

Randomized controlled trial of prenatal zinc supplementation and fetal bone growth¹⁻³

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ABSTRACT

Background: Maternal zinc deficiency is relatively common in developing countries, but its consequences for fetal growth are not established.

Objective: The goal was to examine whether improvement in maternal gestational zinc status is positively associated with fetal growth as assessed by ultrasonography.

Design: We conducted a double-masked, randomized trial among 242 pregnant Peruvian women in an impoverished shantytown in Lima, Peru. At 10–16 wk of gestation, the women were randomly assigned to receive daily supplements containing 60 mg Fe and 250 µg folic acid, with or without 25 mg Zn. We measured fetal head circumference, biparietal diameter, abdominal circumference, and femur diaphysis length at 20, 24, 28, 32, 36, and 38 wk of gestation. Fetal measures were analyzed longitudinally to evaluate differences in trends of fetal growth by supplement type, and within-subject correlations were taken into account.

Results: Femur diaphysis length was greater in fetuses whose mothers received zinc supplements ($P < 0.05$), and the difference tended to increase with gestational age. No significant differences by supplement type were observed for the other anatomical sites measured.

Conclusions: The observed positive effect of prenatal zinc on fetal femur diaphysis length is consistent with the results of experimental studies in animals and in vitro. The supplementation effect represents an upward shift in mean femur diaphysis length at term of about one-quarter of the reference SD. These findings suggest the potential importance of maternal zinc status for fetal bone growth in humans and illustrate the value of ultrasonography for evaluating the effect of prenatal nutritional interventions on components of fetal growth. *Am J Clin Nutr* 2004;79:826–30.

KEY WORDS Zinc, fetus, femur growth, Peru, pregnancy, supplementation

INTRODUCTION

Zinc deficiency during pregnancy is likely to be common worldwide, particularly among women in developing countries or those who consume diets based on cereals, vegetables, and legumes that are of moderate to low zinc bioavailability. On the basis of dietary intake data from several published studies, it has been estimated that 82% of pregnant women worldwide may have inadequate usual intakes of zinc (1). More recent prevalences based on food balance data are lower ($\approx 31\%$) but still raise a concern regarding the potential adverse effects of maternal zinc deficiency on pregnancy outcomes (2).

Despite the likely high prevalence of maternal zinc deficiency, data supporting maternal zinc deficiency as a cause of poor fetal growth are far from conclusive. Zinc is critically involved in several biological mechanisms related to growth, including protein synthesis, gene expression, and hormonal regulation (3), and animal studies have shown that zinc deficiency probably begins to affect the growth process before birth (4). Yet, the results of the randomized controlled trials conducted to date have been mixed with respect to birth weight and duration of pregnancy (5).

The objective of our investigation was to determine the effects on fetal growth and neurobehavioral development of supplementing marginally zinc-deficient mothers. Presented here are the results as assessed by ultrasonography. Ultrasonography provides 2 advantages to the evaluation of the effect of supplemental zinc on fetal growth. First, it permits examination of the pattern of fetal growth both over the entire pregnancy and during specific periods of gestation. Second, it enables the investigation of potential effects of zinc on specific components of growth, such as bone growth and maturation (6). We hypothesized that, despite apparent similarities in size at birth, we would be able to detect zinc-associated differences in fetal growth patterns over time by using ultrasonography.

SUBJECTS AND METHODS

Study design

Between 1998 and 2000, we conducted a double-masked controlled trial of prenatal zinc supplementation. The study was

