GLOBAL HEALTH

Preschool Micronutrient Supplementation Effects on Intellectual and Motor Function in School-aged Nepalese Children

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Objective: To examine intellectual and motor functioning of children who received micronutrient supplementation from 12 to 35 months of age.

Design: Cohort follow-up of children 7 to 9 years of age who participated in a 2 × 2 factorial, placebo-controlled, randomized trial from October 2001 through January 2006.

Setting: Rural Nepal.

Participants: A total of 734 children 12 to 35 months of age at supplementation and 7 to 9 years of age at testing.

Interventions: Children received iron plus folic acid (12.5 mg of iron and 50 µg of folic acid); zinc (10 mg); iron plus folic acid and zinc; or placebo.

Main Outcome Measures: Intellectual, motor, and executive function.

Results: In both the unadjusted and adjusted analyses, iron plus folic acid supplementation had no effect overall or on any individual outcome measures being tested. In the unadjusted analysis, zinc supplementation had an overall effect, although none of the individual test score differences were significant. In the adjusted analysis, the overall difference was not significant.

Conclusion: In rural Nepal, we found that iron plus folic acid or zinc supplementation during the preschool years had no effect on aspects of intellectual, executive, and motor function at 7 to 9 years of age, suggesting no long-term developmental benefit of iron or zinc supplementation during 12 to 35 months of age.

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MICRONUTRIENT DEFICIENCIES represent the most common form of malnutrition in developing countries. Iron and zinc deficiencies are of particular concern among infants, children, and women of childbearing age because of increased physiologic requirements, combined with increased losses and poor dietary intake. The relationship between iron status and cognitive development in children has received significant attention in the past 3 decades, with observational and some experimental evidence indicating that iron-deficiency anemia in infancy impairs mental and motor development and behavior.[1-4] Although zinc has not received as much attention as iron with respect to child development outcomes, evidence has emerged that suggests a relationship between zinc deficiency and alterations in cognitive and motor functioning in children, but the results thus far are inconclusive.[1,2,5] Nearly all of the studies[6-10] performed to date have evaluated the effects of single nutrients on child developmental outcomes; however, children often experience multiple nutrient deficiencies concurrently. Therefore, evaluating single and combined effects of iron and zinc may be relevant.

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Observations of poorer mental and motor development have been repeatedly made in iron-deficient infants and children.[1-4] Timing, severity, and duration of the deficiency have been noted as significant factors. Studies[11,12] on the short-term effect of iron in children older than 2 years provide evidence of a benefit of iron treatment on mental and motor development in iron-deficient anemic children, although longer-term follow-up studies are needed. This benefit in children younger than 2 years is less clear. Studies of longer duration have generally also concluded that iron deficiency in infancy is associated with lower mental and motor scores that do not

improve with iron therapy administered before 2 years of age. It may be that iron deficiency that occurs at this age results in irreversible damage to children's development,[13,14] as supported by the animal literature.[4] More recent, large supplementation trials in infants from developing countries are emerging, with some indicating a benefit of iron for social-emotional and motor outcomes.[7,9,15-17] Clearly, more research is needed in this area, especially concerning the ideal age for supplementation.

Zinc deficiency can lead to primary and secondary alterations in brain development and brain growth.[18,19] Observations of altered activity levels and motor development are reported in zinc-deficient infants and toddlers,[20-24] but results of these studies are mixed. One study[20] reports higher motor scores with zinc supplementation, one study[23] reports more cooperation by the infant, and another study[24] reports higher motor quality but not scores. Our study[10] in Nepal reported no benefits of iron or zinc supplementation on age at first walking unassisted, and a study[25] in Bangladeshi infants reported lower mental index scores in infants treated with zinc. Although the data suggest a relationship between zinc deficiency and altered development and behavior, the findings to date are inconclusive.

The effects of concurrent supplementation with iron and zinc on child development are largely unexplored. Five recent reports have examined this, and 2 reports[6,7] revealed an improvement in motor development when infants were given a combination of iron and zinc supplements, whereas 3 others[8-10] did not. A recent study[26] of the long-term effects of supplementation with iron, zinc, or a combination of the 2 during infancy reported no effect of the supplementation on cognitive performance at 9 years of age. Given the conflicting findings, it is clear that more studies are needed before conclusive statements can be made.

