

Adding zinc to prenatal iron and folate supplements improves maternal and neonatal zinc status in a Peruvian population¹⁻⁴

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ABSTRACT

Background: Maternal zinc deficiency during pregnancy may be widespread among women in developing countries, but few data are available on whether prenatal zinc supplementation improves maternal and neonatal zinc status.

Objective: We studied whether maternal zinc supplementation improved the zinc status of mothers and neonates participating in a supplementation trial in a shantytown in Lima, Peru.

Design: Beginning at gestation week 10–24, 1295 mothers were randomly assigned to receive prenatal supplements containing 60 mg Fe and 250 µg folate, with or without 15 mg Zn. Venous blood and urine samples were collected at enrollment, at gestation week 28–30, and at gestation week 37–38. At birth, a sample of cord vein blood was collected. We measured serum zinc concentrations in 538 women, urinary zinc concentrations in 521 women, and cord zinc concentrations in 252 neonates.

Results: At 28–30 and 37–38 wk, mothers receiving zinc supplements had higher serum zinc concentrations than mothers who did not receive zinc (8.8 ± 1.9 compared with 8.4 ± 1.5 µmol/L and 8.6 ± 1.5 compared with 8.3 ± 1.4 µmol/L, respectively). Urinary zinc concentrations were also higher in mothers who received supplemental zinc ($P < 0.05$). After adjustment for covariates and confounding factors, neonates of mothers receiving zinc supplements had higher cord zinc concentrations than neonates of mothers who did not receive zinc (12.7 ± 2.3 compared with 12.1 ± 2.1 µmol/L). Despite supplementation, maternal and neonatal zinc concentrations remained lower than values reported for well-nourished populations.

Conclusion: Adding zinc to prenatal iron and folate tablets improved maternal and neonatal zinc status, but higher doses of zinc are likely needed to further improve maternal and neonatal zinc status in this population. *Am J Clin Nutr* 1999;69:1257–63.

KEY WORDS Maternal zinc deficiency, pregnancy, zinc status, supplementation, iron, folate, women, neonates, Peru

INTRODUCTION

Iron deficiency anemia is the most prevalent micronutrient deficiency disorder in the world, and pregnant women are particularly at risk. For example, in Peru, 35% of women of child-bearing age and 50% of pregnant women have anemia (1, 2). To treat or prevent anemia during pregnancy, as well as to meet the increased iron needs of pregnancy, Peruvian women—like

women around the world—are prescribed daily iron supplements during pregnancy.

Pregnant women are also at risk of other micronutrient deficiencies and there is interest in the public health community in adding other nutrients to prenatal supplements taken by pregnant women worldwide. In particular, there is growing concern that maternal zinc deficiency may be widespread (3, 4). Adding zinc to prenatal iron and folate tablets, however, may be problematic. Iron and zinc are known to compete for absorption (5) and iron supplements may themselves impair maternal zinc status (6–9). Although less studied, concern that folic acid supplementation may impair zinc absorption has also been raised (9, 10).

The results of the several zinc supplementation studies of pregnant women that have been conducted in developed countries have been mixed (11–14). In 2 studies, zinc was incorporated directly into the prenatal supplements (12, 14); of these studies, only one reported significant improvements in maternal serum zinc concentrations during pregnancy in zinc-supplemented women (14). The women in these studies (11–14), however, had higher intakes of zinc and iron than would be true for most women in developing countries. Because maternal needs for both zinc and iron during pregnancy appear to influence zinc absorption and metabolism (15), the relevance of these findings for women in developing countries is not clear.

As part of a randomized trial of maternal zinc supplementation and pregnancy outcome and maternal and infant health, Peruvian women were randomly assigned to receive prenatal

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iron and folate supplements with or without zinc. Because Peruvian guidelines indicate universal prenatal iron supplementation, we could not directly assess the effect of iron supplementation on zinc status; however, we could examine whether adding zinc to prenatal iron and folate tablets improved the zinc status of these women during pregnancy and of their neonates at delivery.

