstead et al., 2000). An estimated 17% of the world's population is at risk of inadequate zinc intake, while in sub-Saharan Africa and Tanzania specifically, estimates indicate that over a quarter of both populations have inadequate zinc intake (Wessells, 2012). Children are at particular risk of zinc deficiency due to their increased needs for growth and development, as well as the poor quality of complementary foods, especially in sub-Saharan Africa, where households rely on staple grains for the majority of their diet (Wessells, 2012). In Tanzania, the vast majority of home-made weaning foods - primarily comprised of cassava, millet or sourghum porridges - are particularly poor sources of bioavailable zinc as well as other micronutrients (Mosha et al., 2000).

Several randomized controlled trials have been conducted to assess the effect of zinc supplementation on child development (Ashworth A, 1998, Bentley et al., 1997, Black et al., 2004a, Gardner et al., 2005, Hamadani et al., 2001, Lind et al., 2004, Sazawal et al., 1996, Castillo-Durán et al., 2001, Friel et al., 1993, Sandstead, 1998, Cavan et al., 1993, Gibson et al., 1989); however, none of these studies was conducted among infants in sub-Saharan Africa, a population which may have unique risk factors for zinc deficiency and poor developmental outcomes due to the region's low bioavailability of zinc and other micronutrients in common complementary foods, the high rates of growth faltering and micronutrient deficiencies in infants and children, poor maternal nutrition and education, as well the high burden of infectious diseases, all of which are likely to impair early childhood development (Walker et al.). Multiple micronutrient supplements have recently gained significant attention as an intervention due to their potential for improving efficiency in

interventions, and the fact that multiple deficiencies often cluster in the same individuals and communities (Black et al., 2013, Allen et al., 2009, Harrison, 2010). Folate and vitamin B12 play an essential role in the development of the central nervous system - insufficient folate during pregnancy can lead to neural tube defects (Black, 2008), and vitamin B12 plays an essential role in the myelination of nerves, the establishment of a balanced S-adenosylmethionine:S-adenosylhomocysteine ratio and ratio of neurotrophic and neurotoxic cytokines, as well as preventing the build up of lactate in brain cells (Dror and Allen, 2008). While vitamin B12 deficiency may lead to developmental delays, the extent to which supplementation of vitamin B12 and other B complex vitamins can affect the neurodevelopment of children without clinically apparent deficiencies has not been well assessed (Dror and Allen, 2008, Casella et al., 2005, Chalouhi et al., 2008, Schneede et al., 1994, Louwman et al., 2000). In addition to direct effects on development of the central nervous system, deficiencies in vitamins C, B12 and folate can also lead to anemia. Anemia, lethargy and reduced stimulation could also be associated with poorer developmental status(Bhatnagar, 2001, Black, 2003).

Our group has previously shown that supplementation of vitamins B-complex, C, and E to HIV-positive women during pregnancy and lactation improved developmental indices in their children at 6, 12 and 18 months (McGrath et al., 2006). In our current study, we evaluate whether the same micronutrient combination has similar effects when provided directly to infants. Using a 2x2 factorial design, we assess the effect of zinc and/or a high-dose multivitamin (vitamins B-complex, C & E) supplementation initiated early in infancy and continued for over a year, on the development of HIV-unexposed children in Sub-Saharan Africa.

## PARTICIPANTS AND METHODS

Children in this study were participants in a 2x2 factorial, randomized, double-blind, placebo-controlled trial in Dar es Salaam, Tanzania. Details of the trial have previously been published (McDonald et al., 2015). Briefly, the trial was designed to examine the effect of daily administration of zinc and/or multi-vitamin supplements on respiratory and gastrointestinal morbidity among children born to HIV-negative mothers. In all, 2400 singleton, live birth infants born to HIV-negative mothers were randomized at age 5-7 weeks to receive either daily zinc and multivitamin (Zn + MV) supplements, Zn only, MV only, or placebo for 18 months. Infants of multiple births and infants with congenital abnormalities or other severe medical conditions were excluded because their unique medical conditions could interfere with the study results.

At baseline, infant anthropometry was assessed and a thorough clinical examination by a study physician was conducted. The supplements were provided as opaque capsules containing an orange-flavored powder manufactured by Nutriset (Malaunay, France). Nutriset manufactured the nutrient supplements in a good manufacturing practice (GMP)- certified pharmaceutical laboratory, and performed all required analyses of the supplements to confirm nutrient content and stability. Mothers were instructed to push the capsule through the back of the blister pack, open the capsule and empty the contents into a clean, plastic cup, mix with 5ml of sterile water and administer the solution to the child orally. All four regimens were tested to ensure they were indistinguishable in appearance, smell and taste. For infants receiving Zn, each capsule contained 5mg of zinc. For infants in the MV group, each capsule contained 60 mg of vitamin C, 8 mg of vitamin E, 0.5 mg of vitamin B1, 0.6 mg of vitamin B2, 4 mg of niacin, 0.6 mg of B6, 130µg of folic acid and 1 µg of B12. From the time of randomization until 6 months of age, infants received 1 capsule per day, representing between 150-600% of the Recommended Dietary Allowance (RDA) or Adequate

Intake (AI) for the different micronutrients for infants in this age range. From seven months of age until the end of follow-up, infants received two capsules per day representing 150-400% of the RDA or AI for 7-15 month-olds. The dosage of the supplements was selected in order to maximize the likelihood of seeing an impact of supplementation (by providing doses substantially above the RDA), while also staying within a limited range so as to be considered safe for young children. At each monthly follow-up visit, trained study nurses assessed regimen compliance by counting the number of unconsumed capsules. Overall compliance was defined as the mean percentage of capsules a child consumed over the number of capsules he/she should have consumed between visits. At approximately 15 months of age, a sub-sample of 247 children was selected from a single research site (Magomeni Hospital) due to training and space restrictions. We chose to assess the children at 15 months since this was near the conclusion of the study and therefore allowed the near maximum duration of supplementation.

The sub-sample underwent developmental assessment using the cognitive, language (receptive and expressive) and motor (fine and gross) scales of the Bayley Scales of Infant and Toddler Development 3rd Edition (BSID-III) (Bayley, 2006). A Boston-based child development specialist (DB) traveled to Dar es Salaam to train nurses in the administration of BSID-III. The BSID-III was performed in Kiswahili by one of two trained study nurses in a quiet and well-ventilated room, with a parent or guardian present. Because acute illness can impair children's neurobehavioral performance, the assessment was rescheduled if a child was ill or febrile at the time of the visit. All investigators and participants were blinded to treatment group.

**Ethics:** Ethical approval was granted by the Harvard School of Public Health Human Subjects Committee, the Muhimbili University of Health and Allied Science Committee of Research and Publications, the Tanzanian Institute of Medical Research and the Tanzanian Food and Drug

Authority. A Data Safety Monitoring Board (DSMB) also met twice annually over the course of the study.

**Statistical Analysis:**

Power calculations for tests assessing a difference in mean BSID-III scores for infants who received multivitamins versus those who did not (MV+ vs MV-) and for infants who received zinc and those who did not (ZN+ vs ZN-) were based a type 1 error probability of 0.025 because of the 2x2 factorial design of the study. With a sample of 247 infants and a balanced distribution of treatment group, and an estimated standard deviation in each treatment group for the five different BSID-III domains of 2.0 [Manji et al.], we had 80% power to detect a difference in mean BSID-III scores in each domain of 0.78.

Baseline socio-demographic and maternal and child health-related variables were described using frequencies and chi-square tests for categorical variables, and mean $\pm$ SD with t-tests for continuous variables. Raw mean BSID-III scores for cognitive functioning, expressive and receptive language skills, as well as fine and gross motor skills were compared using two-sided t-tests. While it is possible to calculate age-standardized BSID-III scores based on a reference population of US infants, we did not include these standard scores in our analysis because the BSID-III has not been validated in the Tanzanian context. It would be inappropriate to compare our study population's composite scores with those of a US reference population. Quantile-quantile plots and a Shapiro-Wilke test did not reveal violations of normality in any of the raw BSID-III scores.

BSID-III scores across each treatment group were analyzed using the intent-to-treat principle. We first compared scores in each of the 5 domains of the BSID-III across each of the four treatment groups by using univariate and multivariate linear regression models in order to estimate the mean

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difference in raw scores by treatment arm. Multivariate models were adjusted for child's sex, post-conceptual age, and test administrator (administrator 1 vs. administrator 2). Because we did not find that the interaction term for Zn+MV was significant in the univariate or multivariate linear regression models for any of the BSID-III five domains, we collapsed the treatment groups so that we could compare infants who received zinc versus those who did not (Zn+ vs. ZN-) and infants who received multivitamins versus those who did not (MV+ vs MV-). We then re-conducted the univariate and multiple linear regression models with only two treatment arms.

Because improving neurobehavioral outcomes among children on the lower end of the developmental distribution may be of particular biological and policy interest, we also assessed whether supplementation had an effect on the odds of a low developmental scores across all five domains. Quartiles of scores for each of the five domains were created based on the scores in our study sample. We then conducted multivariate logistic regression models using the same covariates as above in order to estimate the effect of supplementation on odds of performing in the lowest quartiles of the five different developmental domains.

We also conducted additional analyses comparing baseline characteristics of all infants who underwent the BSID-III assessment to children in the parent trial who were not selected for assessment using chi-squared tests for categorical outcomes and t-tests for continuous outcomes. In addition, because it is possible the supplements provide the greatest benefit to children who are poorly nourished at baseline, we repeated the main analyses among only infants in the lowest quartile of birth weight (the lowest quartile was selected because the number of infants born <2500g yielded too few infants for this analysis). We also repeated the analyses only among infants with ≥95% compliance with their assigned treatment regimen based on pill counts. All analyses were conducted in SAS system version 9.3 (SAS Institute, Cary, NC USA).

