

# Efficacy of Iron and/or Zinc Supplementation on Cognitive Performance of Lead-Exposed Mexican Schoolchildren: A Randomized, Placebo-Controlled Trial

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## ABSTRACT

**OBJECTIVE.** Lead exposure in children has been associated with both global and specific cognitive deficits. Although chelation therapy is advised for children with blood lead concentrations of  $>44 \mu\text{g/dL}$ , treatment options for children with lower blood lead values are limited. Because lead absorption is related to children's nutritional status, micronutrient supplements may be 1 strategy for combating low-level, chronic lead exposure. This study was designed to test the efficacy of iron and zinc supplementation for lowering blood lead concentrations and improving cognitive performance in schoolchildren who live in a lead-contaminated city.

**METHODS.** This randomized, double-blind, placebo-controlled field trial was conducted in public elementary schools in Torreón, an industrialized city in northern Mexico. A metal foundry, located close to the city center and within 3.5 km of 9 schools, was the main source of lead exposure. A total of 602 children who were aged 6 to 8 years and regularly attending first grade in the study schools were enrolled. Children were given 30 mg of iron, 30 mg of zinc, both, or a placebo daily for 6 months. A total of 527 completed the treatment, and 515 were available for long-term follow-up, after another 6 months without supplementation. Eleven cognitive tests of memory, attention, visual-spatial abilities, and learning were administered at baseline and each follow-up.

**RESULTS.** There were no consistent or lasting differences in cognitive performance among treatment groups.

**CONCLUSIONS.** Daily supplementation with iron and/or zinc may be of limited usefulness for improving cognition in lead-exposed schoolchildren. However, these treatments may be effective in settings with higher prevalence of nutritional deficiencies or in younger children.

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### Key Words

child, cognitive function, lead toxicity, nutritional deficiency

### Abbreviations

PbB—blood lead concentration  
 CDC—Centers for Disease Control and Prevention  
 ID—iron deficiency  
 SZn—serum zinc concentration  
 CI—confidence interval  
 TLC—Treatment of Lead-Exposed Children Trial Group

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**T**HE ADVERSE EFFECTS of lead exposure on cognitive performance in children have been established during the past few decades. There is a range of susceptibilities and responses to lead among children,<sup>1</sup> resulting in a lack of a “behavioral signature.”<sup>2</sup> Nevertheless, adverse effects have been demonstrated in several cognitive domains, with deficits in performance on tests of global functioning, such as IQ, being reported most frequently and consistently.<sup>1</sup> School performance in math and reading, executive functions, visual-spatial abilities, and attention have also shown adverse associations with blood lead concentrations (PbBs).<sup>3</sup> Recent studies have focused on very low-level lead exposure and suggest that a PbB of  $<10 \mu\text{g/dL}$  is not as safe as previously thought. One study found deficits in nonverbal reasoning, memory, and achievement in children who were aged 6 to 16 years at PbBs of  $<10 \mu\text{g/dL}$  and deficits in tests of reading and arithmetic at levels as low as  $5 \mu\text{g/dL}$ .<sup>4</sup> Another group reported adverse associations with full and performance IQ and select measures of attention, also at PbBs of  $<10 \mu\text{g/dL}$ .<sup>5</sup> Finally, a longitudinal assessment of preschool children that was conducted in Rochester, NY, suggested a nonlinear relation between PbB and cognitive outcomes. The lead-cognition association was characterized by steeper slopes below, rather than above, a PbB of  $10 \mu\text{g/dL}$ .<sup>6</sup>

The Centers for Disease Control and Prevention (CDC) recommends that children who are exposed to lead be relocated to safer living conditions or that the source of exposure be removed. However, certain lead sources, such as industrial emissions, cannot be removed easily.<sup>7</sup> Furthermore, although awareness of harm as a result of environmental exposures is growing, many developing countries may not have the infrastructure that would protect vulnerable populations.<sup>8</sup> Even in the United States, where resources for the prevention and treatment of lead exposure are considerable, medical treatment (chelation) is not recommended for children with low to moderate PbBs. Other treatment or prevention measures are needed, and nutritional interventions may provide an attractive solution. If efficacious, supplementation could be scaled into larger programs or adapted by individual families.

There is evidence that iron and zinc are physiologically linked to lead metabolism and that their deficiencies are associated with diminished cognitive performance. In addition, supplementation with iron or zinc has proven successful in reducing certain cognitive impairments that are associated with deficiencies of these micronutrients, even in children who are 6 years or older.<sup>9–12</sup>

Iron deficiency (ID) and elevated PbBs have been reported to occur together in disadvantaged pediatric populations.<sup>13,14</sup> In young children who attended an urban clinic, ID at 1 visit significantly predicted PbBs of  $10 \mu\text{g/dL}$  or higher at a subsequent visit.<sup>15</sup> There is specu-

lation that iron and lead compete for absorption in the small intestine<sup>16,17</sup> and that in children who consume diets with inadequate amounts of iron, lead absorption is increased.<sup>18</sup> Wolf et al<sup>19</sup> found in a nonrandomized trial that 3-month treatment with oral iron lowered PbBs among nonanemic iron-depleted children who were aged 13 to 24 months. The magnitude of the PbB decline was related to the magnitude of change in serum ferritin (SF) concentrations. Zinc also seems to play an important role in lead metabolism. Kumar et al<sup>20</sup> found that 3 months of zinc treatment effectively reduced lead concentrations in kidneys and lungs of rats that were exposed to lead by 20% and 51%, respectively, compared with untreated controls.

