

# Trial of zinc supplements in relation to pregnancy outcomes, hematologic indicators, and T cell counts among HIV-1-infected women in Tanzania<sup>1-3</sup>

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## ABSTRACT

**Background:** In observational studies, the zinc status of HIV-infected persons has been associated with both positive and adverse clinical outcomes. Such endpoints may affect the risk of adverse birth outcomes among HIV-infected women.

**Objective:** We examined the effects of zinc supplements on birth outcomes, hematologic indicators, and counts of T lymphocyte subsets among 400 HIV-infected pregnant women.

**Design:** Eligible women between 12 and 27 wk of gestation were randomly assigned to daily oral supplementation with either 25 mg Zn or placebo between recruitment and 6 wk after delivery. All women received iron, folic acid, and multivitamin supplements irrespective of the experimental assignment.

**Results:** We observed no significant differences in birth weight, duration of gestation, or fetal and neonatal mortality between women in the zinc and placebo groups. Hemoglobin concentrations increased between baseline and 6 wk postpartum in both groups. However, the rise in hemoglobin over this period was significantly lower ( $P = 0.03$ ) in the zinc group ( $\bar{x} \pm SD$ :  $11.5 \pm 17.9$  g/L) than in the placebo group ( $15.2 \pm 18.6$  g/L). Similarly, the changes in red blood cell count and in packed cell volume over the same period were significantly lower in the zinc group ( $P < 0.01$  and  $P = 0.01$ , respectively). Zinc had no effect on CD4<sup>+</sup>, CD8<sup>+</sup>, or CD3<sup>+</sup> cell counts during the follow-up period.

**Conclusion:** Because of the lack of beneficial effects of zinc on adverse pregnancy outcomes and the likelihood of negative effects on hemoglobin concentrations, no compelling evidence exists to support the addition of zinc to prenatal supplements intended for pregnant HIV-infected women. *Am J Clin Nutr* 2005;81:161-7.

**KEY WORDS** Zinc, HIV infection, CD4<sup>+</sup> cells, CD8<sup>+</sup> cells, CD3<sup>+</sup> cells, pregnancy, birth weight, preterm infants, small-for-gestational age infants, Tanzania

## INTRODUCTION

Poor maternal zinc status is associated with adverse pregnancy outcomes in many observational studies and in early randomized trials with various methodologic limitations (1). In a well-designed placebo-controlled trial among African American women with low plasma zinc concentrations, prenatal zinc supplementation resulted in a significant increase in birth weight and in a longer duration of pregnancy (2). This result suggests that zinc supplementation may be beneficial in developing countries,

where pregnant women may be more likely to have suboptimal zinc intakes. In a series of randomized trials in Asia and Latin America, however, supplementation had no beneficial effect on birth weight or the duration of pregnancy (3). It was noted that no trial has been carried out in African settings, where HIV infection is prevalent. Approximately 10-35% of pregnant women in southern Africa are HIV-infected; these women have a higher risk of adverse pregnancy outcomes, including fetal loss (4, 5), low birth weight, preterm birth, and intrauterine growth retardation (6). If zinc supplementation were beneficial for these outcomes, it would constitute a low-cost intervention.

Apart from its potential effects on pregnancy outcomes, the relations between zinc status and HIV-related outcomes are controversial. High zinc intakes were shown to be significantly associated with faster disease progression and higher risks of mortality among men in a prospective cohort study of asymptomatic HIV-infected men in the United States (7, 8). In another US study of HIV-positive men, however, plasma concentrations of zinc were inversely associated with mortality (9). Results from these observational studies are difficult to interpret; confounding of micronutrient status by other variables, such as stage of disease or access to health care, could provide alternative explanations for these observations.

We have shown that multivitamin supplements administered to HIV-infected women during pregnancy significantly decreased the risks of fetal death, preterm delivery, intrauterine growth retardation, and low birth weight and increased hemoglobin and CD4<sup>+</sup> cell counts (10). It is important to examine the potential effects of additional inexpensive nutritional interventions, such as zinc supplementation, on these outcomes. We

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enrolled HIV-infected pregnant women in Dar es Salaam, Tanzania, to examine the efficacy of zinc supplements on birth outcomes, including fetal death, birth weight, and duration of pregnancy. We also examined the effect of the supplements on proxy indicators of HIV disease stage, namely hemoglobin concentrations and T lymphocyte subsets.

