Maternal multivitamin supplementation reduces the risk of diarrhoea among HIV-exposed children through age 5 years

Nasim Khavari^{a,b}, Hongyu Jiang^a, Karim Manji^c, Gernard Msamanga^c, Donna Spiegelman^d, Wafaie Fawzi^d and Christopher Duggan^{a,*}

^aDivision of GI/Nutrition, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA;

^bDepartment of Pediatrics, Stanford University, Palo Alto, CA 94306, USA; ^cDepartments of Community Medicine and Pediatrics, Muhimbili University of Health and Allied Sciences, United Nations Road, Dar es Salaam, Tanzania; ^dDepartments of Nutrition, Epidemiology, Biostatistics, Global Health and Population, Harvard School of Public Health, Boston, MA 02115, USA

*Corresponding author: Present address: Center for Nutrition, Division of GI/Nutrition, Boston Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA. Tel: +1 617 355 7612; Fax +1 617 730 4722; E-mail: Christopher.Duggan@childrens.harvard.edu

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Background: The aim of this study was to determine whether maternal vitamin supplementation affects long-term mortality and morbidity of children born to HIV-infected mothers.

Methods: In total, 1078 HIV-infected pregnant woman were enrolled in a double-blind, 2×2 factorial, randomised, placebo-controlled trial in Tanzania. Data were collected for 874 children at monthly clinic visits through a median age of 51 months.

Results: Maternal receipt of multivitamins (HR=0.93; 95% CI 0.70–1.22) or vitamin A (HR=1.00; 95% CI 0.76–1.32) did not affect all-cause child mortality through age 5 years. Among HIV-negative children, maternal multivitamin supplementation was associated with a lower mortality rate up to 5 years (HR=0.60; 95% CI 0.38–0.95), primarily in children <2 years of age. Maternal vitamin A supplementation did not significantly affect child mortality up to 5 years (HR=0.76; 95% CI 0.48–1.20). Children born to mothers who received multivitamins had a lower risk of all types of diarrhoea (RR=0.86; 95% CI 0.75–0.98) through 5 years of age. The reduced risk of watery diarrhoea persisted in children from 2–5 years of age (RR=0.71; 95% CI 0.54–0.95).

Conclusions: Maternal vitamin supplementation during pregnancy and lactation may be associated with long-lasting effects in HIV-exposed children [ClinicalTrials.gov Identifier: NCT00197743].

Keywords: Child mortality, Diarrhoea, HIV, Infection, Maternal nutrition, Respiratory tract

Introduction

Diarrhoea, respiratory tract infections and malnutrition are among the leading morbidities and causes of death among infants and children in the developing world,¹ and infants born to HIV-infected mothers are at higher risk for these illnesses. Regular antenatal care as well as improvement in the nutritional status of mothers and children prior to 24 months of age can improve infant and childhood outcomes.²

Supplementation with multiple micronutrients in susceptible pregnant women has been noted to have several beneficial perinatal and neonatal effects. Maternal micronutrient supplementation has been associated with reduced infant mortality, reduced morbidities including preterm birth and low birthweight, and higher birth measurements.^{3–5} In children up to 2 years of age,

maternal multivitamin supplementation has been associated with increased length and weight gain.⁶ However, it is unclear how long these effects may persist and/or whether additional beneficial or adverse effects exist.

Among populations with a high prevalence of HIV infection, data are even more limited.⁷ A randomised, placebo-controlled trial was conducted to determine the effects of vitamin A and multivitamin supplementation to HIV-infected mothers during pregnancy. Among infants born to these mothers, we previously found that maternal multivitamin supplementation resulted in higher infant mean CD4 counts (151 cells/μl; *p*=0.001) and a 17% reduction in the risk of diarrhoea (RR=0.83; 95% CI 0.71–0.98) through 24 months of age.⁸ Children born to mothers in the multivitamin arm also had increased birth weight and higher attained weight that persisted at 24 months.⁹ To more

fully evaluate the potential long-term impact of maternal vitamin supplementation on childhood infections and mortality, enrolled children were followed-up to 5 years of age.

Materials and methods

Details regarding the methods and participants of this trial have been published previously.¹⁰ Briefly, from April 1995 through July 1997 pregnant women in Tanzania were recruited and tested for HIV infection for participation in the trial. HIV-infected pregnant women between 12 and 27 weeks gestation were enrolled. Follow-up of mothers and children continued through August 2003.

