Prenatal Micronutrient Supplementation and Intellectual and Motor Function in Early School-aged Children in Nepal

Parul Christian, DrPH
Laura E. Murray-Kolb, PhD
Subarna K. Khatry, MBBS, FRCS
Joanne Katz, ScD
Barbara A. Schaefer, PhD
Pamela M. Cole, PhD
Steven C. LeClerq, MPH
James M. Tielsch, PhD

Context Iron and zinc are important for the development of both intellectual and motor skills. Few studies have examined whether iron and zinc supplementation during gestation, a critical period of central nervous system development, affects children's later functioning.

Objective To examine intellectual and motor functioning of children whose mothers received micronutrient supplementation during pregnancy.

Design, Setting, and Participants Cohort follow-up of 676 children aged 7 to 9 years in June 2007–April 2009 who had been born to women in 4 of 5 groups of a community-based, double-blind, randomized controlled trial of prenatal micronutrient supplementation between 1999 and 2001 in rural Nepal. Study children were also in the placebo group of a subsequent preschool iron and zinc supplementation trial.

Interventions Women whose children were followed up had been randomly assigned to receive daily iron/folic acid, iron/folic acid/zinc, or multiple micronutrients containing these plus 11 other micronutrients, all with vitamin A, vs a control group of vitamin A alone from early pregnancy through 3 months postpartum. These children did not receive additional micronutrient supplementation other than biannual vitamin A supplementation.

Main Outcome Measures Children's intellectual functioning, assessed using the Universal Nonverbal Intelligence Test (UNIT); tests of executive function, including go/no-go, the Stroop test, and backward digit span; and motor function, assessed using the Movement Assessment Battery for Children (MABC) and finger-tapping test.

Results The difference across outcomes was significant (Bonferroni-adjusted $P < .001$) for iron/folic acid vs control but not for other supplement groups. The mean UNIT T score in the iron/folic acid group was 51.7 (SD, 8.5) and in the control group was 48.2 (SD, 10.2), with an adjusted mean difference of 2.38 (95% confidence interval [CI], 0.06-4.70; $P = .04$). Differences were not significant between the control group and either the iron/folic acid/zinc (0.73; 95% CI, –0.95 to 2.42) or multiple micronutrient (1.00; 95% CI, –0.55 to 2.56) groups. In tests of executive function, scores were better in the iron/folic acid group relative to the control group for the Stroop test (adjusted mean difference in proportion who failed, –0.14; 95% CI, –0.23 to –0.04) and backward digit span (adjusted mean difference, 0.36; 95% CI, 0.01-0.71) but not for the go/no-go test. The MABC score was lower (better) in the iron/folic acid group compared with the control group but not after adjustment for confounders (mean difference, –1.47; 95% CI, –3.06 to 0.12; $P = .07$). Finger-tapping test scores were higher (mean difference, 2.05; 95% CI, 0.87-3.24; $P = .001$) in the iron/folic acid group.

Conclusion Aspects of intellectual functioning including working memory, inhibitory control, and fine motor functioning among offspring were positively associated with prenatal iron/folic acid supplementation in an area where iron deficiency is prevalent.

Trial Registration clinicaltrials.gov Identifier: NCT00115271

JAMA. 2010;304(24):2716-2723 www.jama.com

Micronutrient inadequacy is a critical concern among pregnant women and young children throughout the world. Gestation and the early postnatal period are considered sensitive periods for brain development, and nutritional deprivation during this period may lead to functional impairments.

Early iron deficiency can alter neuroanatomy, biochemistry, and metabolism, leading to changes in neurophysiologic processes that support cognitive and sensorimotor development.[1,2] It also has an adverse effect on neurogenesis, altering brain morphology in specific regions such as the hippocampus and striatum.[3,4] In addition, the timing of iron availability for myelin production, especially to oligodendrocytes, is critical[5]; hypomyelination due to iron deficiency persists later in life.[6,7] Iron deficiency also alters neurochemistry, specifically monoamine transmission, reception, and metabolism.[6] Finally, iron is important for energy metabolism in the brain.[2,8] Abnormal cerebral energy metabolism, as measured via loss of cytochrome *c* oxidase in the hippocampus and frontal cortex[9] and via mag-

Author Affiliations are listed at the end of this article.
Corresponding Author: Parul Christian, DrPH, Center for Human Nutrition, Johns Hopkins Bloomberg School of Public Health, 615 N Wolfe St, Room W2041, Baltimore, MD 21205 (pchristi@jhsph.edu).

©2010 American Medical Association. All rights reserved.

Downloaded from jama.ama-assn.org at Tel-Aviv University on 25 December 2010

netic resonance spectroscopy studies,[3,4] has been linked to iron deficiency. Energy-dependent processes such as dendritic arborization and synaptogenesis are thereby impaired[2]; MAP2 (microtubule-associated protein 2) expression required for dendritic scaffolding may be involved.[10] Also in the hippocampus, down-regulation of several genes involved in synaptic plasticity occurs with perinatal iron deficiency.[11]

Evidence from primates or humans that can demonstrate the effects of gestational iron deficiency on cognitive and motor functioning is limited. In rhesus monkeys, infants prenatally deprived of iron exhibited 20% reduced spontaneous activity level, lower inhibitory response to novel environments, and frequent behavioral changes,[12] whereas human infants with cord serum ferritin concentrations below 35 µg/L had lower auditory recognition memory.[13] In a controlled trial in Australia, prenatal iron supplementation (20 mg/d) had no effect on IQ test performance in 4-year-old offspring,[14] but iron deficiency anemia was prevalent in only 11% of the mothers.