**RESULTS:**

A total of 14,901 pregnant women were screened for study enrollment, of whom 3,815 were HIV-negative women who met eligibility criteria and consented to participate in the study. After excluding infants with medical conditions at birth, those with unknown delivery status and/or those who did not return for randomization, 2400 infants were randomized to Zn, MV, Zn + MV, or placebo, 247 of whom underwent neurobehavioral assessments (**Figure 2.1**). In the neurobehavioral sub-sample, there were no significant differences across treatment group in any of the maternal, child or household characteristics at baseline (**Table 2.1**). On average, mothers were 26 years old and about a three-quarters had 7 or fewer years of formal education, which corresponds to the completion of primary school in Tanzania. Nine-tenths of mothers were married or cohabitating with their partners and for about a third of mothers, this was their first pregnancy. One-fifth of households spent less than 1000 Tanzanian shillings (approximately USD 0.75 at the time of the study) on food per day and about a quarter of households had none

**Figure 2.1: Study profile of children who participated in neurobehavioral sub-study of trial of multivitamin and zinc supplementation to children in Dar es Salaam, Tanzania**
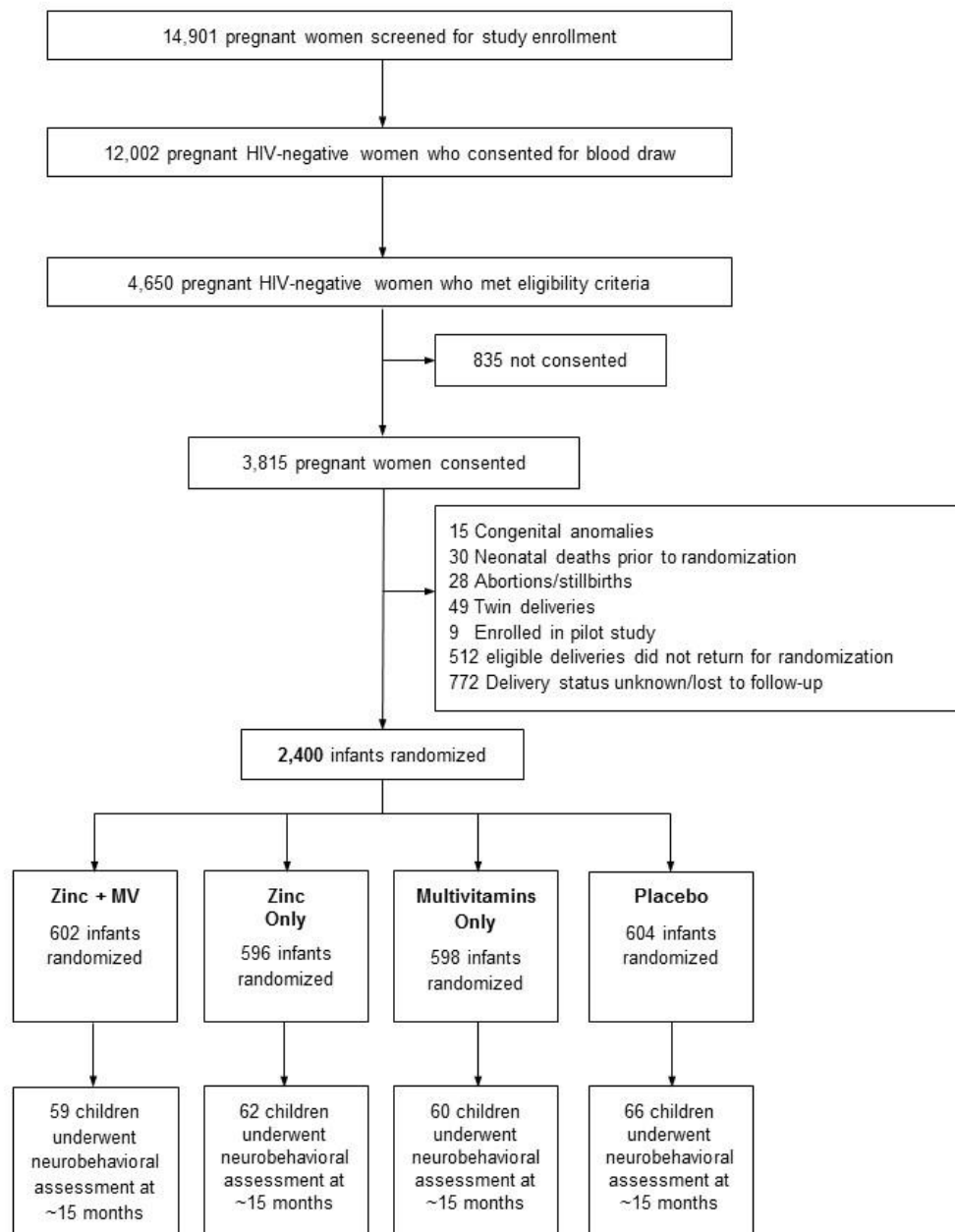
**Table 2.1. Characteristics of mothers & children in neurobehavioral sub-study**

| | Zinc | | Multivitamins | |
|---|---|---|---|---|
| | **Yes** **(n=121)** | **No** **(n=126)** | **Yes** **(n=119)** | **No** **(n=128)** |
| *Maternal characteristics* | | | | |
| Age (y) [1] | 26.1 ± 4.5 | 25.8 ± 5.0 | 26.1 ± 4.7 | 25.8 ± 4.9 |
| Formal education ≤7 y [*n* (%)][3] | 91 (75.2) | 101 (80.8) | 91 (77.1) | 101 (78.9) |
| Employment [*n* (%)] | | | | |
|   Housewife without income | 72 (60.5) | 80 (64.0) | 71 (60.7) | 81 (63.8) |
|   Housewife with income | 41 (34.5) | 39 (31.2) | 42 (35.9) | 38 (29.9) |
|   Other | 6 (5.0) | 6 (4.8) | 4 (3.4) | 8 (6.8) |
| Marital status [*n* (%)] | | | | |
|   Married/cohabitating with partner | 105 (87.5) | 112 (89.6) | 102 (87.2) | 115 (89.8) |
| Prior pregnancies [*n* (%)] | | | | |
|   None | 37 (30.8) | 48 (38.4) | 42 (35.9) | 43 (33.6) |
|   1-4 | 81 (67.5) | 75 (60.0) | 73 (62.4) | 83 (64.8) |
|   ≥5 | 2 (1.7) | 2 (1.6) | 2 (1.7) | 2 (1.6) |
| Height (cm) | 156.5 ± 6.4 | 156.3 ± 5.5 | 156.4 ± 6.0 | 156.4 ± 5.9 |
| Mid-upper arm circumference (cm) | 26.2 ± 3.3 | 26.6 ± 3.2 | 26.5 ± 3.4 | 26.3 ± 3.1 |
| *Socioeconomic characteristics* | | | | |
| Daily food expenditure per person in household < 1000 TSh[4] [*n* (%)] | 26 (22.2) | 22 (19.5) | 21 (19.3) | 27 (22.3) |
| Household possessions[5] [*n* (%)] | | | | |
|   None | 38 (31.4) | 29 (23.2) | 33 (28.0) | 34 (26.6) |
|   1-4 | 61 (50.4) | 75 (60.0) | 64 (54.2) | 72 (56.3) |
|   ≥ 5 | 22 (18.2) | 21 (16.8) | 21 (17.8) | 22 (17.2) |
| *Child characteristics* | | | | |
| Age at randomization (wk) | 5.9 ± 0.3 | 5.9 ± 0.3 | 5.9 ± 0.4 | 5.9 ± 0.3 |
| Male sex [*n* (%)] | 64 (52.9) | 62 (49.2) | 63 (52.9) | 63 (49.2) |
| Birthweight (g) | 3.2 (0.5) | 3.2 (0.5) | 3.3 (0.5) | 3.2 (0.4) |
| Low birth weight, <2500 g [*n* (%)] | 6 (5.0) | 2 (1.6) | 3 (2.5) | 5 (3.9) |
| Born preterm, <37 wk [*n* (%),][6] | 10 (10.6) | 10 (9.7) | 8 (8.6) | 12 (12.5) |
| Weeks at delivery | 39.5 (2.5) | 39.7 (2.5) | 39.8 (2.3) | 39.5 (2.6) |
| Small for gestational age[6, 7] | 8 (7.6) | 13 (14.4) | 8 (8.2) | 13 (13.4) |
| Apgar score ≤ 7 at 5 minutes after birth [*n* (%)] | 1 (0.9) | 1 (0.8) | 1 (0.9) | 1 (0.8) |
| Baseline length-for-age Z-score[8] | -0.43 ± 1.23 | -0.25 ± 1.16 | -0.28 ± 1.30 | -0.39 ± 1.09 |
| Baseline weight-for-length Z-score[8] | 0.06 ± 1.21 | 0.07 ± 1.24 | 0.00 ± 1.32 | 0.13± 1.13 |
| Baseline weight-for-age Z-score[8] | -0.37 ± 1.13 | -0.21 ± 0.97 | -0.28 ± 1.18 | -0.29 ± 0.93 |
| Age in mos. at Bayley assessment | 14.5 ± 0.4 | 14.5 ± 0.4 | 14.5 ± 0.4 | 14.5 ± 0.4 |

1. Mean ± SD (all such values)
2. p-values for continuous variables from t-tests using a pooled SD; categorical variable p-values are from chi-squared tests
3. In Tanzania, 7 years is the duration of most primary schools.
4. At the time of the study, this was roughly equivalent to USD 0.75
5. From a list that includes sofa; television; radio; refrigerator; and fan
6. n=197
7. Based on growth standards developed by Oken et al.(Oken et al., 2003)
8. Anthropometric indicators at randomization (age 5-7 weeks)

of the following possessions: sofa; television; radio; refrigerator; or fan. Children were on average 6 weeks old at baseline and about half were male. About one-tenth of children were born preterm (<37 weeks gestation) and less than 5% were low birth weight (<2500 g). Mean age at the time of neurobehavioral assessment was 14.5 months. Median regimen compliance with the treatment regimen among infants who participated in the early childhood development assessment was 97% (25th and 75th percentiles: 95% and 99%.) of the allocated regimen based on pill counts at clinic visits.

In both the univariate and multivariate linear regression models (adjusted for infant sex and post-conceptual age, as well as treatment examiner), we did not find that the interaction term for Zn + MV was a significant predictor of any of the 5 BSID-III domains. After collapsing treatment groups, we again found that there was no significant difference in any of the five domains of the BSID-III among children who received zinc supplements compared to children who did not receive zinc supplements (**Table 2.2**), or among children who received multivitamin supplements versus those who did not receive multivitamins (**Table 2.3**). We also did not find a significant difference in odds of a low BSID-III score in any of the 5 domains comparing children who received zinc and those who did not, or among children who received multivitamins compared to those who did not in either univariate or multivariate models (**Tables 2.4 and 2.5**). Odds of receiving a low score in any of the five domains, either of the two language domains or either of the two motor domains did not differ significantly across treatment groups.

In our additional analyses, we did not find any significant differences between the sub-sample selected for BSID-III assessment and those excluded from the sub-sample in any of the baseline characteristics with the exception of the amount of Tanzanian Shillings spent on food per day

**Table 2.2: Comparison of mean raw BSID-III[a] scores across zinc treatment groups**

| | Mean Raw Score ± SD Zn+ (n=121) | Mean Raw Score ± SD Zn- (n=126) | Crude difference[b] (95%CI) | p-value | Adjusted difference[c] (95%CI) | p-value |
|---|---|---|---|---|---|---|
| **Cognition** | 50.03 ± 3.33 | 50.19 ± 3.35 | -0.16 (-0.99, 0.68) | 0.711 | -0.19 ( -1.04, 0.65) | 0.652 |
| **Receptive Language** | 19.13 ± 2.03 | 18.94± 2.11 | 0.19 (-0.33, 0.71) | 0.478 | -0.01 (-0.40, 0.38) | 0.956 |
| **Expressive Language** | 20.12 ± 2.63 | 19.84 ± 2.80 | 0.28 (-0.40, 0.96) | 0.415 | -0.02 ( -0.52, 0.48) | 0.947 |
| **Fine Motor** | 35.17 ± 2.84 | 34.89 ± 3.02 | 0.28 (-0.45, 1.02) | 0.446 | -0.09 ( -0.53, 0.36) | 0.698 |
| **Gross Motor** | 48.28 ± 2.41 | 48.06 ± 2.33 | 0.23 (-0.37, 0.82) | 0.455 | -0.06 (-0.43, 0.32) | 0.767 |

a. BSID-III: Bayley Scales of Infant and Toddler Development 3rd Edition
b. Crude differences and CIs obtained from a linear regression models with only zinc supplementation as a predictor.
c. Adjusted differences and CIs from multiple linear regression models adjusted for examiner (examiner 1 vs. examiner 2), post-conceptual age and sex of child.