Women were randomly assigned in a 2×2 factorial design to receive a daily oral dose of one of four regimens from enrolment throughout pregnancy and until follow-up ended: 1. vitamin A alone (30 mg β-carotene plus 5000 international units [IU] of pre-formed vitamin A); 2. multivitamins excluding vitamin A (20 mg of vitamin B₁, 20 mg of vitamin B₂, 25 mg of vitamin B₆, 100 mg of niacin, 50 µg of vitamin B₁₂, 500 mg of vitamin C, 30 mg of vitamin E and 0.8 mg of folic acid); 3. multivitamins including vitamin A in the same doses as above; or 4. placebo. At delivery, women in groups 1 and 3 received an additional oral dose of vitamin A (200 000 IU), whilst women in groups 2 and 4 were given a placebo. Active tablets and placebo were identical in size and colour. Women and children, irrespective of experimental group, were provided with standard prenatal and child care services in Tanzania, including distribution of 100 000 IU of vitamin A to infants at 6 months of age and 200 000 IU at 12, 18 and 24 months. All women were scheduled to receive standard doses of antenatal iron and folic acid throughout pregnancy and lactation periods as per Tanzanian Ministry of Health guidelines. At the time of the study, antiretroviral therapy (ART) was generally not available to HIV-infected individuals in Tanzania.

Sociodemographic characteristics were collected at baseline. Breastfeeding was nearly universally adopted in this population. Morbidity surveillance of children was carried out through monthly clinic visits. Participants were examined by study physicians and nurses at monthly visits. At each visit, mothers were asked about the number of days that the child had signs or symptoms of diarrhoeal or respiratory diseases during the previous month. Diarrhoea was defined as 3 or more watery stools in the prior 24 h. Respiratory signs and symptoms reviewed were fever, cough, difficulty breathing, chest retractions, and difficulties with eating, drinking or breastfeeding. Children's respiratory rates were measured on the day of the monthly visit, and a rapid respiratory rate was defined as ≥50 breaths/min for infants and ≥40 breaths/min for children older than 1 year of age.

Episodes of diarrhoea during each monthly interval were defined as follows: acute diarrhoea included all intervals with ≥1 day of diarrhoea but <14 days. Dysentery was defined as any diarrhoea with visible blood or mucus. Acute diarrhoea was classified as dysentery or non-dysentery (i.e., watery) diarrhoea. Respiratory infection was defined in four ways: the incidence of cough alone; cough and fever; 'cough plus' (defined as cough with at least one of the following symptoms/signs: difficulty breathing; chest retractions; and refusal to eat, drink or breastfeed); or cough with rapid respiratory rate on the day of visit.

At baseline, maternal complete blood count and absolute counts of T-cell subsets (CD4⁺ and CD8⁺) were measured.

Infant T-cell counts were also measured at birth and at 6-monthly intervals thereafter. Details of the assessment of HIV status have been published elsewhere.¹¹

The effects of supplementation on childhood mortality and common morbidities, including symptoms of diarrhoea and respiratory infections, were examined. These endpoints were examined from age 6 weeks to 5 years of age and from age 2–5 years. These time points were chosen since data from 6 weeks to 2 years of age have been published previously.^{8,11} For the mortality analysis, crude mortality incidence rates were calculated by study arm and by HIV status, and Cox proportional hazards (PH) models were used to investigate the effects of the supplements on time to death. Mortality follow-up time ended at 5 years of age or at the last time survival was ascertained, whichever was sooner. Multivariate PH models were also fit to evaluate the effect of supplementation on mortality, adjusting for known or suspected maternal (baseline CD4, ESR, haemoglobin, vitamin A and E levels, CD8, body mass index, viral load, age, education, prior pregnancy and marital status) and child (gender, birth weight, gestational age, HIV infection status, baseline weight- and height-for-age Z-scores) risk factors. In these models, children's HIV infection status was treated as a time-varying covariate. Maternal characteristics were measured at study entry. Child characteristics including gender, birth weight and birth gestational age were collected at birth; weight- and height-for-age Z-scores were obtained at analysis baseline (6 weeks for 6-week to 5-years analysis or 2 years for 2–5-years analysis). Possible interaction between vitamin A and multivitamin supplementation was evaluated with an interaction term in the abovementioned models.

Since child HIV infection status is a strong predictor for mortality and a possible effect modifier for supplementation effects, in further analyses the effect of the supplements on mortality were examined within each HIV infection status stratum for the 6-week to 5 years and 2–5 years mortality analyses (child HIV status was time-varying).

For the morbidity analyses, the mean (SD) number of diarrhoeal and respiratory infection episodes per child-year was calculated. Data were analysed using the generalised estimating equations with repeated binary outcomes as the response variables, with an exchangeable working covariance structure. Covariates used in multiple regression models were defined similarly to those used in the PH model for mortality analyses, with the only exception being child age. To accommodate the potential non-linear effect of child age on morbidity outcomes, child age was categorised into 6-month intervals.