The CDC recommends that parents pay attention to their children’s nutrition (especially when ID is suspected) and provide meals that contain plenty of iron,<sup>7</sup> partly because iron may reduce ID-associated pica. However, to our knowledge, no double-blind, randomized trial of micronutrient supplementation has been conducted among lead-exposed children. We hypothesized that iron and/or zinc would improve cognitive function of schoolchildren, either through their independent actions or by reducing PbBs. We further expected that children with higher PbBs at baseline would benefit more from treatment than would children with lower exposures. We present here the effects of iron and/or zinc treatment on cognitive function of Mexican schoolchildren who were exposed to lead from a metal foundry. These results are part of a large randomized, placebo-controlled trial that also assessed the efficacy of iron and/or zinc supplementation on PbBs<sup>21</sup> and behavior<sup>22</sup> of lead-exposed children.

## METHODS

### Study Design

This was a randomized, double-blind, placebo-controlled trial of iron and/or zinc supplementation, conducted in the city of Torreón, in northern Mexico. The main source of lead exposure was a metal foundry. The study began in February 2001 and was completed in June 2002. The children were evaluated at baseline (T1) with a battery of cognitive tests, and their blood was taken for determination of lead and micronutrient status. Subsequently, they were supplemented for 6 months. Immediately after the end of treatment (T2) and again after another 6 months without supplementation (T3), the children were evaluated with the same measures as at baseline and had blood drawn for measurement of biochemical indicators. The effects of supplementation (from T1 to T2) are referred to as short-term effects. The long-term effects of treatment are those that were evaluated at T3. This study was approved by research ethics committees at the Johns Hopkins Bloomberg School of

Public Health and the National Institute of Medical Sciences and Nutrition in Mexico.

### Study Sample

First-graders ( $n = 724$ ) from 9 public elementary schools that are located within a 3.5-km radius of the foundry were invited to participate (Fig 1). Written parental consent was solicited during informational meetings held at each school before the study began. Parents could give consent at the meetings or return the forms at a later date. Consent was obtained for 602 children. One child with PbB of  $>45 \mu\text{g/dL}$ , an exclusion criterion, was referred for clinical treatment. No children were excluded on the basis of hemoglobin (criterion  $<9 \text{ g/dL}$ ). The sample size needed for the study (500 children overall) was calculated to detect a  $2.2\text{-}\mu\text{g/dL}$  change in mean PbB after supplementation at  $\alpha$  level of .05 and power of 80%. A 15% loss to follow-up was taken into account in the calculations. We overenrolled to give all of the first-graders at the schools a chance to participate. Although the sample size was not calculated on the basis of cognitive outcomes, with 602 children at

baseline, we had 80% power to detect a main effect of 3.7, 4.8, and 3.6 points on the Peabody Picture Vocabulary Test, the Math Achievement Test, and the Freedom From Distractibility factor, respectively, the 3 global measures of cognition that we used in our study.

### Supplementation Procedure

Participants were randomized individually to 1 of 4 treatments: iron only, zinc only, both iron and zinc, or placebo. The randomization was conducted by 2 of the authors (K.K. and L.P.) and was conducted separately for boys and girls and for all of the classrooms in each school. Iron was given as 30 mg of ferrous fumarate and zinc as 30 mg of zinc oxide. The combined treatment had 30 mg of iron and 30 mg of zinc. All tablets were white and indistinguishable from each other. For the purpose of blinding, each supplement was given a letter code, and neither the researchers nor the participants were aware of which letter referred to which formulation. The formulations were kept in a sealed envelope at the National Institute of Medical Sciences and Nutrition in