**Table 2.3: Comparison of mean raw BSID-III[a] scores across multivitamin treatment groups**

| | Mean Raw Score ± SD MV+ (n=119) | Mean Raw Score ± SD MV- (n=128) | Crude difference[b] (95%CI) | p-value | Adjusted difference[c] (95%CI) | p-value |
|---|---|---|---|---|---|---|
| **Cognition** | 50.04 ± 3.19 | 50.18 ± 3.47 | -0.14 (-0.97, 0.69) | 0.746 | -0.19 ( -1.04, 0.65) | 0.649 |
| **Receptive Language** | 19.09 ± 2.09 | 18.98 ± 2.07 | 0.11 (-0.41, 0.63) | 0.683 | -0.11 (-0.50, 0.27) | 0.562 |
| **Expressive Language** | 20.10 ± 2.65 | 19.87 ± 2.78 | 0.23 (-0.45, 0.92) | 0.501 | -0.05 (-0.55, 0.45) | 0.831 |
| **Fine Motor** | 35.14 ± 2.87 | 34.92 ± 2.99 | 0.22 (-0.51, 0.96) | 0.555 | -0.13 (-0.58, 0.31) | 0.555 |
| **Gross Motor** | 48.32 ± 2.33 | 48.02 ± 2.40 | 0.31 (-0.28, 0.91) | 0.301 | 0.03 (-0.34, 0.41) | 0.863 |

a. BSID-III: Bayley Scales of Infant and Toddler Development 3rd Edition
b. Crude differences and CIs obtained from a linear regression model with only multivitamin supplementation as a predictor
c. Adjusted differences and CIs from multiple linear regression models adjusted for examiner (examiner 1 vs. examiner 2), post-conceptual age and sex of child.

**Table 2.4. Effect of Zinc Supplementation on Odds of a Raw BSID-III[a] Score in the Lowest Quartile**

| | Zn+ N (%) | Zn- N (%) | Crude OR (95%CI)[b] | p-value | Adjusted OR (95%CI)[c] | p-value |
|---|---|---|---|---|---|---|
| Cognition | 35 (28.9) | 34 (27.0) | 1.10 (0.63, 1.92) | 0.734 | 1.14 (0.65, 2.00) | 0.652 |
| Receptive Language | 27 (22.3) | 37 (29.4) | 0.69 (0.39, 1.23) | 0.207 | 0.71 (0.37, 1.37) | 0.304 |
| Expressive Language | 35 (28.9) | 41 (32.5) | 0.84 (0.49, 1.45) | 0.539 | 1.10 (0.55, 2.24) | 0.784 |
| Fine Motor | 28 (23.1) | 41 (32.5) | 0.62 (0.36, 1.10) | 0.101 | 0.68 (0.31, 1.49) | 0.334 |
| Gross Motor | 44 (36.4) | 54 (42.9) | 0.76 (0.46, 1.27) | 0.298 | 1.03 (0.37, 2.89) | 0.961 |
| Either language category | 39 (32.2) | 49 (38.9) | 0.75 (0.44, 1.26) | 0.275 | 0.85 (0.43, 1.66) | 0.627 |
| Either motor category | 45 (37.2) | 56 (44.4) | 0.74 (0.45, 1.23) | 0.247 | 0.94 (0.30, 2.93) | 0.915 |
| Any of 5 categories | 68 (56.2) | 71 (56.4) | 0.99 (0.60, 1.64) | 0.981 | 1.55 (0.75, 3.22) | 0.236 |

a. BSID-III Bayley Scales of Infant and Toddler Development 3rd Edition
b. Crude odds ratios and CIs for scoring in the lowest quartile for Zn+ compared to Zn- from logistic regression models with only Zn group as a predictor.
c. Adjusted odds ratios and CIs from logistic regression models adjusted for examiner (examiner 1 vs. examiner 2), post-conceptual age and sex of child.


**Table 2.5. Effect of Multivitamin Supplementation on Odds of a Raw BSID-III[a] Score in the Lowest Quartile**

| | MV+ N (%) | MV- N (%) | Crude OR (95%CI)[b] | p-value | Adjusted OR (95%CI)[c] | p-value |
|---|---|---|---|---|---|---|
| Cognition | 29 (24.4) | 40 (31.3) | 0.71 (0.40, 1.24) | 0.229 | 0.74 (0.42, 1.31) | 0.301 |
| Receptive Language | 30 (25.2) | 34 (26.6) | 0.93 (0.53, 1.65) | 0.809 | 1.07 (0.56, 2.06) | 0.829 |
| Expressive Language | 34 (28.6) | 42 (32.8) | 0.82 (0.48, 1.41) | 0.471 | 1.01 (0.50, 2.04) | 0.978 |
| Fine Motor | 29 (24.4) | 40 (31.3) | 0.71 (0.40, 1.24) | 0.229 | 0.80 (0.36, 1.74) | 0.565 |
| Gross Motor | 44 (37.0) | 54 (42.2) | 0.80 (0.48, 1.34) | 0.403 | 1.15 (0.41, 3.23) | 0.798 |
| Either language category | 39 (32.8) | 49 (38.3) | 0.79 (0.47, 1.33) | 0.367 | 0.92 (0.47, 1.80) | 0.797 |
| Either motor category | 46 (38.7) | 55 (43.0) | 0.84 (0.50, 1.39) | 0.491 | 1.53 (0.48, 4.94) | 0.474 |
| Any of 5 categories | 64 (53.8) | 75 (58.6) | 0.82 (0.50, 1.36) | 0.446 | 1.11 (0.54, 2.29) | 0.773 |

a. BSID-III Bayley Scales of Infant and Toddler Development 3rd Edition
b. Crude odds ratios and CIs for scoring in the lowest quartile for MV+ compared to MV- from logistic regression models with only MV group as a predictor.
c. Adjusted odds ratios and CIs from logistic regression models adjusted for examiner (examiner 1 vs. examiner 2), post-conceptual age and sex of child.

(20.9% of infants in the sub-study came from families spending less than 1000 TSH per day compared to 29.5% among those who were not selected for the sub-study, p=0.006). In our analysis assessing the effect of supplements in infants born in the lowest quartile of birth weight (<3000 g), we did not find a significant effect of supplements on BSID-III scores. Similarly, in our analysis assessing the effect of supplements only among infants with ≥95% compliance with the treatment regimen, we did not see an effect of supplementation.

## DISCUSSION:

In this randomized, 2x2 factorial, clinical trial among infants born to HIV-negative mothers in Dar es Salaam, Tanzania, neither daily zinc nor multivitamin supplements, alone or in combination, had a significant effect on developmental outcomes at 15 months of age. Our findings add to the growing literature on multiple micronutrients and childhood development. To date, there have been a limited number of randomized controlled trials that assessed the effect on development of direct multiple micronutrient supplementation to children. A recent review found 17 trials assessing the effect of three or more micronutrients on cognition in children aged 5-17 years; however, only 3 of the studies were conducted in children under 5 years of age (Eilander et al., 2010). Two of the three studies in young children found significant improvements in motor development (Faber et al., 2005) and time to unassisted walking (Olney et al., 2006); however, both multiple micronutrient combinations contained iron, thus preventing researchers from isolating whether the effect was attributable to improvements in iron status. The third study found no effect of supplements on development (Dhingra et al., 2004). Studies in older children indicate that multiple micronutrient supplements may confer a small benefit for fluid intelligence (reasoning ability) though the effect was not significant, and a significant positive effect on academic performance (Eilander et al., 2010).

While it is difficult to aggregate findings on the effect of multiple micronutrients on early childhood development due to differences across studies in the nutrient combinations and dosages used as well as child age ranges, our group has used this particular combination of multivitamins in previous studies. We previously reported that this multivitamin supplement (Vitamins C, E and B vitamin complex) when provided to HIV-positive women during pregnancy and lactation can improve developmental scores in their children from 6 to 18 months (McGrath et al., 2006). In our most recent study on development, however, we also provided the supplements directly to infants born to HIV-positive mothers (from 6 weeks to 15 months), and found no effect of the vitamins on development at 15 months (Manji et al., 2014). It is worth noting that mothers in the second study received vitamins B-complex, C and E supplementation during pregnancy and lactation, so it is likely that infants in both intervention and placebo groups had better baseline micronutrient status. Our results from the current study, where mothers did not receive supplementation with this particular combination of micronutrients, corroborates the findings that providing this combination of multivitamins directly to infants is unlikely to confer benefits for development.

Our findings on the lack of an effect of zinc supplementation on developmental outcomes, particularly motor development, are surprising given that several other randomized controlled trials have found benefits of zinc supplementation for child development (Ashworth A, 1998, Bentley et al., 1997, Black et al., 2004a, Gardner et al., 2005, Sazawal et al., 1996, Castillo-Durán et al., 2001, Friel et al., 1993, Colombo et al., 2014). In particular, several trials specifically reported an effect of zinc supplementation on motor development – including decreases in frequencies of low psychomotor and mental development scores (Castillo-Durán et al., 2001), increases in time spent sitting or playing versus inactive (Bentley et al., 1997), and increases in time spent in high movement activities (Sazawal et al., 1996). In one study, investigators found that zinc supplements alone

improved hand and eye coordination, but that the effect of supplements was even stronger in children who also received stimulation (Gardner et al., 2005). Several of the studies which found an effect of zinc supplementation specifically targeted children with poor nutritional status at baseline – including studies in small-for-gestational-age infants (Black et al., 2004b), low-birth-weight infants (Friel et al., 1993) or children with low weight-for age at baseline (Gardner et al., 2005) indicating that the baseline nutritional status of our sample may have decreased the likelihood of an effect of zinc supplementation for ECD. However, in the sensitivity analysis in our current study among infants with the lowest quartile of birth weight (<3000g), we still did not find an effect of either supplement.

It is worth noting that several other trials have not demonstrated a significant effect of zinc supplementation to infants and children on mental, psychomotor or behavioral domains (Ashworth A, 1998, Sandstead, 1998, Cavan et al., 1993, Gibson et al., 1989, Lind et al., 2004), and two studies in Bangladesh found that zinc supplements resulted in poorer developmental outcomes – possibly due to a micronutrient imbalance (Hamadani et al., 2001, Hamadani et al., 2002). Interestingly, a recent study in Peru assessed the effect of zinc, iron and copper supplementation compared to iron and copper supplementation and assessed child development using a battery of outcomes including the Bayley Scales of Infant Development, 2nd edition (BSID-II) as well as a visual habituation/recognition memory task, free-play attention tasks and an assessment of inhibitory and memory processes (Colombo et al., 2014). Although the researchers found that the addition of zinc improved development trajectories in attentional variables, they did not find an effect on the BSID-II or on inhibitory and memory processes. This suggests that although zinc may help to maintain normative developmental trajectories for some measures of attention in the first 18 months of life,

global measures of developmental might not be sufficiently sensitive to detect these changes (Colombo and Carlson, 2012).

Our study does not support the hypothesis that zinc supplementation, alone or in combination with multivitamin supplements, improves child development as assessed by the BSID-III, a global test designed to identify developmental delay. The results presented here correspond with our group's previous publication from the same trial that indicated that neither supplement alone nor in combination had a significant effect on stunting, wasting or underweight in this population – indicating that improved growth outcomes were unlikely to be a mediator in the supplement-development relationship (Locks et al., 2015).

