Iron and zinc supplementation promote motor development and exploratory behavior among Bangladeshi infants\textsuperscript{1,3}

Maureen M Black, Abdullah H Baqui, K Zaman, Lars Ake Persson, Shams El Arifeen, Katherine Le, Scot W McNary, Monowara Parveen, Jena D Hamadani, and Robert E Black

ABSTRACT
Background: Iron and zinc deficiency are prevalent during infancy in low-income countries.
Objectives: The objectives were to examine whether a weekly supplementation of iron, zinc, iron+zinc, or a micronutrient mix (MM) of 16 vitamins and minerals would alter infant development and behavior.
Design: The participants were 221 infants from rural Bangladesh at risk of micronutrient deficiencies. Development and behavior were evaluated at 6 and 12 mo of age by using the Bayley Scales of Infant Development II and the Home Observation Measurement of Environment (HOME) scale. In this double-blind trial, the infants were randomly assigned to 1 of 5 treatment conditions: iron (20 mg), zinc (20 mg), iron+zinc, MM (16 vitamins and minerals, including iron and zinc), or riboflavin weekly from 6 to 12 mo. Multivariate analyses were conducted to examine the change in development and behavior for each supplementation group, with control for maternal education, HOME score, months breastfed, anemia, growth at 6 mo, and change in growth from 6 to 12 mo.
Results: Iron and zinc administered together and with other micronutrients had a beneficial effect on infant motor development. Iron and zinc administered individually and in combination had a beneficial effect on orientation-engagement. Two-thirds of the infants were mildly anemic, no treatment effects on hemoglobin concentration were observed, and hemoglobin was not associated with measures of development or behavior.
Conclusion: The beneficial effects of weekly iron and zinc supplementation on motor development and orientation-engagement suggest that infants benefit from these minerals when administered together.

KEY WORDS Iron, zinc, micronutrient supplementation, infant development, functional isolation, Bangladesh

INTRODUCTION
Evidence suggests that micronutrient deficiencies may be associated with problems in early development and behavior (1). Iron deficiency is the most common nutritional deficiency in the world and a major cause of anemia (2, 3), particularly during infancy and toddlerhood when there is rapid growth and a high nutritional demand (4). Less is known about the prevalence of zinc deficiency, but supplementation trials in developing countries suggest low zinc intake among infants and toddlers (5), which leads to the consensus that both iron and zinc deficiency are major public health problems (2, 6). In low-income societies, where dietary resources are inadequate to meet children’s requirements for iron and zinc, micronutrient supplementation or fortification may be necessary.

Most randomized controlled trials of supplements have examined the effect of single nutrients, either iron or zinc, on infants’ development and behavior. Short-term trials (<15 d) of iron supplementation among anemic infants have shown no differences in children’s motor or mental performance (7). Three longer-term iron supplementation trials have shown a significant improvement in children’s development (8, 9) and behavior (10). Most of the prevention trials among infants at risk of iron deficiency are difficult to interpret because methods varied, and limited consideration was given to socioeconomic and caregiving differences (11).

Zinc supplementation trials have shown beneficial effects on growth (12), diarrhea and pneumonia morbidity (13), and mortality (14) among infants and toddlers in low-income countries. However, the effects of zinc supplementation on infant development and behavior have not yielded consistent findings (15, 16).

Although single nutrient evaluations allow investigators to isolate the effects associated with specific nutrients, infants with low-nutrient diets often have multiple deficiencies. Some evidence shows that supplementation with iron and zinc together may interfere with the absorption of both minerals (17, 18). However, a micronutrient mixture containing both iron and zinc, in a 1:1 ratio, was effective in promoting linear growth (19). To evaluate the separate and joint effects of iron and zinc supplements, we conducted a randomized controlled trial in which children received a weekly supplement of iron alone, zinc alone, both iron and zinc, or riboflavin alone. Because deficiencies of other micronutrients have been shown to be associated with behavioral and developmental problems (1, 20), we also evaluated a micronutrient mixture of 16 vitamins and minerals, including iron and zinc.

