The Journal of Nutrition
Community and International Nutrition

Preschool Iron-Folic Acid and Zinc Supplementation in Children Exposed to Iron-Folic Acid in Utero Confers No Added Cognitive Benefit in Early School-Age[1–3]

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Abstract

In Nepal, antenatal iron-folic acid supplementation improved aspects of intellectual, executive, and fine motor function among school-age children. We examined the impact of added zinc to the maternal antenatal supplement (M-IFAZn) and preschool supplementation from 12 to 36 mo with iron-folic acid (C-IFA) ± zinc (C-IFAZn) on cognitive outcomes compared to maternal iron-folic acid (M-IFA) alone. Children 7–9 y old (n = 780) who participated in early childhood micronutrient supplementation trial during 2001–2004 and whose mothers participated in an antenatal micronutrient supplementation between 1999 and 2001 were followed for cognitive assessments in 2007–2009. Using multivariate analysis of variance and adjusting for confounders, M-IFA with child supplementation (either C-IFA or C-IFAZn) did not impact scores on the tests of general intelligence (Universal Nonverbal Intelligence Test), and executive function (Stroop and go/no go tests) relative to the M-IFA alone. However, children in the C-IFAZn group had slightly lower scores on the backward digit span (−0.29, 95% CI: −0.55, −0.04) and Movement Assessment Battery for Children (1.33, 95% CI: 0.26, 2.40) relative to the referent group, whereas both C-IFA (−1.92, 95% CI: −3.12, −0.71) and C-IFAZn (−1.78, 95% CI: −2.63, −0.92) produced somewhat lower finger tapping test scores (fine motor skills). The combination of M-IFAZn and C-IFA or C-IFAZn did not lead to any outcome differences relative to M-IFA alone. Preschool iron-folic acid ± zinc to children exposed to iron-folic acid in utero or addition of zinc to maternal iron-folic acid conferred no additional benefit to cognitive outcomes assessed in early school age. The late timing of supplementation during preschool may explain the lack of impact of iron and/or zinc. J. Nutr. 141: 2042–2048, 2011.

Introduction

More than 200 million young children globally fail to achieve their developmental potential, attributed in part to inadequate nutrition (1). Iron deficiency has been shown to affect cognitive and motor development in the critical prenatal and early childhood periods (2) by altering interactive morphological, neurochemical, and bioenergetic processes such as oligodendrocyte wrapping for myelination, monoamine synthesis in the striatum influencing dopamine metabolism, and neuronal and glial energy metabolism in the hippocampus (3). Similarly, zinc deficiency may deter development by affecting activity in >200 enzymes related to RNA and DNA synthesis, NMDA[9] receptor regulation in the hippocampus, and other structural and functional mechanisms in the brain (4,5).

Although variations in timing, duration, severity of iron deficiency, and mediating factors hinder confirmed causation (6), evidence from animal, case-control, and longitudinal studies almost uniformly identify associations between iron deficiency

[1] This work was done by the Center for Human Nutrition, Department of International Health of the Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, in collaboration with the National Society for the Prevention of Blindness, Kathmandu, Nepal, with funding from the NIH grant R01 HD050254-01 and the Bill and Melinda Gates Foundation, Seattle, WA. The antenatal micronutrient supplementation study was conducted under the Micronutrients for Health Cooperative Agreement No. HRN-A-00-97-00015-00 and the Global Research Activity Cooperative Agreement No. GHS-A-00-03-00019-00 between the Johns Hopkins University and the Office of Health, Infectious Diseases and Nutrition, United States Agency for International Development, Washington, DC. Bill and Melinda Gates Foundation and Sight and Life Research Institute, Baltimore, MD, provided additional support for the study. The preschool child iron and zinc supplementation study was funded by NIH, Bethesda, MD (HD 38753), the Bill and Melinda Gates Foundation, and a Cooperative Agreement between JHU and the Office of Health and Nutrition, U.S. Agency for International Development (HRN-A-00-97-00015-00). The parent trials are registered at ClinicalTrials.gov (NCT00115271 and NCT00109551).
[2] Author disclosures: P. Christian, M. E. Morgan, L. Murray-Kolb, S. C. LeClerq, S. K. Khatry, B. Schaefer, P. M. Cole, J. Katz, and J. M. Tielsch, no conflicts of interest.
[3] Supplemental Figure 1 and Supplemental Table 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
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[9] Abbreviations used: C-IFA, child iron-folic acid; C-IFAZn, child iron-folic acid-zinc supplement; HOME, the Middle Childhood Home Observation for the Measurement of the Environment; MABC, Movement Assessment Battery for Children; M-IFA, maternal iron-folic acid; M-IFAZn, maternal iron-folic acid-zinc supplement; NMDA, N-methyl D-aspartate PL, placebo; UNIT, Universal Nonverbal Intelligence Test.