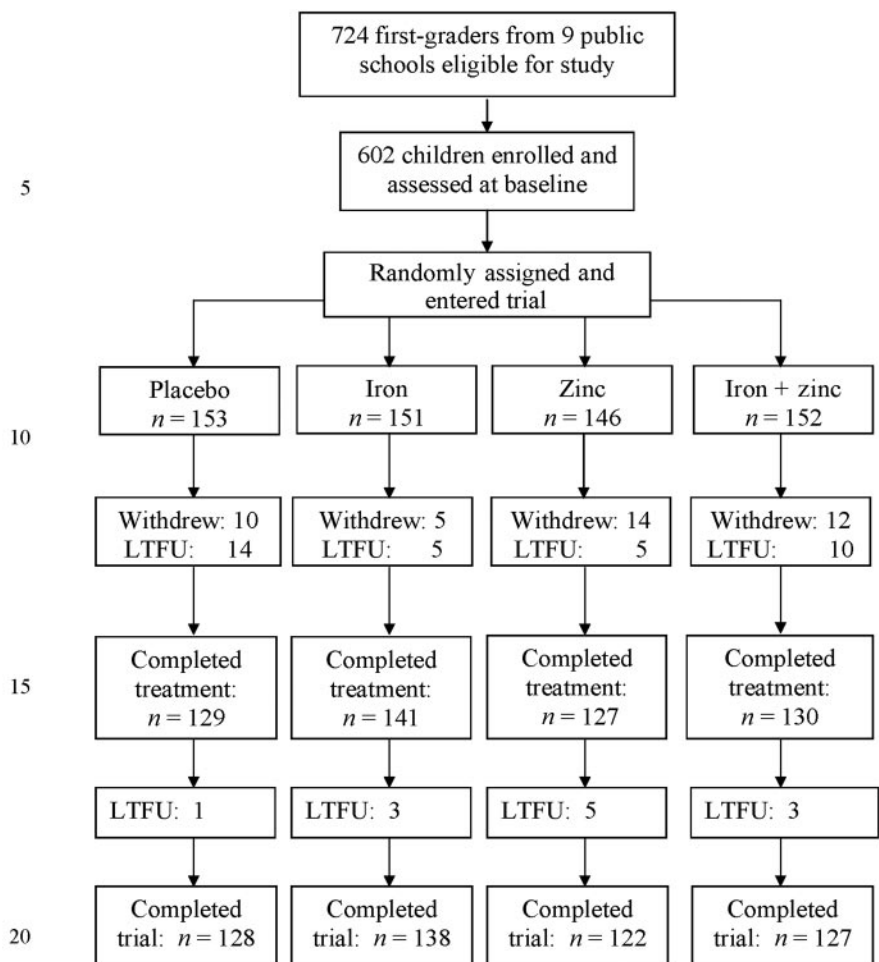


FIGURE 1  
Trial profile. LTFU, lost to follow-up.

Mexico City until the trial had ended. We are unaware of any attempts by parents or children to guess their tablet assignment, but this was not assessed formally.

The supplements were distributed daily in each classroom ( $n = 23$ ) during the school week by nursing students. The tablets were administered in the morning during the first hour of school. Children who experienced nausea received the supplements from their parents after school. The supplement was given individually to each child, and the nurse made sure that the tablet was swallowed. For children who missed school for extended periods of time, parents received an adequate supply of tablets for supplementation at home. During vacations, the tablets were distributed at children's homes every 2 weeks. Tablet consumption was recorded daily at school and during the summer, every 2 weeks, based on parental reports. Except for initial nausea or diarrhea, we did not note any adverse effects of treatment.

### Cognitive Assessment

The testing battery consisted of 14 paper-and-pencil or computer-based tests that covered various aspects of cognitive functioning (Table 1). The tasks were either previously validated with Mexican-American children (Wechsler Intelligence Scale for Children—Revised Mexican version [WISC-RM] and Peabody Picture Vocabulary Test) or pilot-tested by us with first- and second-graders in a public elementary school in Mexico City. The entire WISC-RM<sup>23</sup> battery was not administered because of time constraints; the Coding, Arithmetic, and Digit Span tasks have been used previously in lead-exposed children and can be combined to create the Freedom From Distractibility factor, which, like an IQ score, can be scaled to a mean of 100 and SD of 15 points<sup>24</sup> (p 816). This abbreviated WISC-RM battery was pilot-tested by us but was not validated with a Spanish-speaking sample of similar age range. Two other tasks (Number and Letter Sequencing) were combined into 1