This study was designed to assess intellectual and motor functioning among Nepalese children 7 to 9 years of age who belonged to the control arm of a previous prenatal micronutrient supplementation trial and who received daily micronutrient supplements from the age of 12 to 35 months in a placebo-controlled, cluster-randomized, double-masked, 2 × 2 factorial trial from October 2001 through January 2006.[27,28] We hypothesized that supplementation with iron plus folic acid or zinc would improve intellectual and motor outcomes in these children at school age. For the iron plus folic acid and zinc group, we hypothesized that this supplementation would improve outcomes at least to the same extent or more than with iron plus folic acid or zinc alone.

METHODS

In 2007, we undertook a follow-up study of children who received micronutrient supplementation in utero (through maternal supplementation) and during the preschool years (12-35 months of age) in a southeastern plains district (Sarlahi) of Nepal. In the child supplementation trial, children were eligible to participate starting at 1 month of age. However, children who had participated (in utero) in the maternal supplementation trial were at minimum 12 months of age by the time the child supplementation trial began. As such, children who we are following up were eligible for supplementation from the age of 12 to 35 months during the child supplementation trial. Our goals were to understand the effects of in utero supplementation, preschool supplementation, and the combination of the 2 on later childhood intellectual and motor functioning. The effects of in utero supplementation are reported elsewhere.[29] This analysis focuses on the effects of supplementation that occurred during the preschool years. Therefore, to isolate this effect, only children whose mothers were in the placebo arm of the in utero supplementation trial were eligible for this present analysis. A total of 3675 children were enrolled in the preschool supplementation trial, of whom 772 had mothers who were in the control arm (who received only vitamin A prenatally) of the previous (in utero) trial.[30] These trials took place in 30 Village Development Committees of the district where our research group has been working since 1989. Supplementation given during the preschool years was part of a 4-arm, double-masked, cluster-randomized, controlled trial. The arms were iron plus folic acid (12.5 mg of iron and 50 μg of folic acid), zinc (10 mg), iron plus folic acid and zinc, and placebo. Children 12 to 35 months of age received 1 tablet daily (length of supplementation depended on age at enrollment). Adherence was monitored weekly by counting the number of tablets consumed and was high (75% of all possible doses; interquartile range, 62%-91%) and varied by supplementation group (**Table 1**). As part of the national program, children received a large dose of vitamin A (200 000 IU) once every 6 months throughout the study. Eligible children were prospectively followed up from June 2007 to April 2009, with the specific aims of examining the effect of micronutrient supplementation at 12 to 35 months of age on intellectual and motor functioning at 7 to 9 years of age.

Households with eligible children were invited to participate in the follow-up study; the purpose of the study was explained and parental verbal consent and child assent were obtained. Data collection for the study was performed during 2 home-based visits and 1 clinic-based visit for which children and their mothers were brought to a central site for psychological testing. Details about testing and measurements for this study have been published previously.[29] Briefly, the assessments performed were as follows.

An enrollment interview was conducted at the child's home at which time we collected information on demographics, socioeconomic status, morbidity symptoms of child and mother during the previous 7 and 30 days, dietary intake, iodine content of household salt, and history of child's school enrollment. Psychological testing took place during the clinic-based visit and included measurements of general intellectual functioning, executive functioning, and motor abilities. During the clinic-based testing, the tester recorded any observed abnormality or abnormality reported by a child's mother for vision, hearing, motor function, or behavior problems. These data were collected to ensure there were no differences between the supplementation groups on these variables because they may affect a child's score on the cognitive, motor, or behavioral testing. General intellectual functioning was measured with the Universal Nonverbal Intelligence Test[31] (UNIT); executive functioning was measured with a battery of a go/no-go task, a Stroop (numbers) test, and a Backward Digit Span test; and motor abilities, including gross and fine motor skills, were assessed with the Movement Assessment Battery for Children[32] (MABC) and a finger-tapping test. Choice of tests to administer was based on the capacity of the measure to assess aspects of cognitive or motor functioning previously shown to be sensitive to brain changes attributable to nutritional influences. Tests were not modified for this population, although the analogic reasoning subtest of the UNIT was not administered because it contains stimuli with pictures of objects unfamiliar to most Nepalese children. The

Table 1. Baseline Characteristics of the Enrolled Children, Their Mothers, and Households by Child Supplementation Group in Sarlahi, Nepal (2007-2009)[a]