Our study has several limitations. We only conducted a single neurodevelopmental assessment at ~15 months of age, which prevents us from assessing the effect of micronutrient supplements earlier in infancy or longitudinally as children age. Our sample also has limited generalizability since our results in our urban sample cannot be generalized to rural populations where infants are likely to have worse nutritional status. However, our findings are likely generalizable to other peri-urban settings in Sub-Saharan Africa.  In addition, although our sample size is sufficiently large to assess the main effect of supplementation, we had limited power to assess the effect of supplements in subgroups (such as low-birth weight infants). Finally, it is also worth noting that while the BSID-III is suitable for identifying developmental delays, it is possible that is not sensitive enough to identify smaller changes in developmental outcomes.  Despite these limitations, to our knowledge, our study is the largest randomized controlled trial that assesses the effect of zinc or multiple micronutrients on child development in an African setting. The randomized design allowed us to assess whether there is a causal effect of daily supplementation of zinc or multiple micronutrients on neurodevelopmental outcomes. For instance, providing daily micronutrients at multiple times the

RDA beginning at 6 weeks and continuing for over a year before assessment, provides a particularly strong study design to assess whether direct provision of micronutrients to infants in this urban, African setting improves development. Our finding of a lack of effect of zinc and/or multivitamin supplements on early child development in this randomized, double-blind, placebo controlled trial highlights the importance of pursuing other strategies in vulnerable populations, particularly those that integrate nutrition with responsive caretaking and stimulation activities (Grantham-McGregor et al., 2014, 2014, United Nations Children's Fund (UNICEF) and the World Health Organisation (WHO), 2014).

**KEY MESSAGES**

- An estimated 200 million children under age 5 years fail to fulfill their developmental potential each year. This highlights the importance of building an evidence base for interventions that can improve early childhood development.

- In this randomized, double-blind, placebo controlled trial, we did not find a significant effect of zinc and/or multivitamin supplements on early child development as assessed by the Bayley Scales of Infant and Toddler Development 3rd Edition.

- Our findings highlight the importance of pursuing alternative strategies to promote early childhood development in vulnerable populations, particularly those that integrate nutrition with responsive caretaking and stimulation.

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# Chapter 3

## Mortality, Morbidity and Growth in HIV-Infected, HIV-Exposed-Uninfected and HIV-Unexposed Infants in Tanzania

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**Abstract**

**Background:** Globally, over 3 million children currently live with HIV. While access to prevention-of-mother-to-child-transmission of HIV (PMTCT) services has recently expanded, a new potential global health obstacle has emerged - the increasing number of HIV-exposed-infected (HIV-EU) infants born each year. Appropriate child health policies require a better understanding of the unique health risks of both HIV-infected and HIV-EU children.

**Methods:** The study sample is comprised of 3,554 children in Dar es Salaam, Tanzania who participated in one of two randomized controlled trials of multiple micronutrient supplementation for infants. At 6 weeks of age, 264 infants born to HIV-infected mothers were confirmed to be HIV-infected and 2088 were confirmed to be HIV-EU. An additional 1202 infants born to HIV-uninfected mothers serve as the unexposed controls. Infants were followed-up until 18-months of age, death or loss to follow-up. Morbidity and growth were assessed at monthly nurse visits.

**Results:** Hazard ratios (95% CI) for time to death in HIV-infected and HIV-EU infants compared to HIV-unexposed infants were 28.92 (14.80, 56.52) and 2.78 (1.40, 5.52) respectively, after controlling for baseline socio-demographic characteristics and infant feeding practices. Compared to unexposed infants, HIV-infected infants had higher risks of all measured morbidities (diarrhea, acute respiratory infection, fever, vomiting, anorexia, ear infection, and unscheduled outpatient and hospital visits), while HIV-EU infants were significantly more likely to suffer from acute respiratory infection, fever, unscheduled outpatient visits and hospitalizations, after controlling for socio-demographic and infant feeding characteristics. HIV-infected infants were also more likely to suffer from stunting, wasting and underweight at baseline and during follow-up. HIV-EU infants were significantly more likely to be underweight at baseline [adjusted RR (95%CI) 2.05 (1.45, 2.89)

p<0.01], but on average, they experienced slower declines in HAZ, WAZ and WHZ and a decreased risk of stunting [adjusted HR (95%CI): 0.64 (0.51, 0.81) p<0.001] over-follow-up.

**Conclusion:** HIV-infected infants had the highest rates of mortality, morbidity and growth failure. HIV-EU infants also had an increased risk of mortality and morbidity compared to HIV- infants, but their growth outcomes were mixed - they were smaller at 6 weeks, but ultimately ended up taller, on average, than their unexposed peers at 18 months. In addition to preventing and treating HIV infection in infants, PMTCT and child health services should also target HIV-EU children to improve health outcomes in this vulnerable population.

**Introduction**

Globally, 35.2 million people are estimated to be living with the human immunodeficiency virus (HIV), 3.2 million of whom are children (1). One of the great global health achievements in the past two decades has been the expansion of prevention-of-mother-to-child-transmission of HIV (PMTCT) services across much of the globe. In 2014, coverage of antiretroviral treatment for PMTCT services reached 74% of pregnant women living with HIV, resulting in 48% decline in new pediatric HIV infections between 2009 and 2014(2). As a result of expanded PMTCT services, each year almost a quarter of infants born in several sub-Saharan African countries are HIV-uninfected infants who are born to HIV-infected mothers, the so-called HIV-exposed-but-uninfected (HIV-EU) children (3). Whether HIV-EU children have the potential for similar health outcomes as HIV-unexposed infants, or whether they have outcomes 'between' those of HIV-infected and HIV-uninfected infants has yet to be determined (3).

There are several mechanisms through which maternal HIV infection can lead to sub-optimal child health and growth. If the children become infected by HIV, they have a drastically increased risk of mortality, morbidity and growth failure compared to uninfected infants (4-7). However, even if infants escape infection, pre-natal exposure to HIV may influence developmental programming (8). A handful of studies have documented impaired immune function (9-12) and an increased risk of morbidity and mortality (7, 13-15) among HIV-EU infants compared to HIV-unexposed infants. Additional studies have also documented an increased risk of low-birth-weight – due to both intrauterine growth restriction as well as prematurity compared to HIV-unexposed children (7, 16-19). Fewer studies, however, have assessed the effect of HIV-exposure on long-term health outcomes such as growth and common morbidities (5). Furthermore, much of the research on long-term outcomes in HIV-EU children comes from cohorts in the wealthy nations where anti-retroviral

access and underlying risk factors for poor growth, morbidity and mortality are quite different than in the low-resource settings (5, 20). In order to enhance global understanding of the unique health needs of HIV-infected and HIV-EU infants relative to unexposed infants in low-resource settings, we evaluated mortality, morbidity and growth outcomes in HIV-infected and HIV-EU children in Dar es Salaam, Tanzania, compared to unexposed infants from the same peri-urban community.

## Subjects and Methods

### Subjects

The sample for this study were 3,554 children in urban Dar es Salaam, Tanzania who participated in one of two randomized controlled trials of multiple micronutrient supplementation in infancy. The first trial randomized 2387 infants born to HIV-infected mothers to either daily administration of multivitamins (vitamins B-complex, C & E) or placebo at 6 weeks of age (21). Randomization of infants occurred between August 2004 and November 2007; follow-up ended in May 2008. The supplements did not show an effect on mortality, morbidity or child growth (21, 22). Infants in this trial who were confirmed to be HIV-infected at 6 weeks of age will serve as the HIV-infected sample for our analyses, while infants who were confirmed to be HIV-uninfected at 6 weeks serve as the HIV-EU sample. If HIV-EU infants tested positive for HIV infection at a later clinic visit, they were removed from the analyses after their last negative test. All other infants remain in the analysis until death, loss-to-follow-up or 18-months of age.

The second trial was implemented with a 2x2 factorial design assessing the effect of zinc plus multivitamins (the same combination and dosages of vitamins B-complex, C and E as above), zinc alone, multivitamins alone or placebo among 2400 infants born to HIV-uninfected women (23). Children initiated daily supplementation at 6 weeks and continued for 18 months. In order to

enhance comparability between the two trial samples, we restricted our analyses only to infants who received multivitamins alone or placebo.

In both studies, women attending antenatal clinics in urban Dar es Salaam were invited to participate in each trial starting at 32 weeks gestation, and additional mothers were recruited from labor wards. Women who provided written informed consent were followed up throughout the antenatal, delivery, and postnatal periods to assess whether the mother-infant pair met the eligibility criteria for the child to be randomized at age 5–7 wks. Maternal HIV-status was assessed using enzyme-linked immonosorbent assays (ELISAs) that used the Murex HIV antigen/antibody (Abbot Murex, Dartford, Kent, U.K) followed by the Enzygnost anti-HIV-1/2 Plus (Dade Behring, Marburg, Germany). Discordant results between two ELISAs were resolved by a Western blot assay. Mothers who were identified as HIV-infected during recruitment for the first trial were invited to participate in that study, while mothers confirmed to be HIV-uninfected during recruitment for the second trial were invited to participate in the second trial. The two studies were designed to allow for a pooled analysis – they were conducted in overlapping clinic sites with similar staff, they used identical inclusion/exclusion criteria (other than maternal HIV-status), and they collected the same socio-demographic and clinical data on all mothers and children. Inclusion criteria for both studies included: singleton, healthy infants of women who intended to stay in Dar-es-Salaam for 2 years after delivery.

**Study Protocol**

Children from both studies began supplementation between 5-7 weeks and continued daily supplementation with either placebo or multivitamin supplements (vitamins B-complex, C and E vs. placebo) until the end of follow-up. The supplements for both trials were identical. For more details on supplementation dosages, see the published papers (21, 23). In brief, infants under 6 months of

age who were randomized to multivitamins consumed one capsule per day that contained 150–600% of the US Adequate Intake for children aged 0–6 mo, and two capsules per day after 6 months containing 200–400% of the AI for infants 6-12 months and 133–800% of the US RDA for children aged 1–3 y.

In both trials, mothers were asked to bring children to the clinic for follow-up visits every 4 weeks after randomization. During each monthly follow-up visit, a trained research nurse performed a standardized assessment of child morbidity with the mother based on the mother's symptom diary that she received at the previous visit. The symptom diary was a pictorial aid of illness symptoms (eg, diarrhea, vomiting, the need for unscheduled outpatient or hospital visits, etc.) where mothers were asked to check off which days their child suffered from these symptoms. Diarrhea was defined as ≥3 loose or watery stools within a 24-h period. At each monthly follow-up visit, a trained study nurse also measured child anthropometry using standard techniques (24). Weight was measured on a digital infant balance scale with 10-g precision (Tanita) and length with 1-mm precision using a rigid length board with an adjustable foot piece. Children who missed their monthly follow-up appointment were visited at home by a study nurse, and their vital status was confirmed through contact with immediate family members. A verbal autopsy was performed in cases of child death to determine the cause of death. Coding of the cause of death from the verbal autopsies was performed independently by 2 pediatricians (KPM and CD), and differences were adjudicated by a third pediatrician.

**Standard of Care**

Mothers who enrolled during pregnancy received standard prenatal care including anthropometric assessment, intermittent prophylaxis for malaria, tetanus toxoid immunization, deworming using mebendazole, anemia assessment, and iron-folic acid supplementation. On the basis of earlier

findings of the benefits of prenatal multivitamins among HIV-infected women who were not receiving ARVs (29,30), all HIV-infected women received supplements containing high doses of vitamins B-complex, C, and E during pregnancy and lactation until the study end. Maternal multivitamin doses were generally several times the Recommended Dietary Allowance (RDA) for B-complex vitamins, vitamin C, and vitamin E, but women who were started on antiretroviral therapy (ARV) were changed to single RDA multivitamin dosages.