\textsuperscript{1} From the Department of Pediatrics, University of Maryland School of Medicine, Baltimore (MMB, KL, and SWM); the Johns Hopkins University Bloomberg School of Public Health, Baltimore (AHB and REB); and ICDDR,B: Centre for Health and Population Research, Bangladesh (AHB, KZ, LAP, SEA, MP, and JDH).
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\textsuperscript{3} Address reprint requests to MM Black, Department of Pediatrics, University of Maryland School of Medicine, 655 West Lombard Street, Suite 311, Baltimore, MD 21201. E-mail: mblack@peds.umaryland.edu.

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The trial was conducted among rural Bangladeshi infants from 6 to 12 mo of age. Infants in this setting are at high risk of micronutrient deficiencies, malnutrition, and poor dietary quality (21). In keeping with the functional isolation hypothesis, which posits that the developmental delays often seen in nutritionally deprived infants may be partially explained by limited opportunities for exploration (22), we examined whether micronutrient supplementation would lead to changes in infant exploration and initiative. Our objectives were to examine the effects of micronutrient supplementation on infants’ mental, motor, and behavioral development.

SUBJECTS AND METHODS

Subjects

The infants and mothers recruited to participate in this investigation were a subset of a larger double-blind randomized trial of micronutrient supplementation conducted in the Matlab field research area of the ICDDR,B: Centre for Health and Population Research, Bangladesh (23). Matlab is a rural subdistrict of ≈500 000 people located south of the capital city of Dhaka. The residents’ primary occupations are farming and fishing, and most villages have limited access to electricity, safe water, and sanitary waste disposal. The procedures were approved by the ethical review board of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), and by the University of Maryland, Baltimore.

Methods

Using demographic information from an ongoing Health and Demographic Surveillance System, potentially eligible infants were identified and families were invited to participate (23). Informed consent was obtained from parents, and infants were screened for eligibility. Infants were eligible if they were 6 mo of age, did not receive infant formula, were not severely malnourished (midupper arm circumference ≥ 110 mm), were not severely anemic (hemoglobin ≥ 90 g/L), and did not have obvious neurologic disorders, physical disabilities, or chronic illnesses.

Every alternate infant was invited to participate in the developmental substudy; 43 families refused and 28 families were absent, leaving a sample of 346 infants (Figure 1). At recruitment, research assistants in the field collected socioeconomic and demographic data and measured the infants’ weights, lengths, and hemoglobin concentrations (Table 1). Measurements were repeated at 12 mo.

Before receiving the supplements and when the infants were 6 mo of age, the mothers and infants were brought to a developmental clinic for an individual evaluation with a psychologist. The Bayley Scales of Infant Development, version II (Bayley II) were administered (Table 2). When the infants were 12 mo of age, this procedure was repeated at the clinic. Additionally, a trained examiner visited the participant’s home for ≈40 min. After asking the mother questions about the child and home, she encouraged the family to participate in their routine activities while she observed their interactions and the quality of the home environment and scored the Home Observation Measurement of Environment (HOME) scale.

Intervention

In this double-blind trial, trained community health workers visited infants weekly for 6 mo and fed them a liquid mixture of iron, zinc, iron and zinc, micronutrient mix (MM), or riboflavin. The supplements were prepared as capsules, which were mixed with flavored syrup and fed to the infants. The mixtures were

![Diagram](image-url)
TABLE 1
Demographic and anthropometric scores at 6 and 12 mo of age by supplementation group