**TABLE 1** Description of Cognitive Tasks, in Order of Administration

| Task  | Description   |
|---|---|
| Peabody Picture Vocabulary Test <sup>37</sup>                   | Test of receptive vocabulary. Consists of 111 cards, with 4 black-and-white sketches and 1 stimulus word per card. Children choose the picture that is best described by a given word.  |
| Math Achievement Test   | Measures performance on math curriculum. Consists of 39, 78, and 78 items for the first, second, and third evaluations, respectively. The questions were presented in both numerical and pictorial forms and tested proficiency in counting, 1-to-1 correspondence, addition, subtraction, counting by 10s, multiplication, and division.   |
| Freedom From Distractibility factor <sup>21</sup>               | Composed of 3 subscales from the Wechsler Intelligence Scale for Children—Revised, Spanish Language Version. The Arithmetic subscale measures numerical knowledge and reasoning skills. The Coding subscale tests learning of unfamiliar tasks. It requires attention, short-term memory, cognitive flexibility, and visual-motor speed and accuracy. The Digit Span measures auditory short-term memory and attention.                     |
| Sternberg Memory  | This is a measure of visual short-term memory scanning. A string of capital letters appears briefly on the computer screen, followed by a single capital letter. The child touches the screen to indicate whether the single letter was among the initial string.   |
| Figure Matching   | A set of figures are shown simultaneously on a computer screen. A probe stimulus appears in the upper half of the screen, and several response stimuli appear in the lower half. The child points out the figure in the bottom row that matches the probe on top.   |
| Stimulus Discrimination   | A single capital letter or object is shown briefly on the screen, followed by another screen with a string of different letters or objects. The child touches the letter that appeared initially.   |
| Cognitive Abilities Test: Stimulus Discrimination <sup>38</sup> | Measures the ability to recognize and discriminate a figure from visually similar figures. The child uses a keypad and a touch-sensitive computer monitor to respond. First, a probe stimulus is flashed briefly onto the screen. Then, 6 similar-looking stimuli (including the probe) appear simultaneously on the screen. The child is instructed to touch the stimulus that matches the probe.  |
| Visual Memory Span  | Tests the ability to recall a sequence of spatial locations. An array of 12 squares of different colors appear on the screen. These squares are illuminated briefly in random order. After a brief delay, the child is asked to touch the squares that were illuminated, repeating the exact order. The task begins with 1 square and progresses to 12.   |
| Visual Search   | This is a test of visual selective attention and visual-motor speed. The child views a screen displaying many small rectangular boxes, each containing a single letter "b," "d," or "p" with 0, 1, or 2 dots next to, above, or below the letter (eg, "b." or ".p"). The child touches, as quickly as possible, only the boxes with a "d" and 2 dots anywhere inside the rectangle.   |
| Sequencing <sup>39</sup>  | Adapted from part A of the Trail Making Test, this task involves selective attention and mental flexibility. Both number and letter versions were administered. In the numbers version, children use a marker to draw a line connecting, in proper sequence, as many encircled numbers (4–18) that are randomly distributed on a page as possible in 60 seconds. In the letters version, children connect letters (D–P, including Ñ and Q). |
| Figure Design   | Children are shown an outline of a figure and are asked to construct that figure with a given set of puzzle blocks (squares, triangles, or diamonds). Three levels of assistance are given upon request: showing the outline with 1 block drawn in, outline with all pieces drawn in, or allowing design to be constructed on top of the complete outline. Figures range from 2- (practice) to 7-block designs.                             |

Sequencing score, and the total number of correct responses was analyzed. Altogether, 11 outcomes are presented here.

For administration, the tests were divided into 2 groups, given on 2 different days by a team of 10 Mexican psychologists. There was a  $2.7 \pm 1.7$ -day lapse between the 2 sessions. All testers underwent an extensive training by a child psychologist (J.A.R.). Each tester saw 3 or 4 children every day of testing, and testing sessions lasted 65 to 70 minutes. Testing took place at the schools, in an isolated room, with as little noise and distraction as possible. The testers were unaware of children's lead or micronutrient status.

For repeated assessments at T2 and T3, the Math Achievement Test was designed to have parallel forms and additional questions that reflected the increasing difficulty of the mathematics curriculum. For the other outcomes, the same tests were administered at all 3 times. We expected improved performance at each time based on a learning curve. We also expected this improvement to be equal in all 4 groups, including the placebo, in which case differences in scores would be attributable to the intervention.

### Laboratory Measures

Fasting venous blood samples were obtained from children at their schools. The samples were kept on ice and transported to the laboratory, where they were separated and aliquoted. Whole blood lead and serum zinc concentrations (SZn) were measured using atomic absorption spectrophotometry. The method for PbB assessment is described elsewhere.<sup>25</sup> The method used to measure serum zinc was described by Makino and Tahara.<sup>26</sup> SZn was determined in triplicate, with a coefficient of variation of 5% or less. Serum ferritin was analyzed using an immunoradiographic method (Coat-A-Count Ferritin IRMA, DPC, Los Angeles, CA).

### Covariates

Covariates were chosen to include well-documented predictors of intellectual outcomes in children: child's age, gender, possessions, home ownership, crowding, maternal education, parents' involvement in schooling, birth order, family structure, and school. All information was taken from teacher and parent questionnaires. All models that contained the covariates were checked for multicollinearity by examining variance inflation factors; no multicollinearity was detected.

Economic status was approximated by a family possessions variable, which reflects the household ownership of 3 items: a car, a computer, and a VCR. The variable's construction is described in detail elsewhere.<sup>22</sup> Home ownership was scored 0 or 1 and crowding in the home as the number of family members per room. Maternal education was scored as having or not having any high school education. The frequency with

which children forgot homework (always, sometimes, or never) was a proxy for parents' involvement in children's schooling. Birth order included 3 categories: first child, second child, and third child and beyond. Family structure was coded as 2-parent or other arrangement.

### Data Analysis

The analysis was performed using Stata 6.0 (Stata Corp, College Station, TX). Multiple linear regressions were used to estimate the effects of each treatment on the mean change in cognitive performance. Short- and long-term effects were analyzed separately. Additional analyses were stratified by baseline lead category (PbB  $<10$  or  $\geq 10$   $\mu\text{g}/\text{dL}$ ) and gender. Both of these analyses were specified a priori. Iron by zinc interaction terms were included in all models and reported at  $P < .05$ . For models with nonsignificant interactions, only the main effects were reported. We did not adjust for multiple comparisons. All analyses were adjusted for baseline lead concentration and baseline performance on a given test. The analyses that were stratified by baseline lead concentration were additionally adjusted for the covariates described above. All analyses were performed on an intention-to-treat basis, regardless of compliance; missing values were not imputed.