All analyses were performed with SAS v.9.2 (SAS Institute, Cary, NC, USA). During an interim analysis, the data and safety monitoring board suggested the vitamin A treatment arms be halted due to increased transmission of HIV to children in these arms.¹¹

Results

All children born to HIV-infected mothers who were singletons and first twins and who had available data from 6 weeks to ≤5 years of age were included in the study. The trial profile is depicted in Figure 1. At 6 weeks post-partum, women assigned to the treatment groups had similar baseline characteristics (Table 1). Characteristics of the children in the multivitamin and vitamin A groups were not significantly different with regard to

Figure 1. Flowchart of selection for study population. MVI: multivitamins; FUP: follow-up.

gender and frequency of gestational age <37 weeks. As previously reported, infants born to women who received multivitamins had higher birth weight ($p=0.001$) and a lower frequency of low birth-weight compared with women who did not receive multivitamins ($p=0.002$).¹⁰ Infants born to mothers who received vitamin A had a higher incidence of HIV infection at age 6 weeks ($p=0.05$).¹² Maternal and child characteristics at 2 years of age ($n=649$) were generally similar.

Child follow-up rates were comparable among the study arms. The median (IQR) number of monthly study visits was 20.0 in children whose mothers received multivitamins versus 20.5 in children whose mothers did not receive multivitamins ($p=0.80$). Similar data for the vitamin A versus no vitamin A groups were observed (20.0 vs 22.0; $p=0.60$). Median (IQR) total breastfeeding duration was 18.0 months versus 18.0 months in the multivitamin versus no multivitamin arms ($p=0.47$) and 18.3 months versus 21.2 months in the vitamin A versus no vitamin A arms ($p=0.67$).

From 6 weeks to 5 years of age (Table 2), after adjusting for important maternal and child risk factors, there were no statistically significant differences in child mortality in either the multivitamin (HR=0.93; 95% CI 0.70–1.22 compared with no multivitamins) or vitamin A groups (HR=1.00; 95% CI 0.76–1.32 compared with no vitamin A). From 2–5 years of age, there was no significant difference in overall mortality of children in the multivitamin group (HR=1.24; 95% CI 0.68–2.26 compared with no multivitamins) or vitamin A groups (HR=1.70; 95% CI 0.92–3.15 compared with those not receiving vitamin A).

Mortality data stratified by time-varying child HIV status indicated that the multivitamin effect was modified by HIV status (Table 2). In HIV-negative children aged 6 weeks to 5 years, multivitamins were associated with lower mortality (HR=0.60; 95% CI 0.38–0.95). However, this effect was confined to children <2 years of age and did not appear to extend to the older age group of 2–5 years (HR=1.40; 95% CI 0.47–4.17). Maternal multivitamins had no significant effect among HIV-positive children

Table 1. Baseline maternal and child characteristics of 874 infants at 6 weeks of age born to HIV-infected mothers^a

Variable	Received multivitamins			Received vitamin A		
	Yes (n=450)	No (n=424)	p-value	Yes (n=443)	No (n=431)	p-value
Mothers						
Age (years)	24.7 (4.6)	24.7 (4.8)	NS	24.6 (4.7)	24.8 (4.7)	NS
Education level (n[%])			NS			NS
None	33 (7.3)	30 (7.1)		28 (6.3)	35 (8.1)	
Primary education, 1–4 years	21 (4.7)	20 (4.7)		16 (3.6)	25 (5.8)	
Primary education, 5–8 years	344 (76.4)	339 (80.0)		348 (78.6)	335 (77.7)	
Secondary education, 8 years	52 (11.6)	35 (8.3)		51 (11.5)	36 (8.4)	
CD4 ⁺ cell count (n[%]) ^b			NS			NS
0–199 cells/ μ l	50 (11.9)	52 (12.9)		50 (12.0)	52 (12.8)	
200–499 cells/ μ l	241 (57.1)	224 (55.7)		241 (57.7)	224 (55.2)	
≥ 500 cells/ μ l	131 (31.4)	126 (31.3)		127 (30.4)	130 (32.0)	
CD4 ⁺ cell count (cells/ μ l)	420.4 (196.4)	419.4 (206.1)	NS	413.3 (196.5)	426.8 (205.7)	NS
CD8 ⁺ cell count (cells/ μ l)	731.7 (319.6)	741.5 (309.2)	NS	734.0 (310.0)	739.1 (319.3)	NS
ESR (mm/h)	57.3 (34.7)	59.7 (36.5)	NS	57.1 (34.7)	59.8 (36.5)	NS
Haemoglobin (g/dl)	9.4 (1.6)	9.6 (1.7)	NS	9.4 (1.7)	9.5 (1.7)	NS
Vitamin A (μ g/dl)	24.1 (8.8)	25.2 (10.9)	NS	24.5 (9.1)	24.7 (10.5)	NS
Vitamin E (μ mol/dl)	9.8 (2.8)	9.7 (2.8)	NS	9.9 (2.7)	9.6 (2.9)	NS
Children						
Male sex (n[%]) ^b	218 (48.8)	222 (52.7)	NS	220 (50.1)	220 (51.3)	NS
Birth weight (g)	3080 (471)	2970 (518)	0.001	3027 (505)	3025 (498)	NS
Birthweight <2500 g (n[%]) ^b	30 (7.5)	54 (14.2)	0.002	42 (10.6)	42 (11.0)	NS
Gestational age <37 weeks (n[%]) ^b	104 (23.2)	108 (25.5)	NS	111 (25.1)	101 (23.4)	NS
HIV+ at 6 weeks of age (n[%]) ^b	69 (15.3)	51 (12.0)	NS	71 (16.0)	49 (11.4)	0.05