Manuscript received June 17, 2011. Initial review completed July 30, 2011. Revision accepted August 29, 2011.
First published online September 28, 2011; doi:10.3945/jn.111.146480.

and lower mental and motor development scores as well as more behavioral problems than in nondeficient children < 2 y and suggest that detriments persist throughout childhood (7–10) and even adulthood (11). Randomized controlled trials have found inconclusive evidence of iron on child cognitive function. Of 7 published, randomized, supplementation trials of iron among < 3 y olds, 3 found no effects on cognitive performance (12–14). The 4 that found cognitive outcomes associated with supplementation show effects on varying aspects of children's behavior and function (15–18). For example, in a recent trial in Zanzibar, children who received iron for 1 y had better language acquisition scores as assessed by parental report (18), whereas Lozoff et al. (15) found beneficial effects of iron on visual perceptual skills. A 2009 meta-analysis concluded that preventive zinc did not affect children's mental and psychomotor development (19), yet the results are inconsistent derived from a small number of trials of short supplementation duration. Results from Chile (20), Indonesia (21), Bangladesh (22), and India (23) indicate no global motor benefits, but several high-risk groups such as low-birth weight infants and stunted toddlers showed signs of increased motor activity with zinc supplementation and some motor quality indicators improved (19,24). In one study in Bangladesh, infants who received zinc had poorer mental development scores (25). Our group also showed that supplementation with iron-folic acid, zinc, or their combination was not associated with younger age at walking unassisted in a deficient, rural, Nepali population (26). Black et al. (21) found that combined supplementation with iron-folic acid and zinc protected infants from psychomotor development declines experienced within a placebo group, whereas Lind et al. (22) found no effects from iron-folic acid and zinc.

Few studies have examined, in a prospective or retrospective fashion, the impact of iron intervention in utero alone or in combination with postnatal interventions. Recently, we conducted a prospective study in 2007–2009 to assess developmental outcomes of children whose mothers received IFA or IFAZn and multiple micronutrients and who were themselves supplemented with IFA or IFAZn from 12–36 mo of age as part of 2 controlled, cluster-randomized, double-masked trials in rural Nepal. We already showed that antenatal maternal iron-folic acid (but not other combinations of micronutrients) improved working memory, inhibitory control, and motor functioning of offspring at 7–9 y of age (27). In the present analysis, we examined whether the addition of zinc to antenatal iron-folic acid or subsequent supplementation during preschool with iron-folic acid with or without zinc confers an added benefit for cognitive outcomes beyond that already seen with antenatal iron-folic acid.

Participants and Methods

Study design. In Sarlahi, Nepal, 7- to 9-y-old children from 30 Village Development Committees who had participated in 2 micronutrient supplementation trials indirectly in utero via antenatal supplementation and as 12- to 36-mo-old preschoolers were prospectively followed from June 2007 to April 2009 for assessment of developmental outcomes. Exposure to in utero supplementation was part of a maternal double-masked, cluster-randomized, controlled, antenatal supplementation trial conducted from 1999 to 2001 in which pregnant women received daily folic acid (400 μg), folic acid-iron (60 mg), folic acid-iron-zinc (30 mg), or folic acid-iron-zinc and cholecalciferol (10 μg), tocopherol (10 mg), thiamine (1.6 mg), riboflavin (1.8 mg), niacin (20 mg), pyridoxine (2.2 mg), vitamin B-12 (2.6 μg), ascorbic acid (100 mg), phylloquinone (65 μg), copper (2.0 mg), and magnesium (100 mg), all with 1000 μg retinol equivalents of vitamin A as retinyl palmitate compared to vitamin A alone as the control throughout pregnancy through 3 mo postpartum (28). Offspring of participating mothers from age 12 to 36 mo were then part of a larger 2 × 2 factorial, placebo-controlled, double-masked, cluster-randomized trial conducted from 2001 to 2005 in which they received daily iron-folic acid (12.5 mg), zinc (10 mg), iron-folic acid-zinc, or placebo (29). All children received semiannual doses of vitamin A as prescribed by national policy. In both studies, a cluster was a study-defined area called the sector and was used as a unit of randomization; there were 426 sectors in the study area.

Data collection. Briefly, we visited households with eligible children, invited them to participate in the prospective follow-up study, described the purpose of the study, and obtained parental verbal consent and child assent. Two home-based visits provided demographic data and home environment data while psychological testing was performed during a clinic-based visit. Although a previously published study detailed the testing and measurement procedures (27), they are summarized below.

Information about demographics, socioeconomic status, child's school enrolment history, child's and mother's morbidity over the previous 7 and 30 d, dietary intake, and household salt iodine content was collected during the home-based enrolment interview. At the central testing site, we measured intellectual, executive, and motor functioning as well as measured anthropometry and hemoglobin levels. Because our hypotheses related to iron and zinc supplementation are primarily driven by their effects on central nervous system development and function derived from both the animal and human literature, each test was selected based on its previously found sensitivity to changes in brain function attributed to nutritional influences, keeping in mind the neural basis for the tasks. Thus, we were be able to assess whether children in any of the supplementation groups showed faster processing of information and greater inhibitory control, cognitive functions that rely on dopaminergic pathways, which are sensitive to iron deficiency. However, it is also possible that the cumulative effect of supplementation could have a more general effect on intellectual functioning, which we assessed independently. Studies have consistently shown a decrease in motor activity accompanied by iron and zinc deficiency, which was also examined. Thus, general intelligence was assessed with the UNIT (30). The UNIT provides an assessment of general intelligence using entirely nonverbal administration and response formats. The UNIT was developed to test children irrespective of race, ethnicity, sex, language, country of origin, or hearing status and the materials and stimuli were designed to be universal and cross-cultural. A (numbers) Stroop test (31), backward digit span (Wechsler and Psychological), and go/no-go tasks (32) were utilized to assess inhibitory control and processing speed, components of executive functioning. The MABC and a finger tapping test were administered to evaluate gross and fine motor abilities (33). The MABC scores on failures and thus higher scores reflect worse performance. Height and weight were measured and used to calculate standardized Z-scores using the 2006 WHO reference standard (34). We estimated stimulation at home with the HOME and the mother's reasoning capacity with the Raven's Colored Matrices (35,36).