SUBJECTS AND METHODS

Between 1995 and 1997, a double-blind, randomized controlled trial of prenatal zinc supplementation to improve maternal and infant health was conducted at the Hospital Materno Infantil "Cesar Lopez Silva" in Villa El Salvador, an impoverished shantytown in Lima, Peru. Previous information indicated some degree of maternal zinc deficiency in this area. A dietary survey of pregnant women described the typical diet as bread or oatmeal with coffee or milk for breakfast; broth and rice or noodles with a stew (containing beans, potatoes, and perhaps chicken entrails), perhaps a salad (lettuce, tomato, and onion), and lemonade or a corn-based drink at midday; bread with coffee or tea in the late afternoon; and coffee or tea with leftovers from midday in the evening (16). On average, 12% of the energy from this dietary pattern came from protein, 20% from fat, and 68% from carbohydrate; intakes of iron, calcium, and vitamin A were 13 mg/d, 475 mg/d, and 600 retinol equivalents/d, respectively (16). Although not presented in this report, usual intake of zinc from this diet is estimated to be 7 mg/d and the dietary pattern is consistent with one of low-to-moderate mineral bioavailability (N Zavaleta and M Berlanga, unpublished observations, 1996). Moreover, as part of a representative survey (2), it was determined that 60% of pregnant women had serum zinc concentrations $<9.18 \mu\text{mol/L}$ during pregnancy, indicating some degree of maternal zinc deficiency (N Zavaleta, M Berlanga, B Lonnerdal, KH Brown, unpublished observations, 1993).

Women were considered eligible for the study if they had a low-risk pregnancy (uncomplicated and eligible for vaginal delivery), were carrying a singleton fetus, and had lived in coastal Peru for ≥ 6 mo before becoming pregnant. The women gave their signed consent to participate after the protocol was fully explained to them. The study protocol was approved by the institutional review boards of the Instituto de Investigación Nutricional (IIN) and The Johns Hopkins School of Hygiene and Public Health.

On entry into prenatal care between gestation weeks 10 and 24, women were randomly assigned by parity (nullipara or multipara) and week of gestation at enrollment (<17 or ≥ 17 wk) strata to receive daily supplements containing 60 mg Fe (as ferrous sulfate) and 250 μg folate with or without an additional 15 mg Zn (as zinc sulfate). Per Peruvian guidelines, women were asked to take one pill daily midmorning with a vitamin C-containing drink or water. Supplementation began at gestation week 10–24 and continued until 4 wk postpartum. The supplements were produced by a local pharmaceutical company (Instituto Quimioterápico, SA, Lima, Peru) in coded blister packages. To verify the formulation of the supplements and the integrity of the coding scheme, samples of each supplement type were analyzed by the IIN laboratory 2 times during the study. Despite this, the investigators remained blinded as to the coding scheme until data analyses for this paper were almost complete.

At enrollment, sociodemographic information was collected by interview. Duration of pregnancy was calculated based on

maternal reporting of date of last menses, as well as by clinical indications of pregnancy duration at enrollment, and a best estimate of gestational age was determined. Women were followed up monthly (or as necessary) by the hospital staff as well as by the project obstetrician. Maternal anthropometric measures and venous blood samples for monitoring concentrations of serum zinc, serum ferritin, and hemoglobin were taken at enrollment, at gestation week 28–30, and at gestation week 37–38. At each time point, a urine sample was also collected to assess urinary zinc concentrations. At birth, a sample of cord vein blood was taken to determine newborn serum zinc concentrations. Compliance with the supplementation was monitored by pill counts and was checked biweekly by health care workers who interviewed the women in their homes. For these analyses, we considered compliance as the number of pills provided to each mother for the number of days she was in the study during pregnancy. Infants were weighed at birth by hospital personnel and crown-heel length and various circumferences and skinfold thicknesses were measured on day 1 by project personnel.