The evidence for similar effects associated with zinc supplementation in pregnancy is even more inconclusive. A trial in Peru[15] reported improvements in fetal neurobehavioral development associated with maternal supplementation, whereas in Bangladesh, infants of zinc-supplemented mothers had lower scores on the Bayley Scales of Infant Development than those whose mothers received a placebo during pregnancy; this may be due to an inhibitory effect of zinc on iron status.[16] Zinc supplementation in African American women had no effect on cognitive or psychomotor functioning of their 5-year-old offspring.[17]

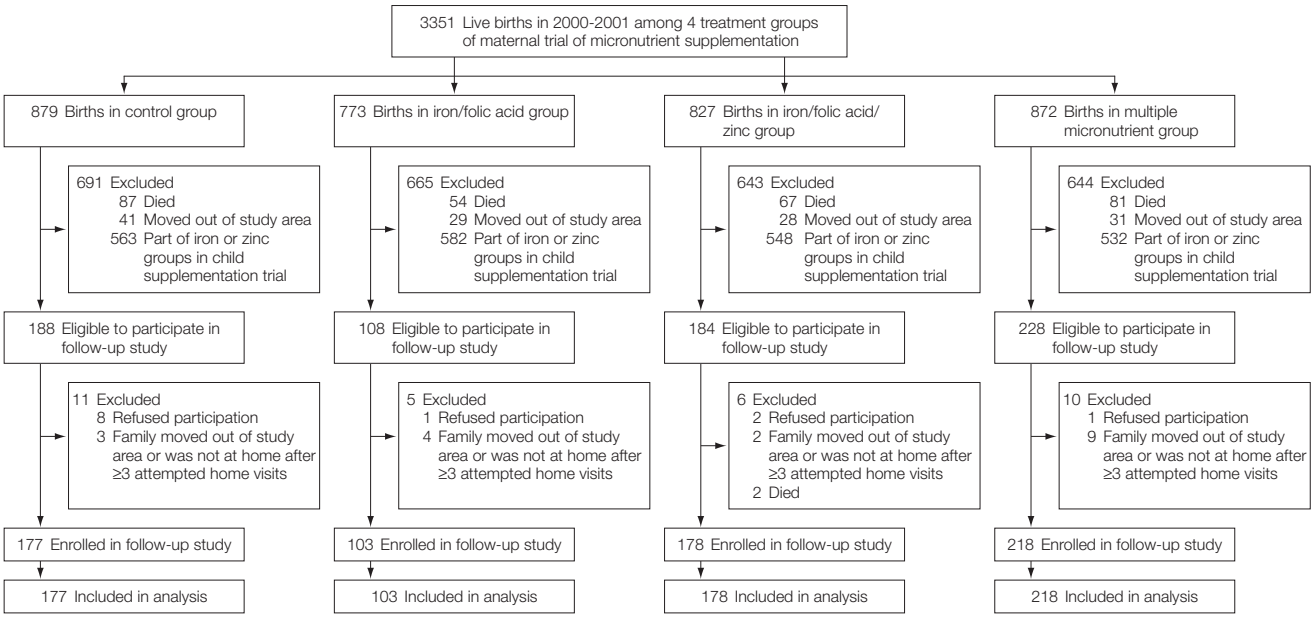
This study was designed to assess intellectual, executive, and motor functioning in a cohort of Nepalese children aged 7 to 9 years in 2007-2009 whose mothers received daily prenatal and postnatal micronutrient supplements in a controlled, cluster-randomized, double-blind trial conducted between 1999 and 2001.[18,19] Additionally, in 2001-2005, these children were enrolled in a randomized, placebo-controlled trial of iron and/or zinc supplementation during preschool age.[20,21] To isolate the effect of maternal supplementation alone, data from children in the placebo group of the preschool supplementation trial were included in the present analysis.

METHODS

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.

From June 2007 to April 2009, we prospectively followed up 7- to 9-year-old offspring of women who had participated in a trial of prenatal supplementation with different combinations of micronutrients in a southeastern plains district (Sarlahi) of Nepal (FIGURE and eFigure [available at http://www.jama.com]). The study was conducted in 30 Village Development Committees (VDCs) of the district. Of a total of 48 VDCs, 30 VDCs were defined as the study area based on their geographic location. The VDCs located in the hills toward the north, where access was harder, and toward the border with India, where the population was expected to be transient, were not included. Village Development Committees in the far east or west of the district were also excluded. The entire 30-VDC study area was then divided into 426 sectors, or smaller community clusters, to create randomization units. Each sector had an area

Figure. Study Participation by Treatment Group



©2010 American Medical Association. All rights reserved.

Downloaded from jama.ama-assn.org at Tel-Aviv University on 25 December 2010

small enough to be covered by a local female worker who walked to all the households with participants. The parent 5-group, double-masked, cluster-randomized controlled trial had as its goal to examine effects on birth weight and infant mortality of daily supplementation with alternative combinations of micronutrients during pregnancy through 12 weeks postpartum.[18,19] The mean gestational age at enrollment was 11 weeks (SD, 5.1 weeks) and adherence to supplements was high (88% [interquartile range, 67%-97%] of all possible doses); neither of these differed by supplementation group. The intervention groups (with micronutrient amounts per daily supplement) were folic acid (400 µg), folic acid plus iron (60 mg), folic acid/iron plus zinc (30 mg), and folic acid/iron/zinc plus vitamins D (10 µg), E (10 mg), B_1 (1.6 mg), B_2 (1.8 mg), B_6 (2.2 mg), B_{12} (2.6 µg), C (100 mg), and K (65 µg); niacin (20 mg); copper (2.0 mg); and magnesium (100 mg), all with 1000-µg retinol equivalents of vitamin A. Vitamin A supplementation alone was the control. Children born to those in the folic acid–only group were not included in this follow-up study because of the low likelihood of folic acid alone having any effect on study outcomes. Also, to isolate effects of maternal supplementation, only children from the placebo group of a subsequent iron and/or zinc supplementation trial among preschoolers[20,21] were included in this analysis. Per Nepalese government policy, children received 200 000 IU of vitamin A biannually from 6 to 60 months of age. To our knowledge, the study children had no other exposure to other forms of micronutrient supplementation.

Households with eligible children were invited to participate in the follow-up study. The purpose of the study was explained and parental oral consent and child assent were obtained.

Enrollment Interview and Assessment

Eligible households were visited to collect information about household demographics and socioeconomic status, a detailed history of the child's enrollment in school, type of school, and number of completed and repeated years of schooling.

Local terms were used to obtain histories of morbidity symptoms in study children during the previous 7 and 30 days. Assessment of diet at the time of follow-up was performed using a 7-day food frequency questionnaire. Household salt used for cooking was tested with a semiquantitative kit (MBI Kits, Madras, India). Iodine content was recorded as 0, less than 15 ppm, or 15 ppm or more.