NS: not significant.

^a Data are the mean (SD) unless otherwise stated.^b Data were not available for some mothers/children for these variables, therefore the denominators are not the same as the column totals.

(HR=1.03; 95% CI 0.72–1.47). There was no statistically significant effect of maternal vitamin A to HIV-infected children on mortality from 6 weeks to 5 years of age (HR=1.11; 95% CI 0.77–1.58). From 2–5 years of age, HIV-infected children tended to have a higher death rate when their mothers received vitamin A (HR=2.30; 95% CI 0.98–5.40), but this effect was not statistically significant nor was it observed in HIV-negative children (HR=1.32; 95% CI 0.46–3.75).

In HIV-negative children from 2–5 years of age there was a significant interaction ($P_{\text{int}}=0.02$) noted between the multivitamin and vitamin A groups (Table 2). With the placebo arm as a reference group, and examining the groups separately, the adjusted HRs for mortality were as follows: multivitamin only (HR=0.29; 95% CI 0.05–1.68); vitamin A only (HR=0.19; 95% CI 0.02–1.67); and multivitamin and vitamin A (HR=1.74; 95% CI 0.51–5.94). However, the number of deaths in these categories was very small (range 1–7).

The effects of maternal vitamin supplementation on child diarrhoea and respiratory infections from 6 weeks through 5 years of age are shown in Table 3. After adjusting for maternal and child risk factors, children of mothers who received multivitamins had a 14% reduced risk of any type of diarrhoea (RR=0.86; 95% CI 0.75–0.98), a 14% reduced risk of acute diarrhoea (RR=0.86;

95% CI 0.75–0.98) and a 21% reduced risk of watery diarrhoea (RR=0.79; 95% CI 0.67–0.93). There was no statistically significant reduction in the risk of dysentery or signs of respiratory infection in the multivitamin group. Children born to mothers who received vitamin A supplementation had no significant reduction in the risk for diarrhoea or respiratory infections compared with children whose mothers did not receive vitamin A.

Between 2–5 years of age, the effects of multivitamin supplementation in reducing the risk of diarrhoea tended to persist, although the power to detect these effects was limited due a lower incidence in diarrhoea in this age range. Children of mothers who received multivitamins had a 13% reduced risk of overall diarrhoea (RR=0.87; 95% CI 0.69–1.08), a 14% reduced risk of acute diarrhoea (RR=0.86; 95% CI 0.69–1.08) and a 29% reduced risk of watery diarrhoea (RR=0.71; 95% CI 0.54–0.95). Multivitamins did not affect the risk of dysentery or respiratory infections. From age 2–5 years, children born to mothers who received vitamin A supplementation had no significant difference in the risk of diarrhoea or respiratory infections compared with children whose mothers did not receive vitamin A.

Stratified analysis showed that the effects of vitamin A and multivitamins on the risk of diarrhoea and respiratory infections were comparable both among HIV-infected and non-infected

Table 2. Effects of maternal supplementation with multivitamins or vitamin A on mortality outcomes stratified by time-varying child HIV status

