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ORIGINAL ARTICLES

# Iron and Zinc Supplementation Does Not Impact Urinary Arsenic Excretion in Mexican School Children

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**Objective** To examine the role of iron and zinc in arsenic excretion and metabolism in children.

**Study design** An analysis of urinary arsenic (UAs) concentrations from a double-blind randomized trial originally testing the efficacy of iron and zinc for lowering blood lead levels in children. A 2 × 2 factorial design was used, with children randomized individually, stratified by sex and classroom, to receive 30 mg ferrous fumarate (n = 148), 30 mg zinc oxide (n = 144), iron and zinc together (n = 148), or placebo (n = 151). Of the 602 children enrolled, 527 completed the 6-month treatment, and 485 had both baseline and final UAs values. The baseline total UAs concentration ranged from 3.2 to 215.9 μg/L.

**Results** At baseline, children in the highest tertile of serum ferritin concentration had higher excretion of dimethylarsinic acid (DMA; 1.93 ± 0.86%; $P < .05$), but lower excretion of monomethylarsonic acid (-0.91 ± 0.39%; $P < .05$), compared with children in the lowest tertile. In an intention-to-treat analysis, iron had no effect on arsenic methylation or UAs excretion, but children receiving zinc had lower %DMA in urine (-1.7 ± 0.8; $P < .05$).

**Conclusions** Iron and zinc status are not related to arsenic metabolism in children, and supplementation with these minerals has limited application in lowering arsenic concentrations. *(J Pediatr 2017;185:205-10).*

**Trial registration** ClinicalTrials.gov: NCT02346188.

Children are exposed to a number of chemicals, often in the context of underlying nutritional deficiencies, such as iron deficiency (ID).[1,2] Mixtures of metals are linked to poorer developmental outcomes and cognitive deficits.[3] Nutrient-based interventions designed to target a single metal toxicity might not consider the potential benefits or unintended consequences for other toxic metals. In 2000-2001, we conducted a randomized controlled trial to test the efficacy of a 6-month course of iron and zinc supplementation in lowering children's blood lead level (BLL).[4-6] The children were also exposed to arsenic, which we did not investigate in the original trial.

Arsenic affects growth and development.[7-9] Inorganic arsenic (iAs) is absorbed in the gut[10] and then undergoes methylation to monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in the liver.[11] Urinary excretion of MMA and DMA is the primary route of arsenic elimination[12]; thus, factors that affect the methylation and urinary elimination of arsenic may affect its toxicity. Investigations of the role of nutrients in arsenic excretion have focused mainly on folate, B vitamins, and selenium,[13-16] which, through involvement in one-carbon metabolism, affect the methylation of arsenic.

The role of minerals in iAs exposure or metabolism has received less attention.[17] In one of the few epidemiologic studies reported to date, higher intake of iron and zinc was associated with lower urinary %iAs and %MMA, but higher %DMA in a nationally representative sample of US adults.[18] Indian adults exposed to arsenic and presenting with skin lesions reported lower zinc intake, but not iron intake, compared with arsenic-exposed healthy controls.[19] In contrast, higher iron intake was not associated with urinary iAs or arsenic metabolites in Bangladeshi adults.[20] Basu et al[21] likewise found no association between iron and zinc intake and urinary arsenic (UAs) concentration in individuals from West Bengal. There are little available data in children, but a recent study found associations between ID and lower %MMA, but slightly higher %DMA.[22] Several animal studies have evaluated the effects of iron and zinc supplementation on arsenic exposure and toxicity,[23-26] and have suggested an effect on arsenic accumulation.

By leveraging data from Mexican school children who had participated in an iron and zinc supplementation trial, we had the unique opportunity to clarify

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| BLL | Blood lead concentration | ID | Iron deficiency |
| BMI | Body mass index | MMA | Methylarsonic acid |
| DMA | Dimethylarsinic acid | SES | Socioeconomic status |
| Hb | Hemoglobin | UAs | Urinary arsenic |
| iAs | Inorganic arsenic | | |

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potential links among children's micronutrient status, supplementation, and arsenic exposure and metabolism.

## Methods

This study was a secondary analysis of a double-blind randomized trial of iron and zinc supplementation conducted in 2000-2001 among Mexican schoolchildren to test the efficacy of micronutrients in lowering children's BLL. The design and main findings from that trial have been reported elsewhere.[4-6] In brief, all first-grade children (aged 6-7 years) attending 9 public elementary schools located in the vicinity of a metal foundry were invited to participate. Arsenic exposure likely occurred from drinking water, given the elevated levels of water arsenic detected in this region.[27] Children whose parents provided consent to participate in the study were assessed at baseline for total UAs, iAs, MMA, DMA, BLL, serum ferritin, and serum zinc concentrations. One child with a BLL ≥45 μg/dL was excluded; no children were excluded owing to hemoglobin (Hb) <9 g/dL.