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Iron (n = 49)</th>
<th>Zinc (n = 49)</th>
<th>Iron+Zinc (n = 43)</th>
<th>MM (n = 35)</th>
<th>Riboflavin (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>27.7 ± 6.0&lt;sup&gt;2&lt;/sup&gt;</td>
<td>27.9 ± 5.5</td>
<td>28.5 ± 5.6</td>
<td>28.7 ± 6.1</td>
<td>27.8 ± 6.1</td>
</tr>
<tr>
<td>Education (y)</td>
<td>3.9 ± 3.5</td>
<td>4.0 ± 3.8</td>
<td>4.5 ± 3.7</td>
<td>5.1 ± 4.0</td>
<td>4.4 ± 4.0</td>
</tr>
<tr>
<td>Married (%)</td>
<td>100</td>
<td>98</td>
<td>97.7</td>
<td>94.4</td>
<td>100</td>
</tr>
<tr>
<td>Socioeconomic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monthly income (Taka)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3459 ± 2068</td>
<td>3851 ± 3731</td>
<td>3688 ± 3721</td>
<td>3722 ± 2586</td>
<td>3149 ± 1662</td>
</tr>
<tr>
<td>Household size</td>
<td>6.8 ± 2.2</td>
<td>6.3 ± 2.4</td>
<td>6.4 ± 3.5</td>
<td>6.6 ± 2.8</td>
<td>6.0 ± 2.1</td>
</tr>
<tr>
<td>HOME score</td>
<td>27.3 ± 4.1</td>
<td>28.7 ± 5.3</td>
<td>28.0 ± 4.9</td>
<td>29.9 ± 3.8</td>
<td>28.0 ± 4.5</td>
</tr>
</tbody>
</table>

Infant characteristics

<table>
<thead>
<tr>
<th>Sex (% male)</th>
<th>45</th>
<th>49</th>
<th>40</th>
<th>50</th>
<th>47</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at 6 mo (mo)</td>
<td>6.5 ± 0.3</td>
<td>6.5 ± 0.3</td>
<td>6.5 ± 0.3</td>
<td>6.4 ± 0.3</td>
<td>6.5 ± 0.2</td>
</tr>
<tr>
<td>Age at 12 mo (mo)</td>
<td>12.8 ± 0.5</td>
<td>12.8 ± 0.4</td>
<td>12.7 ± 0.4</td>
<td>12.6 ± 0.3</td>
<td>12.7 ± 0.4</td>
</tr>
<tr>
<td>Breastfeeding duration at 12 mo (d)</td>
<td>126.3 ± 53.9</td>
<td>115.1 ± 58.2</td>
<td>130.2 ± 51.7</td>
<td>110.7 ± 60.3</td>
<td>112.0 ± 58.4</td>
</tr>
<tr>
<td>Hemoglobin at 6 mo (g/dL)</td>
<td>10.3 ± 0.8</td>
<td>10.5 ± 1.0</td>
<td>10.5 ± 1.0</td>
<td>10.5 ± 0.8</td>
<td>10.8 ± 1.4</td>
</tr>
<tr>
<td>Hemoglobin at 12 mo (g/dL)</td>
<td>10.6 ± 1.2</td>
<td>10.3 ± 0.7</td>
<td>10.3 ± 0.7</td>
<td>10.7 ± 0.6</td>
<td>10.7 ± 0.5</td>
</tr>
<tr>
<td>Length-for-age z score at 6 mo</td>
<td>-1.2 ± 0.8</td>
<td>-1.2 ± 0.8</td>
<td>-1.2 ± 0.7</td>
<td>-1.2 ± 0.8</td>
<td>-1.2 ± 0.9</td>
</tr>
<tr>
<td>Length-for-age z score at 12 mo</td>
<td>-1.6 ± 0.9&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-1.6 ± 0.9&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-1.8 ± 0.9&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-1.7 ± 0.8&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-1.7 ± 1.0&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight-for-length z score at 6 mo</td>
<td>0.1 ± 0.9</td>
<td>0.2 ± 0.9</td>
<td>0.0 ± 0.9</td>
<td>0.1 ± 0.9</td>
<td>0.1 ± 0.8</td>
</tr>
<tr>
<td>Weight-for-length z score at 12 mo</td>
<td>-0.9 ± 0.8&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-0.8 ± 0.8&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-0.8 ± 0.8&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-0.8 ± 0.8&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-0.7 ± 0.9&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weight-for-age z score at 6 mo</td>
<td>-1.0 ± 1.0</td>
<td>-0.9 ± 1.1</td>
<td>-1.1 ± 1.0</td>
<td>-1.1 ± 0.8</td>
<td>-1.1 ± 1.0</td>
</tr>
<tr>
<td>Weight-for-age z score at 12 mo</td>
<td>-2.2 ± 1.0&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-2.0 ± 1.2&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-2.2 ± 1.3&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-2.1 ± 1.0&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-2.0 ± 1.2&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> MM, micronutrient mix; HOME, Home Observation Measurement of Environment. There were no significant differences between supplementation groups (univariate analysis).