Psychological Testing

Four master's-level graduate students in psychology were the psychometrists who administered the Universal Nonverbal Intelligence Test (UNIT) and the Movement Assessment Battery for Children (MABC). They were trained by methods used in child clinical and school psychology PhD programs. When each performed a fully accurate test administration and scoring, they were certified to collect data. Test sessions were video-recorded and approximately 20% of the video records of each psychometrist's work were selected randomly and reviewed for test administration and scoring accuracy by Pennsylvania State University graduate students supervised by the Pennsylvania State University coinvestigators.

Each test was selected for its capacity to measure aspects of cognitive or motor functioning previously known to be sensitive to brain function changes attributed to nutritional influences. The UNIT[22] is a nonverbal test of general intelligence using hand gestures to maximize fairness for all children irrespective of race, ethnicity, sex, language, country of origin, or hearing status.[23] The UNIT has 6 subtests assessing symbolic memory (0-30 possible raw score points), cube design (0-53), spatial memory (0-27), analogic reasoning (0-31), object memory (0-30), and mazes (0-93). The analogic reasoning subtest was culturally inappropriate because the stimuli include pictures of objects that are unfamiliar to most Nepalese children; it was therefore not administered.

Three tasks of executive function were administered that assessed inhibitory control and processing speed: a Stroop numbers test, backward digit span, and go/no-go. The Stroop test is commonly used to test inhibitory control,[24] and the backward digit span from the Wechsler memory scale[25] is used to test working memory and inhibitory control. A computerized go/no-go task was used to further assess inhibitory control. The task was a series of 210 "go" trials (press computer key when "go" stimulus is present) and 70 "no-go" trials (do not press if a "no-go" stimulus is present).[26]

Motor development was assessed with the MABC,[27] which consists of a series of tasks to assess manual dexterity, ball skills, and balance. As an additional assessment of fine motor skills, a finger-tapping test was performed to assess index finger motor speed of each hand.

Children were brought to a central site for testing. On arrival, they were given a snack and a drink, and the psychometrist spent 30 minutes building rapport before taking the children to a testing room.

Anthropometry and Hemoglobin

At the central site, trained anthropometrists measured height, weight, and mid upper arm circumference using standard procedures. Hemoglobin was estimated using fingerstick with a B-Hemoglobin Analyzer (HemoCue, Lake Forest, California). Children with anemia (hemoglobin <115 g/L) received iron/folic acid supplements as treatment.

Environment

Environmental influences on the selected child outcomes were indexed with the Middle Childhood Home Observation for the Measurement of the Environment (HOME) Inventory[28] and the Raven Coloured Progressive Matrices,[29] administered to mothers at the central site visit.

 ©2010 American Medical Association. All rights reserved.

Downloaded from jama.ama-assn.org at Tel-Aviv University on 25 December 2010

Data Analysis

In this study, the sample size was fixed by the prior study designs. Hence, we calculated the smallest detectable differences in means of tests of cognition and motor functioning planned for the study between groups of interest that could be detected with a given power (80% and 90%) and type I error ($\alpha = .05$). Testing was 2-sided and differences are estimated for varying levels of estimated correlations in outcomes within a cluster given the cluster-randomized design of the study as shown in eTable 1.

Weight-for-age, height-for-age, and body mass index *z* scores were calculated using the international reference standard.[30] Following basic exploratory analysis and asset score creation for socioeconomic status (asset score ranged from 0-11 and was made up of any household ownership of goats, cattle, cart, bicycle, mortorcycle, electricity, radio, television, telephone, mobile phone, or watches), baseline comparisons of the 4 treatment groups were performed with analysis of variance to assess imbalance on potentially confounding variables. Variables that were unbalanced between the treatment groups and associated with outcomes were controlled for in the adjusted analyses. Intellectual functioning outcomes were UNIT total scores, failure on the Stroop practice test, longest number of digits remembered backward correctly (backward digit span), and no-go percentage correct. Motor function was assessed with the MABC total and the mean number of finger taps of both hands.

Although UNIT is a standardized IQ test, there are no norms for Nepal, where it has never been used. Its factor structure for the Nepal sample was assessed using exploratory and confirmatory factor analyses of UNIT subscales. After omitting items with no variability, α was calculated for each subtest total raw score. The coefficients indicated satisfactory reliabilities, ranging from 0.76 for symbolic memory to 0.88 for mazes. Exploratory factor analyses suggested a 2- or 3-factor structure. Confirmatory analyses using EQS, version 6.0 (Multivariate Software Inc, Encino, California) compared the 2- and 3-factor solutions and found the 2-factor model to have a superior fit, with fit indexes of 0.995 (vs 0.927 for the 3-factor model). The subtests and their raw mean scores comprising the UNIT factors were as follows: for factor 1, symbolic memory (3.64 [SD, 2.73]), object memory (9.11 [SD, 4.56]), cube design (5.94 [SD, 5.13]), and spatial memory (4.36 [SD, 3.62]); and for factor 2, mazes subtest (22.16 [SD, 13.25]). Raw scores were converted to T scores (mean, 50 [SD, 10]) based on a child's age (7, 8, or 9 years) and were used as the outcome. The MABC is scored for failures so that higher scores reflect higher motor impairment. Raw scores on each task were converted to scaled scores provided in the manual, based on the standardization sample. Scaled score ranges on each of the subtests were as follows: manual dexterity, 0 to 15; ball skills, 0 to 10; and balance skills, 0 to 15. The total scaled score had a range of 0 to 40. Children whose score is less than the fifth percentile are considered to definitively have a motor problem. Those who score between the fifth and 15th percentiles are considered to be borderline in terms of motor problems. For the total scaled score, the 15th percentile cutoff is greater than 10. Generally, the above-mentioned cutoffs are used for clinical assessment.