Outcome	Multivitamins						Vitamin A						P _{int} ^b	P _{int} ^c
	Yes n (# pyear) ^a	No n (# pyear) ^a	HR (95% CI) ^b	P ^b	HR (95% CI) ^c	P ^c	Yes n (# pyear) ^a	No n (# pyear) ^a	HR (95% CI) ^b	P ^b	HR (95% CI) ^c	P ^c		
6 weeks–5 years														
Total mortality	115 (1888)	119 (1752)	0.89 (0.69–1.15)	NS	0.93 (0.70–1.22)	NS	128 (1803)	106 (1837)	1.20 (0.93–1.55)	NS	1.00 (0.76–1.32)	NS	NS	NS
Mortality by HIV status														
HIV-positive	82 (364)	73 (315)	0.94 (0.69–1.29)	NS	1.03 (0.72–1.47)	NS	93 (385)	62 (294)	1.13 (0.82–1.56)	NS	1.11 (0.77–1.58)	NS	NS	NS
HIV-negative	33 (1524)	46 (1437)	0.67 (0.43–1.05)	NS	0.60 (0.38–0.95)	0.03	35 (1418)	44 (1543)	0.83 (0.53–1.30)	NS	0.76 (0.48–1.20)	NS	NS	NS
2–5 years														
Total mortality	29 (1159)	24 (1089)	1.15 (0.67–1.98)	NS	1.24 (0.68–2.26)	NS	35 (1100)	18 (1148)	2.04 (1.16–3.61)	0.01	1.70 (0.92–3.15)	NS	NS	NS
Mortality by HIV status														
HIV-positive	20 (325)	17 (274)	1.07 (0.56–2.05)	NS	1.19 (0.53–2.69)	NS	27 (338)	10 (261)	2.18 (1.05–4.50)	0.04	2.30 (0.98–5.40)	0.06	NS	NS
HIV-negative	9 (834)	7 (815)	1.22 (0.45–3.27)	NS	1.40 (0.47–4.17)	NS	8 (762)	8 (887)	1.11 (0.42–2.95)	NS	1.32 (0.46–3.75)	NS	0.02	0.02

NS: not significant; # pyear: number of person-years follow-up.

P_{int} represents the interaction between multivitamins and vitamin A.

^a n=number of death events.

^b HR represents the hazard ratio of death from the Cox model without adjusting for other covariate effect. P represents the corresponding unadjusted p-value.

^c HR represents the hazard ratio of death from the Cox multiple regression model adjusting for the following baseline maternal characteristics: CD4, ESR, haemoglobin, vitamin A level, vitamin E level, CD8, body mass index, viral load, age, education, prior pregnancy and marital status; and child characteristics: gender, birth weight, birth gestational age, HIV infection status (time-varying), weight-for-age Z-score and height-for-age Z-score at 6 week or 2 years.

Table 3. Effects of maternal supplementation with multivitamins or vitamin A on the incidence of diarrhoea and respiratory infection

Morbidity outcome	Mean no. of episodes per child-year (SD)	Multivitamins		Vitamin A		P _{int}
		RR (95% CI) ^a	RR (95% CI) ^b	RR (95% CI) ^a	RR (95% CI) ^b	
6 weeks–5 years (n=794)						
Diarrhoea						
All	1.20 (1.64)	0.86 (0.75–0.98)	0.86 (0.75–0.98)	1.07 (0.94–1.23)	1.02 (0.90–1.16)	0.02
Acute	1.12 (1.50)	0.86 (0.75–0.98)	0.86 (0.75–0.98)	1.05 (0.91–1.20)	0.99 (0.88–1.13)	0.009
Watery	0.52 (1.12)	0.80 (0.68–0.95)	0.79 (0.67–0.93)	1.02 (0.86–1.21)	0.97 (0.83–1.14)	0.01
Dysentery	0.60 (0.99)	0.90 (0.75–1.08)	0.90 (0.75–1.08)	1.07 (0.89–1.29)	1.00 (0.84–1.20)	NS
Cough	4.56 (2.89)	1.03 (0.96–1.11)	1.04 (0.96–1.11)	1.03 (0.96–1.11)	1.00 (0.94–1.08)	NS
Cough and fever	1.56 (1.96)	1.00 (0.88–1.13)	0.99 (0.88–1.11)	1.07 (0.85–1.22)	1.00 (0.89–1.13)	NS
Cough plus ^c	0.72 (1.30)	0.90 (0.75–1.09)	0.89 (0.74–1.06)	1.11 (0.92–1.33)	1.01 (0.84–1.22)	NS
Cough and rapid respiratory rate	0.16 (0.64)	1.19 (0.87–1.63)	1.19 (0.88–1.63)	0.83 (0.61–1.14)	0.76 (0.56–1.03)	NS
2–5 years (n=524)						
Diarrhoea						
All	0.88 (1.65)	0.85 (0.68–1.07)	0.87 (0.69–1.08)	1.27 (1.01–1.60)	1.19 (0.96–1.48)	NS
Acute	0.85 (1.62)	0.85 (0.68–1.07)	0.86 (0.69–1.08)	1.27 (1.01–1.60)	1.20 (0.97–1.49)	NS
Watery	0.36 (1.00)	0.70 (0.52–0.93)	0.71 (0.54–0.95)	1.24 (0.93–1.66)	1.21 (0.92–1.59)	NS
Dysentery	0.49 (1.28)	0.98 (0.72–1.34)	0.99 (0.74–1.33)	1.29 (0.94–1.76)	1.17 (0.88–1.57)	NS
Cough	4.78 (3.08)	1.03 (0.94–1.14)	1.05 (0.96–1.15)	1.00 (0.91–1.11)	0.98 (0.89–1.08)	NS
Cough and fever	1.32 (1.80)	0.96 (0.80–1.15)	0.99 (0.83–1.18)	1.07 (0.89–1.28)	0.98 (0.83–1.15)	NS
Cough plus ^c	0.66 (1.30)	0.84 (0.64–1.11)	0.90 (0.69–1.16)	1.06 (0.81–1.39)	0.99 (0.75–1.30)	NS
Cough and rapid respiratory rate	0.08 (0.72)	1.10 (0.53–2.29)	1.13 (0.56–2.27)	1.38 (0.67–2.83)	1.13 (0.54–2.38)	0.01

NS: not significant.