### RESULTS

The mean  $\pm$  SD baseline PbB was  $11.5 \pm 6.1$   $\mu\text{g}/\text{dL}$ , and 51% had levels  $\geq 10$   $\mu\text{g}/\text{dL}$ . The overall prevalence of children with depleted iron stores (SF  $<15$   $\mu\text{g}/\text{L}$ ) and zinc deficiency (SZn  $\leq 65$   $\mu\text{g}/\text{dL}$ ) was 21.7% and 28.9%, respectively. The supplementation groups did not differ on the prevalence of nutritional deficiencies or other demographic characteristics.<sup>22</sup> However, the iron group had a slightly higher PbB ( $12.7 \pm 7.3$   $\mu\text{g}/\text{dL}$ ) at baseline than the placebo ( $10.8 \pm 5.5$   $\mu\text{g}/\text{dL}$ ;  $P = .056$ ) or the zinc group ( $10.9 \pm 5.4$   $\mu\text{g}/\text{dL}$ ;  $P = .096$ ). All analyses of treatment effects were adjusted for baseline PbB. Lead at baseline was associated with cognitive performance (Table 2) and explained 0.6% to 4.6% of variability in test scores of the entire sample.

### Supplementation Diagnostics

The duration of supplementation varied for individual children, ranging from 107 to 175 days. On average, children received supplementation for  $147 \pm 15$  days, with a 91% compliance. The treatment groups did not differ on these indicators. Of 602 children enrolled, 527 completed treatment (T2), and 515 were evaluated for long-term effects (T3; Fig 1).

### Summary of Supplementation Effects on Blood Lead

There was a significant main effect of iron on blood lead at T2, such that children who had received any iron experienced a mean decline in PbB of 0.3  $\mu\text{g}/\text{dL}$  (95% confidence interval [CI]:  $-0.6$  to  $-0.04$ ). The main ef-

**TABLE 2** Baseline Association Between Cognitive Performance and Lead for 594 Children

| Task <sup>a</sup>  | Mean (SD)    | Range    | $\beta^b$ | $P^b$ |
|--|--------------|----------|-----------|-------|
| General Abilities and Learning                                       |              |          |           |       |
| PPVT Standard score  | 103.2 ± 15.6 | 55–145   | -.56      | <.01  |
| MAT Final score  | 31.3 ± 7.5   | 3–52     | -.22      | <.01  |
| Freedom From Distractibility factor                                  | 94.4 ± 15.9  | 52–133   | -.33      | <.01  |
| Attention and Memory   |              |          |           |       |
| Sternberg Memory   | 12.1 ± 2.9   | 4–20     | -.05      | <.01  |
| Figure Matching  | 24.7 ± 3.7   | 8–32     | -.07      | <.01  |
| Stimulus Discrimination  | 17.6 ± 3.8   | 0–20     | -.04      | .14   |
| Cognitive Abilities Test, Stimulus Discrimination mean decision time | 6.0 ± 1.7    | 3.0–16.3 | .02       | .14   |
| Visual Memory Span   | 6.5 ± 3.5    | 2–24     | -.05      | .034  |
| Visual-spatial abilities   |              |          |           |       |
| Visual Search  | 19.1 ± 7.3   | 0–37     | -.07      | .13   |
| Sequencing   | 7.3 ± 4.2    | 0–26     | -.09      | <.01  |
| Figure Design, final score   | 19.7 ± 6.0   | 2–40     | -.06      | .13   |

PPVT indicates Peabody Picture Vocabulary Test; MAT, Math Achievement Test.

<sup>a</sup> Unless specified, number correct was the measure of interest and was used in regression analysis.

<sup>b</sup> Unstandardized  $\beta$  coefficient from an unadjusted bivariate regression model; lead entered as a continuous variable.

fect of zinc was not significant (0.1; 95% CI: -0.2 to 0.3). There were also no significant interactions.<sup>21</sup>

### Treatment Effects on Cognitive Performance

With time, performance on all tasks improved significantly. However, increase in scores was not related to the supplementation. With 11 outcomes and 2 follow-

ups, we generated 44  $P$  values for the planned factorial analyses (40  $P$  values for main-effects analyses and 2 interaction  $P$  values), most of which were nonsignificant. In fact, only 3 (7%) of the treatment effects examined were significant at the conventional  $P = .05$  (Table 3); all other estimates had wide CIs that included 0 (Fig 2).