Data were analyzed as for intention to treat and testing was 2-sided. We estimated the difference between test scores for each of the 3 active treatment groups compared with the placebo group using multivariate analysis of variance (MANOVA) across outcomes to account for correlated multiple outcomes. Treatment groups were coded as indicator variable. In addition, we applied a Bonferroni correction to the *P* values to account for the multiple comparisons being made between 3 treatment groups vs a single control. Only 643 records were used in the multivariate analysis as records with missing data for individual outcomes were excluded. As part of the multivariate analysis of covariance procedure, a bootstrap method was used to estimate 95% confidence intervals (CIs) and *P* values accounting for independent correlation of responses among children from the same sector due to the cluster-randomized design.[31,32] Because age, sex, schooling, and asset score were strongly associated with UNIT and MABC scores, these variables were included in the adjusted models. In addition, because diet and morbidity were different by treatment group, we ran models adjusted for these variables as confounders along with other variables. We also examined age and sex for effect modification across the outcomes using MANOVA with bootstrapped 95% CIs. Data were analyzed using SAS software, version 9.2 (SAS Institute Inc, Cary, North Carolina) and STATA software, version 11 (Stata Corp, College Station, Texas).

RESULTS

In 2000-2001, 3351 live births were recorded in the 4 treatment groups of the maternal trial of micronutrient supplementation[18,19] (Figure and eFigure). By March 2007, 129 had moved out of the study area and 289 had died, leaving a total of 2933 known to be alive and residing in the study area. In this analysis, we excluded all children (500-600 per group) who received active supplements as part of a preschool iron and zinc supplementation trial carried out by our group from 2001 to 2005[20,21] (Figure). Of the remaining 708 children eligible for inclusion in this analysis, the number of participants by treatment group ranged between 108 and 228. Of this, a total of 676 consented to participate and were enrolled, corresponding to a loss to follow-up of approximately 5%.

The mean age of children at follow-up was 8.4 (SD, 0.65) years and differed by treatment group ($P = .003$) (**TABLE 1**). A majority (80%) had started school. Treatment groups differed with respect to dairy and meat intake in the past 7 days, percentage reporting symptoms of lower respiratory tract and gastrointestinal tract infection, and socioeconomic status. The percentages of

©2010 American Medical Association. All rights reserved.

Downloaded from jama.ama-assn.org at Tel-Aviv University on 25 December 2010

children with stunting, wasting, or anemia did not differ by treatment group. Maternal age, schooling, literacy, and Raven score did not differ by treatment group.

In the control group, the mean UNIT T score was 48.2 (SD, 10.2); the proportion who failed the Stroop test was 0.45 (SD, 0.50); the mean longest number of digits correctly recalled in a backward digit span was 1.72 (SD, 0.96); the percentage correct no-go trials was 45.2 (SD, 21.0); the mean MABC standard score was 9.82 (SD, 6.99); and the mean number of finger taps was 35.3 (SD, 5.7) (TABLE 2). MANOVA revealed a significant difference (Bonferroni-adjusted $P<.001$) across tests for the iron/folic acid group compared with the control group. On individual tests, scores were better for intellectual function, tests of executive function (except for the go/no-go test), and motor function among children in the iron/folic acid group relative to the control group (TABLE 3). These differences were attenuated or absent in the iron/folic acid/zinc and multiple micronutrient groups across tests. Adjusting for age, sex, having ever been sent to school, asset score, diet, and morbidity did not change the results of the MANOVA test for the iron/folic acid group ($P<.002$) but P values were higher and no longer significant for some individual tests (TABLE 4). Adjusted mean differences in the iron/folic acid group relative to the control group were 2.38 (95% CI, 0.06-4.70; $P=.04$) for the UNIT T score, –0.14 (95% CI, –0.23 to –0.04; $P=.005$) for failure on the Stroop test; 0.36 (95% CI, 0.01-0.71; $P=.02$) for the backward digit span; –1.47 (95% CI, –3.06 to 0.12; $P=.07$) for the MABC (lower scores indicating better motor function); and 2.05 (95% CI, 0.87-3.24; $P=.001$) for the finger-tapping test.

Stratified analysis by age and sex and adjusted for confounders showed some differences, with older and female children benefiting more from the iron/folic acid intervention, but interaction effects were largely not statistically significant (eTable 2).

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

Characteristics	Control (n = 177)	Iron/Folic Acid (n = 103)	Iron/Folic Acid/Zinc (n = 178)	Multiple Micronutrients (n = 218)	*P* Value[b]
Child characteristics					
Age, mean (SD), y	8.3 (0.65)	8.6 (0.60)	8.3 (0.63)	8.4 (0.66)	.002
Male	83 (46.9)	49 (47.6)	100 (56.2)	103 (47.2)	.24
Primary caretaker mother	170 (96.0)	94 (91.3)	168 (94.4)	207 (94.9)	.39
Ever sent to school	131 (74.0)	87 (84.5)	149 (83.7)	172 (78.9)	.08
Diet in the past 7 d (any intake)					
Milk and dairy products[c]	138 (78.0)	71 (68.9)	122 (68.5)	139 (64.1)	.03
Meat, chicken, or fish	107 (60.4)	64 (62.1)	122 (68.5)	114 (52.5)	.01
Dark green leafy vegetables	113 (63.8)	79 (76.7)	123 (69.1)	141 (65.0)	.12
Citrus fruits[d]	64 (36.2)	45 (43.7)	67 (37.6)	100 (46.1)	.16
Yellow fruits and vegetables[e]	75 (42.4)	47 (45.6)	58 (32.6)	94 (43.3)	.08
Tea	61 (34.5)	47 (45.6)	59 (33.2)	78 (35.9)	.18
Morbidity in the past 7 d					
Lower respiratory tract infection[f]	1 (0.6)	4 (3.9)	5 (2.8)	12 (5.5)	.05
Diarrhea/dysentery[g]	2 (1.1)	9 (8.7)	5 (2.8)	6 (2.8)	.01
Child anthropometry and anemia					
Stunting (height-for-age z score <–2)	81 (46.0)	40 (39.6)	108 (50.0)	70 (39.6)	.14
Wasting (BMI *z* score <–2)[h]	27 (15.3)	16 (15.8)	29 (16.4)	34 (15.7)	.99
Anemia (hemoglobin <115 g/L)	40 (23.0)	23 (23.0)	43 (24.4)	42 (19.6)	.70
Mid upper arm circumference, mean (SD), cm	15.6 (1.2)	15.8 (1.3)	15.6 (1.2)	15.7 (1.4)	.57
Maternal characteristics					
Age at enrollment, mean (SD), y	31.2 (5.4)	32.0 (5.0)	31.6 (5.4)	32.0 (5.6)	.50
Raven score, mean (SD)	15.7 (4.6)	16.6 (5.4)	16.6 (5.1)	15.9 (5.0)	.28
Literacy	21 (12.0)	22 (21.4)	32 (18.0)	43 (19.8)	.14
Adult education in past 3 y	14 (8.0)	11 (11.2)	19 (10.9)	25 (11.7)	.68
Household characteristics					
Household salt iodine level ≥15 ppm	115 (65.3)	80 (78.4)	115 (66.1)	149 (69.0)	.07
Walls made with stone or cement	50 (28.3)	20 (19.4)	41 (23.0)	42 (19.3)	.16
Cement roof	8 (4.5)	7 (6.8)	19 (10.7)	11 (5.1)	.08
Asset score, mean (SD)[i]	4.5 (2.3)	5.0 (2.1)	4.8 (2.3)	4.2 (2.0)	.02
HOME Inventory score, mean (SD)	23.0 (5.7)	24.6 (6.5)	23.5 (6.9)	23.2 (5.7)	.16