P_{int} represents the interaction between multivitamins and vitamin A.

^a RR represents the relative risk of a morbidity event from the generalised estimating equation model unadjusted for other risk factors.

^b RR represents the relative risk of a morbidity event from the generalised estimating equation model adjusting for the following baseline maternal characteristics: CD4, ESR, haemoglobin, vitamin A level, vitamin E level, CD8, body mass index, viral load, age, education, prior pregnancy and marital status; and child characteristics: gender, birth weight, birth gestational age, current age, HIV infection status (time-varying), weight-for-age Z-score and height-for-age Z-score at 6 weeks or 2 years.

^c Cough plus is defined as cough with at least one of the following episodes: difficulty breathing; chest retractions; and refusal to eat, drink or breastfeed.

children. None of the interaction tests between HIV status and supplementation factor were significant for any of the morbidity outcomes.

Discussion

In this long-term follow-up study of HIV-infected and -exposed children, maternal micronutrient supplementation during pregnancy and lactation was associated with several important and lasting health effects. These included an estimated 40% lower risk of all-cause mortality among HIV-uninfected children through age 5 years (with the majority of the reduction prior to 2 years of age) and an estimated 14% lower risk of diarrhoeal disease among all children through age 5 years. These data extend our previous findings^{8,9,11} of improved infant and young child (age 2 years) outcomes with maternal multivitamin supplementation through age 5 years. We also found that maternal vitamin A supplementation does not have an effect on mortality among children born to HIV-infected mothers.

A number of trials of maternal micronutrient supplementation during pregnancy have been performed in poor countries and the results have generally shown an increase in mean birth weight and a reduction in the frequency of low birthweight.¹³ In addition, several trials have evaluated the effect of maternal supplementation on neonatal and/or infant mortality rates, demonstrating either no or little effect in some studies, and decreased mortality in infants of mothers with HIV-infection in others.^{14,15} Studies that have extended follow-up of these cohorts into childhood to measure the effect of supplementation on childhood mortality and morbidity, however, have been uncommon. In rural Nepal, 4926 pregnant women were randomised to one of four maternal micronutrient regimens (all containing vitamin A). Compared with controls, children of women who received folic acid and iron supplementation had a 31% lower risk of mortality between birth and age 7 years. No significant effect on long-term child mortality was seen in the groups who received folic acid alone, folic acid-iron-zinc or multiple micronutrients.¹⁶ Two trials of multivitamin supplementation, also in presumably HIV-uninfected mothers, in Nepal and Hungary found no difference in the frequency of

respiratory or digestive infections in their children at 2 years^{6,17} and 6 years of age.¹⁷ Similarly, among 646 women recruited to 5 London centres at risk for pre-eclampsia, supplementation with high-dose vitamins C and E was not associated with improved respiratory outcomes in their children through age 2 years.¹⁸ In contrast to these studies, the current trial enrolled only HIV-infected women whose nutritional status and requirements likely differed from these presumably HIV-negative women, used a regimen consisting of multiples of the Recommended Dietary Allowances (RDA) for various nutrients, and maintained continuous follow-up with the cohort as opposed to re-contacting previously enrolled women and children. These and other factors may explain the differences in the current findings and these earlier studies.

The exact mechanism by which multivitamin supplementation may affect child health remains unclear, however possible mechanisms may include effects on the mothers,¹⁹ their children, or both. Improved maternal health and well-being may improve postnatal care to the child. Higher infant birth weight and better nutritional status in infants in the multivitamin group may improve child immunological status.²⁰ However, even when controlling for birth weight and nutritional status, we found that vitamin supplementation reduced the risk of diarrhoea. Improved maternal nutrition during gestation and/or lactation may lead to intrauterine and/or early infancy effects on childhood immunity by altering thymus or other immune development. Prenatal undernutrition may be associated with altered development of lymphoid tissue, decreased thymus development and decreased immune response during adolescence,^{21,22} an illustration of metabolic or nutritional programming.^{23,24} Vitamin supplementation may ameliorate these effects.²⁵