<sup>2</sup> ± SD (all such values).

<sup>3</sup> 59.05 Bangladesh Taka = 1 US dollar (2004).

<sup>4</sup> Significantly different from 6-mo value; P < 0.001 (repeated-measures ANOVA).

Similar in taste and appearance. The capsules and the syrup were prepared by ACME laboratories (Dhaka, Bangladesh). The iron group received 20 mg elemental Fe in the form of ferrous sulfate and 1 mg riboflavin, and the zinc group received 20 mg elemental Zn in the form of zinc acetate and 1 mg riboflavin. The iron+zinc group received 20 mg elemental Fe, 20 mg elemental Zn, and 1 mg riboflavin. The MM group received ≈2 times the recommended dietary allowance (based on World Health Organization standards) (24) of thiamine, niacin, folic acid, pantethenic acid, iodine, copper, manganese, selenium, and vitamins C, D, E, B-6, and B-12 in addition to 20 mg elemental Fe, 20 mg elemental Zn, and 1 mg riboflavin. The control group received 1 mg riboflavin.

TABLE 2
Developmental and behavioral scores at 6 and 12 mo of age by supplementation group

<table>
<thead>
<tr>
<th>Supplementation group</th>
<th>Iron (n = 49)</th>
<th>Zinc (n = 49)</th>
<th>Iron+Zinc (n = 43)</th>
<th>MM (n = 35)</th>
<th>Riboflavin (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental Developmental Index at 6 mo</td>
<td>103.2 ± 8.6</td>
<td>104.0 ± 6.5</td>
<td>103.0 ± 5.9</td>
<td>104.5 ± 6.5</td>
<td>103.5 ± 6.8</td>
</tr>
<tr>
<td>Mental Developmental Index at 12 mo</td>
<td>104.3 ± 9.5</td>
<td>104.7 ± 8.3</td>
<td>105.4 ± 11.6</td>
<td>104.3 ± 13.0</td>
<td>102.7 ± 13.5</td>
</tr>
<tr>
<td>Psychomotor Developmental Index at 6 mo</td>
<td>106.7 ± 13.9</td>
<td>108.8 ± 11.1</td>
<td>108.4 ± 11.7</td>
<td>108.7 ± 10.7</td>
<td>108.6 ± 14.4</td>
</tr>
<tr>
<td>Psychomotor Developmental Index at 12 mo</td>
<td>99.5 ± 16.6&lt;sup&gt;2&lt;/sup&gt;</td>
<td>101.2 ± 16.6&lt;sup&gt;2&lt;/sup&gt;</td>
<td>103.7 ± 16.2&lt;sup&gt;3&lt;/sup&gt;</td>
<td>104.5 ± 16.5&lt;sup&gt;4&lt;/sup&gt;</td>
<td>95.4 ± 16.3&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Orientation-engagement percentile at 6 mo</td>
<td>74.8 ± 25.1</td>
<td>73.5 ± 25.9</td>
<td>73.0 ± 26.4</td>
<td>74.0 ± 27.1</td>
<td>79.1 ± 26.2</td>
</tr>
<tr>
<td>Orientation-engagement percentile at 12 mo</td>
<td>77.9 ± 22.6&lt;sup&gt;4&lt;/sup&gt;</td>
<td>75.8 ± 22.5</td>
<td>79.7 ± 24.6&lt;sup&gt;4&lt;/sup&gt;</td>
<td>66.6 ± 35.3</td>
<td>67.0 ± 31.0&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Emotional regulation percentile at 6 mo</td>
<td>70.4 ± 26.3</td>
<td>68.3 ± 27.6</td>
<td>61.2 ± 28.2</td>
<td>65.3 ± 30.9</td>
<td>68.8 ± 27.7</td>
</tr>
<tr>
<td>Emotional regulation percentile at 12 mo</td>
<td>61.8 ± 26.3</td>
<td>54.8 ± 31.8</td>
<td>58.8 ± 29.7</td>
<td>47.5 ± 29.7</td>
<td>58.0 ± 25.8</td>
</tr>
<tr>
<td>Motor quality percentile at 6 mo</td>
<td>53.1 ± 28.0</td>
<td>55.0 ± 25.8</td>
<td>57.3 ± 27.8</td>
<td>57.0 ± 24.9</td>
<td>51.1 ± 24.5</td>
</tr>
<tr>
<td>Motor quality percentile at 12 mo</td>
<td>34.0 ± 29.6</td>
<td>36.3 ± 24.5&lt;sup&gt;4&lt;/sup&gt;</td>
<td>41.5 ± 31.0&lt;sup&gt;4&lt;/sup&gt;</td>
<td>38.1 ± 31.0&lt;sup&gt;4&lt;/sup&gt;</td>
<td>22.1 ± 20.2</td>
</tr>
</tbody>
</table>