## SUBJECTS AND METHODS

### Recruitment and randomization

Pregnant women who were HIV-infected, who resided in Dar es Salaam at the time of the baseline interview, and who intended to stay in the city until delivery and for 1 y thereafter were eligible to enroll in this trial. Eligibility also required an estimated gestational age at randomization of between 12 and 27 wk. HIV-1 serostatus was ascertained from consenting women with the use of 2 enzyme-linked immunoassays (11), and discrepant results were resolved by Western Blot test (Genetic Systems, Redmond, WA). We used a two-stage informed consent procedure. Consent was initially sought for HIV testing as part of prenatal screening at antenatal clinics. HIV-positive women were not asked to enroll in the randomized trial immediately at the time of posttest counseling; instead, we sought their consent to be randomly assigned at a follow-up visit that was scheduled about 3 d later. In this manner, women were allowed time to cope with their positive HIV result and carefully consider participation in the trial, thereby decreasing the probability of dropout after enrollment. Randomization and all subsequent visits took place at a study clinic located at the Muhimbili National Hospital, Dar es Salaam, the main tertiary care hospital in Tanzania. Participants were recruited between September 2000 and October 2002, during which time the prevalence of HIV-1 among prenatal clinic attendees was 11.5%. The study protocol was approved by the Research and Publications Committee at Muhimbili University College of Health Sciences and the Institutional Review Board of the Harvard School of Public Health.

A randomization list was prepared in blocks of 20, and, at enrollment, each eligible woman was assigned to the next numbered bottle of regimen. Women received a daily oral dose of 1 of the 2 interventions from enrollment until the end of the study at 6 wk postpartum: 1) 25 mg Zn as zinc sulfate included in an effervescent citrus-flavored tablet or 2) a similarly constituted zinc-free placebo. All of the experimental tablets were identical in color, taste, and appearance. Women were instructed to dissolve one tablet in water every day. The resulting solutions were colorless and clear; the strong lemon-lime flavor of the solution effectively concealed the metallic taste of zinc and prevented the participants from determining which regimen they were assigned to. The doses used are safe during pregnancy (12). Both active and placebo regimens were prepared by Hermes Arzneimittel GmbH, Munich, Germany.

### Standard of care

All women received ferrous sulfate (400 mg, equivalent to 120 mg ferrous Fe) and folate (5 mg) daily and prophylactic chloroquine phosphate (500 mg, equivalent to 300 mg chloroquine base) weekly as per the standard of prenatal care in Tanzania. Given our earlier findings on the benefits of multivitamin supplements among HIV-positive pregnant women (10), all women

also received these supplements starting from the time of randomization until the time of delivery, irrespective of zinc treatment assignment. These supplements contained 20 mg thiamine, 20 mg riboflavin, 25 mg vitamin B-6, 100 mg niacin, 50  $\mu$ g vitamin B-12, 0.8 mg folate, 500 mg vitamin C, and 30 mg vitamin E. As a measure against mother-to-child transmission of HIV, all women were offered a 200-mg dose of nevirapine to be taken at the onset of labor and 2 mg nevirapine/kg to their infant within 72 h of delivery (13).

### Study procedures

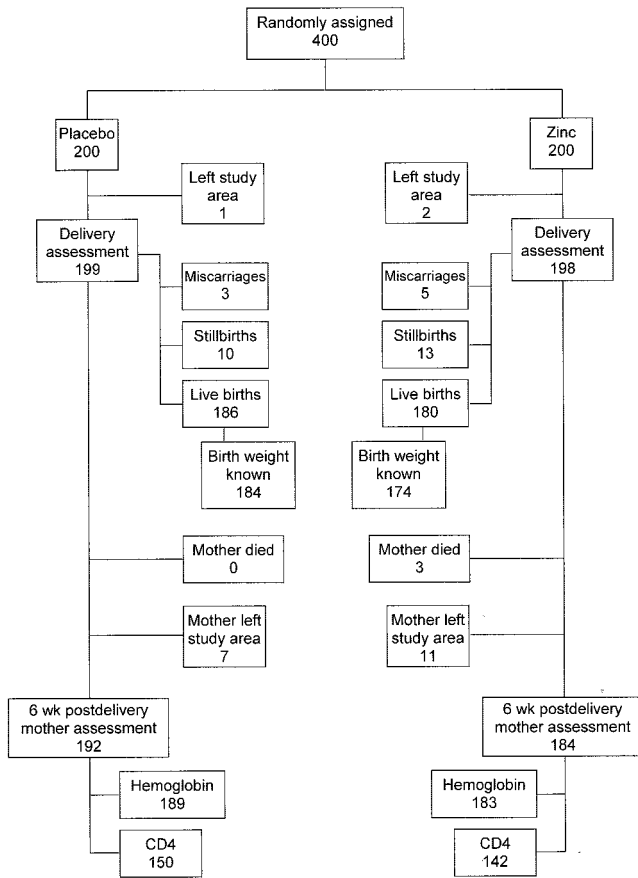
At the first visit, information on the woman's age, education level, marital status, socioeconomic conditions, and obstetric history was collected. Also, at baseline and every month thereafter, a study physician conducted a full physical examination, and a research nurse gathered information on health problems during the prior month and obtained anthropometric measurements. Additional clinical assessment and continued counseling and support were provided as needed. The women who had psychosocial problems were referred to a full time research social worker, who had backup support from 2 psychiatrists. The women were asked to bring their regimen bottles at their monthly clinic visits. Nurses counted the remaining pills, and the women's supply was replenished. Compliance was evaluated on the basis of the number of tablets absent from the returned bottles divided by the total number of tablets the woman should have taken.