We also found that maternal vitamin A supplementation does not have an effect on mortality in HIV-infected children up to 5 years of age. While there is no statistically significant effect of vitamin A on child mortality, we observed a trend of increased mortality in children from 2–5 years of age. Limited studies have investigated the effects of vitamin A supplementation to HIV-infected mothers on their infants, and even fewer have investigated its effects on childhood outcomes. Similar to our findings, the Zimbabwe group (ZVITAMBO), which provided both antenatal and postnatal vitamin A to mothers and infants in a 2x2 factorial design, established that maternal, maternal and infant, and infant only vitamin A supplementation had a time-sensitive effect on HIV transmission. It is likewise possible that in our patient population vitamin A not only increased mother-to-child HIV transmission but also worsened prognosis for these children. We should note that our design differed from childhood postnatal vitamin A supplementation trials to HIV-infected children, which have been associated with lower mortality²⁶ and lower morbidities including cough and diarrhoeal episodes.^{26–28}

This study has several limitations. Although the sample size was large, the size of the parent trial was determined to provide sufficient power to detect differences in vertical HIV transmission rather than long-term childhood outcomes. Larger studies may be needed to confirm some of the effects observed. Universal postnatal vitamin A supplementation to all children, which is standard of care, may have decreased the occurrence of the outcomes. Also, the study design precludes us from determining whether supplementation during both pregnancy and lactation is required to reduce child mortality and morbidity, since both

were administered. Another limitation is the lack of comparison with HIV-negative women and their unexposed children; thus, it is unknown whether the findings in this study are generalisable to HIV-unexposed children in resource-limited settings.

Finally, the effects of maternal and child ART on the outcomes of interest were not assessed, since at the time of the study these medications were not generally available in Dar es Salaam (Tanzania). Although current WHO guidelines recommend universal ART be given to pregnant woman with HIV,²⁹ access to ART therapy continues to be extremely limited.³⁰ While multivitamin supplementation is not a replacement for ART therapy, the cost of multivitamin therapy is less than US\$0.05/day, making it an ideal alternative in countries where access to universal ART implementation may be delayed. The interactions between ART therapy and vitamin supplementation have not yet been well studied, however this study supports the potential health outcomes that vitamin supplementation may provide and provides impetus for future investigations examining the effects of combination therapy with ART and vitamin supplementation.

Conclusions

To our knowledge, this is the first study to evaluate long-term childhood outcomes of maternal vitamin supplementation in HIV-infected women. Many studies have shown promising effects of antenatal micronutrient supplementation on birth outcomes, but the long-term effects we observed are notable. Multiple areas of research still need to be addressed, including the timing, dose and duration of micronutrient supplementation as well as whether their effects persist in the setting of more widespread availability of ART. These data reveal a potentially promising intervention that may reduce childhood mortality and morbidity among HIV-exposed infants.

Authors' contributions: NK participated in the literature review, analysis of the data, development of tables and writing the majority of the manuscript; HJ and DS performed the majority of the statistical analysis and statistical overview of the project; KM and GM were the on-site directors of the project; WF assisted with oversight of and implementation of the project; CD, the principal investigator, assisted with development of the project idea, oversight and review of statistical analysis, and extensive revisions of the manuscript. All authors read and approved the final manuscript. NK and CD are guarantors of the paper.

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Competing interests: None declared.

Ethical approval: This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Human Subjects Committee of the Harvard School of Public Health (Boston, MA), the Committee of Research and Publications of Muhimbili University of Health and Allied Sciences (Dar es Salaam, Tanzania) and the Tanzanian National Institute of Medical Research. Written informed consent was obtained from all subjects.