Abbreviations: BMI, body mass index, calculated as weight in kilograms divided by height in meters squared; HOME, Home Observation for the Measurement of the Environment.
[a] Data are expressed as No. (%) unless otherwise indicated. Data are missing for diet and morbidity (n=1), child anthropometry (n=6), anemia (n=12), maternal age (n=10), Raven score (n=28), literacy (n=3), household salt (n=8), asset score (n=4), and HOME score (n=7).
[b] Comparisons 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 at least 4 times a day or blood in stool.
[h] Body mass index calculated as weight in kilograms divided by height in meters squared.
[i] 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, or watches in the household.

COMMENT

In a rural South Asian population, overall outcomes of general intellectual test performance and aspects of executive and motor function in 7- to 9-year-old children were better among those whose mothers had received prenatal iron and folic acid supplementation compared with controls. We previously reported high prevalence rates of iron deficiency and anemia during pregnancy in this rural area of Nepal.[33,34] In general, the differences in test scores between the other intervention groups and controls were not statistically significant.

We found evidence linking prenatal iron/folic acid supplementation with working memory and inhibitory control test results. On the Stroop and backward digit span tests, children in the iron/folic acid group did the best and significantly

©2010 American Medical Association. All rights reserved.

Downloaded from jama.ama-assn.org at Tel-Aviv University on 25 December 2010

better than control children, although children scored similarly across treatment groups on correct response to the no-go stimulus, which measures inhibitory control. The development of executive functioning is protracted, beginning in infancy and extending into early adulthood. Thus, future examination of this domain at an older age may reveal larger differences that may have been too early to detect in this analysis.

General intellectual functioning was higher with prenatal iron/folic acid supplementation. We observed an adjusted 2.4-point or one-quarter SD difference overall in the UNIT score, which, although of low clinical significance at the individual level, may be a meaningful difference at the population level. Examination of the UNIT score distributions for the control and iron/folic acid groups suggests a larger difference in the lower tail. The differences in intellectual and executive function tasks suggest that iron/folic acid, when provided during critical periods of development, may make an important difference in children's ability to learn tasks that are related to their academic achievement.

The higher scores of motor function, specifically related to fine motor control and speed, that are apparent in

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

	Mean (SD) Score			
Test	Control (n = 177)	Iron/Folic Acid (n = 103)	Iron/Folic Acid/Zinc (n = 178)	Multiple Micronutrients (n = 218)
UNIT	48.2 (10.2)	51.7 (8.5)	50.2 (9.3)	49.3 (9.7)
Stroop test, proportion who failed	0.45 (0.50)	0.20 (0.40)	0.35 (0.48)	0.29 (0.46)
Backward digit span test	1.72 (0.96)	2.21 (1.23)	1.84 (1.06)	1.80 (1.08)
Go/no-go test, % no-go correct	45.2 (21.0)	47.1 (21.0)	42.6 (20.5)	47.0 (20.9)
MABC	9.82 (6.99)	6.78 (4.93)	8.59 (5.81)	8.65 (6.14)
Finger-tapping test	35.3 (5.7)	38.5 (4.5)	36.5 (5.0)	36.3 (5.0)

Abbreviations: MABC, Movement Assessment Battery for Children; UNIT, Universal Nonverbal Test of Intelligence.
[a] Data are missing for the UNIT test (n=16), Stroop test (n=8), backward digit span test (n=6),go/no-go test (n=7), MABC (n=19), and finger-tapping test (n=9).

Table 3. Differences in Test Scores by Maternal Supplementation Group Relative to Controls Among Children Aged 7 to 9 Years in Sarlahi, Nepal (2007-2009)

	Iron/Folic Acid (n=98)[a]		Iron/Folic Acid/Zinc (n=174)[a]		Multiple Micronutrients (n=200)[a]	
Test	Mean Difference (95% CI)[b]	*P* Value[c]	Mean Difference (95% CI)[b]	*P* Value[c]	Mean Difference (95% CI)[b]	*P* Value[c]
UNIT	3.36 (0.90 to 5.81)	.02	1.99 (0.01 to 3.98)	.15	1.62 (–1.22 to 4.46)	.79
Stroop test, proportion who failed	–0.23 (–0.41 to –0.06)	.03	–0.09 (–0.23 to 0.05)	.66	–0.17 (–0.31 to –0.04)	.04
Backward digit span test	0.51 (0.14 to 0.88)	.02	0.12 (–0.14 to 0.38)	>.99	0.13 (–0.20 to 0.46)	>.99
Go/no-go test, % no-go correct	1.42 (–5.57 to 8.42)	>.99	–1.97 (–8.78 to 4.83)	>.99	2.57 (–3.91 to 9.05)	>.99
MABC	–2.78 (–4.98 to –0.58)	.04	–0.93 (–3.17 to 1.31)	>.99	–1.28 (–3.82 to 1.25)	>.99
Finger-tapping test	3.16 (1.26 to 5.07)	.003	1.11 (–0.93 to 3.14)	.86	1.14 (0.74 to 3.04)	.70
P value[d]		<.001		.54		.058

Abbreviations: CI, confidence interval; MABC, Movement Assessment Battery for Children; UNIT, Universal Nonverbal Test of Intelligence.
[a] Missing data were excluded from analysis. Total n=171 in control group.
[b] Estimated using multivariate regression with bootstrapping to estimate 95% CI, adjusted for design effect.
[c] Bonferroni-adjusted *P* values to adjust for multiple comparisons.
[d] *P* values for overall treatment effect using the Wilks λ and Lawley-Hotelling trace test derived from multivariate analysis of variance with Bonferroni correction applied to *P* values to adjust for multiple comparisons.