Characteristic	Control (n=177)	Iron Plus Folic Acid (n=171)	Zinc (n=146)	Iron, Folic Acid, and Zinc (n=200)	*P* Value[b]
Child characteristics					
Age, mean (SD), y	8.3 (0.65)	8.3 (0.76)	8.4 (0.63)	8.5 (0.61)	<.001
Male sex	90 (47.9)	92 (51.7)	74 (46.3)	96 (45.9)	.68
Primary caretaker mother	170 (96.1)	167 (97.7)	142 (97.3)	189 (94.5)	.44
Ever sent to school	131 (74.0)	124 (72.5)	117 (80.1)	155 (77.5)	.37
Adherence, mean (SD), %	75.2 (20.6)	73.4 (23.3)	79.7 (18.0)	72.1 (21.5)	.02
Adherence, median (IQR), %	82.6 (65.5-90.1)	83.2 (61.6-89.9)	86.5 (75.1-91.1)	79.6 (61.6-88.1)	.01
Diet in the past 7 days (any intake)					
Milk and dairy products[c]	138 (78.0)	128 (74.9)	110 (75.3)	133 (66.5)	.07
Meat, chicken, or fish	107 (60.5)	97 (56.7)	80 (54.8)	116 (58.0)	.77
Dark green leafy vegetables	113 (63.8)	119 (69.6)	103 (70.6)	157 (78.5)	.02
Citrus fruits[d]	64 (36.2)	78 (45.6)	58 (39.7)	75 (37.5)	.28
Yellow fruits and vegetables[e]	75 (42.4)	60 (35.1)	51 (34.9)	88 (44.0)	.17
Tea	61 (34.5)	84 (49.1)	65 (44.5)	89 (44.5)	.04
Morbidity in the past 7 days					
Lower respiratory tract infection[f]	1 (0.7)	3 (2.0)	1 (0.8)	3 (1.7)	.66
Diarrhea or dysentery[g]	2 (1.1)	10 (5.9)	8 (5.5)	7 (3.5)	.09
Child anthropometry and anemia					
Weight for age *z* score, mean (SD)	−2.09 (0.91)	−2.07 (0.93)	−1.99 (0.87)	−2.08 (0.93)	.80
Height for age *z* score, mean (SD)	−1.89 (0.90)	−1.85 (0.91)	−1.97 (0.84)	−1.93 (0.87)	.65
BMI *z* score, mean (SD)	−1.25 (0.86)	−1.26 (0.84)	−1.03 (0.77)	−1.20 (0.86)	.06
MUAC, mean (SD), cm	15.62 (1.19)	15.66 (1.44)	15.83 (1.39)	15.79 (1.26)	.40
Hemoglobin, mean (SD), g/dL	1.28 (0.59)	1.29 (0.60)	1.30 (0.46)	1.30 (0.55)	.98
Anemia (hemoglobin) <12 g/dL	72 (41.4)	62 (36.9)	42 (28.0)	66 (33.3)	.08
Any reported abnormality					
Vision	0	0	0	2 (1.0)	.09
Hearing	1 (0.6)	1 (0.6)	1 (0.7)	2 (1.0)	.15
Motor function	2 (1.1)	4 (2.4)	3 (2.1)	2 (1.0)	.27
Behavior	9 (5.1)	7 (4.2)	6 (4.2)	14 (7.0)	.24
Maternal characteristics					
Raven's score, mean (SD)	15.7 (4.6)	16.4 (5.2)	16.9 (5.5)	16.9 (4.9)	.10
Literacy	21 (11.9)	41 (24.1)	42 (29.0)	42 (21.0)	.002
Maternal educational level, years of schooling					.01
None	157 (88.7)	132 (77.7)	111 (76.6)	165 (82.5)	
1-5	8 (4.5)	10 (5.9)	12 (8.3)	12 (6.0)	
>6	12 (6.8)	28 (16.5)	22 (15.2)	23 (11.5)	
Household characteristics					
Salt iodine level >15 ppm	115 (65.3)	110 (65.5)	106 (72.6)	145 (72.9)	.23
Walls made with stone or cement	50 (28.3)	33 (19.3)	29 (19.9)	45 (22.5)	.18
Cement roof	8 (4.5)	10 (5.9)	3 (2.1)	11 (5.5)	.37
Asset score, mean (SD)[h]	4.5 (2.4)	4.5 (2.2)	4.8 (2.2)	4.4 (2.3)	.30
HOME score, mean (SD)	23.0 (5.7)	25.6 (6.2)	24.6 (5.7)	24.2 (6.3)	.001

Abbreviations: BMI, body mass index; HOME, Home Observation for the Measurement of the Environment; IQR, interquartile range; MUAC, middle upper arm circumference.