References

- Black RE, Allen LH, Bhutta ZA et al. Maternal and Child Undernutrition Study Group. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008;371:243–60.
- World Bank. Repositioning Nutrition as Central to Development. Washington, DC: World Bank; 2006:1–272.
- Shankar AH, Jahari AB, Sebayang SK et al. Effect of maternal multiple micronutrient supplementation on fetal loss and infant death in Indonesia: a double-blind cluster-randomised trial. *Lancet* 2008;371:215–27.
- Friis H, Gomo E, Nyazema N et al. Effect of multimicronutrient supplementation on gestational length and birth size: a randomized, placebo-controlled, double-blind effectiveness trial in Zimbabwe. *Am J Clin Nutr* 2004;80:178–84.
- Bhutta ZA, Ahmed T, Black RE et al. What works? Interventions for maternal and child undernutrition and survival. *Lancet* 2008;371:417–40.
- Vaidya A, Saville N, Shrestha BP et al. Effects of antenatal multiple micronutrient supplementation on children's weight and size at 2 years of age in Nepal: follow-up of a double-blind randomised controlled trial. *Lancet* 2008;371:492–9.
- Humphrey JH, Iliff PJ, Marinda ET et al. Effects of a single large dose of vitamin A, given during the postpartum period to HIV-positive women and their infants, on child HIV infection, HIV-free survival, and mortality. *J Infect Dis* 2006;193:860–71.
- Fawzi WW, Msamanga GI, Wei R et al. Effect of providing vitamin supplements to human immunodeficiency virus-infected, lactating mothers on the child's morbidity and CD4⁺ cell counts. *Clin Infect Dis* 2003;36:1053–62.
- Villamor E, Saathoff E, Bosch RJ et al. Vitamin supplementation of HIV-infected women improves postnatal child growth. *Am J Clin Nutr* 2005;81:880–8.
- Fawzi WW, Msamanga GI, Spiegelman D et al. Randomised trial of effects of vitamin supplements on pregnancy outcomes and T cell counts in HIV-1-infected women in Tanzania. *Lancet* 1998;351:1477–82.
- Fawzi WW, Msamanga GI, Hunter D et al. Randomized trial of vitamin supplements in relation to transmission of HIV-1 through breastfeeding and early child mortality. *AIDS* 2002;16:1935–44.
- Fawzi W, Msamanga G, Spiegelman D et al. Transmission of HIV-1 through breastfeeding among women in Dar es Salaam, Tanzania. *J Acquir Immune Defic Syndr* 2002;31:331–8.
- Kawai K, Spiegelman D, Shankar AH et al. Maternal multiple micronutrient supplementation and pregnancy outcomes in developing countries: meta-analysis and meta-regression. *Bull World Health Organ* 2011;89:402–11.
- Christian P, Tielsch JM. Evidence for multiple micronutrient effects based on randomized controlled trials and meta-analyses in developing countries. *J Nutr* 2012;142:173S–7S.
- Siegfried N, Irlam JH, Visser ME et al. Micronutrient supplementation in pregnant women with HIV infection. *Cochrane Database Syst Rev* 2012;(3):CD009755.
- Christian P, Stewart CP, LeClerq SC et al. Antenatal and postnatal iron supplementation and childhood mortality in rural Nepal: a prospective follow-up in a randomized, controlled community trial. *Am J Epidemiol* 2009;170:1127–36.
- Dobo M, Czeizel AE. Long-term somatic and mental development of children after periconceptional multivitamin supplementation. *Eur J Pediatr* 1998;157:719–23.
- Greenough A, Shaheen SO, Shennan A et al. Respiratory outcomes in early childhood following antenatal vitamin C and E supplementation. *Thorax* 2010;65:998–1003.
- Baum MK, Campa A, Lai S et al. Effect of micronutrient supplementation on disease progression in asymptomatic, antiretroviral-naïve, HIV-infected adults in Botswana: a randomized clinical trial. *JAMA* 2013;310:2154–63.
- Kupka R, Msamanga GI, Aboud S et al. Patterns and predictors of CD4 T-cell counts among children born to HIV-infected women in Tanzania. *J Trop Pediatr* 2009;55:290–6.
- McDade TW, Beck MA, Kuzawa CW et al. Prenatal undernutrition and postnatal growth are associated with adolescent thymic function. *J Nutr* 2001;131:1225–31.
- McDade TW, Beck MA, Kuzawa C et al. Prenatal undernutrition, postnatal environments, and antibody response to vaccination in adolescence. *Am J Clin Nutr* 2001;74:543–8.
- Langley-Evans SC, Carrington LJ. Diet and the developing immune system. *Lupus* 2006;15:746–52.
- Entringer S, Buss C, Wadhwa PD. Prenatal stress and developmental programming of human health and disease risk: concepts and integration of empirical findings. *Curr Opin Endocrinol Diabetes Obes* 2010;17:507–16.
- Koletzko B, Aggett PJ, Bindels JG et al. Growth, development and differentiation: a functional food science approach. *Br J Nutr* 1998;80(Suppl 1):S5–45.
- Semba RD, Ndugwa C, Perry RT et al. Effect of periodic vitamin A supplementation on mortality and morbidity of human immunodeficiency virus-infected children in Uganda: a controlled clinical trial. *Nutrition* 2005;21:25–31.
- Fawzi WW, Mbise RL, Hertzmark E et al. A randomized trial of vitamin A supplements in relation to mortality among human immunodeficiency virus-infected and uninfected children in Tanzania. *Pediatr Infect Dis J* 1999;18:127–33.
- Coutsoudis A, Bobat RA, Coovadia HM et al. The effects of vitamin A supplementation on the morbidity of children born to HIV-infected women. *Am J Public Health* 1995;85:1076–81.
- WHO. Antiretroviral Drugs for Treating Pregnant Women and Preventing HIV Infection in Infants: Recommendations for a Public Health Approach (2010 Version). Geneva: World Health Organization; 2010.
- WHO. Global Update on HIV Treatment 2013: Results, Impact and Opportunities: WHO Report in Partnership with UNICEF and UNAIDS. Geneva: World Health Organization; 2013.