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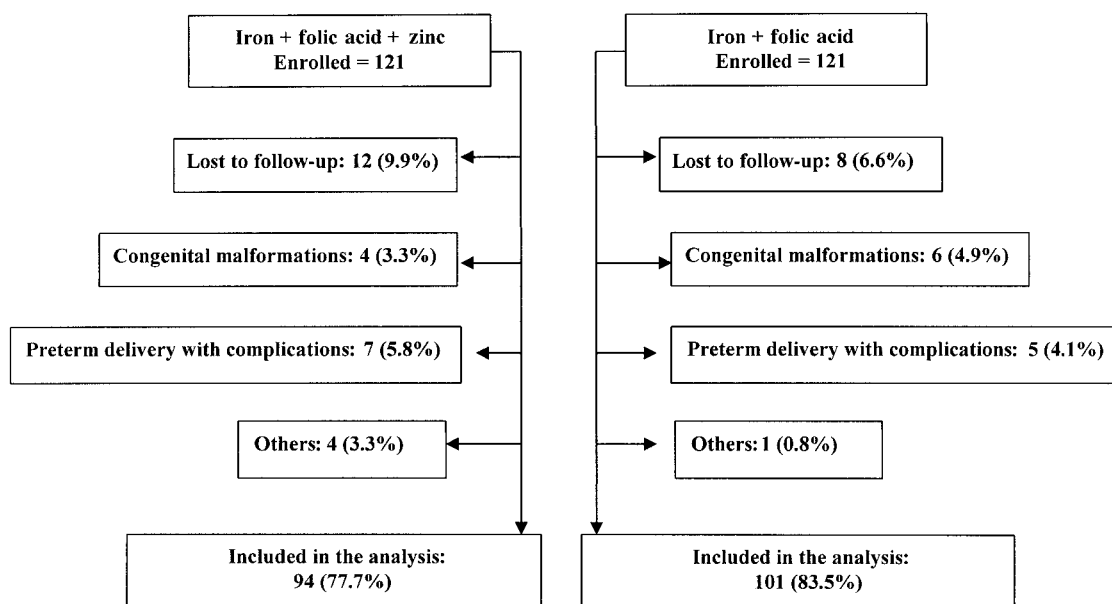


FIGURE 1. Trial profile.

conducted among 242 women receiving prenatal care at the Hospital Materno Infantil San José in Villa El Salvador, an impoverished peripheral district in Lima, Peru. Previously, we showed that the dietary zinc intake of Peruvian women during pregnancy is ≈ 8 mg/d (7), much lower than the recommended intake (at that time) of 15 mg/d (US recommended dietary allowance; 8). In this population, plasma and urinary zinc concentrations are lower than those observed in more zinc-replete gravid populations, and we showed previously that improvements in maternal and neonatal zinc status are detectable with 15 mg supplemental Zn/d (9).

Eligibility

Women were eligible for the study if they were considered to be at low risk (eligible for vaginal delivery), carrying a singleton fetus, a resident of Villa El Salvador, and had been living in coastal Peru for ≥ 6 mo before becoming pregnant. Women were enrolled in the study at 10–16 wk of gestation as determined by the date of their last menses and confirmed by ultrasonography (10). Signed consent to participate was obtained. The Institutional Review Boards of the Instituto de Investigación Nutricional and the Bloomberg School of Public Health approved the study protocol.

Randomization and treatment allocation

At entry into prenatal care, between 10 and 16 wk of gestation, the women were randomly assigned in blocks of 2 within 4 strata based on parity (primipara or multipara) and week of gestation at enrollment (10–13 or 14–16 wk) to receive a daily supplement containing 60 mg Fe (ferrous sulfate) and 250 μ g folic acid, with or without 25 mg Zn (zinc sulfate). The allocation sequence and randomization lists were computer generated by the investigators at the Bloomberg School of Public Health.

Supplementation began at entry into the study and continued until 1 mo postpartum. The supplements had the same appearance and taste, and both study personnel and participants were masked to treatment assignment. The supplements were produced by a local manufacturer (Instituto Quimioterapico, SA,

Lima, Peru), packaged as blister packs, and distributed to participants monthly. Adherence with supplementation was assessed during home visits by observing the number of tablets remaining in each blister pack.

Data collection

Socioeconomic information was collected via interview at enrollment. At 20, 24, 28, 32, 36, and 38 wk gestation, the women underwent an electronic fetal monitoring session to record patterns of fetal heart rate and fetal movement. These are indexes of fetal neurobehavioral development and were the primary outcomes of the study. The women also underwent fetal ultrasound examinations to assess fetal growth, and these outcomes are the focus of the present report. In addition, maternal anthropometric measures and iron and zinc status were assessed at enrollment, 28 wk, and 36 wk. Newborn iron and zinc status was determined at birth. Birth weight, length, and head circumference were assessed by hospital personnel.