When the first trial began, routine medical care for pregnant women with HIV infection included diagnosis and treatment of sexually transmitted infections and prophylaxis, and diagnosis and treatment of opportunistic infections. ARV medication was initially limited to nevirapine prophylaxis for maternal to child transmission (one dose given to the mother at the onset of labor and one dose given to the infant within 72 h of birth) (21). As the study progressed, the availability of ARVs increased substantially through programs such as the President's Plan for AIDS Relief and other governmental and nongovernmental programs. Beginning in July 2005, women and children in the study were screened for ARV eligibility and treated according to Tanzanian Ministry of Health guidelines. For adults, eligibility was based on WHO stage IV HIV disease, or CD4 cell count ≤200 cells/mL, or WHO stage III and CD4 cell count ≤350 cells/mL. For children aged <18 mo, eligibility was based on CD4%<20 or Pediatric WHO Stage III; for children aged 18 mo, eligibility was based on Pediatric WHO Stage III or CD4%<15%. The standard first-line regimen was stavudine, lamivudine, and nevirapine for adults and zidovudine, lamivudine, and nevirapine for children; alternative drugs were available for specific circumstances. All children were tested for HIV infection at 6 wk of age by using the Amplicor HIV-1 DNA assay version 1.5 (Roche Molecular Systems Inc). Tests at 18 mo of age were performed by using HIV ELISAs followed by Enzygnost anti-HIV-1/2 Plus (Dade Behring); discordant results were resolved by using a Western blot test.

Samples from children who tested positive at 18 mo were then back tested via polymerase chain reaction to estimate time of transmission.

In accordance with the WHO recommendations and Tanzanian Ministry of Health and Social Welfare guidelines at the time, HIV-infected mothers were counseled on the risks and benefits of exclusive breastfeeding. HIV-uninfected mothers were encouraged to exclusively breastfeed for the first 6 months followed by complementary feeding and continued breastfeeding until 2 years. All children received growth monitoring, immunizations, and routine medical care for illnesses. As standard of care, children in both trials received periodic high-dose vitamin A supplementation, as per Tanzanian Ministry of Health guidelines (100,000 IU at 9 mo and 200,000 IU at 15 and 21 mo). Infants born to HIV-infected mothers also received cotrimoxazole prophylaxis until age 6 months; after that, only breastfeeding or HIV-infected children continued to receive cotrimoxazole.

### Ethical Approval

Ethical approval for both trials was granted by the Harvard T.H. Chan School of Public Health Human Subjects Committee, the Muhimbili University of Health and Allied Science Committee of Research and Publications, the Tanzanian Institute of Medical Research and the Tanzanian Food and Drug Authority. Separate Data Safety Monitoring Boards also met twice annually over the course of the studies. All mothers provided written informed consent to enroll themselves and their infants in the studies.

### Data Management and Analyses

Details on data collection and management from the two trials has been previously published (21-23, 25). In brief, data were double entered by using Microsoft Access software and converted to SAS software (version 9.1; SAS Institute) for analysis. For growth analyses, we calculated age- and sex-specific z-scores for three anthropometric indexes: weight-for-height (WHZ), height-for-age (HAZ)

and weight-for-age (WAZ) using the 2006 WHO growth standards.(26) In accordance with WHO recommendations, we set all extreme HAZ (<-6 or >6), WHZ (<-5 or >5), and WAZ (<-6 or >5) values to missing (27).

Descriptive statistics were used to summarize baseline maternal, child and household characteristics of the three groups of infants (HIV-infected, HIV-EU and HIV-unexposed). For categorical variables, frequencies in absolute numbers and percentages were assessed and compared using chi-squared tests. For continuous variables mean and standard deviations were derived and compared using ANOVA.

Cox proportional hazards models with the exact method for ties were used to compare rates of mortality across the three groups, with the HIV-unexposed infants designated as the reference group. Covariates for the adjusted models were selected by first conducting univariate Cox models for mortality using common predictors of child mortality: maternal education (0, 1-7, or ≥8 years), height (cm), marital status, parity (0, 1-3, ≥4) and occupation (housewife with income, housewife without income or other); household asset score (0, 1 or 2 from list that included television and refrigerator); frequency of household consumption of meat or fish (at least once a week or less); and child's sex and treatment group (multivitamin vs. placebo). All variables that were significant at the p=0.20 level were retained in the final adjusted model. *A priori,* we determined that infant sex and treatment group (multivitamin vs. placebo) would be retained in all models. Because infant feeding is of particular interest in the context of HIV and because it is a time-varying covariate, we added infant feeding to each adjusted model separately to assess the extent to which accounting for infant feeding changed the relationship between HIV-exposure group and each outcome. For the cox proportional hazard models for mortality, infant feeding was defined as time-updating covariates for the duration of breastfeeding and the duration of exclusive breastfeeding. The assumption of proportional hazards was assessed by comparing plots of the survival function over time and by

including an interaction term between HIV-exposure group (HIV-infected, HIV-EU and HIV-unexposed) and time (infant age in months) in the Cox models.

Generalized estimating equations (GEE) with the binomial distribution, log link, and exchangeable covariance structure were used to compare the relative risk of common morbidities at each nurse visit across the three groups (diarrhea, fever, cough, vomiting, ear infection, anorexia as well as unscheduled outpatient visits and hospitalizations). We determined *a priori* that in addition to treatment group and infant sex, we would also include infant age (<26 weeks, 26-52 weeks and ≥52 weeks) in all multivariate morbidity models. We then individually assessed the same baseline covariates considered in the mortality analyses for each morbidity outcome – each model contained a single baseline covariate and infant age as predictors. To enhance consistency and interpretability, we selected the same set of covariates for all multivariate models for morbidity outcomes. Any covariate that was a significant predictor at the 0.20 level for at least two morbidity outcomes was retained in the adjusted morbidity models. For the fully-adjusted models, we also included a time-varying covariate for infant feeding mode, defined as exclusive, partial or no breastfeeding at each nurse visit.

Relative risks of stunting, wasting and underweight at baseline were compared using generalized estimating equations with the logarithm as the link function, empirical variance and a binomial distribution; while time to stunting, wasting and underweight over follow-up were compared using Cox proportional hazards models with the exact method for ties. To select covariates for adjustment, we conducted univariate models with the above-mentioned covariates for each growth outcome. To enhance consistency, we selected the same covariates for all growth outcomes. In addition to infant sex and treatment group, any covariate that was significant at the 0.20 level for at least two growth outcomes were retained in the multivariate growth models. Similar to the cox

proportional hazard models for mortality, in the fully-adjusted models, we included time-varying covariate for the duration of exclusive breastfeeding and the duration of any breastfeeding. The assumption of proportional hazards was assessed by comparing plots of the survival function over time and by including an interaction term between baseline infant HIV-status (HIV-infected, HIV-EU and HIV-unexposed) and time (infant age in months) in the Cox models. Mixed effects models with restricted cubic splines (with set knots at 10 weeks, 3, 6, 9 and 15 months) were used to model mean HAZ, WAZ and WHZ in infants from 6 weeks through 18 months. Covariates in the mixed efffects models were identical to those used in the models for stunting, wasting and underweight.

Because the guidelines for the initiation of ARVs changed in the middle of our study, we were able to assess ARV use during pregnancy as an effect modifier of the relationship between HIV-exposure group and all of our outcomes of interest. We first created three categories for all HIV-infected mothers: mothers who received ARVs during pregnancy, mothers with early-stage HIV (who would not qualify for ARVs either before or after the change of guidelines) and mothers with late-stage HIV who did not receive ARVs during pregnancy. In order to ensure that maternal ARV usage preceding child health outcomes, and also because maternal ARV use during pregnancy may be particularly important for child health outcomes, we first identified mothers who received ARVs during pregnancy. For mothers who did not receive ARVs during pregnancy, we stratified mothers as having "late stage HIV" based on the Tanzanian guidelines for ARV initiation which were introduced during our study (WHO stage IV HIV disease, or CD4 cell count ≤200 cells/mL, or WHO stage III and CD4 cell count ≤350). Early stage HIV was defined as the inverse of late-stage HIV. For infants whose mothers did not receive ARVs during pregnancy but initiated treatment after birth, we censored the children once the mothers initiated ARVs. To assess effect modification in the cox proportional hazard models for mortality, stunting, wasting and underweight, we conducted likelihood ratio tests comparing full models that contained both infant HIV-exposure

group as well as interaction terms for infant HIV-exposure group and maternal HIV-stage/ARV group versus reduced models that only contained infant HIV-exposure group. When overall test for effect modification yielded a p-value <0.05, maternal HIV-stage/ARV use was considered a significant effect modifier, and we repeated our primary analyses stratified by maternal HIV-stage/ARV group. All analyses were conducted in SAS version 9.3 (SAS Institute, Cary, NC USA).

## Results

2387 infants of HIV-infected mothers were randomized to multivitamins or placebo in the first trial at 6 weeks of age. 264 of these infants were confirmed to be HIV-infected at baseline and 2088 were confirmed HIV-uninfected, and thus served as the HIV-EU sample. 2400 infants born to HIV-uninfected mothers were randomized to zinc + multivitamins, zinc alone, multivitamins alone or placebo at 6 weeks of age; 1202 of whom received either multivitamins alone or placebo, and thus serve as the HIV-unexposed sample. With the exception of infant sex, all baseline variables were significantly different across the three HIV-exposure groups (**Table 3.1)**. On average, HIV-uninfected mothers were younger, taller, had larger middle-upper arm circumferences, were less likely to be a housewife without income, more likely to be married or cohabitating with their partner, and had fewer previous pregnancies. They also lived in households that were more likely to own refrigerators and/or televisions. Approximately half of all infants in each exposure group were male. Infants born to HIV-infected mothers were more likely to be low birth weight, particularly if they were HIV-infected themselves. HIV-infected infants were also significantly more likely to be born pre-term. Breastfeeding practices among HIV-infected and HIV-uninfected mothers were notably different. HIV-infected mothers were more likely to exclusively breastfeed for longer than HIV-uninfected mothers (mean duration of 3.5 versus 1.9 months); however, HIV-infected mothers ceased