Blood and urine samples were collected from women in a fasted state between 0800 and 1000. Blood samples were centrifuged immediately at room temperature for 5 min at 3000–3500 rpm, frozen at -20°C , and then transported on ice during a 45-min drive to the IIN laboratory. At IIN, serum and urinary zinc concentrations were measured by atomic absorption spectrophotometry (model 3100; Perkin-Elmer, Norwalk, CT) and serum ferritin was measured in duplicate by an enzyme-linked immunosorbent assay with reagents purchased from DAKO (Santa Barbara, CA). Bovine liver from the National Institute of Standards and Technology (Gaithersburg, MD) and ferritin controls (Diagnostic Products Corporation, Los Angeles) were used as standards and values were within expected ranges. Hemoglobin concentrations were measured at the hospital by the cyanomethemoglobin method under the supervision of IIN laboratory staff.

It was not feasible to collect 24-h urine samples for all women to estimate urinary zinc excretion, or to reduce some of the random variation in excretion by adjusting for creatinine. Zlotkin and Casselman (17) showed a strong correlation ($r = 0.87$) between urinary zinc excretion estimated from a single urine sample and estimates of total urinary zinc excretion over a 24-h period and estimated that creatinine adjustment reduces the variance in urinary zinc excretion by 24%. Thus, our data on urinary zinc excretion from single urine samples should provide accurate (unbiased) estimates of the mean or median of the distributions of total urinary zinc excretion for comparison by supplement type, but inflated estimates of the variances of the distributions.

A total of 1295 women were enrolled in the supplementation trial. We attempted to collect biological samples from all women and neonates, but cord samples could be collected at only 70% of the births at the project hospital and costs prohibited analyses of all blood and urine samples. Therefore, to examine the effect of zinc supplementation on indicators of zinc status, we decided to measure maternal serum zinc and urinary zinc concentrations in a subsample of ≈ 500 women with serial blood and urine samples. This decision was made after ≈ 1000 serum samples (from all 3 time points) had been analyzed. Therefore, women whose samples had already been analyzed were included in this subsample; if samples could not be collected at all 3 time points for women with ≥ 1 sample already analyzed (for example, if they gave birth before gestation week 37), the women were replaced in the cohort. The goal was to include women in the



TABLE 1

Selected characteristics of 538 Peruvian women at entry into prenatal care and the study at gestation week 10–24

Characteristic	Supplement type	
	Iron + folate + zinc (<i>n</i> = 270)	Iron + folate (<i>n</i> = 268)
Age (y)	24.3 ± 5.3 ^{1,2}	25.5 ± 5.7
Education (%)		
Primary incomplete	5.2	10.2
Secondary incomplete	43.1	35.0
Secondary complete	26.2	29.0
Secondary plus	25.5	25.9
Parity (%)		
0	48.9	45.9
1	28.5	24.3
2–3	17.8	19.4
≥4	4.8	10.5
Single (%)	17.0	13.4
In-home electricity (%)	72.3 ²	79.9
Housing material (%)		
Cardboard	23.7	27.6
Wood	17.0	17.5
Brick	59.3	54.9
Toilet (%)		
Installed	63.7	60.5
Latrine	27.0	29.5
Open air	9.3	10.1
Height (cm)	151.6 ± 5.3	151.3 ± 5.9
Body mass index (kg/m ²)	24.0 ± 3.2	24.2 ± 3.4
Gestational age (wk)	16.0 ± 4.7	16.0 ± 4.6
Iron status (<i>n</i> = 525)		
Hemoglobin (g/L)	116 ± 13	115 ± 14
Serum ferritin (μg/L)	22 (9, 53) ³	20 (8, 50)

¹ $\bar{x} \pm$ SD.

²Significantly different from iron + folate, *P* < 0.05.

³Geometric mean (–1 SD, +1 SD).

subsample throughout the study enrollment period; however, because the sample size of 500 women was reached before the end of enrollment, few of the final 200 women enrolled in the study were included in the subsample.

Ultimately, 3 serum zinc determinations were available for 538 women (270 and 268 per supplementation group), representing 42% of all women enrolled in the study, 49% of the first 1100 women enrolled in the study, and 60% of all women delivering after gestation week 38. For 521 of these women (97%), serial urinary zinc determinations (256 and 265 per supplementation group) were also available. Overall, 363 determinations of cord zinc concentration were made, 256 of them were in infants born to women in this cohort (129 and 127 per supplementation group). For consistency, we present the data on 252 (98%) of the infants born to women with serial zinc determinations for whom anthropometric data at birth were also available.