Laboratory results were available to the managing physicians, who prescribed treatment if indicated. Using specimens collected at the randomization visit, we carried out routine stool and urine examinations and obtained a complete blood count. Hemoglobin was measured with a CBC5 Coulter Counter (Coulter Corporation, Miami). The FACScount system (Becton-Dickinson, San Jose, CA) was used to obtain absolute counts of CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> T lymphocyte subsets. Blood count and lymphocyte subsets analyses were repeated at 6 wk postpartum.

Women were encouraged to deliver at Muhimbili National Hospital, where research midwives were available 24 h a day. A research midwife weighed the infants to the nearest 10 g on a standard beam balance immediately after birth. She also measured birth length on an infant length board, measured head circumference with a nonstretchable tape (both to the nearest 0.1 cm), and weighed placentas after removal of blood clots. Gestational age was based on recall of the date of the last menstrual period at recruitment.

Women who did not come for their monthly appointments were visited at home when possible and were asked to come to the study clinic if their condition allowed. For women who traveled out of Dar es Salaam, we attempted to maintain contact with neighbors and relatives to collect information on the outcome of pregnancy, ie, whether the woman had a live birth or experienced a miscarriage or a stillbirth.

The primary endpoint of the trial was to examine the effect of zinc supplementation on hemoglobin concentrations between enrollment and 6 wk postpartum. A sample size of 150 subjects in each group was calculated to detect a difference in mean hemoglobin concentration based on a two-sample *t* test for the difference between 2 regimens. We enrolled an additional 100 women for a total of 400 women. An intent-to-treat analysis of treatment effects was used. Of the 400 women who were enrolled in the study, data on birth outcomes were not available for 3



**FIGURE 1.** Trial profile. The numbers represent the number of subjects.

women (2 in the zinc group and 1 in the placebo group) (**Figure 1**). Of the 397 women with known birth outcomes (miscarriage, still birth, or live birth), 366 had live births and were eligible for the analyses of birth weight and prematurity. We had a specific date of delivery for all 366 women; we did not have a birth weight for 8 of the infants because of delivery at home or at another medical facility. There were no differences in the availability of data on gestational age or birth weight between treatment groups. We examined the effects of the supplements on the continuous birth outcomes, namely gestational age and birth weight, length, head circumference, and placental weight. We also examined the effects of the supplements on the risk of miscarriage (defined as delivery before 28 wk of gestation), stillbirth (defined as delivery of a dead baby at or after 28 wk of gestation), and fetal death (all miscarriages and stillbirths). An infant born with any evidence of life, such as breathing or beating of the heart, was considered live born. Perinatal mortality included stillbirths and deaths in the first 7 d of life. Neonatal mortality was defined as deaths among live births in the first 28 d of life. Other categorical outcomes evaluated included a birth weight <2500 g (low birth weight; LBW), a birth weight <2000 g, preterm delivery (<37 wk of gestation), severe preterm delivery (<34 wk of gestation), and small-for-gestational age (SGA), which was defined as a birth weight less than the 10th percentile for gestational age on the basis of the reference of Brenner et al (14).