## Table 3.1: Sociodemographic Characteristics of Study Sample

| | HIV-Unexposed<br>n=1202 | HIV-EU<br>n=2088 | HIV-Infected<br>n=264 | P value[1] |
|---|---|---|---|---|
| ***Maternal characteristics*** | | | | |
| Age (y)[2] | 26.4 ± 5.0[1] | 28.2 ± 5.0 | 28.2 ± 4.9 | <0.001 |
| Height (cm) | 156.7 ± 5.7 | 156.3 ± 6.1 | 155.6 ± 6.8 | 0.034 |
| Middle-upper arm circumference (cm) | 27.0 ± 3.1 | 26.0 ± 3.2 | 25.2 ± 3.3 | <0.001 |
| Formal education [*n* (%)] | | | | <0.001 |
| None | 19 (1.6) | 143 (6.9) | 12 (4.6) | |
| 1-7 y [3] | 869 (72.7) | 1475 (71.3) | 202 (77.1) | |
| ≥ 8 y | 307 (25.7) | 451 (21.8) | 48 (18.3) | |
| Employment [*n* (%)] | | | | 0.002 |
| Housewife without income | 723 (60.6) | 1328 (66.0) | 180 (70.9) | |
| Housewife with income | 388 (32.5) | 439 (21.8) | 40 (15.8) | |
| Other | 82 (6.9) | 245 (12.2) | 34 (13.4) | |
| Marital status [*n* (%)] | | | | |
| Married or cohabitating with partner | 1079 (90.7) | 1803 (87.2) | 220 (84.6) | 0.001 |
| Prior pregnancies [*n* (%)] | | | | <.0001 |
| None | 392 (33.0) | 477 (23.1) | 56 (21.5) | |
| 1-4 | 725 (61.0) | 1424 (68.9) | 182 (69.7) | |
| ≥ 5 | 72 (6.1) | 166 (8.0) | 23 (8.8) | |
| ***Socioeconomic characteristics*** | | | | |
| Household appliances[4] [*n* (%)] | | | | <.0001 |
| None | 407 (34.1) | 1143 (55.3) | 137 (52.5) | |
| Either a television or refrigerator | 384 (32.1) | 495 (24.0) | 63 (24.1) | |
| Both a television and refrigerator | 404 (33.8) | 429 (20.8) | 61 (23.4) | |
| Household consumes meat/fish more than once per week | 777 (65.4) | 1459 (70.8) | 179 (68.6) | 0.006 |
| Spouse education ≤ 7 years | 627 (57.4) | 1044 (61.0) | 136 (63.6) | 0.090 |
| ***Child characteristics*** | | | | |
| Male sex [*n* (%)] | 627 (52.2) | 1141 (54.7) | 131 (49.6) | 0.169 |
| Age at baseline (weeks) | 5.9 ± 0.4 | 5.8 ± 0.5 | 5.9 ± 0.4 | <0.001 |
| Low birth weight, < 2500 g [*n* (%)] | 39 (3.3) | 114 (5.7) | 42 (17.0) | <0.001 |
| Born preterm, <37 wk [*n* (%)][5] | 143 (13.0) | 299 (14.5) | 208 (19.4) | 0.032 |
| Duration of exclusive breastfeeding (months) | 1.9 (1.5) | 3.6 (2.1) | 3.1 (2.1) | <0.001 |
| Duration of any breastfeeding (months) | 13.2 (5.9) | 4.4 (2.4) | 4.5 (3.2) | <0.001 |

1. P-values for continuous variables are from ANOVA; p-values for categorical variables are from chi-squared tests
2. Mean ± SD (all such values), unless otherwise shown
3. In Tanzania, 7 years is the duration of most primary schools.
4. From a list that includes television & refrigerator

breastfeeding much earlier than HIV-uninfected mothers (mean duration of 4.5 months versus 13.2 months).

Infants born to HIV-infected mothers experienced substantially higher rates of mortality in crude and adjusted models compared to infants born to HIV-uninfected mothers, even when the infants remained uninfected (**Table 3.2**). Compared to unexposed infants, HIV-infected infants experienced a thirty-fold increase in mortality, while HIV-EU infants had a three-fold increase in rate of mortality, even after adjusting for socio-demographic characteristics and infant feeding. Infants born to HIV-infected mothers also had higher rates of most common infectious morbidities in crude and adjusted models compared to infants born to HIV-uninfected mothers (**Table 3.3**). In models adjusted for baseline socio-demographic covariates and mode of infant feeding at each nurse visit, HIV-infected infants had an increased risk of all morbidities assessed (diarrhea, cough, fever,

**Table 3.2:**
**Mortality Rates in HIV-Unexposed, HIV-Exposed-Uninfected and HIV-infected Infants**

|  | HIV-Unexposed n=1202 | HIV-EU n=2088 | HIV-Infected n=264 |
|---|---|---|---|
| Observed # deaths before age 18 months (%) | 16 (1.3%) | 119 (5.7%) | 113 (42.8%) |
| 1-year mortality rate (95% CI)[1] | 1.2% (0.8, 2.0) | 4.6% (3.9, 5.6) | 46.5% (38.7, 55.9) |
| Crude hazard ratio (95% CI)[2] | ref | 3.76 (2.23, 6.33) | 37.96 (22.49, 64.10) |
| P-value | - | <0.001 | <0.001 |
| Hazard ratio adjusted for baseline covariates (95%CI)[3] | ref | 3.56 (2.07, 6.13) | 36.29 (21.00, 62.71) |
| P-value | - | <0.001 | <0.001 |
| Hazard ratio adjusted for baseline covariates and infant feeding (95%CI)[4] | ref | 2.78 (1.40, 5.52) | 28.92 (14.80, 56.52) |
| P-value | - | 0.003 | <0.001 |

1. 1-year mortality rate estimated from Poisson regression models.
2. All hazard ratios, 95% confidence intervals and p-values are from cox proportional hazards models with the exact method for ties.
3. Adjusted for maternal education (0, 1-7 or 8+ years), occupation (housewife with income, housewife without income, or other), height (cm), number of household assets (0, 1 or 2 from a list that included television and refrigerator), and infant sex and treatment group (multivitamin vs. placebo).
4. Adjusted for the same baseline covariates above as well as time-updating covariates for duration of exclusive breastfeeding and duration of any breastfeeding.

**Table 3.3: Comparing common morbidities in HIV-infected, HIV-exposed-uninfected and HIV-unexposed infants**

| | HIV-Unexposed (reference) | HIV-Exposed-Uninfected | | | | HIV-Infected | | | |
|---|---|---|---|---|---|---|---|---|---|
| | n=14,823[1] | n=36,561[1] | | | | n=3,214[1] | | | |
| | # events (%)[2] | # events (%) | Crude RR (95%CI)[3] | Partially-Adjusted RR (95% CI)[4] | Fully-Adjusted RR (95% CI)[5] | # events (%) | Crude RR (95%CI) | Partially-Adjusted RR (95% CI)[4] | Fully-Adjusted RR (95% CI)[5] |
| Diarrhea | 566 (4.01) | 1,380 (4.81) | 1.21 (1.09, 1.35)**** | 1.22 (1.09, 1.36)**** | 1.18 (1.00, 1.39)* | 154 (5.86) | 1.50 (1.23, 1.81)**** | 1.49 (1.23, 1.82)**** | 1.46 (1.16, 1.84)** |
| Cough | 3322 (23.39) | 8,672 (30.26) | 1.31 (1.25, 1.37)**** | 1.26 (1.20, 1.33)**** | 1.28 (1.19, 1.37)**** | 942 (35.90) | 1.53 (1.41, 1.67)**** | 1.49 (1.37, 1.63)**** | 1.52 (1.38, 1.68)**** |
| Fever | 1471 (10.36) | 3348 (11.68) | 1.14 (1.07, 1.23)**** | 1.08 (1.01, 1.16)** | 1.16 (1.03, 1.29)** | 543 (20.69) | 2.03 (1.82, 2.27)**** | 1.97 (1.75, 2.21)**** | 2.11 (1.83, 2.43)**** |
| Anorexia | 346 (2.44) | 800 (2.79) | 1.16 (1.01, 1.34)** | 1.08 (0.94, 1.25) | 1.02 (0.81, 1.29) | 118 (4.50) | 1.90 (1.50, 2.41)**** | 1.93 (1.51, 2.48)**** | 1.85 (1.35, 2.52)**** |
| Vomiting | 237 (1.67) | 502 (1.75) | 1.07 (0.90, 1.27) | 1.06 (0.89, 1.26) | 0.97 (0.74, 1.28) | 70 (2.67) | 1.64 (1.22, 2.21)** | 1.68 (1.24, 2.28)**** | 1.58 (1.11, 2.23)** |
| Ear infection | 71 (0.50) | 198 (0.69) | 1.45 (1.05, 2.01)** | 1.46 (1.06, 2.02)** | 1.54 (0.94, 2.52)* | 75 (2.86) | 5.88 (3.99, 8.67)**** | 5.49 (3.59, 8.40)**** | 5.85 (3.37, 10.15)**** |
| Unscheduled outpatient visits | 223 (1.62) | 870 (3.03) | 1.87 (1.59, 2.21)**** | 1.77 (1.50, 2.10)**** | 1.74 (1.35, 2.25)**** | 113 (4.30) | 2.70 (2.13, 3.43)**** | 2.70 (2.11, 3.47)**** | 2.67 (1.96, 3.63)**** |
| Hospitalizations | 20 (0.14) | 176 (0.61) | 4.32 (2.66, 6.99)**** | 4.35 (2.62, 7.25)**** | 3.56 (1.80, 7.05)**** | 55 (2.09) | 15.07 (8.69, 26.13)**** | 15.68 (8.80, 27.93)**** | 12.96 (6.42, 26.17)**** |

1. n is the sum of the number of nurse visits for all children.
2. Number of events defined as maternal report of morbidity in 28 days (4 weeks) prior to each nurse visit. Denominator for percentage calculations was the total number of nurse visits.
3. Crude RR, 95% CI, and corresponding P-values obtained from generalized estimating equations with the binomial distribution, log link, and exchangeable covariance structure.
4. Partially-adjusted RR, 95% CI, and corresponding P-values were obtained from generalized estimating equations with the binomial distribution, log link, and exchangeable covariance structure. Partially-adjusted models were adjusted for child's sex and age (<6 months, 6-12 months and >12 months), treatment group (multivitamin supplement vs. placebo) and maternal education (0, 1-7, or 8 years), occupation (housewife with no income, housewife with income, other), marital status (cohabitating with partner, yes vs. no), parity (0, 1-4, 5+), height (tertiles) and age (tertiles).
5. Fully-adjusted RR, 95% CI, and corresponding p-values were obtained from generalized estimating equations with the binomial distribution, log link, and exchangeable covariance structure. Fully-adjusted models contain all the same covariates as partially-adjusted models, but also include infant feeding mode at each nurse visit (exclusive breastfeeding, mixed feeding, or no breastfeeding).
*p<0.10;    **p<0.05;    ***p<0.01;    ****p<0.001

vomiting, pus draining from their ears, unscheduled outpatient visits and hospitalizations), while HIV-EU infants experienced a significantly increased risk of cough, fever, unscheduled outpatient visits and hospitalizations compared to unexposed infants, though their risks remained substantially lower than that of HIV-infected infants.

With regard to growth outcomes, HIV-infected infants experienced significantly impaired growth relative to their HIV-unexposed peers at all time periods in both crude and multivariate models **(Figures 3.1 and 3.2 and Table 3.4).** Of particular note, HIV-infected infants already had an increased risk of stunting [RR (95% CI): 2.63 (1.83, 3.78), p<0.001], wasting [RR (95% CI): 2.13 (1.40, 3.25), p<0.001] and underweight [RR (95% CI): 5.63 (3.81, 8.31), p<0.001] by 6 weeks of age in models adjusted for baseline sociodemographic characteristics and infant feeding mode at 6 weeks. They also continued to have significantly higher rates of stunting, wasting and underweight for the duration of follow-up.

At 6-weeks of age, HIV-EU infants had lower mean HAZ, WAZ and WHZ than HIV-unexposed infants; however only the difference in risk of underweight was significantly different at baseline [Adjusted RR (95%CI): 2.05 (1.45, 2.89), p<0.001]. The mixed effects models with restricted cubic splines show that HIV-EU infants experienced slower declines in HAZ, WAZ and WHZ than their unexposed peers; however the difference was most marked in HAZ where the curves crossed in the first few months of life. In the cox proportional hazard models assessing rates of stunting, wasting and underweight from 6 week through 18 months, HIV-EU infants had significantly higher rates of wasting and underweight in the crude models; however neither remained significant in models adjusted for sociodemographic characteristics and infant feeding. By contrast, HIV-EU infants had lower rates of stunting over follow-up compared to HIV-unexposed infants in crude and adjusted models [Adjusted HR (95%CI): 0.64 (0.51, 0.81), p<0.001].