Baseline characteristics in the supplementation groups were compared by *t* tests or chi-square analyses to assess comparability. Maternal serum zinc concentrations at each of the 3 time points and cord serum zinc concentrations in the newborns were compared by *t* tests. Between-group comparisons of urinary zinc concentrations were also performed by *t* tests, after the distributions were normalized by using a cube-root transformation. Analysis of variance techniques were then used to estimate the effects of zinc supplementation on maternal serum zinc and (transformed) uri-

nary zinc concentrations during pregnancy and to estimate supplementation effects on neonatal zinc status, adjusted for covariates and potentially confounding factors (18). For presentation, the values for the adjusted median and ±1 SD of urinary zinc concentrations were transformed to their original units (μmol/L). The effect of zinc supplementation on maternal serum and urinary zinc concentrations was also estimated after adjustment for initial zinc concentration (at gestation week 10–24). Furthermore, we investigated whether the effect of maternal zinc supplementation varied depending on selected maternal characteristics including initial zinc concentration. To characterize the magnitude of the change in maternal and newborn zinc status in response to zinc supplementation, we calculated the responsiveness of each indicator, which is the difference in the postsupplementation means (or medians) of the indicator divided by the pooled SD (19, 20). Responsiveness can also be calculated as the response per unit dose of the supplement to compare the responsiveness of each indicator across different levels of supplementation (19). Statistical significance was defined as *P* < 0.05 and data analyses were performed with SAS (SAS Institute Inc, Cary, NC).

RESULTS

The characteristics of the 538 participants for whom serum samples were analyzed are presented in **Table 1**. There were no significant differences in the presented variables between the 2 groups at enrollment, except that the mothers receiving zinc in addition to iron and folate were significantly younger and less likely to have electricity in their homes than those receiving iron and folate alone. These differences were adjusted for in subsequent analyses.

Maternal serum and urinary zinc concentrations adjusted for maternal age and in-home electricity at each time point are presented in **Table 2**. Maternal serum zinc concentrations declined during pregnancy. Maternal urinary zinc concentrations were uniformly low, but appeared to be somewhat lower at gestation week 28–30 than at enrollment and to rise by gestation week 37–38. There were no significant differences in serum or urinary zinc concentrations at enrollment between the supplementation groups, but at each time point postsupplementation, women receiving zinc in addition to iron and folate had higher serum and urinary zinc concentrations than did women receiving iron and folate alone. Adjustment for exact week of gestation at assessment and compliance (%) did not affect these results, and adjustment for initial zinc concentration had only a minimal effect on the estimated differences between groups. The effect of zinc supplementation on serum or urinary zinc concentrations did not differ significantly depending on initial zinc concentration (*P* > 0.10).

The adjusted serum zinc concentrations of the participating women are shown by supplement type in **Figure 1**; for comparison, we calculated the simple average of plasma or serum zinc concentrations by trimester of pregnancy reported in the literature for well-nourished women, most of whom were taking prenatal iron supplements (21–23). Two results are apparent. First, there was a much steeper decline in serum zinc concentration with pregnancy in the Peruvian population than in the population taken from the literature. Second, although the zinc supplement improved serum zinc concentrations, serum zinc concentrations in our zinc-supplemented group were still well below the average of values reported in the literature.