Sample size

As shown in **Figure 1**, the analyses pertain to 195 of the 242 women enrolled in the supplementation trial. Of these women, 94 received zinc supplements and 101 did not. Of the 47 excluded subjects, 20 were lost to follow-up because of a change of address, declination to participate, or travel, and 27 developed obstetric or medical complications. There were no significant differences in the distribution of complications by supplement type. We excluded subjects with complications according to a protocol of fetal neurobehavioral assessment developed by one of the investigators (JAD), which was used in presenting the neurobehavioral outcomes of this study (11). Exclusions were justified to maintain consistency in the number of study subjects during statistical analysis across neurobehavioral and fetal growth outcomes. Decisions to exclude subjects were made before statistical analysis and were based on a review of the medical records by the 2 study obstetricians (AF and MM), who were masked to treatment assignment. A priori sample size calcula-

tions indicated that a sample size of 100 women in each group would have provided 0.8 power with 0.05 significance level to detect statistically significant cross-sectional differences of the magnitude of ≈ 0.4 SD. Analysis was by intention to treat.

Fetal biometry

Overall, the presented analyses include data from 1142 fetal ultrasonography sessions performed at the scheduled gestational age (± 0.5 wk). One hundred sixty-eight women had 6 evaluations at these scheduled gestational ages, 26 women had 5 evaluations (19 had no week 38 evaluation, 1 had no week 24 evaluation, and 6 had no week 20 evaluation), and 1 woman had 4 evaluations (no week 24 or week 28 evaluations).

At each examination, head circumference, biparietal diameter, abdominal circumference, and femur diaphysis length were measured according to published techniques (12). Circumferences were calculated by using the transverse and anteroposterior diameters of the head and abdomen. Head circumference was calculated by using the biparietal and occipitofrontal diameters. Each image was photographed and stored in the patient's record. Fetal biometry examinations were performed by 2 obstetricians (AF and MM) who had been trained by personnel of The Johns Hopkins Fetal Assessment Center. Intraobserver and interobserver measurement errors were assessed according to a published protocol (13). Briefly, 42 fetuses were examined at different gestational ages ranging from 20 to 38 wk. Each examiner obtained 2 images of each fetal anatomical variable under study at ≈ 5 min apart. Differences between the 2 measurements were expressed as the percentage of the measurement obtained from the technically best image of the 2. Percentage differences were used to take into account differences in the magnitude of the fetal anatomical variables at different gestational ages. Percentage differences for the 2 examiners were averaged, and the mean values were compared with zero and with each other by using a *t* test. Mean percentage differences between 2 measurements of fetal anatomical variables made by each examiner on the same subject at 5-min intervals were not statistically different from zero or between examiners. The magnitude of the observed differences was contained in the margin of error reported in previously published studies (13). In addition, measurement error was evaluated by calculating the intraclass correlation coefficients between the pairs of repeated measurements (14). Intraclass correlation coefficients were essentially 1.0 and were comparable with published data (15, 16).

Statistical analysis

Baseline characteristics were evaluated by supplement type by means of *t* test or chi-square analysis. Fetal growth measures were analyzed by using repeated-measures analysis of variance to examine differences by supplement type over time and interactions between supplement type and time. To describe the pattern of growth and the contrasts by supplement type, we also calculated *z* scores by comparing the mean of the variable for each supplement type at each time point with the median and SD around the median of the variable in a reference population (17). We applied polynomial regression methods, using the generalized estimating equations method, to test for differences in growth trends by supplement type, taking into account correlations between repeated measurements on the same subjects (18). This technique also enabled us to include subjects with some

TABLE 1
Characteristics of Peruvian newborns by prenatal supplement type¹

| | Iron + folic acid + zinc (<i>n</i> = 94) | Iron + folic acid (<i>n</i> = 101) |
|-------------------------|--|--|
| Gestational age (wk) | 39.8 \pm 1.1 ² | 39.7 \pm 1.0 |
| Female (%) | 51.1 | 49.5 |
| Birth weight (g) | 3351 \pm 427 | 3316 \pm 389 |
| Length (cm) | 49.9 \pm 1.7 [94] ³ | 49.7 \pm 1.9 [100] |
| Head circumference (cm) | 34.0 \pm 1.35 [91] | 33.8 \pm 1.3 [96] |

¹ There were no significant differences by supplement type.

² $\bar{x} \pm$ SD (all such values).