SI conversion factors: To convert hemoglobin to grams per liter, multiply by 10.

[a] Data are given as number (percentage) of children, mothers, or households unless otherwise indicated.

[b] Using analysis of variance for continuous variables and the χ^2 test for categorical variables.

[c] Includes milk, yogurt, and buttermilk.

[d] Includes oranges and guava.

[e] Includes ripe mango, papaya, jackfruit, and pumpkin.

[f] Productive cough or rapid breathing and fever.

[g] Watery stools 4 or more times per day or blood in stool.

[h] Asset score ranges from 0 to 11 and is made up of any ownership of goats, cattle, cart, bicycle, motorcycle, electricity, radio, television, telephone, mobile telephone, and watches in the household.

testers were graduate students with master's degrees in psychology trained by methods used in child clinical and school psychology PhD degree programs. They were certified to collect data once each of them performed a fully accurate test administration and scoring. All test sessions were video-recorded, and approximately 20% of the recordings for each tester were randomly selected and reviewed for accuracy by doctoral students at The Pennsylvania State University (supervised by the coinvestigators from The Pennsylvania State University). At the clinic visit, we also took measurements of anthropometry and hemoglobin levels. The anthropometry measures included height, weight, and middle upper arm circumference. From these, we calculated weight-for-age, height-for-age, and weight-for-height *z* scores using the international reference standard.[33] In addition, we collected information on factors known to affect child cognition, such as the quality and quantity of

stimulation available to the child in his or her home environment (assessed at the home) and the mother's reasoning ability (assessed at the clinic). The instruments used for these purposes were the middle childhood Home Observation for the Measurement of the Environment[34] (HOME) Inventory and Raven's Colored Progressive Matrices,[35] respectively.

Our first step in data analysis included creating an index of socioeconomic status and conducting basic exploratory analyses. Next, we ran baseline comparisons of the treatment groups to check for any significant differences among the groups on potentially confounding variables. The variables considered included demographic and socioeconomic characteristics, dietary intake, child schooling, HOME scores, morbidity prevalence, anthropometric measurements, adherence to supplementation, maternal literacy, maternal educational level, and maternal reasoning ability. Variables that were significantly different among the treatment groups or associated with outcomes were controlled for in the adjusted analyses.

Details of the outcome variables and analytic approach can be found in our previous article.[29] Briefly, for UNIT, the raw scores on the subtests were added and converted to T scores (mean [SD] 50 [10]) based on the child's age of 7, 8, or 9 years. For the go/no-go test, the percentage correct on no-go trials (an index of inhibitory control) was used. For the Backward Digit Span test, the longest number of digits remembered correctly was used. For the Stroop test, children were categorized as having failed the practice test if they could not complete it. Motor function was assessed with the MABC total and the mean number of finger taps of both hands. Because MABC scores on failure, a higher score shows poorer motor performance.

Testing was 2-sided with data analyzed as intent to treat using a 2×2 factorial analytic approach. The difference between scores for the 2 treatment groups and their interaction was assessed using multivariate analysis of variance (MANOVA) across outcomes to account for correlated multiple outcomes. Treatment groups were coded as iron plus folic acid vs no iron plus folic acid, zinc vs no zinc, and a cross-product of the 2. Because the interaction terms were insignificant, we reran the models excluding the interaction term and only report these models in the results. A bootstrap method was used as part of the MANOVA procedure to estimate 95% CIs and *P* values, accounting for correlation of responses among children from the same sector due to the cluster-randomized design.[36,37] Age at testing, sex, schooling, HOME[10] score, adherence with supplementation, maternal literacy, dark green leafy vegetable intake, and tea intake were associated with UNIT and MABC scores and/or differed among groups and were therefore included in the adjusted analyses. Age at testing and sex were also examined for effect modification along with age at enrollment using MANOVA. Age at enrollment was stratified into 3 categories: 12 months to younger than 18 months, 18 months to younger than 24 months, and 24 months or older. Data were analyzed using SAS statistical software, version 9.2 (SAS Institute, Inc), and Stata, version 11.1 (StataCorp).

Ethical approval for the 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, Kathmandu, Nepal. The parent trial is registered at clinicaltrials.gov NCT00109551.