Relative to the variability in serum zinc concentrations at each time point postsupplementation during pregnancy, zinc supplementation resulted in an upward shift of 0.21–0.23 SD in the distribution

TABLE 2

Adjusted serum and urinary zinc concentrations of Peruvian women at enrollment (gestation week 10–24) and at gestation weeks 28–30 and 37–38 by type of prenatal supplement¹

Gestation (wk)	Serum zinc concentration		Urinary zinc concentration	
	Iron + folate + zinc (n = 270)	Iron + folate (n = 268)	Iron + folate + zinc (n = 256)	Iron + folate (n = 265)
	$\mu\text{mol/L}$		$\mu\text{mol/L}$	
10–24	10.6 \pm 2.1 ²	10.4 \pm 2.1	2.02 (0.67, 4.50) ³	1.92 (0.62, 4.64)
28–30	8.8 \pm 1.9 ⁴	8.4 \pm 1.5	1.59 (0.55, 3.48) ⁴	1.35 (0.44, 3.06)
28–30	8.7 \pm 1.8 ^{4,5}	8.4 \pm 1.2	1.57 (0.60, 3.25) ^{5,6}	1.37 (0.48, 2.97)
37–38	8.6 \pm 1.5 ⁴	8.4 \pm 1.4	1.83 (0.69, 3.82) ⁴	1.58 (0.60, 3.29)
37–38	8.6 \pm 1.5 ^{4,5}	8.3 \pm 1.4	1.82 (0.72, 3.68) ^{4,5}	1.59 (0.61, 3.29)

¹Adjusted for maternal age (y) and presence of electricity in the home by multiple regression.

² $\bar{x} \pm \text{SD}$.

³Normalized median (–1 SD, +1 SD).

⁴Significantly different from iron + folate, $P < 0.05$ (multiple regression).

⁵Also adjusted for initial concentration (at gestation week 10–24).

⁶Difference by type of prenatal supplement: $P = 0.06$.

of maternal serum zinc concentrations (eg, 0.4 $\mu\text{mol/L}$ \div 1.7 $\mu\text{mol/L}$ at 28–30 wk). The responsiveness of urinary zinc concentrations to zinc supplementation was of a somewhat smaller magnitude, on the order of 0.16–0.21 SD. When considered per mg supplemental Zn/d, the responsiveness of both serum and urinary zinc concentrations to supplementation was 0.02–0.04 $\mu\text{mol/L}$.

The characteristics of 252 of the infants born to the women studied are presented in **Table 3**. No significant differences in infant size or gestational age at birth by supplement type were found. The cord vein serum zinc concentrations of the newborns are compared by supplement type in **Table 4**, before and after

adjustment for covariates and potentially confounding factors. The difference in cord zinc concentration by type of prenatal supplement was only marginally significant initially, but became significant after adjustment for maternal age and parity, in-home electricity, and infant birth weight and sex. As shown, adjustment for these factors did not greatly influence the estimated difference in mean cord zinc concentration by supplement type, but rather reduced the variance in cord zinc concentration around each mean. This is because cord zinc concentration was positively associated with birth weight, male sex, and maternal parity, and each contributed substantially to the variability in observed