Table 4. Differences in Test Scores by Maternal Supplementation Group Relative to Controls, Adjusted for Confounders, Among Children Aged 7 to 9 Years in Sarlahi, Nepal (2007-2009)

	Iron/Folic Acid (n=98)[a]		Iron/Folic Acid/Zinc (n=174)[a]		Multiple Micronutrients (n=197)[a]	
Test	Mean Difference (95% CI)[b]	*P* Value[b]	Mean Difference (95% CI)[b]	*P* Value[b]	Mean Difference (95% CI)[b]	*P* Value[b]
UNIT	2.38 (0.06 to 4.70)	.04	0.73 (–0.95 to 2.42)	.39	1.00 (–0.55 to 2.56)	.20
Stroop test, proportion who failed	–0.14 (–0.23 to –0.04)	.005	–0.00 (–0.07 to 0.06)	.93	–0.11 (–0.20 to –0.02)	.02
Backward digit span test	0.36 (0.01 to 0.71)	.02	0.04 (–0.17 to 0.26)	.70	0.07 (–0.11 to 0.25)	.44
Go/no-go test, % no-go correct	–0.54 (–7.44 to 6.35)	.88	–2.12 (–6.51 to 2.26)	.34	1.36 (–3.17 to 5.89)	.56
MABC	–1.47 (–3.06 to 0.12)	.07	–0.27 (–1.72 to 1.18)	.72	–0.54 (–1.89 to 0.79)	.42
Finger-tapping test	2.05 (0.87 to 3.24)	.001	0.70 (–0.60 to 2.00)	.29	0.61 (–0.60 to 1.83)	.32
P value[c]		.002		>.99		.49

Abbreviations: CI, confidence interval; MABC, Movement Assessment Battery for Children; UNIT, Universal Nonverbal Test of Intelligence.
[a] Missing data were excluded from analysis. Total n=169 in control group.
[b] Estimated using multivariate regression with bootstrapping to estimate 95% CI, adjusted for design effect, age, sex, ever having been sent to school, asset score, milk/dairy intake, meat/chicken/fish intake, lower respiratory tract infection, and diarrhea/dysentery in the past week.
[c] *P* value for overall treatment effect, using the Wilks λ and Lawley-Hotelling trace test derived from multivariate analysis of variance with Bonferroni correction applied to *P* values to adjust for multiple comparisons, and adjusted for age, sex, ever having been sent to school, asset score, milk/dairy intake, meat/chicken/fish intake, lower respiratory tract infection, and diarrhea/dysentery in the past week.

©2010 American Medical Association. All rights reserved.

Downloaded from jama.ama-assn.org at Tel-Aviv University on 25 December 2010

this study also show the possible importance of iron in utero. Both myelination and dopamine functioning may be related to the development of the motor system. Furthermore, motor-related areas of the brain also contain high concentrations of iron.[35] There is not much information concerning meaningful differences in MABC scores, but the mean adjusted non–statistically significant difference of 1.5 in the total score represents a one-quarter SD difference.

The combination of iron/folic acid/zinc was not different from controls. As noted, previous studies of prenatal zinc supplementation have failed to establish any benefit to mental or motor outcomes in children.[16,17] Addition of zinc to iron and folic acid attenuated or negated the positive association with outcomes, which may be related to the inhibitory role of zinc in iron absorption described in the literature.[36,37] We also noted a similar attenuation with added zinc of the positive effect of iron and folic acid on the original trial outcomes of birth weight[18] and maternal anemia.[34] Outcomes in the multiple micronutrient supplement group were also not different than the control. Other studies have found small changes in cognitive outcomes with this intervention, mostly in infants or young children. In China, prenatal multiple micronutrient supplementation was associated with increases in mental development scores of 1.00 and 1.22 points compared with folic acid and folic acid/iron supplementation, respectively, although not in psychomotor scores at 1 year of age.[38] In a stratified analysis, infants of mothers with low body mass index who received multiple micronutrients had small but significant increments in motor scores and activity ratings than those whose mothers received iron/folic acid in Bangladesh.[39] Similarly, among children of women infected with human immunodeficiency virus, small albeit significant increases were observed with multivitamin supplementation relative to a placebo in psychomotor but not mental development indexes.[40]

It is unlikely that outcome differences in the study were due to vitamin A present in the supplement. In a separate study in which we followed up children of women who received either vitamin A or placebo before, during, and after pregnancy, we found no differences between supplementation with vitamin A and placebo in either UNIT or MABC scores.[41] However, a positive interaction between these 2 nutrients (iron and vitamin A) cannot be ruled out. We are unable to separate the folic acid–induced effects from those of iron. However, a systematic review that included 4 randomized clinical trials of the effects of dietary supplements of folic acid found no beneficial effect of 750 μg/d of folic acid on measures of cognition or mood in older healthy women,[42] although these data are not directly relevant for school-aged children. Prenatal folic acid use in a US population was positively associated with gross motor development but negatively associated with personal-social outcomes.[43] Similarly, maternal folic acid use was associated with fewer child behavioral problems.[44] Data from these studies are unconvincing regarding the role of folic acid; however, a synergistic interaction between iron and folic acid may exist.