Statistical analysis. The present analyses included children from a combination of 5 maternal-child supplementation arms of the 2 trials and included 107 sectors. These were children in the maternal iron-folic acid and child placebo group as the referent category, which previously showed a beneficial impact, compared to those who were in the maternal iron-folic acid plus either in the child iron-folic acid or child iron-folic acid plus zinc groups, or in the maternal iron-folic acid with zinc plus either in the child iron-folic acid or iron-folic acid plus zinc groups (**Supplemental Fig. 1**). The 5 groups were as follows: *1*) M-IFA C-PL (referent group); *2*) M-IFA C-IFA; *3*) M-IFA C-IFAZn; *4*) M-IFAZn C-IFA; and *5*) M-IFAZn C-IFAZn.

Analytical steps of our 2-sided testing, intent-to-treat approach included basic data exploration, socioeconomic status index creation, baseline data comparison among the 5 groups, potential confounder identification and adjustment, conversion of raw test scores to scales or *t* scores (for the UNIT), estimation of outcome differences, and examination of effect modification. The Stroop test was expressed as proportion of failures (i.e. those who failed the practice test) and the go/no-go task, which assesses inhibitory control, was examined using the percentage of no-go stimuli on which the children's response was correct. A MANOVA model was used to

assess differences between the referent group and each other intervention arm to account for correlated multiple outcomes using the overall Wilks' λ and Lawley-Hotelling trace test. SE and *P* values were generated through the bootstrap method to adjust for the cluster-randomized study design and account for correlations among children in the same sector (37,38). Child age, sex, asset score, whether child had ever started school, child compliance with treatment, previous 7-d intake of dark green leafy vegetables and yellow fruit and vegetables, and history of diarrhea/dysentery, maternal literacy, maternal compliance to treatment, and household salt iodine $\geq$15 μg/g were adjusted for in the analysis due to significant differences among intervention groups, an association with UNIT or MABC outcomes, or previously documented disparities among categories. Effect modifications by age at supplementation initiation and assessment along with sex and treatment compliance were investigated using interaction terms and stratification with MANOVA and bootstrapped SE. Stata version 11.0 was used to analyze data (StataCorp).

Ethical approval for the follow-up study was obtained from Institutional Review Boards at the Johns Hopkins Bloomberg School of Public Health, The Pennsylvania State University, and the Institute of Medicine, Tribhuvan University.

Results

Of the 3675 children born between 2000 and 2001 who participated in the child supplementation study and whose mothers participated in a maternal supplementation trial, 2863 were not in the 5 arms in this analysis (Supplemental Fig. 1). Of the remaining 812 eligible children, 32 children had died or migrated out of the study area or their status was unknown after the completion of the original trial. Thus, 780 were visited, of which 745 (96%) were enrolled after 4 had refused to participate and 31 were not met after repeated visits or had permanently moved.

The age of children at follow-up was 8.4 $\pm$ 0.65 y and differed ($P < 0.05$) by treatment group (**Table 1**). Slightly more boys than girls participated in the study and most children (83%) had started school. Treatment groups differed with respect to children's percent compliance to treatment, intake of dark green leafy vegetables and yellow fruits and vegetables, symptoms of diarrhea/dysentery during the past 7 d, maternal literacy, maternal percent compliance to treatment, proportion of households with salt iodine levels $\geq$15 μg/g, household wall building materials, and household asset scores. Percentages of children ever in school, with symptoms of lower respiratory infection, stunting, wasting, or anemia, did not differ by treatment group. The groups did not differ on maternal characteristics of schooling, Raven's score, or HOME inventory score.

Test scores by treatment group are presented in **Table 2**. Relative to the referent group (M-IFA), each arm, except the M-IFAZn/C-IFA group, produced an overall Wilks' λ and Lawley-Hotelling trace test that was significant ($P < 0.001$) (Supplemental Table 1). However, few of the treatment group differences for individual tests were significant. After controlling for confounders, the overall MANOVA test was significant only for the M-IFA/C-IFA group, with finger tapping test score being worse in this group relative to the control (**Table 3**). Relative to the control group, individual scores on backward digit test, MABC, and finger tapping test were also significantly worse in the M-IFA/C-IFAZn group. There was no consistent effect modification (data not shown).