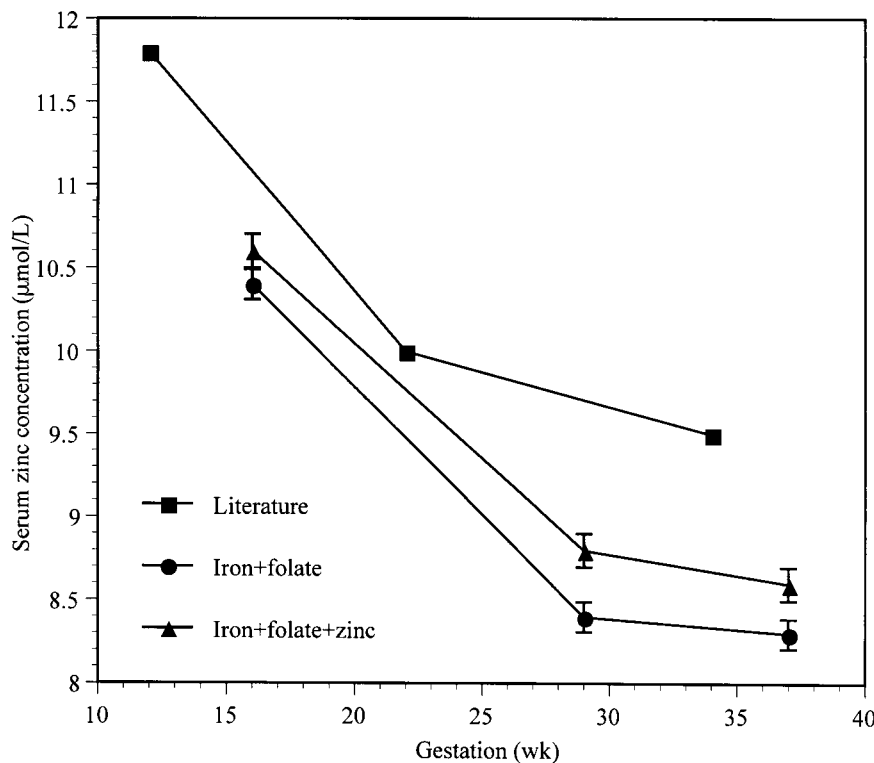


FIGURE 1. Mean (\pm SE) serum zinc concentrations of Peruvian women at enrollment in the study and at gestation weeks 28–30 and 37–38 by supplement type and the simple average of plasma or serum zinc concentrations during each trimester of pregnancy reported in the literature (21–23).

TABLE 3Selected characteristics of Peruvian neonates by type of maternal prenatal supplement¹

	Prenatal supplement type	
	Iron + folate + zinc (n = 127)	Iron + folate (n = 125)
Gestational age (wk)	39.9 ± 1.3 ²	39.9 ± 1.5
Female infant (%)	52.8	53.6
Birth weight (g)	3361 ± 394	3349 ± 399
Crown-heel length (cm)	50.1 ± 1.9	50.3 ± 1.7
Head circumference (cm)	34.3 ± 1.3	34.2 ± 1.3

¹n = 252. There were no significant differences between groups.² $\bar{x} \pm SD$.

cord zinc concentrations in neonates. The responsiveness of cord zinc concentration to maternal zinc supplementation was 0.22 SD before adjustment and 0.27 SD after adjustment. Neonatal zinc concentrations responded 0.03 $\mu\text{mol/L}$ per mg supplemental Zn/d provided to the mother.

During the analyses, we also adjusted neonatal cord zinc concentrations for maternal serum zinc concentrations at gestation week 37–38 (not shown). Differences in cord zinc concentrations by supplement type were diminished considerably, but there was still some evidence that infants of mothers supplemented with zinc had higher cord zinc concentrations than did those born to mothers supplemented with iron and folate alone ($P = 0.08$).

DISCUSSION

In this Peruvian population, adding 15 mg Zn to daily prenatal supplements containing 60 mg Fe and 250 μg folate improved indicators of maternal and newborn zinc status. Serum zinc concentrations were higher in zinc-supplemented mothers at both gestation week 28–30 and gestation week 37–38 and despite low urinary zinc concentrations in general, women supplemented with zinc had higher urinary zinc concentrations at each time point than did mothers supplemented with iron and folate only. Cord zinc concentrations were also higher in the neonates whose mothers received zinc supplements, after adjustment for potentially confounding factors and extraneous sources of variability in cord zinc concentration among newborns.

The responsiveness of the indicators of maternal and neonatal zinc status to zinc supplementation indicated shifts in the distributions of 0.16–0.21 SD for maternal urinary zinc concentrations, 0.21–0.23 SD for maternal serum zinc concentrations, and 0.23–0.27 SD for newborn cord zinc concentrations. In other zinc supplementation studies in which women were supplemented with 15–30 mg elemental Zn/d (11–14), the responsiveness of maternal plasma or serum zinc concentrations to zinc supplementation varied between 0.0 and 0.89 SD (Table 5). Although slight, there was some evidence of a positive association between responsiveness and dosage in these studies; supplementation-associated shifts in plasma or serum zinc concentrations were 0–0.1, 0.16, 0.11–0.35, and 0.12–0.89 SD for daily supplements containing 15, 20, 25, and 30 mg Zn, respectively. The responsiveness of maternal serum zinc concentrations in our study of Peruvian women was within the range of estimates of responsiveness calculated from the literature, but the magnitude was similar to estimates from studies in which higher doses of zinc were provided. When considered per mg supplemental Zn/d (Table 5), the data across studies become