We conducted the analyses with and without data from twins ( $n = 10$  pairs), and the results were virtually the same. Thus, the

results included data from the twins. Twin pregnancies were analyzed as a single outcome; for continuous variables, such as birth weight, the mean of the 2 twin values was used. For categorical variables, such as stillbirth, if either infant had the outcome, the pregnancy was considered to have that outcome. Treatment effects on categorical variables were assessed by calculating relative risks with 95% CIs based on the exact binomial distribution (15). Wilcoxon's rank-sum test was used for continuous variables (16).

We examined the effect of the vitamin supplements on maternal T cells (absolute counts of CD4<sup>+</sup>, CD8<sup>+</sup>, and CD3<sup>+</sup> cells), total white blood cells, total lymphocytes, and hematologic measurements (hemoglobin, red blood cell count, and packed cell volume). We observed no differences between treatment arms in the availability of data on these measurements. For each endpoint, we examined the difference between treatment groups at baseline and at 6 wk postpartum as well as the change between these visits.

We also assessed whether treatment effects on birth outcomes were modified by baseline CD4<sup>+</sup> cell counts in 3 strata (<200, 200–499, and  $\geq 500$  cells/mm<sup>3</sup>) and within tertiles of baseline body mass index (BMI; defined as weight at baseline in kilograms divided by the square of height in meters). We used the Breslow-Day test for homogeneity (17) to examine whether treatment effects were significantly different between strata of potential modifiers. All *P* values reported are two-sided; statistical significance in this study was defined as  $P < 0.05$ . All statistical analyses were carried out using the SAS system (version 8.0; SAS Institute, Cary, NC).

## RESULTS

We observed no significant differences at baseline between women in the zinc and placebo groups in sociodemographic characteristics (including age, parity, and marital status) or nutritional variables (including weight and midupper arm circumference) (**Table 1**). Women in the 2 regimen groups were also not significantly different with respect to baseline height, past history of adverse pregnancy outcomes, or malaria. The mean ( $\pm$ SD) duration of follow-up from the time of randomization was  $8.6 \pm 7.2$  mo (median: 6.4 mo). Compliance with the study regimen was high between the time of randomization and delivery ( $94 \pm 10\%$ ; median: 99%; interquartile range: 93–100%) and between the time of delivery and the end of follow-up at 6 wk postpartum ( $80 \pm 27\%$ ; median: 92%; interquartile range: 69–100%). There were no significant differences in the duration of follow-up or compliance between the 2 experimental groups.

Zinc supplements had no effect on birth weight ( $P = 0.96$ ); the mean ( $\pm$ SD) weight in this group was  $3071 \pm 526$  g compared with  $3085 \pm 507$  g in the placebo group (**Table 2**). There were also no significant differences between the 2 groups in birth length ( $P = 0.87$ ), head circumference ( $P = 0.27$ ), or duration of gestation ( $P = 0.99$ ). Zinc had no significant effect on the categorical pregnancy outcomes, including low birth weight ( $P = 0.87$ ), prematurity <37 wk ( $P = 0.78$ ), or small-for-gestational age ( $P = 0.32$ ) (**Table 3**). There were also no significant effects of the supplements on birth weight <2000 g or gestation age at birth <34 wk (data not shown).

We examined the effect of the supplements on the risks of death during the fetal and early postpartum periods (**Table 4**). There were 31 observed fetal deaths: 18 in the zinc group and 13

**TABLE 1**

Baseline characteristics of the study participants according to treatment regimen

Baseline characteristic	Zinc group (n = 200)	Placebo group (n = 200)	P <sup>1</sup>
Week of gestation at first visit (wk)	23.0 ± 3.5 <sup>2</sup>	22.5 ± 3.5	0.12
Age (y)	26.7 ± 4.9	27.0 ± 5.0	0.45
No. of previous pregnancies	1.6 ± 1.5	1.8 ± 1.8	0.46
Midupper arm circumference (cm)	26.0 ± 2.9	26.3 ± 3.2	0.38
Weight (kg)	58.9 ± 9.5	60.6 ± 10.6	0.09
Lacks formal education [% (n)]	6.6 ± 13	6.0 ± 12	0.82
Has secondary education [% (n)]	19.2 ± 38	16.5 ± 33	0.48
Is housewife [% (n)]	67.0 ± 132	68.0 ± 136	0.83
Has male partner [% (n)]	86.4 ± 171	84.0 ± 168	0.51
Has her own income [% (n)]	33.3 ± 66	32.5 ± 65	0.86
Has had miscarriage [% (n)]	21.9 ± 43	25.1 ± 50	0.46
Has had stillbirths [% (n)]	4.6 ± 9	4.0 ± 8	0.78
Primiparous [% (n)]	25.5 ± 50	23.6 ± 47	0.66