Discussion

Within a high micronutrient-deficient population (39), successive supplementation with iron-folic acid or iron-folic acid plus zinc at 12–36 mo of age after maternal iron-folic acid supplementation or the addition of zinc to maternal supplementation with iron-folic acid did not confer any added benefits for cognitive outcomes in early school-aged children. The group that received successive supplementation in preschool with iron-folic acid alone or with zinc may have even had diminished positive effects of only maternal iron-folic acid supplementation on functions of working memory and inhibitory control as well as motor development. The differences relative to the referent group, although significant, were small and likely not very clinically meaningful.

We previously found that maternal iron-folic acid supplementation during pregnancy through 12 wk postpartum improved intellectual and aspects of executive and motor function in offspring compared to the control group (27), suggesting that the intrauterine period was critical for neurocognitive developmental effects of iron and folic acid. In contrast, in a separate analysis, child supplementation alone between 12 and 36 mo of age (without any maternal supplementation) with iron-folic acid, zinc, or their combination had a limited impact on intellectual, executive, or motor function (40). We also found that supplementation with iron-folic acid or zinc and their combination from 1 to 36 mo of age did not result in younger age at first walking and in the iron-folic acid group may even have delayed it by 28.0 d (95% CI = 11.3, 44.7) (26).

Data from the original trials reveal that preschool children had high levels of both iron and zinc deficiency (39,41). In the placebo group, 16% of children had serum zinc concentrations $<$ 9.7 μmol/L and an additional 32% had concentrations between 9.7 and $<$10.7 μmol/L (41). Similarly, $>$ 50% were anemic (Hb $<$ 110g/L) and 28% had serum ferritin $<$ 12 μg/L (39). In the present analysis, our cohort of children, comprising those who had also been exposed to maternal supplementation in utero and subsequently child supplementation, the mean age of starting supplementation was 22.5 mo. Thus, it is possible that the timing of our preschool supplementation was too late to counter the effects of iron deficiency on cognitive and motor function. Given that our supplementation began at 12 mo, age at entry stratification should exhibit the trend of less effect as the age of supplementation initiation increases. However, we found no effect modification or differences among strata. Alternative explanations for the lack of added benefit of childhood iron-folic acid supplementation include inadequate levels of other limiting nutrients. Children were also stunted and wasted and possibly the addition of micronutrients without energy during the preschool years was therefore ineffective. It is also important to recognize that nutrition is one of many factors that influence children's cognitive performance and that other environmental deficits (e.g. lack of language and cognitive stimulation, abject poverty) may be severe enough in this setting that they are hard to overcome.

Unlike the predominantly null effect seen among children who received iron-folic acid in utero and added supplementation with iron-folic acid in early childhood, the combination of iron-folic acid plus zinc in children had significantly lower scores in aspects of working memory, inhibitory control, and motor skills relative to maternal antenatal iron-folic acid supplementation alone. Several trials among infants and preschoolers have shown increased activity and motor development among those who received zinc supplements relative to placebo controls (20,42–44), but did not demonstrate a change in cognitive function (20,42,45). One study in Bangladesh found that zinc-supplemented infants achieved lower scores on the mental development index of the Bayley's scales at 12 mo than the control infants (25). In our study, even if childhood zinc supplementa-

TABLE 1 Baseline characteristics of the enrolled children, their mothers, and households by maternal and child supplementation group in Sarlahi, Nepal (2007–2009)[1]

	M-IFA C-PL,[2] *n* = 103	M-IFA C-IFA,[2] *n* = 164	M-IFA C-IFAZn,[2] *n* = 217	M-IFAZn C-IFA,[2] *n* = 137	M-IFAZn C-IFAZn,[2] *n* = 124	*P*[3]
Child characteristics						
Child age, *y*	8.6 ± 0.60	8.2 ± 0.54	8.5 ± 0.63	8.5 ± 0.66	8.0 ± 0.61	<0.0001
Age at supplementation initiation, *mo*	22.8 ± 3.7	22.9 ± 3.5	22.3 ± 3.8	22.2 ± 3.8	22.1 ± 4.0	0.32
Male, *n (%)*	49 (47.6)	83 (50.6)	117 (53.9)	84 (61.3)	54 (43.6)	0.05
Ever sent to school, *n (%)*	87 (84.5)	136 (82.9)	177 (81.6)	123 (89.8)	96 (77.4)	0.10
Treatment compliance, *%*	81.6 ± 16.9	69.6 ± 22.0	71.9 ± 23.3	74.7 ± 22.6	72.9 ± 22.0	0.0002
Diet in the past 7 d (any intake), *n (%)*						
Milk and dairy products[4]	71 (68.9)	117 (71.3)	160 (73.7)	106 (77.4)	85 (68.6)	0.48
Meat, chicken, fish	64 (62.1)	105 (64.0)	120 (55.3)	85 (62.0)	70 (56.5)	0.39
Dark green leafy vegetables	79 (76.7)	104 (63.4)	166 (76.5)	101 (73.7)	73 (58.9)	0.001
Citrus fruits[5]	45 (43.7)	82 (50.0)	87 (40.1)	65 (47.8)	53 (42.7)	0.34
Yellow fruits and vegetables[6]	47 (45.6)	58 (35.7)	71 (32.7)	58 (42.3)	60 (48.4)	0.02
Tea	47 (45.6)	68 (41.5)	96 (44.2)	66 (48.2)	39 (31.5)	0.07
Morbidity in the past 7 d, *n (%)*						
Lower respiratory infection[7]	4 (3.9)	2 (1.2)	4 (1.9)	1 (0.7)	5 (4.0)	0.23
Diarrhea/dysentery[8]	9 (8.7)	8 (4.9)	5 (2.3)	2 (1.5)	5 (4.0)	0.03
Height-for-age Z-score < −2, *n (%)*	40 (39.6)	73 (44.8)	83 (38.6)	63 (46.0)	56 (45.5)	0.53
BMI Z-score < −2, *n (%)*	16 (15.8)	27 (16.6)	28 (13.0)	21 (15.3)	23 (18.7)	0.72
Hemoglobin < 115 g/L, *n (%)*	23 (23.0)	44 (27.0)	37 (17.3)	22 (16.1)	32 (26.5)	0.05
Maternal characteristics						
Raven's score	16.6 ± 5.4	17.2 ± 5.3	16.8 ± 5.4	16.9 ± 5.3	16.4 ± 5.5	0.77
Literacy, *n (%)*	22 (21.4)	49 (30.1)	40 (18.6)	34 (25.0)	20 (16.3)	0.03
Ttreatment compliance, *%*	80.2 ± 24.5	74.1 ± 29.4	81.8 ± 21.9	79.8 ± 26.7	82.5 ± 23.2	0.03
Household characteristics						
Household salt iodine level ≥15 μg/g, *n (%)*	80 (78.4)	114 (69.5)	151 (69.9)	93 (68.4)	63 (52.9)	0.001
Walls made with stone or cement, *n (%)*	20 (19.4)	35 (21.3)	60 (27.7)	18 (13.1)	27 (21.8)	0.03
Asset score[9]	5.0 ± 2.1	4.6 ± 2.3	4.6 ± 2.0	4.5 ± 2.1	4.0 ± 2.3	0.02
HOME score	24.7 ± 6.5	24.8 ± 6.3	24.1 ± 6.2	23.9 ± 5.7	22.8 ± 6.1	0.07