**TABLE 3** Selected Effects of Supplementation on Cognitive Performance in Lead-Exposed Children

| Task  | Main Effect Iron              | Main Effect Zinc                | Interaction                   |
|---|-------------------------------|---------------------------------|-------------------------------|
| Short-term effects <sup>a,b,c</sup>         |                               |                                 |                               |
| PPVT  | -0.5 (-2.3 to 1.3)            | 1.1 (-0.7 to 2.9)               | —                             |
| MAT   | -0.3 (-2.7 to 2.0)            | -1.5 (-3.8 to 0.9)              | —                             |
| Freedom From Distractibility                | -1.3 (-3.2 to 0.5)            | -0.4 (-2.2 to 1.5)              | —                             |
| Sternberg                                   | -0.8 (-1.5 to -0.1)           | -0.4 (-1.1 to 0.3)              | 1.0 (0.1 to 2.0) <sup>d</sup> |
| Figure Matching                             | 0.1 (-0.4 to 0.7)             | -0.1 (-0.7 to 0.4)              | —                             |
| Stimulus Discrimination, correct            | 0.6 (0.2 to 1.0) <sup>d</sup> | 0.0 (-0.4 to 0.4)               | —                             |
| Stimulus Discrimination, mean decision time | -0.1 (-0.2 to 0.1)            | -0.1 (-0.3 to 0.1)              | —                             |
| Visual Memory Span                          | 0.1 (-0.6 to 0.9)             | 0.1 (-0.6 to 0.9)               | —                             |
| Visual Search                               | -0.1 (-1.3 to 1.1)            | 0.2 (-1.0 to 1.4)               | —                             |
| Sequencing                                  | 0.2 (-0.5 to 1.0)             | 0.1 (-0.7 to 0.8)               | —                             |
| Figure Design                               | 0.0 (-0.8 to 0.7)             | 0.7 (-0.04 to 1.4) <sup>e</sup> | —                             |
| Long-term effects <sup>c</sup>              |                               |                                 |                               |
| PPVT  | -0.6 (-2.4 to 1.1)            | -0.2 (-1.9 to 1.5)              | —                             |
| MAT   | -0.7 (-3.4 to 1.9)            | -2.2 (-4.8 to 0.4)              | —                             |
| Freedom From Distractibility                | -0.6 (-2.5 to 1.3)            | -0.4 (-2.5 to 1.5)              | —                             |
| Sternberg                                   | -0.3 (-0.8 to 0.1)            | 0.2 (-0.2 to 0.7)               | —                             |
| Figure Matching                             | -0.3 (-0.8 to 0.2)            | 0.0 (-0.4 to 0.6)               | —                             |
| Stimulus Discrimination, correct            | 0.0 (-0.4 to 0.3)             | -0.1 (-0.4 to 0.3)              | —                             |
| Stimulus Discrimination, mean decision time | 0.0 (-0.2 to 0.1)             | -0.1 (-0.2 to 0.1)              | —                             |
| Visual Memory Span                          | 0.3 (-0.5 to 1.2)             | -0.1 (-0.9 to 0.8)              | —                             |
| Visual Search                               | 0.4 (-1.0 to 1.8)             | 0.0 (-1.4 to 1.3)               | —                             |
| Sequencing                                  | -0.4 (-1.3 to 0.5)            | 0.9 (0.03 to 1.8) <sup>d</sup>  | —                             |
| Figure Design                               | 0.0 (-0.4 to 1.1)             | 0.4 (-0.4 to 1.1)               | —                             |

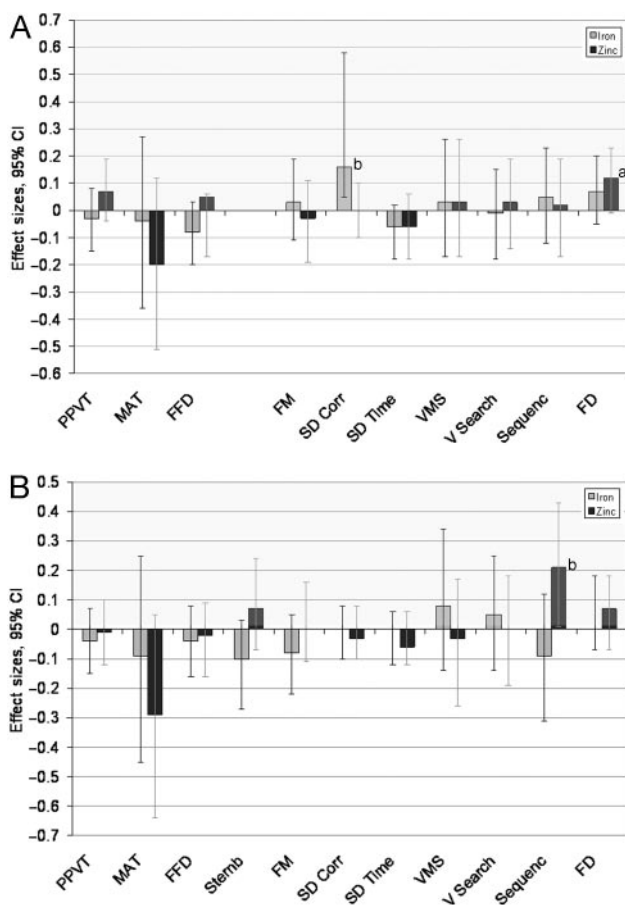
<sup>a</sup> Reported as  $\beta$  (95% CI); dashes signify lack of interactions.

<sup>b</sup> Adjusted for baseline performance and baseline lead concentration.

<sup>c</sup> Short-term analysis was based on a sample of 519 children, and long-term analysis was based on a sample of 506 children.