Our study has several strengths and some limitations. Because of the original randomized study design and a high rate of follow-up, we can report some linkage between the role of iron supplementation in utero and cognitive and motor outcomes. However by excluding the folic acid–alone group and including only the placebo group of the child supplementation trial, we deviated from the original designs of the trials and, therefore, have refrained from making any causal inferences. We used tests of general intelligence and motor function that are well known and standardized for use in different cultures and settings. Yet, factors such as a child's sex and ever having been sent to school were strong predictors of the outcomes, especially for the UNIT score. It is possible that in this setting, having been to school better prepares a child for testing. Separating out the effect of better achievement on test scores due to schooling is important, but there were some differences by treatment group in the proportion of children sent to school, and the adjustment for this variable did alter the study results somewhat, suggesting that schooling may have been in the causal pathway; this warrants further study. Sex difference in test scores may be related to the emphasis on proficiency in household chores and taking care of younger siblings among girls, not allowing them sufficient time for schoolwork and study. Schoolwork proficiency may itself be valued differently by sex.

In conclusion, our study found evidence that maternal prenatal supplementation with iron and folic acid was positively associated with general intellectual ability, some aspects of executive function, and motor function, including fine motor control, in offspring at 7 to 9 years of age in a rural area of Nepal where iron deficiency is highly prevalent. Antenatal iron/folic acid use per international guidelines should be expanded in many low- and middle-income settings where program coverage continues to be poor. Further follow-up studies are required to examine whether the observed benefits in early school age persist into adolescence and adulthood.

Author Affiliations: Center for Human Nutrition, Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland (Drs Christian, Murray-Kolb, Katz, and Tielsch and Mr LeClerq); Departments of Nutritional Sciences (Dr Murray-Kolb), Educational and School Psychology and Special Education (Dr Schaefer), and Psychology (Dr Cole), Pennsylvania State University, University Park; and Nepal Nutrition Intervention Project–Sarlahi, Nepal Eye Hospital Complex, Kathmandu, Nepal (Dr Khatry and Mr LeClerq).

Author Contributions: Dr Christian had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Christian, Murray-Kolb, Katz, Cole, LeClerq, Tielsch.
Acquisition of data: Christian, Murray-Kolb, Khatry, Cole, LeClerq.
Analysis and interpretation of data: Christian, Murray-Kolb, Katz, Schaefer, Cole, Tielsch.
Drafting of the manuscript: Christian.
Critical revision of the manuscript for important intellectual content: Christian, Murray-Kolb, Khatry, Katz, Schaefer, Cole, LeClerq, Tielsch.

 ©2010 American Medical Association. All rights reserved.

Downloaded from jama.ama-assn.org at Tel-Aviv University on 25 December 2010

Statistical analysis: Christian, Katz, Schaefer.
Obtained funding: Christian, Murray-Kolb, Cole.
Administrative, technical, or material support: Christian, Murray-Kolb, Khatry, Schaefer, Cole, LeClerq.
Study supervision: Christian, Khatry, Schaefer, LeClerq.
Financial Disclosures: None reported.
Funding/Support: This work was carried out by the Center for Human Nutrition, Department of International Health of the Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, in collaboration with the National Society for the Prevention of Blindness, Kathmandu, Nepal, with funding from National Institutes of Health grant R01 HD050254-01 and the Bill and Melinda Gates Foundation, Seattle, Washington. The antenatal micronutrient supplementation study was conducted under Micronutrients for Health Cooperative Agreement HRN-A-00-97-00015-00 and Global Research Activity Cooperative Agreement GHS-A-00-03-00019-00 between Johns Hopkins University and the Office of Health, Infectious Diseases, and Nutrition, US Agency for International Development, Washington, DC. Bill and Melinda Gates Foundation and Sight and Life Research Institute, Baltimore, Maryland, provided additional support for the study. The preschool child iron and zinc supplementation study was funded by the National Institutes of Health (HD 38753), the Bill and Melinda Gates Foundation, and a cooperative agreement between Johns Hopkins University and the Office of Health, Infectious Diseases, and Nutrition, US Agency for International Development (HRN-A-00-97-00015-00).
Role of the Sponsor: The funding agencies had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.
Online-Only Materials: eTables 1 and 2 and the eFigure are available online at http://www.jama.com.
Additional Contributions: Apart from the authors, all members of the Nepal study team helped in the successful implementation of the study, including field manager, coordinator, supervisors, psychology research assistants (Keshav Mishra, Nar Bahadur Thapa, Mona Lisa Pradhan, Sumitra Dhakal, and Bikram Sherchan), and team leader interviewers. Those named here received compensation as salaried staff.