<sup>1</sup> Wilcoxon's rank-sum and chi-square tests for continuous and categorical characteristics, respectively.<sup>2</sup>  $\bar{x} \pm SD$  (all such values).

in the placebo group. Compared with the placebo, zinc had no significant effect on fetal loss (relative risk: 1.39; 95% CI: 0.58, 3.86;  $P = 0.36$ ). The supplements also had no significant effects on perinatal (relative risk: 1.36; 95% CI: 0.64, 3.14;  $P = 0.32$ ) or neonatal (relative risk: 1.45; 95% CI: 0.39, 7.22;  $P = 0.46$ ) death. There were no significant differences in the effects of zinc on pregnancy outcomes within the strata of baseline CD4<sup>+</sup> cells or BMI (data not shown).

Women in both the zinc and placebo groups were not significantly different at baseline with respect to various hematologic indicators, including hemoglobin, red blood cell count, and packed cell volume (**Table 5**). Both groups experienced an increase in hemoglobin concentrations between baseline and 6 wk postpartum. However, the increase in hemoglobin over this period was significantly lower in the zinc group ( $11.5 \pm 17.9$  g/L) than in the placebo group ( $15.2 \pm 18.6$  g/L) ( $P = 0.03$ ). Similarly, the increase in red blood cell count over the same period was significantly smaller in women in the zinc group ( $0.59 \times 10^6/\text{mm}^3 \pm 0.75 \times 10^6/\text{mm}^3$ ) than in women in the placebo group ( $0.78 \times 10^6/\text{mm}^3 \pm 0.70 \times 10^6/\text{mm}^3$ ) ( $P < 0.01$ ). The change in packed cell volume was also significantly lower in the zinc group ( $P = 0.01$ ). After adjustment for baseline values of hemoglobin, red blood cell count, or packed cell volume in respective mixed-effects models, the effects of zinc on the change in each of these outcomes at 6 wk were not significantly different from those observed in unadjusted analyses. The adjusted result for hemoglobin was  $-3.7$  g/L (95% CI:  $-7.4, 0.1$ ;  $P = 0.06$ ), for red blood cell counts was  $-0.19 \times 10^6$  cells/mm<sup>3</sup> (95% CI:  $-0.35, -0.04$ ;

$P = 0.01$ ), and for packed cell volume was  $-1.21\%$  (95% CI:  $-2.29, -0.12$ ;  $P = 0.03$ ).

We next examined the effect of the supplements on T cell counts (**Table 6**). Zinc had no effect on CD4<sup>+</sup>, CD8<sup>+</sup>, or CD3+ cell counts during the follow-up period. As expected during pregnancy, all cell counts increased in both the zinc and placebo groups. At baseline, women in both the zinc and placebo groups had comparable CD4<sup>+</sup> cell counts; the rise in cell count was not significantly different in the 2 groups (mean: 95 and 101 cells/mm<sup>3</sup>, respectively;  $P = 0.97$ ).

## DISCUSSION

Zinc supplements in combination with other prenatal supplements, including iron, folate, and other vitamins, did not affect the duration of pregnancy or anthropometric indexes of fetal growth in the population of HIV-positive Tanzanian women studied. The supplements had no effect on CD4<sup>+</sup> and other T cell counts but resulted in an adverse effect on concentrations of hemoglobin and other hematologic indicators. Our study had several strengths. Given the study's randomized double-blind design, differences in background characteristics between the 2 experimental groups were reduced to a minimum; hence, confounding by other variables was unlikely. The high compliance with the regimen and the minimal loss to follow-up strengthened the validity of the findings.