<sup>d</sup>  $P < .05$ .

<sup>e</sup>  $P < .1$ .



**FIGURE 2** Normalized short-term (A) and long-term (B) effects of iron and zinc on cognitive performance of lead-exposed children. Scores that fall in the shaded area show positive main effects of iron and zinc; scores that fall in the white area represent micronutrients that performed worse than placebo. The main effect of iron was calculated as score  $\Delta$  in children who received any iron – score  $\Delta$  in children who did not receive any iron. Main effect of zinc was calculated similarly. For obtaining effect sizes, the main effects of iron and zinc with their 95% CIs were divided by the SD of each cognitive measure at baseline. PPVT indicates Peabody Picture Vocabulary Test; MAT, Math Achievement Test; FFD, Freedom From Distractibility; Sternb, Sternberg Memory Test; FM, Figure Matching; SD Corr, Stimulus Discrimination, number correct; SD Time, Stimulus Discrimination Task, mean decision time; VMS, Visual Memory Span; V Search, Visual Search; Sequenc, Sequencing; FD, Figure Design. Short-term main effects on Sternberg Memory test not shown—for interaction see Table 3. <sup>a</sup> $P < .1$ ; <sup>b</sup> $P < .05$ .

The short-term analysis revealed a positive main effect of iron on the number of correct responses on the Stimulus Discrimination task ( $P = .007$ ; Table 3). There was also a significant treatment interaction on the Sternberg memory task ( $P = .036$ ), such that children who received iron only performed worse than any other group (predicted values: iron only  $1.7 \pm 0.2$  points; zinc only:  $2.2 \pm 0.2$ ; both:  $2.4 \pm 0.2$ ; placebo:  $2.6 \pm 0.2$ ). The positive main effect of zinc on Figure Design did not reach statistical significance ( $P = .064$ ; Table 3). At a subsequent follow-up, these effects were no longer present. However, a beneficial main effect of zinc on the Sequencing Task (number correct) did emerge at T3 ( $P = .043$ ; Table 3).

## Subgroup Analyses

### Boys Versus Girls

Boys and girls responded differently to supplementation on select tasks (data not shown). In the short term, girls experienced a negative main effect of iron on Peabody Picture Vocabulary Test performance ( $-2.6$  points; 95% CI:  $-5.3$  to  $-0.02$ ) and a positive main effect of zinc on the Visual Memory Span ( $1.3$  points; 95% CI:  $0.2$  to  $2.4$ ). In boys, zinc had a negative effect on the Math Achievement Test ( $-3.5$  points; 95% CI:  $-6.7$  to  $-0.3$ ). There was also an iron by zinc interaction on the Sternberg Memory test ( $P = .022$ ), in that the iron-only group performed worst (predicted value:  $1.6 \pm 0.3$  points), whereas the zinc-only ( $2.2 \pm 0.3$ ) and the combined group ( $2.8 \pm 0.3$ ) did not differ from placebo ( $2.6 \pm 0.3$ ). Over the long term, the negative effect of zinc on math performance in boys became stronger ( $-4.5$  points; 95% CI:  $-4.1$  to  $-0.9$ ). The group differences on the Sternberg Memory Test remained ( $P = .044$ ). The long-term analysis revealed new significant effects for girls: a positive main effect of zinc on the Sequencing performance ( $1.4$  points; 95% CI:  $-0.004$  to  $2.8$ ) and 2 interactions. Girls who received iron or zinc alone performed more poorly on the Math Achievement Test than girls who received either the placebo or the combined treatment. Conversely, the combined treatment resulted in worse performance than placebo on the Visual Search task, whereas iron-only and zinc-only groups performed better compared with placebo ( $P = .04$ ).

### Baseline PbB Categories ( $<10$ vs $\geq 10$ $\mu\text{g/dL}$ )

There were no consistent supplementation effects by baseline lead status. In the short term, select tasks that require attention and visual spatial abilities benefited from supplementation. For example, among children who had baseline PbB of  $<10$   $\mu\text{g/dL}$ , iron improved the mean decision time on the Stimulus Discrimination task ( $-0.3$ ; 95% CI:  $-0.5$  to  $0.0005$ ), and zinc improved scores on the Figure Design task ( $1.6$  points; 95% CI:  $0.5$  to  $2.6$ ). Among children with baseline PbB of  $\geq 10$   $\mu\text{g/dL}$ , iron improved the Stimulus Discrimination score ( $0.7$  points; 95% CI:  $0.1$  to  $1.4$ ). However, there was a negative main effect of iron on the Freedom From Distractibility score ( $-2.6$  points; 95% CI:  $-5.1$  to  $-0.04$ ). Over the long term, zinc had a beneficial effect on the Sequencing Score ( $1.8$  points; 95% CI:  $0.4$  to  $3.5$ ) and the mean decision time on the Stimulus Discrimination task ( $-0.3$ ; 95% CI:  $-0.5$  to  $-0.1$ ) for children with baseline PbB of  $<10$   $\mu\text{g/dL}$ . Among children with baseline PbB of  $\geq 10$   $\mu\text{g/dL}$ , there was a negative main effect of iron on the Figure Matching score ( $-0.8$  points; 95% CI:  $-1.6$  to  $-0.1$ ).