**Figure 3.1: Height-for-age, weight-for-age and weight-for-height z-scores over time in HIV-infected, HIV-Exposed-Uninfected and HIV-Unexposed Females (adjusted for baseline covariates)**
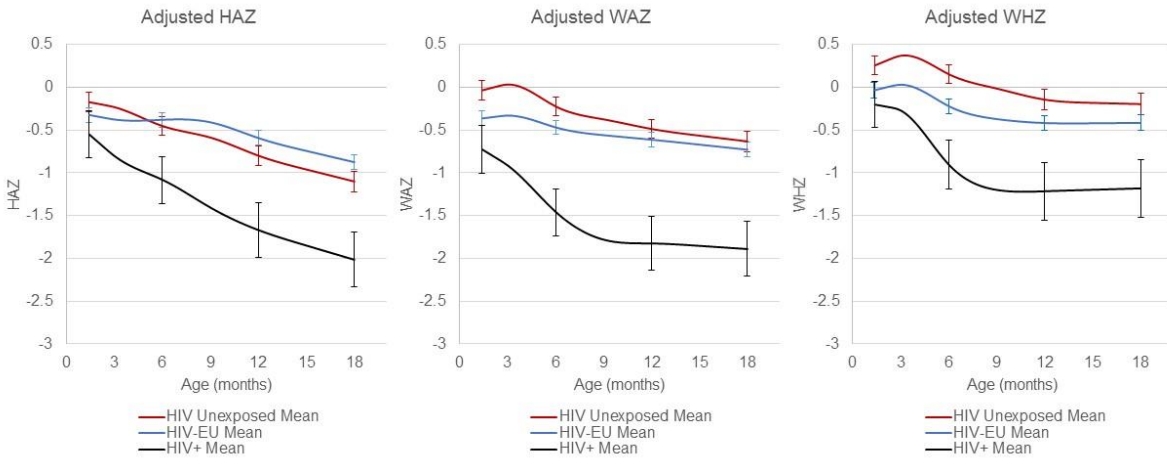


Curves were creating using mixed effects models with restricted cubic splines with knots at 10 weeks and 3, 6, 9 12 & 15 months of age. The models are adjusted for maternal height, education and occupation, household asset score, and infant sex and treatment group. The adjusted means presented here are for female children who received placebo in the parent trial, live in a household with one major household appliance (either a television or a refrigerator), and whose mother is the median height (156cm), is a housewife with no income and has 1-7 years of education. Bars represent 95% confidence intervals for each of the three groups at age 6 weeks and 6, 12 and 18 months.

**Figure 3.2: Height-for-age, weight-for-age and weight-for-height z-scores over time in HIV-infected, HIV-Exposed-Uninfected and HIV-Unexposed Males (adjusted for baseline covariates)**
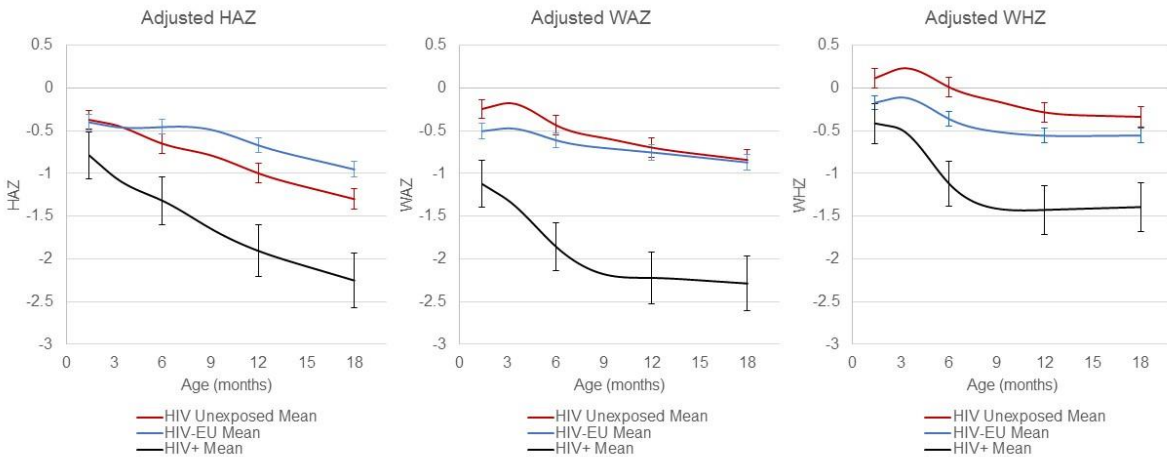


Curves were creating using mixed effects models with restricted cubic splines with knots at 10 weeks and 3, 6, 9 12 & 15 months of age. The models are adjusted for maternal height, education and occupation, household asset score, and infant sex and treatment group. The adjusted means presented here are for male children who received placebo in the parent trial, live in a household with one major household appliance (either a television or a refrigerator), and whose mother is the median height (156cm), is a housewife with no income and has 1-7 years of education. Bars represent 95% confidence intervals for each of the three groups at age 6 weeks and 6, 12 and 18 months.

**Table 3.4: Comparing Stunting, Wasting and Underweight among HIV-unexposed, HIV-exposed and uninfected, and HIV-infected infants**

| | | HIV-Unexposed (reference) | HIV-EU | HIV-Infected |
|---|---|---|---|---|
| | | *Relative Risks at Baseline (6 weeks)[1]* | | |
| **Stunting** | N | 1178 | 2052 | 258 |
| | n (%) | 72 (6.1) | 149 (7.3) | 42 (16.3) |
| | Crude RR (95% CI) | - | 1.19 (0.91, 1.56) | 2.66 (1.87, 3.80)**** |
| | Partially-Adjusted RR (95% CI)[2] | - | 1.12 (0.84, 1.48) | 2.48 (1.73, 3.56)**** |
| | Fully-Adjusted RR (95% CI)[3] | | 1.19 (0.89, 1.59) | 2.63 (1.83, 3.78)**** |
| **Wasting** | N | 1177 | 2043 | 256 |
| | n (%) | 69 (5.9) | 139 (6.8) | 30 (11.7) |
| | Crude RR (95% CI) | | 1.16 (0.88,1.53) | 2.00 (1.33, 3.00)**** |
| | Partially-Adjusted RR (95% CI)[2] | - | 1.16 (0.87, 1.54) | 1.96 (1.29, 2.97)*** |
| | Fully-Adjusted RR (95% CI)[3] | | 1.26 (0.94, 1.70) | 2.13 (1.40, 3.25)**** |
| **Underweight** | N | 1182 | 2065 | 261 |
| | n (%) | 46 (3.9) | 154 (7.5) | 54 (20.7) |
| | Crude RR (95% CI) | - | 1.92 (1.39, 2.64)**** | 5.32 (3.67, 7.69)**** |
| | Partially-Adjusted RR (95% CI)[2] | - | 1.82 (1.31, 2.54)**** | 5.05 (3.46, 7.36)**** |
| | Fully-Adjusted RR (95% CI)[3] | | 2.05 (1.45, 2.89)**** | 5.63 (3.81, 8.31)**** |
| | | *Hazard Ratios from 6 weeks – 18 months[4]* | | |
| **Stunting** | N | 1090 | 1871 | 205 |
| | n (%) | 278 (25.5) | 456 (24.4) | 94 (45.9) |
| | Crude HR (95% CI) | - | 0.80 (0.69, 0.93)*** | 2.21 (1.75, 2.80)**** |
| | Partially-Adjusted HR (95% CI)[5] | - | 0.72 (0.62, 0.85)**** | 1.89 (1.47, 2.41)**** |
| | Fully-Adjusted HR (95% CI)[6] | - | 0.64 (0.51, 0.81)**** | 1.72 (1.31, 2.27)**** |
| **Wasting** | N | 1092 | 1881 | 214 |
| | n (%) | 180 (16.5) | 510 (27.1) | 104 (48.6) |
| | Crude HR (95% CI) | - | 1.52 (1.28, 1.80)**** | 4.03 (3.16, 5.14)**** |
| | Partially-Adjusted HR (95% CI)[5] | - | 1.35 (1.13, 1.62)** | 3.68 (2.86, 4.73)**** |
| | Fully-Adjusted HR (95% CI)[6] | - | 1.16 (0.90, 1.50) | 3.21 (2.38, 4.34)**** |
| **Underweight** | N | 1114 | 1869 | 197 |
| | n (%) | 185 (16.6) | 441 (23.6) | 107 (54.3) |
| | Crude HR (95% CI) | - | 1.29 (1.09, 1.54)*** | 4.46 (3.51, 5.67)**** |
| | Partially-Adjusted HR (95% CI)[5] | - | 1.09 (0.91, 1.30) | 3.37 (2.63, 4.31)**** |
| | Fully-Adjusted HR (95% CI)[6] | - | 1.17 (0.90, 1.51) | 3.54 (2.63, 4.77)**** |

1. All relative risks and corresponding 95% Cis and p-values are from generalized estimating equations with the logarithm as the link function, empirical variance and a binomial distribution.
2. Partially-adjusted models are adjusted for treatment group (multivitamin vs. placebo), child's sex, maternal education (0, 1-7 or 8+ yrs), maternal height (tertiles), household asset score (0, 1 or 2 from a list of television & refrigerator).
3. Fully-adjusted models for baseline relative risks are adjusted for the same covariates as in the partially-adjusted models, but also include infant feeding mode at baseline (exclusively breastfeeding, mixed feeding, no breastfeeding).
4. Hazard ratios are from cox proportional hazards models with the exact method for ties.
5. Partially-adjusted cox proportional hazards are adjusted for treatment group (multivitamin vs. placebo), child's sex, maternal education (0, 1-7 or 8+ yrs), maternal height (tertiles), household asset score (0, 1 or 2 from a list of television & refrigerator), and the corresponding anthropometric z-score at baseline.
6. Fully-adjusted cox proportional hazard models contain the same covariates as the partially-adjusted models, but also include time-varying covariates for breastfeeding duration and exclusive breastfeeding duration.

*p<0.10;   **p<0.05;   ***p<0.01;   ****p<0.001

We found that maternal HIV-stage/ARV use was a significant effect modifier of the relationship between HIV-exposure and mortality **(Table 3.5).** Specifically, HIV-EU infants born to mothers with late-stage HIV who did not receive ARVs during pregnancy had substantially higher rates of mortality than infants of mothers with early stage HIV or mothers who received ARVs during pregnancy. Hazard ratios (95% CIs) comparing each group to HIV-EU children adjusted for sociodemographic characteristics were: 4.48 (1.81, 11.09), 1.78 (0.92, 3.44) and 2.72 (1.36, 5.47) respectively. This trend was also replicated in models adjusting for infant feeding, and in models for n HIV-infected infants, where HIV-infected infants born to mothers with late-stage HIV who did not receive ARVs during pregnancy had the highest rates of mortality.