[1] Values are mean ± SD, unless otherwise specified. C-IFA, child iron-folic acid; C-IFAZn, child iron-folic acid-zinc supplement; M-IFA, maternal iron-folic acid; M-IFAZn, maternal iron-folic acid-zinc supplement; PL, placebo.
[2] Difference in *n* from eligibility to enrollment due to: 1 refused/moved and 4 not met in M-IFA/C-placebo, 2 refused/moved and 4 not met in M-IFA/C-IFA, 15 not met in M-IFA/C-IFAZn, 3 not met in M-IFAZn/C-IFA, and 1 refused/moved and 5 not met in M-IFAZn/C-IFAZn.
[3] Using ANOVA for continuous variables and the chi-square test for categorical variables.
[4] Includes milk, yogurt, and buttermilk.
[5] Includes oranges and guava.
[6] Includes ripe mango, papaya, jackfruit, and pumpkin.
[7] Productive cough or rapid breathing and fever.
[8] Watery stools ≥4 times/d or blood in stool.
[9] Asset score ranges from 0 to 11 and is made up of any ownership of goats, cattle, cart, bicycle, motorcycle, electricity, radio, TV, telephone, mobile phone, or watches in the household.

TABLE 2 Scores on psychometric tests by maternal and child supplementation group assessed among children 7–9 y of age in Sarlahi, Nepal (2007–2009)[1]

Maximum number	M-IFA C-PL $n = 101$	M-IFA C-IFA $n = 163$	M-IFA C-IFAZn $n = 212$	M-IFAZn C- IFA $n = 137$	M-IFAZn C-IFAZn $n = 122$
UNIT	51.7 ± 8.5	51.5 ± 10.6	49.8 ± 10.5	50.4 ± 10.3	50.2 ± 9.8
Proportion failed, Stroop test	0.20 ± 0.40	0.32 ± 0.47	0.33 ± 0.47	0.21 ± 0.41	0.38 ± 0.49
Backward digit span	2.21 ± 1.23	1.91 ± 1.18	1.86 ± 1.02	2.06 ± 1.19	1.70 ± 1.07
No-go correct, %	47.1 ± 21.0	47.2 ± 21.5	41.6 ± 20.2	47.2 ± 19.8	42.3 ± 22.7
MABC[2]	6.78 ± 4.93	8.28 ± 6.60	8.44 ± 6.21	7.79 ± 5.61	8.70 ± 6.14
Finger tapping test	38.5 ± 4.5	36.0 ± 4.9	36.8 ± 5.0	38.0 ± 5.4	36.1 ± 5.8

[1] Values are mean ± SD, $n = 705$. C-IFA, child iron-folic acid; C-IFAZn, child iron-folic acid-zinc supplement; MABC, Movement Assessment Battery for Children; M-IFA, maternal iron-folic acid; M-IFAZn, maternal iron-folic acid-zinc supplement; PL, placebo; UNIT, Universal Nonverbal Intelligence Test.
[2] Higher MABC score is worse.

tion did attenuate the protective potential of maternal iron-folic acid, the mechanisms that led to poorer developmental outcomes remain unclear.