Many other factors need to be considered when interpreting the results. There are no published data on plasma zinc status among adults in Tanzania; however, the women in the study were typical of other urban residents in Dar es Salaam with low intakes of animal products and are thus expected to suffer from zinc deficiency given the predominance of staple foods with a low bioavailability of zinc. Using national data on stunting rates and the adequacy of zinc in the national food supply, investigators of the International Zinc Nutrition Consultative Group estimate that 44% of Tanzanians are at risk of inadequate zinc intake (18). It is unlikely that the null effect noted on pregnancy outcomes was due to the concurrent existence of other micronutrient deficiencies given that we provided all women with a multivitamin supplement, including vitamin B complex and vitamins C and E. The latter supplement was provided given beneficial effects observed

**TABLE 2**

Effect of zinc supplements on continuous outcomes in newborns

Outcome <sup>1</sup>	Zinc group	Placebo group	P <sup>2</sup>
Duration of pregnancy (wk)	39.6 ± 2.9 [180] <sup>3</sup>	39.5 ± 3.2 [186]	0.99
Birth weight (g)	3071 ± 526 [174]	3085 ± 507 [184]	0.96
Birth length (cm)	48.6 ± 2.5 [153]	48.5 ± 3.1 [162]	0.87
Head circumference (cm)	34.4 ± 1.5 [154]	34.6 ± 1.4 [162]	0.27
Placental weight (g)	517 ± 111 [141]	515 ± 115 [147]	0.70

<sup>1</sup> For twin pregnancies, the average value for both twins was used.<sup>2</sup> Wilcoxon's rank-sum test.<sup>3</sup>  $\bar{x} \pm SD$  (all such values); *n* in brackets.

**TABLE 3**  
Effect of zinc supplements on low birth weight and prematurity

Outcome <sup>1</sup>	Zinc group <sup>2</sup>	Placebo group <sup>2</sup>	Relative risk (95% CI) <sup>3</sup>	P <sup>4</sup>
	%	%		
Preterm < 37 wk	17.2 [31/180]	15.6 [29/186]	1.11 (0.63, 2.04)	0.78
Low birth weight, < 2500 g	10.9 [19/174]	10.3 [19/184]	1.06 (0.47, 2.38)	0.87
Small-for-gestational age <sup>5</sup>	14.4 [25/176]	18.5 [34/184]	0.78 (0.41, 1.38)	0.32

<sup>1</sup> For twin pregnancies, the outcome was positive when at least one of the twins had it.

<sup>2</sup> Values in brackets represent the number of infants at risk out of the total number of infants.

<sup>3</sup> 95% CI based on the exact binomial distribution.

<sup>4</sup> Fisher's exact test.

<sup>5</sup> <10th Percentile of weight for gestational age according to Brenner's reference

in an earlier trial among HIV-positive women in Dar es Salaam (10). The dose of zinc used was most likely adequate for having an effect, if such an effect existed. We chose a supplementation dose of 25 mg Zn/d because it was only slightly higher than the recommended dietary allowance for adult women in the United States of 15 mg (19, 20) and because studies using doses of zinc between 20 and 30 mg/d have not reported side effects (2, 12). It is possible that doses higher than those used in the above trials may have had a beneficial effect. However, the dose used is already within close range to the tolerable upper intake level for adults (40 mg). Women were enrolled in the study at their first prenatal visit, which was at 22 wk of gestation on average. Although it is possible that a longer duration of supplement use may have had a significant effect on pregnancy outcomes, the first antenatal visit in Dar es Salaam was relatively earlier than visits in other countries in sub-Saharan Africa (21), and we were interested in enrolling typical pregnant women in Dar es Salaam.

The lack of effect of zinc supplements on birth weight and duration of pregnancy does not agree with the results of a placebo-controlled trial conducted in pregnant African American women who were presumably mostly HIV-negative (2). In the latter group of women, who were apparently healthy but had plasma zinc concentrations below the median at enrollment, daily zinc supplements (25 mg, as used in the Tanzania trial) resulted in greater birth weights and head circumferences. This effect was observed predominantly in women with a BMI < 26. However, similar to our results, 6 of 7 trials in Asia and Latin America showed no effect of zinc supplementation on birth weight; a modest benefit was noted in the Chilean trial. None of the 7 trials indicated an effect on duration of gestation (3).

The effects of zinc (30 mg zinc sulfate) and iron (60 mg as ferrous fumarate) were examined in a recently completed large trial among pregnant women from Nepal; the women were randomly assigned to 1 of 5 groups: 1) folate; 2) folate and iron; 3) folate, iron, and zinc; 4) folate, iron, zinc, and multivitamins; and 5) placebo. All women received daily vitamin A supplements. None of the supplements had a significant effect on fetal loss or perinatal or neonatal deaths (22). Compared with the placebo group, the women who received iron and folate alone had a 14% reduction in LBW and a higher birth weight; however, the women who received zinc in addition to iron and folate had birth weights that were not significantly different from those of the placebo group. The authors attributed this apparent adverse effect of zinc to possible interference with iron absorption by zinc. Although the women who received zinc in our study in Tanzania had a nonsignificantly higher risk of fetal and early postpartum mortality, the study had limited statistical power to examine the efficacy of the supplements on these endpoints.