RESULTS

Children in the present study represent a subset of children (n=3675) whose mothers participated in a micronutrient supplementation trial during pregnancy (**Figure**). Eligibility for the present analysis included children whose mothers were in the control arm of that trial, leaving 772 children as potentially eligible. Of the 772 children who met criteria for this analysis, 7 died and 30 had moved out of the study area during the preschool child supplementation trial. Between the end of the preschool child supplementation trial and the beginning of our follow-up assessment measurements at 7 to 9 years of age, 1 child died, leaving 734 children as eligible for our intellectual and motor assessments. Of these, 33 children could not be located and 7 refused to participate. Therefore, 694 children enrolled in this study, with the number of participants by treatment arm ranging from 146 to 200. Our loss to follow-up rate was approximately 5%.

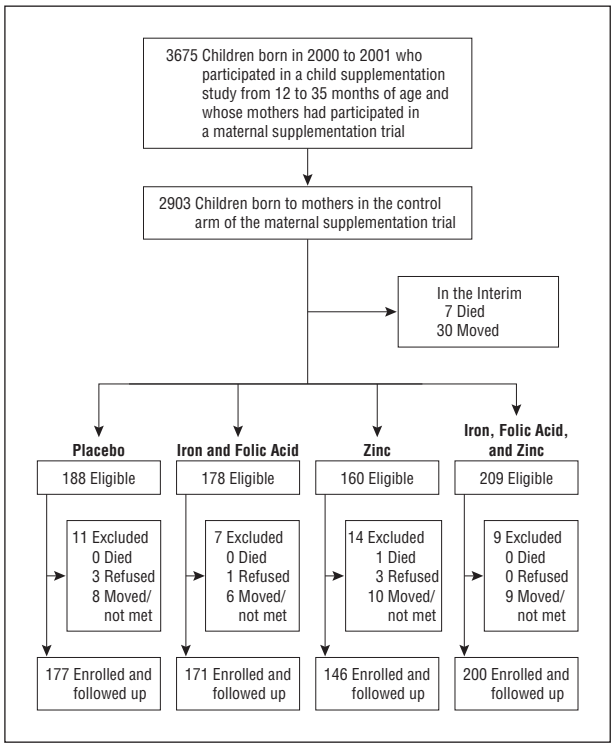

Figure. Study participation and follow-up by treatment group.

The mean (SD) age of children at follow-up was 8.4 (0.7) years and differed ($P < .001$) by treatment group (Table 1). Most (76.0%) had started school. Treatment groups differed with respect to intakes of dark green leafy vegetables and tea in the past 7 days and the score on the HOME inventory, maternal literacy, maternal educational level, and supplementation adherence. Maternal literacy was low across treatment groups but was significantly different ($P=.002$), with the prevalence being approximately half that of the other groups in the controls. No differences by treatment groups were found for anthropometric measurements, hemoglobin levels, morbidity in the past 7 days, asset score, or maternal Raven's score.

In the placebo group, the mean (SD) UNIT T score was 48.2 (10.2), the proportion who failed the Stroop test was 0.45 (0.50), the longest number of digits correctly recalled in on the Backward Digit Span test was 1.72 (0.96), the percentage correct on no-go trials was 45.2 (21.0), the MABC standard score was 9.82 (6.99), and the number of finger taps was 35.3 (5.7) (**Table 2**).

Table 2. Mean (SD) Scores on Psychometric Tests by Child Supplementation Group Assessed Among Children Aged 7 to 9 Years in Sarlahi, Nepal (2007-2009)

Test	Control (n=176)	Iron Plus Folic Acid (n=169)	Zinc (n=144)	Iron, Folic Acid, and Zinc (n=199)
UNIT score	48.2 (10.2)	49.7 (9.7)	51.1 (10.4)	49.3 (10.2)
Stroop test, proportion who failed	0.45 (0.50)	0.32 (0.47)	0.26 (0.44)	0.31 (0.46)
Backward Digit Span test	1.72 (0.96)	1.95 (1.05)	2.05 (1.18)	1.91 (1.09)
Go/no-go test, % correct on the no-go test	45.2 (21.0)	44.9 (20.3)	47.8 (21.5)	42.3 (18.8)
MABC	9.82 (6.99)	9.13 (6.32)	7.76 (6.05)	9.03 (6.06)
Finger-tapping test	35.3 (5.7)	35.6 (5.5)	37.4 (5.5)	36.3 (5.2)

Abbreviations: MABC, Movement Assessment Battery for Children; UNIT, Universal Nonverbal Intelligence Test.