## DISCUSSION

This study examined the efficacy of treating lead-exposed schoolchildren with iron, zinc, or both. Specifi-

cally, the effects of iron and zinc on cognitive performance were examined immediately after a 6-month supplementation period and again after another 6 months without treatment. At both the short- and the long-term follow-ups, there was an overall improvement in scores on cognitive tests, as expected with age and practice. However, very few changes could be attributable to iron, zinc, or both (Table 3, Fig 2). Where the effects of treatment were significant or approaching significance, they were small in magnitude. There were also no consistent differences in the effects of treatment on cognition by gender or baseline lead status. In general, when viewed in light of the number of tasks and comparisons that were conducted (main effects and interactions), the few significant effects that were found in this study do not demonstrate convincingly that micronutrients could meaningfully improve cognitive performance in lead-exposed schoolchildren. Our findings suggest that lead exposure in children should be avoided through primary prevention because lead-associated cognitive deficits may not be reduced easily.

There are several possible explanations for these negative findings. It is possible that our treatment was ineffective because the children in our sample had been exposed to lead for a long time, many since birth, and the negative effects of lead exposure are irreversible by the early school age. In fact, supplementation had a very small effect on PbB, with an iron-attributable decrease of only 2.6%, which is smaller than the effect sizes (6–25%) reported in environmental intervention studies.<sup>27</sup> Furthermore, after treatment was discontinued, most effects on lead and other biochemical indicators were not lasting and became attenuated. Animal studies show that significant reductions in blood lead precede reductions in brain lead, and although PbBs drop to very low concentrations, significant elevations of brain lead may still persist.<sup>28,29</sup> In our study, lack of any substantial or lasting effects on PbB suggests that iron and/or zinc treatment in this age group, at the doses that we gave (30 mg of each micronutrient) and during the time period that we tested (6 months), was unlikely to have changed lead concentrations in the brain. We could speculate that without significant changes in brain lead levels, cognitive effects might not be achievable.

Our study was based in part on the premise that lead utilizes iron's absorptive pathway,<sup>16</sup> the divalent metal transporter 1<sup>17,30</sup> to enter the gastrointestinal track, and that higher intake of iron would compete with lead for absorption. However, recent *in vitro* evidence suggests that lead is able to enter the enterocyte (an absorptive cell in the small intestine) even when the divalent metal transporter 1 is knocked out.<sup>31</sup> Without an antagonism or with a separate lead absorptive pathway that could be used even if iron and lead do compete for absorption, a displacement of lead with micronutrients would be significantly lower than expected.

Our population was relatively well off in terms of micronutrient status. The prevalence of anemia and iron deficiency was low. Zinc deficiency was moderately high. Whereas epidemiologic evidence linking iron and lead exposure suggests that lead absorption is greater in children with ID,<sup>15,18</sup> some studies of iron supplementation in school-aged children suggest that treatment is most effective for improving cognitive performance in children who have ID anemia.<sup>9,32</sup> It is possible, therefore, that in a population with more prevalent iron and zinc deficiencies, supplementation would have been more successful.

Another recent intervention trial for lead-exposed children was unsuccessful in improving cognitive outcomes of young children (Treatment of Lead-Exposed Children Trial Group [TLC]<sup>33,34</sup>). In the original study, 780 children who were aged 12 to 33 months were randomly assigned to receive a 26-day course of either succimer or placebo. Despite an initial drop in PbB in the succimer group, PbBs rebounded and were indistinguishable from the placebo group by week 50 of the study. No significant group differences were found on a battery of neuropsychological tests 3 years after the treatment was discontinued. The children in the TLC study were also followed until school entry, when they were given tests similar to those administered in our study. By then, PbBs in the 2 TLC groups were similar and had declined with age, but no treatment effects on cognitive performance were detected. The TLC findings, together with our own results, suggest that treatment of lead-exposed children may produce limited benefits if exposure is prolonged or children's PbBs are pronounced. This may be attributable to a stable nature of lead's adverse effects or to the incomplete removal of lead from tissues, thus creating a situation of endogenous exposure, manifested as rebounding of PbBs after chelation therapy.<sup>35</sup> Therefore, findings from this and other intervention trials underscore the urgency of preventing lead exposure in children.

## CONCLUSIONS

Iron, zinc, or combined supplementation for 6 months in lead-exposed schoolchildren produced modest effects that were not consistently beneficial and largely not sustained over the long term. In light of these limited findings, we do not recommend iron or zinc supplementation as the sole treatment of lead-exposed children. This is especially the case for children with higher lead exposures, for whom provision of micronutrient supplements could provide a false sense of protection. Ballew and Bowman<sup>36</sup> reached a similar conclusion for calcium on the basis of a review of animal and human feeding studies and of calcium supplementation trials in lead-exposed infants. Additional studies should be conducted to examine the recommendation of giving iron supple-



ments to lead-exposed children, currently included in the CDC guidelines.

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