Concerns about the safety of zinc supplementation in HIV-infected persons were raised on the basis of findings from an observational study among asymptomatic HIV-infected men from the United States. Dietary zinc intake was associated with significantly higher risks of progression to AIDS (7) and mortality (8). In contrast, normalization of plasma zinc concentrations was associated with higher CD4<sup>+</sup> cell counts among men who participated in another prospective cohort study (23). Low plasma zinc was also a significant predictor of AIDS mortality in a third study conducted in the United States (9). We found no effect of zinc supplementation on CD4<sup>+</sup> or CD8<sup>+</sup> cell counts among women in Tanzania. The effects of the supplements on

**TABLE 4**  
Effect of zinc supplements on fetal loss and early child mortality

Outcome <sup>1</sup>	Zinc group <sup>2</sup>	Placebo group <sup>2</sup>	Relative risk (95% CI) <sup>3</sup>	P <sup>4</sup>
Miscarriage	2.5 [5/198]	1.5 [3/199]	1.68 (0.17, 40.3)	0.50
Stillbirth <sup>5</sup>	6.6 [13/198]	5.0 [10/199]	1.31 (0.44, 4.55)	0.53
Fetal death <sup>6</sup>	9.1 [18/198]	6.5 [13/199]	1.39 (0.58, 3.86)	0.36
Perinatal death <sup>7</sup>	11.6 [23/198]	8.5 [17/199]	1.36 (0.64, 3.14)	0.32
Neonatal death <sup>8</sup>	5.6 [10/180]	3.8 [7/186]	1.45 (0.39, 7.22)	0.46

<sup>1</sup> For twin pregnancies, the outcome was positive when at least one of the twins had it.

<sup>2</sup> Values in brackets represent the number of infants at risk out of the total number of infants.

<sup>3</sup> 95% CI based on the exact binomial distribution.

<sup>4</sup> Fisher's exact test.

<sup>5</sup> Delivery of a dead child at or after 28 wk of gestation.

<sup>6</sup> Included stillbirths and miscarriages.

<sup>7</sup> Included stillbirths and child deaths between birth and 28 d postpartum for all women with known pregnancy outcomes.

<sup>8</sup> Included events between birth and 28 d postpartum among live births.

**TABLE 5**  
Effect of zinc supplements on hematologic indicators

Outcome	Zinc group	Placebo group	<i>P</i> <sup>1</sup>
Hemoglobin (g/L)			
Baseline	100 ± 12 [192] <sup>2</sup>	98 ± 14 [193]	0.07
6 wk postpartum	112 ± 16 [183]	114 ± 17 [189]	0.29
Change	11.5 ± 17.9 [175]	15.2 ± 18.6 [182]	0.03
Red blood cell count (× 10 <sup>6</sup> /mm <sup>3</sup> )			
Baseline	3.55 ± 0.52 [189]	3.48 ± 0.56 [190]	0.14
6 wk postpartum	4.16 ± 0.69 [182]	4.26 ± 0.65 [187]	0.12
Change	0.59 ± 0.75 [172]	0.78 ± 0.70 [177]	<0.01
Packed cell volume (%)			
Baseline	30.9 ± 3.4 [189]	30.4 ± 4.0 [190]	0.05
6 wk postpartum	34.5 ± 4.7 [182]	35.1 ± 4.8 [187]	0.21
Change	3.4 ± 5.2 [172]	4.6 ± 5.2 [177]	0.01

<sup>1</sup> Wilcoxon's rank-sum test.