³ *n* in brackets (all such values).

missing data. To study the effect of zinc and advancing gestational age, we introduced into the model an interaction term between zinc supplementation and gestational age. Statistical significance was defined as $P < 0.05$, and data analysis was performed by using STATA statistical software, version 7.0 (Stata Corporation, College Station, TX).

RESULTS

The average woman enrolled in the study was 23 y of age, was a high school graduate, was 152 cm tall, had a body mass index (in kg/m^2) of 23.6, and had a plasma zinc concentration at 13 wk of gestation of 10.0 $\mu\text{mol}/\text{L}$. More than one-half of the women were having their first child. Previously, we showed that there were no significant differences in maternal characteristics at baseline by supplement type (11).

On average, the participants took 157 ± 27 tablets during pregnancy over a 26.3 ± 2.3 -wk period. On the basis of the number of days in the study, the median degree of compliance (10th, 90th percentiles) was 87% (71%, 97%), and no significant difference was found by supplement type: 86% (68%, 97%) in the iron + folic acid + zinc group and 88% (72%, 97%) in the iron + folic acid only group. On the basis of these calculations, the median frequency of supplement use was 6 d/wk, and 90% of the women took their supplements ≥ 5 d/wk.

Duration of pregnancy and neonatal size at birth did not differ significantly by supplement type (Table 1). Furthermore, no significant differences by supplement type were observed for biparietal diameter, head circumference, or abdominal circumference. However, as shown in Table 2, femur diaphysis length was greater in the iron + folic acid + zinc group across gestation ($P < 0.05$). The supplement \times time interaction was not significant ($P = 0.13$).

Shown in Table 3 are the coefficients from the generalized estimating equations models for the effect of maternal zinc supplementation on the average change in size of the 4 fetal anatomical variables. The coefficient for the effect of zinc was significant and positive only for femur diaphysis length, thus confirming an effect of zinc on this variable throughout gestation. The inclusion in the model of an interaction term between zinc supplementation and gestational age was significant ($P < 0.05$), suggesting that the effect of zinc on femur length increased with advancing gestational age. However, because the fit of this model was similar to the simple model (Table 3), the models are equivalent in describing the data.



TABLE 2Femur diaphysis length measurements at 20, 24, 28, 32, 36, and 38 wk of gestation in Peruvian fetuses by prenatal supplement type¹

| Supplement type | 20 wk (n = 89/100) ² | 24 wk (n = 94/99) | 28 wk (n = 94/100) | 32 wk (n = 94/101) | 36 wk (n = 94/101) | 38 wk (n = 84/92) |
|---------------------------------------|------------------------------------|----------------------|-----------------------|-----------------------|-----------------------|----------------------|
| | <i>mm</i> | | | | | |
| Iron + folic acid + zinc ³ | 33.1 ± 1.4 | 44.2 ± 1.3 | 53.7 ± 1.5 | 62.0 ± 1.7 | 68.9 ± 2.0 | 71.3 ± 2.1 |
| Iron + folic acid | 33.0 ± 1.3 | 43.7 ± 1.4 | 53.2 ± 1.5 | 61.7 ± 1.6 | 68.3 ± 2.1 | 70.6 ± 1.9 |

¹ All values are $\bar{x} \pm SD$.² Number of subjects in each group: iron + folic acid + zinc/iron + folic acid only.³ Values at all time points were significantly different from those in the iron + folic acid group, $P < 0.05$ (repeated-measures ANOVA); the supplement \times time interaction was not significant ($P = 0.13$).

DISCUSSION

Our results indicate that supplementing zinc-deficient mothers with 25 mg Zn/d may be beneficial to fetal bone growth. Femur diaphysis length was greater in fetuses whose mothers received zinc supplements ($P < 0.05$). The observed effect became apparent at 24 wk of gestation and represented at term an upward shift in mean femur diaphysis length of about one-quarter of the SD in femur length in a reference population (17). This effect may have important biological and clinical implications, because fetuses in the studied population showed a tendency in the second half of pregnancy to grow more slowly than the ultrasound-based reference fetal growth curves (17). This was also true for the other anatomical sites measured; however, no significant differences by supplement type were observed.