TABLE 4Serum zinc concentrations in cord vein blood of Peruvian neonates by type of maternal prenatal supplement¹

	Prenatal supplement type	
	Iron + folate + zinc (n = 127)	Iron + folate (n = 125)
	$\mu\text{mol/L}$	
Cord vein zinc		
Unadjusted	12.7 ± 2.4 ²	12.2 ± 2.2
Adjusted ³	12.7 ± 2.3 ⁴	12.1 ± 2.1

¹ $\bar{x} \pm SD$ before and after adjustment for covariates by multiple regression; n = 252.²Difference by type of prenatal supplement: $P = 0.08$.³Adjusted for maternal age and parity, in-home electricity, and infant birth weight and sex.⁴Significantly different from iron + folate, $P = 0.03$.

more consistent, suggesting that maternal serum zinc concentrations respond 0.00–0.10 $\mu\text{mol/L}$ for each mg supplemental Zn/d provided during pregnancy.

Only one other study (14) examined the effects of daily prenatal zinc supplementation on cord zinc concentrations as an indicator of neonatal zinc status. Thauvin et al (14) supplemented 48 women with 30 mg Zn/d (as zinc gluconate) or placebo and found a nonsignificant increase in cord plasma zinc concentrations on the order of 0.34 SD in infants born to mothers supplemented with zinc. Although this degree of responsiveness is larger than the effect we observed (of 0.22–0.27 SD), the data in the 2 studies become similar when the responsiveness is estimated per mg supplemental Zn/d.

The absolute difference in cord zinc concentration by supplement type was nearly 2 times that observed in maternal serum late in pregnancy, and the cord zinc concentrations of neonates born to zinc-supplemented mothers were somewhat higher ($P = 0.08$) after adjustment for maternal serum zinc concentrations late in pregnancy. Thus, the fetuses may have benefited more from zinc supplementation than the mothers. However, both maternal and cord zinc concentrations increased by $\approx 5\%$ in response to supplementation and, regardless of supplement type, cord zinc concentrations were $\approx 50\%$ higher than maternal serum zinc concentrations during late pregnancy, a finding consistent with reports from both well-nourished and zinc-deficient populations (14, 24). Thus, although the fetuses may have benefited slightly more than the mothers from prenatal zinc supplementation, greater improvement in maternal zinc status before or during pregnancy is likely required to bring about greater improvements in fetal zinc status as assessed by cord zinc concentrations.

Maternal serum zinc concentrations declined more rapidly in our population than in more zinc-replete populations (Figure 1). Congruent with this, maternal urinary zinc concentrations were uniformly low during pregnancy, declined at 28–30 wk, and then increased only slightly at 37–38 wk. This pattern is in contrast with the progressive increases in urinary zinc excretion that typically occur during pregnancy, particularly during the third trimester (11, 15). Moreover, the cord zinc concentrations were similar to values reported for Zairian newborns (24), but much lower than typical cord zinc concentrations of 15–16 $\mu\text{mol/L}$ (25). These findings suggest continued zinc deficiency in these women and their neonates despite daily prenatal zinc supplementation with 15 mg Zn from gestation week 10–24 until delivery.

TABLE 5

Estimated responsiveness of maternal and neonatal zinc status indicators to prenatal zinc supplementation as reported in the literature