Participants were randomized individually, stratified by sex and classroom, to receive 1 of 4 treatments: only iron (30 mg/day of ferrous fumarate), only zinc (30 mg/day of zinc oxide), iron plus zinc (30 mg/day of each), or placebo. The participants and researchers were blinded to the formulation and treatment assignments. Each child received the supplement daily at school. Parents were provided with an adequate supply of the supplement for extended absences and during summer vacation. Assessments of UAs metabolites and other biochemical and anthropometric measurements were repeated after 6 months of supplementation.

Ethics approval for all aspects of the randomized trial was obtained from the Human Research Committees at the Johns Hopkins Bloomberg School of Public Health and the Mexican National Institute of Medical Sciences and Nutrition.

Fasting blood draws for measurements of biochemical indicators and anthropometric measurements were performed at each school. After the blood draws, the children were given snacks. Children also provided first morning urine samples. The samples were kept on ice and later stored at −70 °C at the University of Juarez at Durango. Information on family socioeconomic status (SES) was obtained via a self-administered parental questionnaire.

For arsenic analysis, stored urine samples were thawed and warmed to 37 °C. Samples were then digested with HCl at 80 °C for 5 hours to release methylated compounds of arsenic.[28] IAs, MMA, and DMA were measured by hydride generation coupled with atomic absorption spectroscopy (AAnalyst 3100; PerkinElmer, Waltham, Massachusetts) following published protocol,[29] with coefficients of variation of 1%-9%. Arsenic analysis was carried out at the Center for Research and Advanced Studies, National Polytechnic Institute, Mexico City. The laboratory personnel were blind to treatment allocation. Values for %iAS, %MMA, and %DMA in urine were calculated based on the proportion of these species relative to UAs.

Hb was measured at the time of the blood draw in a drop of venous blood using a portable hemoglobinometer (HemoCue; Ängelholm, Sweden). Serum ferritin concentration was measured via an immunoradiometric assay using a commercially available kit (Coat-A-Count Ferritin IRMA; DPC, Los Angeles, California). Serum zinc level concentration was measured by atomic absorption spectrometry at the University of Queretaro. Anemia was defined as Hb <12.4 g/dL (altitude-adjusted cutoff value for this age group), iron deficiency (ID) was defined as serum ferritin <12 μg/L, and zinc deficiency was defined as serum zinc <65 μg/dL.

Although water arsenic concentrations were not measured at the individual household level, arsenic concentrations in water were available for the study period (1999, 2000/2001, and 2002) for the participating schools from the Torreón Municipal System of Water Administration (G.G. Vargas, personal communication). Because drinking water to private homes is supplied from the same source, we assumed that the school concentrations approximated household exposures.

### Statistical Analyses

The children were classified according to tertiles of Hb, serum ferritin, and serum zinc concentrations. These tertiles were modeled in ordinary least squares regressions as independent predictors of UAs, %iAs, %MMA, and %DMA. In addition, ID, anemia, and zinc deficiency were tested as predictors of UAs concentration. All regressions were adjusted for age (continuous variable), sex, body mass index (BMI; continuous), years living in Torreón (continuous), school, and family SES. We adjusted the models for BMI to account for any differences in children's ability to methylate arsenic based on body size or growth. It is important to note that the results did not differ when BMI-for-age *z*-scores were entered into the models instead of BMI. SES was estimated from the ownership of "luxury" items, such as automobile, computer, and video cassette recorder, and entered into regression models as a categorical variable. These covariates were chosen based on the literature and biological plausibility.[8,30]

### Main Effects of Iron and Zinc Supplementation on Arsenic Concentrations

We initially compared the distributions of various baseline characteristics among the 4 supplementation groups using 1-way ANOVA and $\chi^2$ statistics. With the 2 × 2 factorial design, we tested the main effects of iron and zinc supplementation, together with an interaction term. If the interaction was not significant, models were rerun, testing for main effects only. Intention-to-treat analysis was used, and each arsenic metabolite was tested separately. To account for potential differences in arsenic methylation due to nutritional status and growth, analyses were adjusted for BMI. As indicated above, there was no difference in findings when the models were adjusted for BMI-for-age *z*-score instead of BMI. Statistical significance was considered at $P < .05$.

### Sensitivity Analyses

School water served as a proxy for household arsenic exposure. Variation in iAs concentrations in the water supply could have influenced the study results. Four schools had a ≥20%