**Table 3.5:**

**Effect Modification of the Relationship between HIV-Exposure and Mortality by Maternal Disease Progression and ARV Use**

| | HIV-Unexposed | HIV-EU | | | HIV-Infected | | |
|---|---|---|---|---|---|---|---|
| | (ref.) | Early-stage maternal HIV[1] no ARVs | Late-stage maternal HIV[2] no ARVs | Maternal ARVs during pregnancy | Early-stage maternal HIV[1] no ARVs | Late-stage maternal HIV[2] no ARVs | Maternal ARVs during pregnancy |
| N | 1202 | 791 | 117 | 370 | 65 | 28 | 51 |
| Observed # deaths (%) | 16 (1.3%) | 26 (3.3%) | 8 (6.8%) | 19 (5.1%) | 18 (27.7%) | 13 (46.4%) | 17 (33.3%) |
| 1-year Morality Rate[3] | 1.2% (0.8, 2.0) | 2.5% (1.7, 3.7) | 6.5% (3.3, 13.0) | 3.9% (2.5, 6.1) | 23.9% (15.0, 37.9) | 51.8% (30.0, 89.1) | 29.8% (18.5, 47.9) |
| **Crude HR (95%CI)[4]** with HIV-unexposed as referent | Ref | 2.03 (1.09, 3.94) | 5.05 (2.06, 12.39) | 3.10 (1.56, 6.16) | 19.96 (9.96, 39.96) | 48.48 (23.03, 102.07) | 23.81 (11.77, 48.19) |
| P-value | - | 0.026 | <0.001 | 0.001 | <0.001 | <0.001 | <0.001 |
| **Crude HR (95%CI)** with infants whose mothers had early-stage HIV (no ARVs) as referent | - | Ref. | 2.44 (1.05, 5.63) | 1.49 (0.82, 2.74) | Ref. | 2.43 (1.18, 5.02) | 1.19 (0.60, 2.36) |
| P-value | - | - | 0.038 | 0.194 | - | 0.016 | 0.612 |
| **Crude HR (95%CI)** with infants whose mothers had late-stage HIV (no ARVs) as referent | - | - | Ref. | 0.61 (0.23, 1.47) | - | - | 0.49 (0.24, 1.03) |
| P-value | - | - | - | 0.273 | - | - | 0.058 |
| **Partially-Adjusted HR (95%CI)[5]** with HIV-unexposed as referent | Ref | 1.78 (0.92, 3.44) | 4.48 (1.81, 11.09) | 2.72 (1.36, 5.47) | 19.37 (9.52, 39.40) | 40.40 (20.67, 95.34) | 21.42 (10.35, 44.32) |
| P-value | - | 0.085 | <0.001 | 0.005 | <0.001 | <0.001 | <0.001 |
| **Partially-Adjusted HR (95%CI)** with infants whose mothers had early-stage HIV (no ARVs) as referent | - | Ref. | 2.12 (1.08, 5.85) | 1.53 (0.83, 2.82) | Ref. | 2.29 (1.09, 4.81) | 1.11 (0.54, 2.24) |
| P-value | - | - | 0.033 | 0.176 | - | 0.028 | 0.781 |
| **Partially-Adjusted HR (95%CI)** with infants whose mothers had late-stage HIV (no ARVs) as referent | - | | Ref. | 0.61 (0.25, 1.46) | | Ref. | 0.48 (0.23, 1.03) |
| P-value | - | - | - | 0.267 | - | - | 0.058 |
| **Fully-Adjusted HR (95%CI)[6]** with HIV-unexposed as referent | Ref | 1.55 (0.63, 3.79) | 3.92 (1.30, 11.78) | 2.24 (0.88, 5.74) | 17.57 (7.02, 43.94) | 35.75 (13.43, 95.18) | 19.30 (7.73, 48.22) |
| P-value | - | 0.339 | 0.015 | 0.092 | <0.001 | <0.001 | <0.001 |
| **Fully-Adjusted HR (95%CI)** with infants whose mothers had early-stage HIV (no ARVs) as referent | - | Ref. | 2.53 (1.09, 5.89) | 1.45 (0.78, 2.69) | Ref. | 2.04 (0.96, 4.30) | 1.10 (0.54, 2.23) |
| P-value | - | - | 0.031 | 0.239 | - | 0.063 | 0.795 |
| **Fully-Adjusted HR (95%CI)** with infants whose mothers had late-stage HIV (no ARVs) as referent | - | - | Ref. | 0.57 (0.24, 1.38) | - | Ref. | 0.54 (.025, 1.16) |
| P-value | - | - | - | 0.215 | - | - | 0.114 |

1. Early stage defined as WHO stage I or II and CD4 cell count >200 cells/mL, or WHO stage III and CD4 cell count >350 cells/mL.
2. Based on criteria for ARV initiation in Tanzania as of July 2005, late-stage defined as WHO stage IV disease, or CD4 count ≤200 cells/mL, or WHO stage III and CD4 count ≤350).
3. 1-year mortality rate estimated from generalized estimating equations with a Poisson distribution offset by the logarithm of time.
4. All hazard ratios, 95% confidence intervals and p-values are from cox proportional hazards models with the exact method for ties.
5. Partially-adjusted Models were adjusted for maternal education (0, 1-7 or 8+ years), occupation (housewife with income, housewife without income, or other), height (cm), number of household assets (0, 1 or 2 from a list that included television and refrigerator), and infant sex and treatment group (multivitamin vs. placebo).
6. Fully-adjusted models contain the same covariates as the partially-adjusted models, but also include time-varying covariates for breastfeeding duration and exclusive breastfeeding duration.

**Discussion**

In this longitudinal study of infants born to HIV-infected and HIV-uninfected mothers in Dar es Salaam, Tanzania, we found that HIV-infected infants had the highest rates of mortality, morbidity and all three types of growth failure from 6 weeks through 18 months of age. HIV-EU infants also had higher rates of morbidity and mortality than HIV-unexposed infants, but mixed outcomes with regards to growth. HIV-EU infants were more likely to be underweight at 6 weeks of age; however, they experienced slower declines in HAZ, WAZ and WHZ over follow-up, and by 18 months they tended to be taller than their HIV-unexposed counterparts.

Our findings on the increased likelihood of mortality(4, 6), morbidity(28) and poor growth(29) among HIV-infected infants is consistent with the literature on pediatric HIV-infection in sub-Saharan Africa. Similarly, the higher rates of morbidity and mortality among HIV-EU children compared to HIV-unexposed children are also consistent with other studies. Two additional studies in East Africa also documented an increased risk of child mortality among infants born to HIV-infected mothers(30, 31) – though one study did not differentiate between HIV-infected and HIV-EU infants(30). HIV-EU children likely experience pre-natal programming of their immune systems as well as an increased exposure to infections postnatally due to maternal HIV status(3). Several studies have documented immune abnormalities in HIV-EU children compared to unexposed infants including lower naive CD4 counts and reduced thymic output(11), as well as reductions in CD4/CD8 ratios and CD41, CD81 and naive T-cell percentages (12). In addition, studies from sub-Saharan Africa have documented higher rates of tuberculosis(13) and *pneumonocystis jiroveci* (15) in HIV-EU children compared to their unexposed peers.

Reduced breastfeeding may also be an important cause of increased morbidity and mortality in HIV-EU infants. In our study, we found that on average, HIV-infected mothers exclusively breastfed

their infants longer than HIV-uninfected mothers, but discontinued breastfeeding much earlier than HIV-uninfected mothers. This is consistent with the counselling that HIV-infected mothers received focusing on the benefits of exclusively breastfeeding over mixed feeding, but also on the continued risk of transmitting the virus to their children with prolonged breastfeeding. Adjusting for infant feeding in our analyses explained a small amount of the difference in mortality and growth outcomes, but did not fully explain the effect of HIV-exposure group on morbidity and mortality.

Our finding that infants of mothers with late-stage HIV who do not receive ARVs have the highest rates of mortality is consistent with other studies that have found maternal HIV disease progression is inversely related to health outcomes in their children (32, 33). Interestingly, we also found that infants of mothers who received ARVs had lower rates of mortality than infants of mothers with late-stage HIV who did not receive ARVs; though, our power to compare these two groups was limited. While there is strong evidence that maternal ARV use during pregnancy and lactation can lower the risk of HIV-transmission to her child (34), our findings add to the growing body of evidence that maternal ARVs may confer health benefits to their children beyond HIV-transmission (35-37). It is worth noting that although maternal ARV use among mothers with late-stage HIV was associated with lower rates of mortality, HIV-EU infants still had substantially higher rates of mortality than HIV-unexposed infants regardless of maternal HIV-stage and ARV use. These findings indicate that while provision of ARVs for mothers during pregnancy and lactation may partially mitigate the effects on child health of HIV exposure, they are likely insufficient for improving the health outcomes of HIV-EU infants to the level of unexposed infants.

Our findings on growth outcomes were surprising. Specifically, we found that HIV-EU infants had lower HAZ, WAZ and WHZ at 6 weeks of age, which is consistent with the few other studies that have assessed birth size in HIV-EU and HIV-unexposed infants in low-resource settings(5). Taken

together, the evidence indicates that pre-natal exposure to HIV has the potential to impair fetal growth. More surprising; however, was that the HIV-EU infants in our study experienced slower declines in HAZ, WAZ and WHZ than their unexposed peers over follow-up, and that ultimately, HIV-EU infants had lower rates of stunting from 6 weeks to 18 months, and higher mean HAZ scores by 18 months of age. There are several potential explanations for this counterintuitive result. Firstly, the rate of mortality was much higher in the HIV-EU population, and thus there is likely survival bias in that the infants who were most likely to become stunted in the HIV-EU sample are the same infants who were most likely to die during follow-up. There were also two important systematic differences between our two trial populations that may explain these results. Because a previous trial in urban Dar es Salaam found that multivitamin supplementation (vitamins B-complex, C and E) for HIV-infected mother during pregnancy and lactation can slow disease progression and delay maternal mortality (38), all HIV-infected mothers in our current study received multivitamin supplementation during pregnancy and lactation, while HIV-uninfected mothers did not. Previous work from our group has shown that the provision of these supplements to HIV-infected women during pregnancy and lactation improves post-natal growth in their offspring (39). In addition, in accordance with the Tanzanian Ministry of Health and Social Welfare guidelines at the time of the trial, all HIV-EU infants all received prophylactic cotrimoxazole as a strategy to potentially reduce maternal-to-child transmission of HIV. A recent meta-analysis of 10 randomized controlled trials (40) has indicated that antibiotic use, and particularly cotrimoxazole as given as a prophylactic for HIV(41), has the potential to improve linear and ponderal growth in young children.

There are several limitations of our study. Given our study's observational design, and particularly that our data come from two separate trials, we cannot rule-out residual confounding. Although we adjusted for several socioeconomic and demographic characteristics in our analyses, we cannot

eliminate the possibility that an unmeasured confounder is driving the observed relationships. In addition, the majority of our participants enrolled and completed study follow-up in the pre-ARV era, so we have limited capacity to extrapolate our findings to populations where ARV access is more common. Due to the change in national guidelines on ARV use during our study, we were able to conduct sub-analyses accounting for maternal HIV-progression and ARV use; however, we had missing data for several study participants and ultimately limited power for these analyses. It is worth noting that even after stratifying by maternal disease progression and ARV use, our findings on worse health outcomes for HIV-EU and HIV-infected infants did not change substantially. Our study also has several strengths. To our knowledge, it is the largest analysis of the mortality, morbidity and growth of HIV-infected, HIV-EU and HIV-uninfected infants in sub-Saharan Africa. The prospective, longitudinal study design and the collection of comprehensive data on socio-demographic characteristics, infant feeding practices, and maternal HIV disease progression and ARV usage allowed us to conduct rigorous analyses accounting for important confounders and effect modifiers.

Our study contributes to a growing body of evidence that indicates that infants born to HIV-infected mothers have worse health outcomes than infants born to HIV-uninfected mothers, regardless of infant HIV infection. While HIV-infected infants suffer dramatically poorer heath and growth outcomes, HIV-EU infants in our study also experienced an increased risk of mortality and morbidity compared to their unexposed peers. These poor health outcomes remained even after accounting for socio-demographic characteristics, infant feeding practices and maternal disease progression and ARV use, thus highlighting the importance of targeting not only HIV-infected, but also HIV-EU infants in HIV care and treatment and child health programs.

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