The impact of maternal zinc supplementation was previously examined. For example, in Bangladesh, infants of zinc-supplemented mothers had lower mental and psychomotor development index scores using the Bayley's scale compared to those whose mothers received a placebo during pregnancy (46). The authors postulated a possible inhibitory role of zinc on iron status, resulting in the negative effects on infant outcomes. Zinc supplementation of African American women in the latter half of pregnancy did not affect the mental and psychomotor development of their children at 5 y of age (47). In the present study, adding zinc to the maternal supplement with iron-folic acid in combination with child iron-folic acid produced neither negative nor positive effects.

In addition to investigating the unique question of successive maternal and child supplementation effects, this study offers several strengths. We examined components of development that are supposedly responsive to both iron and zinc deficiency, thereby reducing the loss of differential treatment outcomes compared to global assessments. The timing of assessment, 7–9 y of age, also allowed us to utilize more sensitive developmental measurements than is possible during infancy and early childhood (48). Lastly, we controlled for various factors known to influence cognitive development, such as maternal education and home and family environment. However, the current study would have been more comprehensive if we had had a maternal iron-folic acid and child zinc supplementation-only group to examine the results of combining the 2 potentially most beneficial treatments of the 2 randomized trials. Further, as mentioned before, earlier child supplementation would have been helpful in determining critical windows of iron and zinc deficiencies.

In conclusion, adding other nutrients or child supplementation to maternal iron-folic acid supplementation appeared to add little benefit for combating the deleterious effects of micronutrient deficiency on intelligence, executive function, and motor development. Fortunately, fewer risks and logistical barriers are involved in maternal iron-folic acid supplementation than combined maternal and child supplementation (49). More research is needed to explore the mechanisms underlying iron and zinc use during crucial periods of development, the timing, and interactive issues related to successive iron and zinc supplementation in deficient mothers and children. Until more information is available, antenatal iron-folic acid supplementation policies remain appropriate for areas in which iron deficiency exists.

TABLE 3 Differences in test scores by maternal and child supplementation group relative to reference adjusted for confounders among children 7–9 y of age in Sarlahi, Nepal (2007–2009)[1]

	M-IFA C-IFA	M-IFA C-IFAZn	M-IFAZn C-IFA	M-IFAZn C-IFAZn
UNIT	−0.52 (−3.17, 2.13)	−1.52 (−3.51, 0.47)	−1.98 (−4.74, 0.78)	−0.32 (−2.80, 2.16)
Proportion failed, Stroop test	0.10 (−0.01, 0.21)	0.10 (−0.01, 0.20)	0.04 (−0.05, 0.14)	0.11 (−0.003, 0.22)
Backward digit test	−0.29 (−0.58, −0.01)	−0.29 (−0.55, −0.04)	−0.19 (−0.53, 0.15)	−0.24 (−0.55, 0.06)
% correct no_go	1.78 (−4.43, 7.99)	−4.45 (−9.70, 0.79)	0.57 (−5.64, 6.78)	−0.78 (−7.40, 5.84)
MABC[3]	0.80 (−0.93, 2.52)	1.33 (0.26, 2.40)	1.29 (−0.01, 2.59)	0.21 (−1.32, 1.74)
Finger Tapping Test	−1.92 (−3.12, −0.71)	−1.78 (−2.63, −0.92)	−0.86 (−2.27, 0.55)	−1.44 (−3.12, 0.24)
P[2]	0.04	0.20	0.77	0.18

[1] Values are differences (95% CI) from the reference population of M-IFA/C-PL using boot strapping to estimate standard error adjusted for design effect and for child age, sex, ever started school, child compliance to treatment, intake of dark green leafy vegetables and yellow fruit and vegetables in past 7 d, diarrhea/dysentery in past 7 d, maternal literacy, maternal compliance to treatment, household salt iodine, and asset index, $n = 698$. C-IFA, child iron-folic acid; C-IFAZn, child iron-folic acid-zinc supplement; MABC, Movement Assessment Battery for Children; M-IFA, maternal iron-folic acid; M-IFAZn, maternal iron-folic acid-zinc supplement; PL, placebo; UNIT, Universal Nonverbal Intelligence Test.
[2] P for the overall treatment effect using Wilks' λ and Lawley-Hotelling trace test derived from the MANOVA.
[3] Higher MABC score is worse.

Acknowledgments

We thank Field Manager Tirthraj Shakya, coordinators Uma Shankar Sah and Gokarna Subedi, supervisors Shishir Bar Shrestha and Keshav Dhaka, and psychology research assistants Keshav Mishra, Nar Bahadur Thapa, Mona Lisa Pradhan, Sumitra Dhakal, and Bikram Sherchan. P.C. was the principal investigator; P.C., L.M-K., S.C.L., P.M.C., and J.M.T. were involved with the study concept and design; P.C., L.M-K., S.C.L., S.K.K., and P.M.C. were responsible for acquisition of data; P.C. and S.C.L. were responsible for study supervision; B.S., J.K., and J.M.T analyzed the data; P.C., B.S., J.K., and J.M.T. interpreted the data; M.E.M. conducted the analyses, prepared the tables, and wrote the first draft of the paper; S.C.L., B.S., P.M.C., J.K., and J.M.T. reviewed the manuscript; P.C. and L.M-K. edited the manuscript; S.C.L. and S.K.K. were responsible for administrative/technical/material support. All authors read and approved the final manuscript.