Site and reference, prenatal supplement, and indicator	Typical values ¹		Responsiveness (SD) ²	Responsiveness per mg/d ³
	+ Zinc	No added zinc		
Colorado (11): 15 mg Zn (zinc sulfate) consumed at night and a prenatal supplement containing 40–240 mg Fe consumed in morning				
Plasma zinc (μmol/L)	8.7 ± 1.1	8.7 ± 2.1	0.0–0.10	0.0–0.10
Urinary zinc (μmol/24 h)	6.7 ± 4.1	6.1 ± 2.9	0.0–0.97	0.04–0.22
California (12): 20 mg Zn (zinc acetate) as part of multivitamin and mineral tablet containing 20 mg Fe and 1 mg folic acid				
Serum zinc (μmol/L)	10.0 ± 1.5	9.8 ± 1.5	0.16	0.02
Alabama (13): 25 mg Zn (zinc sulfate) and a prenatal supplement				
Plasma zinc (μmol/L)	9.3 ± 2.6	8.8 ± 2.5	0.11–0.35	0.02–0.08
France (14): 30 mg Zn (zinc gluconate) as part of a multivitamin and mineral supplement containing 50 mg Fe and 1 mg folic acid				
Plasma zinc (μmol/L)	10.2 ± 2.5	9.3 ± 1.4	0.12–0.89	0.02–0.06
Cord zinc (μmol/L)	16.5 ± 4.2	15.3 ± 2.6	0.34	0.04

¹ Postsupplementation $\bar{x} \pm SD$ by supplement type. Data from Colorado (11) and Alabama (13) were estimated from published graphs. Data from Colorado (11) and California (12) were transformed from $\mu\text{g/dL}$ to $\mu\text{mol/L}$ by using the conversion factors of 0.1530 for serum zinc and 0.01530 for urinary zinc.


² Calculated as the difference in postsupplementation means (or medians) between the zinc-supplemented and control groups divided by the pooled SD to express the response given the variability inherent in the indicator.

³ Calculated as the difference in postsupplementation means (or medians) between the zinc-supplemented and control groups divided by the dose of supplemental zinc in mg/d to compare responses across different dosages. Formulas for each are found in reference 19.

It is possible that some of the differences in maternal serum and urinary zinc concentrations between our study and others reported in the literature (Figure 1) resulted from iron and folate supplementation rather than zinc deficiency per se. Because all women in this project received daily iron and folate supplements, we cannot address this possibility. However, the women participating in most of the studies reported in the literature of maternal zinc status during pregnancy also consumed prenatal iron supplements, some of which also contained up to 1 mg folate/d (11–14).

The primary limitation of our analysis is that we did not assess the effect of prenatal zinc supplementation in all participants or in a representative subsample of participants and their newborns. Instead, we analyzed samples from a subsample of 538 women who provided 3 serum samples during their pregnancy and cord zinc concentrations in a subsample of 252 of their neonates. Because of the sampling scheme, it is clear that women who were able to provide 3 samples had longer pregnancies than women not providing 3 samples. Furthermore, the analysis consisted of women who participated in the project earlier as opposed to near the end because we stopped analyzing serum samples when the number of women with 3 analyzed samples reached ≈ 500 . To examine potential selection bias, we compared the characteristics of eligible women (among the first 1100 participants giving birth after 38 completed weeks of gestation) by whether they contributed serum samples to the analysis. These analyses revealed no significant differences by supplement type, but women whose serum samples were included in the analysis were more likely to be older, of greater parity, more educated, and less likely to have in-home electricity. They were also more likely to have given birth in the project hospital, perhaps indicating lower obstetric risk. Maternal age was negatively associated with serum zinc concentrations at enrollment and differences by supplement type in age and in-home electricity among participants were noted at enrollment. In sum, it appears that the women described here were somewhat better off than eligible women whose sera were not analyzed, and that eligible women were somewhat better off than the rest of the project participants.

Despite this, we found no evidence that such differences were related to supplement type, and during analyses we adjusted for maternal age and in-home electricity. Thus, the zinc status of the sample and the effect of supplementation on zinc status described here can likely be generalized to all project participants.

Despite this limitation, we showed the efficacy of adding zinc to prenatal iron and folate tablets to improve maternal and neonatal zinc status, and that maternal serum and urinary zinc concentrations as well as cord zinc concentrations at birth are responsive to prenatal zinc supplementation. The data, however, indicate continued maternal and neonatal zinc deficiency in this population and suggest that higher doses of zinc should be added to the prenatal supplements. Future studies should investigate the effect of higher doses of zinc incorporated into prenatal supplements on maternal and neonatal zinc status in this and similar populations. 

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