Table 3. Unadjusted Differences in Test Scores by Child Supplementation Among Children 7 to 9 Years of Age in Sarlahi, Nepal (2007-2009)

	Iron Plus Folic Acid vs No Iron Plus Folic Acid		Zinc vs No Zinc	
Test	Difference (95% CI)[a]	*P* Value	Difference (95% CI)[a]	*P* Value
UNIT	−0.15 (−2.31 to 2.05)	.89	1.21 (−1.48 to 3.90)	.38
Stroop test, proportion who failed	−0.05 (−0.15 to 0.05)	.33	−0.09 (−0.23 to 0.04)	.17
Backward Digit Span test	0.06 (−0.15 to 0.28)	.57	0.14 (−0.14 to 0.43)	.33
Go/no-go test, % correct on the no-go test	−2.90 (−7.25 to 1.50)	.19	0.11 (−4.19 to 4.42)	.96
MABC	0.35 (−0.69 to 1.38)	.51	−0.91 (−2.62 to 0.79)	.29
Finger-tapping test	−0.33 (−1.80 to 1.15)	.66	1.27 (−0.52 to 3.06)	.16
P value[b]	.14		.04	

Abbreviations: MABC, Movement Assessment Battery for Children; UNIT, Universal Nonverbal Intelligence Test.
[a] Using boot strapping to estimate SE adjusted for design effect.
[b] *P* value for the overall treatment effect using Wilks λ and Lawley-Hotelling trace test derived from the multivariate analysis of variance (n=667).

Table 4. Differences in Test Scores by Child Supplementation Group Adjusted for Confounders Among Children 7 to 9 Years of Age in Sarlahi, Nepal (2007-2009)

	Iron Plus Folic Acid vs No Iron Plus Folic Acid		Zinc vs No Zinc	
Test	Difference (95% CI)[a]	*P* Value	Difference (95% CI)[a]	*P* Value
UNIT	−0.74 (−2.07 to 0.59)	.28	0.40 (−0.95 to 1.76)	.56
Stroop test, proportion who failed	−0.04 (−0.10 to 0.03)	.26	−0.04 (−0.10 to 0.02)	.22
Backward Digit Span test	−0.01 (−0.16 to 0.14)	.89	0.02 (−0.14 to 0.18)	.80
Go/no-go test, % correct on the no-go test	−3.27 (−7.33 to 0.80)	.12	−2.03 (−5.09 to 1.02)	.19
MABC	0.76 (−0.08 to 1.60)	.08	−0.16 (−1.13 to 0.81)	.74
Finger-tapping test	−0.62 (−1.59 to 0.34)	.21	0.47 (−0.38 to 1.32)	.28
P value[b]	.10		.35	

Abbreviations: MABC, Movement Assessment Battery for Children; UNIT, Universal Nonverbal Intelligence Test.
[a] Using boot strapping to estimate SE adjusted for design effect and adjusted for child age, Home Observation for the Measurement of the Environment score, maternal literacy, child adherence to treatment, sex, ever sent to school, dark green leafy vegetable intake, and tea intake during previous 7 days.
[b] *P* value for the overall treatment effect using Wilks λ and Lawley-Hotelling trace test derived from the multivariate analysis of variance (n=667).

Multivariate analysis of variance revealed a significant overall difference across tests for the zinc group (*P*=.04) vs no zinc but not for the iron plus folic acid group (*P*=.14) compared with the no iron plus folic acid group (**Table 3**). On individual tests, the zinc group scored higher than the reference group on intellectual function, executive function (Stroop test), and fine motor function (finger-tapping test), but none of the differences were significant (Table 3).

When adjusted for age, sex, having ever been sent to school, HOME score, maternal literacy, adherence to treatment, and diet, the differences overall and across tests for both zinc and iron plus folic acid were not significant (**Table 4**). Stratified analysis by age at entry into the preschool supplementation trial adjusted for confounders showed that the overall MANOVA test for the iron plus folic acid effect was significant (*P*=.02) in the 12-month to younger than 18-month age group, with the no-go test and finger-tapping test scores being lower (data not shown). This negative effect was not observed in the other age categories (18 months to <24 months and ≥24 months).