Literature Cited

1. Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B. Developmental potential in the first 5 years for children in developing countries. Lancet. 2007;369:60–70.
2. Lozoff B, Beard J, Connor J, Barbara F, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev. 2006;64:S34–43.
3. Beard J. Recent evidence from human and animal studies regarding iron status and infant development. J Nutr. 2007;137:524S–30S.
4. Hess SY, Lonnerdal B, Hotz C, Rivera JA, Brown KH. Recent advances in knowledge of zinc nutrition and human health. Food Nutr Bull. 2009;30 Suppl 1:S5–11.
5. Benton D. The influence of dietary status on the cognitive performance of children. Mol Nutr Food Res. 2010;54:457–70.
6. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr. 2007;85:931–45.
7. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr. 2001;131: S649–66.
8. Lozoff B. Iron deficiency and child development. Food Nutr Bull. 2007;28 Suppl 4:S560–71.
9. Shafir T, Angulo-Barroso R, Jing Y, Angelilli ML, Jacobson SW, Lozoff B. Iron deficiency and infant motor development. Early Hum Dev. 2008;84:479–85.
10. Corapci F, Calatroni A, Kaciroti N, Jimenez E, Lozoff B. Longitudinal evaluation of externalizing and internalizing behavior problems following iron deficiency in infancy. J Pediatr Psychol. 2010;35:296–305.
11. Lukowski AF, Koss M, Burden MJ, Jonides J, Nelson CA, Kaciroti N, Jimenez E, Lozoff B. Iron deficiency in infancy and neurocognitive functioning at 19 years: evidence of long-term deficits in executive function and recognition memory. Nutr Neurosci. 2010;13:54–70.
12. Heywood A, Oppenheimer S, Heywood P, Jolley D. Behavioral effects of iron supplementation in infants in Madang, Papua New Guinea. Am J Clin Nutr. 1989;50 Suppl 3:630–7.
13. Walter T, De A, I, Chadud P, Perales CG. Iron deficiency anemia: adverse effects on infant psychomotor development. Pediatrics. 1989; 84:7–17.
14. Morley R, Abbott R, Fairweather-Tait S, MacFadyen U, Stephenson T, Lucas A. Iron fortified follow on formula from 9 to 18 months improves iron status but not development or growth: a randomised trial. Arch Dis Child. 1999;81:247–52.
15. Lozoff B, Brittenham GM, Wolf AW, McClish DK, Kuhnert PM, Jimenez E, Jimenez R, Mora LA, Gomez I, Krauskoph D. Iron deficiency anemia and iron therapy effects on infant developmental test performance. Pediatrics. 1987;79:981–95.
16. Moffatt ME, Longstaffe S, Besant J, Dureski C. Prevention of iron deficiency and psychomotor decline in high-risk infants through use of iron-fortified infant formula: a randomized clinical trial. J Pediatr. 1994;125:527–34.
17. Williams J, Wolff A, Daly A, MacDonald A, Aukett A, Booth IW. Iron supplemented formula milk related to reduction in psychomotor decline in infants from inner city areas: randomised study. BMJ. 1999;318: 693–7.
18. Stoltzfus RJ, Kvalsvig JD, Chwaya HM, Montresor A, Albonico M, Tielsch JM, Savioli L, Pollitt E. Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: double blind, placebo controlled study. BMJ. 2001;323:1389–93.
19. Brown KH, Peerson JM, Baker SK, Hess SY. Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. Food Nutr Bull. 2009;30 Suppl 1:S12–40.
20. Castillo-Durán C, Perales CG, Hertrampf ED, Marin VB, Rivera FA, Icaza G. Effect of zinc supplementation on development and growth of Chilean infants. J Pediatr. 2001;138:229–35.
21. Lind T, Lonnerdal B, Stenlund H, Gamayanti IL, Ismail D, Seswandhana R, Persson LA. A community-based randomized controlled trial of iron and zinc supplementation in Indonesian infants: effects on growth and development. Am J Clin Nutr. 2004;80:729–36.
22. Black MM, Baqui AH, Zaman K, Ake PL, El AS, Le K, McNary SW, Parveen M, Hamadani JD, Black RE. Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants. Am J Clin Nutr. 2004;80:903–10.
23. Taneja S, Bhandari N, Bahl R, Bhan MK. Impact of zinc supplementation on mental and psychomotor scores of children aged 12 to 18 months: a randomized, double-blind trial. J Pediatr. 2005;146:506–11.
24. Olney DK, Pollitt E, Kariger PK, Khalfan SS, Ali NS, Tielsch JM, Sazawal S, Black R, Allen LH, Stoltzfus RJ. Combined iron and folic acid supplementation with or without zinc reduces time to walking unassisted among Zanzibari infants 5- to 11-mo old. J Nutr. 2006;136: 2427–34.
25. Hamadani JD, Fuchs GJ, Osendarp SJ, Khatun F, Huda SN, Grantham-McGregor SM. Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. Am J Clin Nutr. 2001;74:381–6.
26. Katz J, Khatry SK, Leclerq SC, Mullany LC, Yanik EL, Stoltzfus RJ, Siegel EH, Tielsch JM. Daily supplementation with iron plus folic acid, zinc, and their combination is not associated with younger age at first walking unassisted in malnourished preschool children from a deficient population in rural Nepal. J Nutr. 2010;140:1317–21.
27. Christian P, Murray-Kolb LE, Khatry SK, Katz J, Schaefer BA, Cole PM, Leclerq SC, Tielsch JM. Prenatal micronutrient supplementation and intellectual and motor function in early school-aged children in Nepal. JAMA. 2010;304:2716–23.
28. Christian P, Khatry SK, Katz J, Pradhan EK, LeClerq SC, Shrestha SR, Adhikari RK, Sommer A, West KP Jr. Effects of alternative maternal micronutrient supplements on low birth weight in rural Nepal: double blind randomized community trial. BMJ. 2003;326:571–76.
29. Tielsch JM, Khatry SK, Stoltzfus RJ, Katz J, Leclerq SC, Adhikari R, Mullany LC, Shresta S, Black RE. Effect of routine prophylactic supplementation with iron and folic acid on preschool child mortality in southern Nepal: community-based, cluster-randomised, placebo-controlled trial. Lancet. 2006;367:144–52.
30. Bracken BA. UNIT: universal nonverbal intelligence test. Itasca (IL): Riverside Pub; 1998.
31. Bull R, Scerif G. Executive functioning as a predictor of children's mathematics ability: inhibition, switching, and working memory. Dev Neuropsychol. 2001;19:273–93.
32. Konishi S, Nakajima K, Uchida I, Sekihara K, Miyashita Y. No-go dominant brain activity in human inferior prefrontal cortex revealed by functional magnetic resonance imaging. Eur J Neurosci. 1998;10:1209–13.
33. Henderson S, Sugden D. Movement assessment battery for children. London: Psychological Corporation; 1992.
34. WHO Multicentre Growth Reference Study Group. WHO child growth standards based on length/height, weight and age. Acta Paediatr Suppl. 2006;450:76–85.
35. Caldwell BM, Bradley RH. Home observation for the measurement of the environment. Little Rock (AR): University of Arkansas; 1984.
36. Raven J, Court J. Manual for Raven's progressive matrices and vocabulary scales. Section 3, standard progressive matrices. Oxford: Oxford Psychologists Press, LTD; 1992.
37. Snedecor G, Cochran WG. Statistical methods. Ames (IA): Iowa State University Press; 1980.