REFERENCES

1. Lozoff B. Iron deficiency and child development. *Food Nutr Bull*. 2007;28(4)(suppl):S560-S571.
2. Georgieff MK. The role of iron in neurodevelopment: fetal iron deficiency and the developing hippocampus. *Biochem Soc Trans*. 2008;36(pt 6):1267-1271.
3. Rao R, Tkac I, Townsend EL, Gruetter R, Georgieff MK. Perinatal iron deficiency alters the neurochemical profile of the developing rat hippocampus. *J Nutr*. 2003;133(10):3215-3221.
4. Ward KL, Tkac I, Jing Y, et al. Gestational and lactational iron deficiency alters the developing striatal metabolome and associated behaviors in young rats. *J Nutr*. 2007;137(4):1043-1049.
5. Todorich B, Pasquini JM, Garcia CI, Paez PM, Connor JR. Oligodendrocytes and myelination: the role of iron. *Glia*. 2009;57(5):467-478.
6. Beard JL. Why iron deficiency is important in infant development. *J Nutr*. 2008;138(12):2534-2536.
7. Ortiz E, Pasquini JM, Thompson K, et al. Effect of manipulation of iron storage, transport, or availability on myelin composition and brain iron content in 3 different animal models. *J Neurosci Res*. 2004;77(5):681-689.
8. 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(5 pt 2):S34-S43.
9. de Deungria M, Rao R, Wobken JD, Luciana M, Nelson CA, Georgieff MK. Perinatal iron deficiency decreases cytochrome c oxidase (CytOx) activity in selected regions of neonatal rat brain. *Pediatr Res*. 2000;48(2):169-176.
10. Clardy SL, Wang X, Zhao W, et al. Acute and chronic effects of developmental iron deficiency on mRNA expression patterns in the brain. *J Neural Transm Suppl*. 2006;(71):173-196.
11. Carlson ES, Stead JD, Neal CR, Petryk A, Georgieff MK. Perinatal iron deficiency results in altered developmental expression of genes mediating energy metabolism and neuronal morphogenesis in hippocampus. *Hippocampus*. 2007;17(8):679-691.
12. Golub MS, Hogrefe CE, Germann SL, Capitanio JP, Lozoff B. Behavioral consequences of developmental iron deficiency in infant rhesus monkeys. *Neurotoxicol Teratol*. 2006;28(1):3-17.
13. Siddappa AM, Georgieff MK, Wewerka S, Worwa C, Nelson CA, deRegnier RA. Iron deficiency alters auditory recognition memory in newborn infants of diabetic mothers. *Pediatr Res*. 2004;55:1034-1041.
14. Zhou SJ, Gibson RA, Crowther CA, Baghurst P, Makrides M. Effect of iron supplementation during pregnancy on the intelligence quotient and behavior of children at 4 y of age: long-term follow-up of a randomized controlled trial. *Am J Clin Nutr*. 2006;83(5):1112-1117.
15. Merialdi M, Caulfield LE, Zavaleta N, Figueroa A, DiPietro JA. Adding zinc to prenatal iron and folate tablets improves fetal neurobehavioral development. *Am J Obstet Gynecol*. 1999;180(2 pt 1):483-490.
16. Hamadani JD, Fuchs GJ, Osendarp SJM, 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(9329):290-294.
17. 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(6):1512-1516.
18. Christian P, Khatry SK, Katz J, et al. Effects of alternative maternal micronutrient supplements on low birth weight in rural Nepal: double blind randomised community trial. *BMJ*. 2003;326(7389):571-576.
19. Christian P, West KP, Khatry SK, et al. Effects of maternal micronutrient supplementation on fetal loss and infant mortality: a cluster-randomized trial in Nepal. *Am J Clin Nutr*. 2003;78(6):1194-1202.
20. Tielsch JM, Khatry SK, Stoltzfus RJ, et al. 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(9505):144-152.
21. Tielsch JM, Khatry SK, Stoltzfus RJ, et al. Effect of daily zinc supplementation on child mortality in southern Nepal: a community-based, cluster randomised, placebo-controlled trial. *Lancet*. 2007;370(9594):1230-1239.
22. Bracken BA, McCallum RS. *Universal Nonverbal Intelligence Test.* Itasca, IL: Riverside; 1998.
23. McCallum S, Bracken B, Wasserman J. *Essentials of Nonverbal Assessment.* New York, NY: John Wiley & Sons Inc; 2001.
24. Bull R, Scerif G. Executive functioning as a predictor of children's mathematics ability: inhibition, switching, and working memory. *Dev Neuropsychol*. 2001;19(3):273-293.
25. Wechsler D. *The Wechsler Intelligence Scale for Children—Third Edition (WISC-III).* San Antonio, Texas: Psychological Corp; 1991.
26. 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(3):1209-1213.
27. Henderson SE, Sugden DA. *Movement Assessment Battery for Children.* London, England: Psychological Corp; 1992.
28. Caldwell B, Bradley R. *Home Observation for the Measurement of the Environment.* Little Rock: University of Arkansas; 1984.
29. Raven JC, Court JH, Raven J. *Manual for Raven's Progressive Matrices and Vocabulary Scales.* Oxford, England: Oxford Psychologists Press Ltd; 1992.
30. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bull World Health Organ*. 2007;85(9):660-667.
31. Snedecor GW, Cochran WG. *Statistical Methods.* 7th ed. Ames: Iowa State University Press; 1980: chap 13.
32. Diggle PJ, Liang KY, Zeger SL. *Analysis of Longitudinal Data.* Oxford, England: Oxford University Press; 1994:chap 3, 7.
33. Dreyfuss ML, Stoltzfus RJ, Shrestha JB, et al. Hookworms, malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. *J Nutr*. 2000;130(10):2527-2536.
34. Christian P, Shrestha J, LeClerq SC, et al. Supplementation with micronutrients in addition to iron and folic acid does not further improve the hematologic status of pregnant women in rural Nepal. *J Nutr*. 2003;133(11):3492-3498.
35. Connor JR. Evidence for iron mismanagement in the brain in neurological disorders. In: Connor JR, ed. *Metals and Oxidative Damage in Neurological Disorders.* New York, NY: Plenum Press; 1997:23-39.
36. Olivares M, Pizarro F, Ruz M. New insights about iron bioavailability inhibition by zinc. *Nutrition*. 2007;23(4):292-295.
37. Whittaker P. Iron and zinc interactions in humans. *Am J Clin Nutr*. 1998;68(2)(suppl):442S-446S.
38. Li Q, Yan H, Zeng L, et al. Effects of maternal multimicronutrient supplementation on the mental development of infants in rural western China: follow-up evaluation of a double-blind, randomized, controlled trial. *Pediatrics*. 2009;123(4):e685-e692.
39. Tofail F, Persson LA, El Arifeen S, et al. Effects of prenatal food and micronutrient supplementation on infant development: a randomized trial from the Maternal and Infant Nutrition Interventions, Matlab (MINIMat) study. *Am J Clin Nutr*. 2008;87(3):704-711.
40. McGrath N, Bellinger D, Robins J, Msamanga GI, Tronick E, Fawzi WW. Effect of maternal multivitamin supplementation on the mental and psychomotor development of children who are born to HIV-1-infected mothers in Tanzania. *Pediatrics*. 2006;117(2):e216-e225.
41. Gillian BJ. *The Impact of Vitamin A Supplementation In Utero and in Infancy on the Psycho-motor Development of School-Aged Children in Rural Nepal [thesis].* Baltimore, MD: Johns Hopkins University; 2009.
42. Malouf M, Grimley EJ, Areosa SA. Folic acid with or without vitamin B12 for cognition and dementia. *Cochrane Database Syst Rev*. 2003;4(4):CD004514.
43. Wehby GL, Murray JC. The effects of prenatal use of folic acid and other dietary supplements on early child development. *Matern Child Health J*. 2008;12(2):180-187.
44. Roza SJ, van Batenburg-Eddes T, Steegers EA, et al. Maternal folic acid supplement use in early pregnancy and child behavioural problems: the Generation R Study. *Br J Nutr*. 2010;103(3):445-452.

©2010 American Medical Association. All rights reserved.

Downloaded from jama.ama-assn.org at Tel-Aviv University on 25 December 2010