COMMENT

Our study followed up children who were part of a prenatal cohort at 7 to 9 years of age to assess the effect of supplementation from 12 to 35 months of age with iron plus folic acid, zinc, or the combination of the 2 on intellectual, executive, and motor functioning. We hypothesized that supplementation with iron plus folic acid or zinc would improve outcomes and that the iron plus folic acid and zinc group would improve outcomes at least to the same extent or more than with either alone. Our results suggest that these hypotheses were not accepted.

In unadjusted analyses, the overall MANOVA test revealed a significant effect of the zinc supplementation, but individual test score differences were not statistically significant. In the adjusted analyses, even the overall difference disappeared. Studies[8,10,20-25] that have examined zinc supplementation (given alone) in early childhood and development report inconsistent findings, although a finding of a relationship between zinc supplementation and motor development seems to be the most consistent among the studies.[20-22,24] Iron plus folic acid supplementation also did not result in improvements for general intellectual, executive, or motor functioning when compared with a placebo. Our findings are similar to those of a recent, prospective, follow-up study in Thailand that evaluated iron and/or zinc supplementation in infancy from 4 to 6 months for 6 months and found no effect on intellectual or motor function at 9 years of age.[26] We recently reported that in utero iron plus folic acid supplementation had a significant beneficial effect on intellectual, executive, and some aspects of motor functioning.[29] Adding other nutrients to the maternal supplement, including zinc, or supplementation during preschool years with iron and zinc supplementation followed by maternal iron plus folic acid had no additional benefit to any of the developmental outcomes.[38] Many studies have examined the effects of iron supplementation on cognitive and motor development in children. A main difference between our study and most of the studies to date is the timing of supplementation. One study,[17] which found a beneficial effect on language development, provided supplements to older children during the preschool years (6-59 months of age). Had we continued the supplementation beyond 35 months of age, perhaps we would have found a benefit of the supplements.

In an adjusted analysis, children who started supplementation with iron plus folic acid from 12 to 18 months of age performed worse on a test of executive function (correct no-go test result) examining inhibitory control and a finger-tapping test compared with those who did not get iron plus folic acid, which is consistent with a Chilean cohort study[39] of children at 10 years of age who as infants were randomly assigned to receive a low-iron (2.3 mg/L) vs high-iron (12.7 mg/L) infant formula. Scores on spatial memory and visual-motor integration were lower at 10 years of age in the group receiving more iron. Thus, the finding of a small negative effect on a test of executive function and fine motor control in those who were the youngest when supplementation began in the Nepalese cohort is similar to the results observed in the Chilean study. This small negative effect was not observed in our Nepalese children who started their supplementation at older ages. These findings should be interpreted with caution because, as pointed out in an editorial,[40] a Thai study[26] testing iron supplementation in infancy did not find such negative results.

Our study has several strengths and limitations. First, we had small loss to follow-up because we were able to test approximately 95% of the original study participants. Second, our sample size was more than adequate to assess our main outcomes of interest. Third, the trial from which this sample was drawn was a randomized, double-blind, controlled trial. Fourth, this is a long-term follow-up trial assessing children 7 to 9 years after supplementation. This approach not only represents something done in few studies but also allowed us to assess outcomes at an age where such testing is easier and proves more stable than at younger ages. Finally, we assessed outcomes in several domains, allowing us to test the effects of supplementation on multiple skills. Limitations include the fact that we did not continuously follow up these children between supplementation in preschool and the present follow-up study. Therefore, there may be unmeasured variables that occurred in the interim for which we cannot account. Although we were powered to detect our primary outcomes of interest, subanalyses, such as interaction effects of age at entry by treatment, were underpowered. Finally, by design, the cohort of children we followed up in this study had not started supplementation during infancy; thus, our study was unable to examine supplementation effects in that critical period for central nervous system development.

In conclusion, our study found no beneficial effect of either iron plus folic acid or zinc supplementation among children from 12 to 35 months of age on intellectual, executive, or motor function at 7 to 9 years of age in this rural Nepalese population, where both iron and zinc deficiencies were common. These findings suggest the need to examine, especially through long-term follow-up, the effect of preschool nutrient interventions on developmental outcomes. The timing, type, and duration of supplementation and other factors known to affect the outcomes of interest are important to evaluate. Currently, there is little evidence to suggest routine prophylactic supplementation of children with iron or zinc in Nepal or other similar regions of South Asia at least for improving developmental outcomes.

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