38. Diggle PJ, Liang KY. Analysis of longitudinal data. Oxford: Oxford University Press; 1994.
39. Jiang T, Christian P, Khatry SK, Wu L, West KP Jr. Micronutrient deficiencies in early pregnancy are common, concurrent, and vary by season among rural Nepali pregnant women. J Nutr. 2005;135: 1106–12.
40. Murray-Kolb LE, Christian P, Schaefer B, Cole P, Katz J, Khatry SK, LeClerq S, Tielsch JM. Long-term effects of micronutrient supplementation on intellectual and motor functions. FASEB. 2011;25: abstract no. 236.7.
41. Tielsch JM, Khatry SK, Stoltzfus RJ, Katz J, LeClerq SC, Adhikar RK, Mullany L, Black RE, Shrestha SR. Effect of daily zinc supplementation on child mortality in southern Nepal: a community-based, cluster randomised, placebo-controlled trial. Lancet. 2007;370:1230–9.
42. Friel JK, Andrews WL, Matthew JD, Long DR, Cornel AM, Cox M, McKim E, Zerbe GO. Zinc supplementation in very-low-birth-weight infants. J Pediatr Gastroenterol Nutr. 1993;17:97–104.
43. Sazawal S, Bentley M, Black RE, Dhingra P, George S, Bhan MK. Effect of zinc supplementation on observed activity in low socioeconomic Indian preschool children. Pediatrics. 1996;98:1132–7.
44. Bentley ME, Caulfield LE, Ram M, Santizo MC, Hurtado E, Rivera JA, Ruel MT, Brown KH. Zinc supplementation affects the activity patterns of rural Guatemalan infants. J Nutr. 1997;127:1333–8.
45. Ashworth A, Morris SS, Lira PI, Grantham-McGregor SM. Zinc supplementation, mental development and behaviour in low birth weight term infants in northeast Brazil. Eur J Clin Nutr. 1998;52:223–7.
46. Hamadani JD, Fuchs GJ, Osendarp SJ, Huda SN, Grantham-McGregor SM. Zinc supplementation during pregnancy and effects on mental development and behaviour of infants: a follow-up study. Lancet. 2002;360:290–4.
47. Tamura T, Goldenberg RL, Ramey SL, Nelson KG, Chapman VR. Effect of zinc supplementation of pregnant women on the mental and psychomotor development of their children at 5 y of age. Am J Clin Nutr. 2003;77:1512–6.
48. Pollitt E. The developmental and probabilistic nature of the functional consequences of iron-deficiency anemia in children. J Nutr. 2001;131: S669–75.
49. Iannotti LL, Tielsch JM, Black MM, Black RE. Iron supplementation in early childhood: health benefits and risks. Am J Clin Nutr. 2006;84: 1261–76.