To our knowledge, this is the first study to show an effect of maternal zinc deficiency on fetal bone growth in humans. The observed positive effect of zinc on fetal femur diaphysis length agrees with the results of experimental studies in vitro and in animals that showed that zinc has a stimulatory effect on bone growth and that fetal bone metabolism and growth are negatively affected by maternal zinc deficiency. Zinc likely plays several roles in relation to bone metabolism (6). Zinc stimulates bone metabolism in rats and bone protein synthesis and bone formation in tissue cultures by increasing the activity of critical enzymes, such as alkaline phosphatase (19–21). Zinc has been shown to augment the anabolic effect of insulin-like growth factor I on osteoblasts, which are responsible for the formation and mineralization of the extracellular matrix of bone during endochondral ossification (22). In addition, zinc was shown to exert an inhibitory effect on the activity of the osteoclasts responsible for bone resorption (23, 24).

TABLE 3

Estimated average differences in growth measures over gestation between fetuses of zinc-supplemented women and fetuses of non-zinc-supplemented women


| | Difference ¹ | <i>P</i> |
|-------------------------|-------------------------|----------|
| | <i>mm</i> | |
| Head circumference | 0.18 ± 0.82 | 0.87 |
| Biparietal diameter | 0.05 ± 0.28 | 0.83 |
| Abdominal circumference | 0.28 ± 1.07 | 0.80 |
| Femur diaphysis length | 0.46 ± 0.19 | 0.01 |

¹ Polynomial regression coefficient (b_3) \pm SE for the polynomial regression model $y = b_0 + b_1(\text{gestational age}) + b_2(\text{gestational age})^2 + b_3(\text{zinc})$ (assuming exchangeable correlations between repeated measurements).

As shown, the effect of zinc was limited to fetal femur length. Evidence from animal studies suggests that maternal zinc deficiency negatively affects fetal skeletal growth and mineralization in long bones such as the femur by producing structural and functional modifications in the epiphyseal growth plate, where the process of bone growth occurs and where zinc is present in high concentrations (25–27). Golub et al (4) followed rhesus monkey infants born from moderately zinc-deficient mothers and given a zinc-deficient diet during the first year of life. When compared with infants of mothers who received a complete diet, those in the zinc-deficiency group showed significantly shorter femur diaphysis lengths at birth and during the first year of life. When the same rhesus monkey infants were examined by X-ray to evaluate skeletal maturation, they showed, compared with controls, retarded skeletal maturation and defective mineralization, which persisted up to 3 y of age (24, 25). These results were confirmed in an experiment by da Cunha Ferreira et al (26) in which maternal zinc deficiency was associated with histologic and histochemical abnormalities in the tibia of fetal rats. Both Leek et al's (27, 28) and da Cunha Ferreira et al's (26) results are consistent with defective endochondral ossification associated with maternal zinc deficiency. Thus, our finding that supplemental zinc did not affect other anatomical sites (biparietal diameter, head circumference, or abdominal circumference) that are related to somatic rather than bone growth may not be unexpected. A limitation of our study is that we did not assess the growth of other long bones of the fetus, nor did we assess bone mineralization and formation in the fetuses and neonates. Thus, we could not investigate specific biological mechanisms or associations that might have contributed to our observations, such as the association of defective endochondral ossification at the epiphyseal growth plate and maternal zinc deficiency observed in animal studies.

We did not observe any significant differences in birth length or weight by supplement type. These results agree with those of a previous study conducted in this same population (29). It is unlikely that the effects of maternal zinc supplementation on fetal femoral growth would be sufficient to effect differences in birth length detectable with standard neonatal anthropometric assessment techniques. We did not assess skeletal proportions of the neonates in general or measure leg length or its components in particular. There is growing interest in examining the nature of environmental influences on fetal femur length, skeletal proportions, and leg length in humans because of observed inverse associations with blood pressure, cardiovascular disease risk factors, and metabolic disorders (30–32). Thus, our initial finding of an effect of supplemental zinc on fetal femur length in a popu-



lation with marginal zinc intakes is provocative and worthy of future research. 

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LEC, JAD, MM, and NZ contributed to the study design, data collection, data analysis, and writing of the manuscript. KAC and AF contributed to the data collection and data analysis. FD contributed to the analysis and interpretation of the longitudinal models and the writing of the manuscript. None of the authors had any financial or personal interest in any company or organization sponsoring the research.

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