<sup>2</sup>  $\bar{x} \pm SD$  (all such values); *n* in brackets.

viral load or clinical outcomes were not examined. We provided iron supplements to all of the women in our trial as per the standard of prenatal care in Tanzania. Iron deficiency anemia, a major public health problem among pregnant women in Tanzania and other developing countries, is a result of low prepregnancy stores, continued inadequate iron intake, malaria and hookworm infections, and increased iron requirements during pregnancy. Prenatal iron supplementation during pregnancy is routine practice given that it has been shown to raise hemoglobin concentrations (24, 25). Although all of the women in our trial,


**TABLE 6**  
Effect of zinc supplements on immune cell counts

Outcome	Zinc group	Placebo group	<i>P</i> <sup>1</sup>
CD4 <sup>+</sup> cell count (/mm <sup>3</sup> )			
Baseline	401 ± 203 [140] <sup>2</sup>	415 ± 210 [148]	0.80
6 wk postpartum	500 ± 251 [142]	525 ± 290 [150]	0.73
Change	95 ± 126 [106]	101 ± 137 [116]	0.97
CD8 <sup>+</sup> cell count (/mm <sup>3</sup> )			
Baseline	716 ± 282 [140]	763 ± 347 [148]	0.40
6 wk postpartum	1004 ± 452 [142]	965 ± 389 [150]	0.71
Change	279 ± 308 [106]	207 ± 282 [116]	0.11
CD4 <sup>+</sup> :CD8 <sup>+</sup>			
Baseline	0.63 ± 0.39 [140]	0.64 ± 0.37 [148]	0.65
6 wk postpartum	0.57 ± 0.37 [142]	0.61 ± 0.36 [150]	0.29
Change	-0.07 ± 0.19 [106]	-0.05 ± 0.18 [116]	0.23
CD3 <sup>+</sup> cell count (/mm <sup>3</sup> )			
Baseline	1186 ± 382 [140]	1254 ± 456 [148]	0.34
6 wk postpartum	1610 ± 630 [142]	1588 ± 557 [150]	0.89
Change	410 ± 420 [106]	330 ± 400 [116]	0.25
Total lymphocyte count (/mm <sup>3</sup> )			
Baseline	1731 ± 539 [189]	1831 ± 656 [190]	0.17
6 wk postpartum	1924 ± 686 [182]	1895 ± 607 [187]	0.79
Change	168 ± 799 [172]	48 ± 842 [177]	
White blood cell count (/mm <sup>3</sup> )			
Baseline	5166 ± 1619 [192]	5329 ± 1814 [193]	0.38
6 wk postpartum	4972 ± 1698 [183]	4951 ± 1540 [189]	0.74
Change	-322 ± 2252 [175]	-413 ± 2196 [182]	0.86

<sup>1</sup> Wilcoxon's rank-sum test.

<sup>2</sup>  $\bar{x} \pm SD$  (all such values); *n* in brackets.

irrespective of experimental group, experienced an increase in hemoglobin and other hematologic indicators, this increase was significantly less among women who received zinc than in those who received placebo. It is possible that zinc supplementation resulted in a negative effect on iron absorption, as previously proposed (26). Women who participated in a randomized trial in Peru who received iron and folic acid alone or iron, folic acid, and zinc had similar hematologic responses (27). In 2 other trials, however, zinc supplementation was apparently associated with a reduced response to iron supplementation. In a trial in Mexico, pregnant women who received multimicronutrient supplements containing iron (60 mg as ferrous sulfate) and zinc (15 mg) experienced a slight decrease in hemoglobin concentrations compared with those who received iron only (28). In the perinatal trial from Nepal mentioned above, increases in hemoglobin concentrations were smaller in the group randomly assigned to receive folic acid plus iron and zinc than in the group who received folic acid and iron only (22). Concerns about a possible adverse effect of zinc supplements on iron absorption were also raised in 2 trials among children in Indonesia, in whom iron and zinc were less efficacious than was iron alone in improving hemoglobin concentrations and iron status (29, 30).

This was a community-based study of women receiving primary health care during pregnancy. The results are generalizable to HIV-infected women attending prenatal care clinics in Tanzania. In light of the lack of beneficial effects of zinc on adverse pregnancy outcomes and the potential for adverse effects of zinc supplementation on hematologic indicators, there is no compelling evidence to add zinc to prenatal supplements intended for pregnant women. 

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WWF was the Harvard Principal Investigator of the project and contributed to the study design, study implementation, and data analyses and was primarily responsible for writing the draft of the manuscript. EV contributed to the data management in the field, data analyses, and writing of the paper. GIM contributed to the study design and the day-to-day running of the study in the field. GA supervised the data entry and management in the field. SA and WU oversaw the laboratory aspects of the study. DH contributed to the study design and provided advice on technical and practical issues during the implementation of the study. All authors contributed to the editing of the final version of the manuscript. None of the sponsors of the study had any role in the study design, data collection, data analysis, data interpretation, or writing of the report.

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