<sup>1</sup> All values are ± SD. MM, micronutrient mix.

<sup>2</sup> Significantly different from 6-mo value, P < 0.05 (repeated-measures ANOVA).

<sup>3</sup> Change from 6 mo significantly different from the riboflavin group, P < 0.05 (Dunnett’s test).

<sup>4</sup> Significantly different from the riboflavin group, P < 0.05 (univariate analysis).
The number of doses each infant received was recorded. All infants received 100,000 IU vitamin A at the beginning of the study, in line with national policy in Bangladesh.

**Measures**

Weight was measured to 0.1 kg with a Salter scale and length to 0.1 cm with a length board. Hemoglobin concentration was measured by the HemoCue B-Hemoglobin System (25). A paramedic obtained capillary blood from the finger, ensuring that the initial drop of blood was not used for measurement.

Motor, mental, and behavioral development were assessed with the Bayley II (26). The Bayley II was chosen because it provides an assessment of complex integrated functioning that is empirically derived, on the basis of recent findings from infant development, and has been used in many cultures (27). The Bayley II was administered by an examiner with a master’s degree in psychology who was trained and supervised by a clinical psychologist with extensive experience in the administration of the Bayley II. Interrater reliability was established during training ($r = 0.90$) and maintained during monthly supervisory field visits. After the administration of the mental and motor scales, the psychologist completed the Behavior Rating Scale, which includes 3 factors: orientation-engagement, emotional regulation, and motor quality. Orientation-engagement served as the measurement of exploration. It includes 11 items that tap the infant’s level of arousal, positive affect, energy, initiative, enthusiasm, exploration, social engagement, and lack of fearfulness. Emotional regulation includes 8 items, such as negative affect, hypersensitivity, frustration tolerance, cooperation, and frenetic movement. Motor quality includes 7 items, such as motor control and tone. For this sample, the coefficient $\alpha$ for each factor ranged from 0.74 to 0.88, indicating that the factors were internally consistent. Raw scores for the mental and motor scales were converted to age-normalized Mental Developmental Index and Psychomotor Developmental Index (PDI) scores, with a population mean of 100 and a SD of 15, and raw scores for the behavioral factors were converted to percentile scores, with high scores representing optimal behavior. US norms were used to facilitate comparisons with published literature.

The quality and quantity of stimulation and support available to a child in the home were measured by using the HOME scale (28). The HOME is an observation scale that has been widely used in international child development research and has shown a strong relation with intellectual development and achievement performance (29). In collaboration with an anthropologist, we adapted and pilot tested the HOME scale to ensure that the items were culturally appropriate. We added items to describe the physical conditions of the homes, for a modified total of 48 items. The internal consistency of the scale, defined by coefficient $\alpha$, was 0.72. Training and interrater reliability were conducted to ensure agreement on the items.

**Data analysis**

The outcomes of interest were the changes in mental, motor, and behavioral development (the slopes) from 6 to 12 mo. In the first phase of the data analysis, we used an overall repeated-measures multivariate analysis of variance to compare the 6 to 12 mo slope of the supplementation groups with the 6 to 12 mo slope of the riboflavin group on a composite of 5 developmental outcomes. A significant time effect suggests a change in scores over time, and a significant group-by-time interaction suggests differences in slopes between the supplementation and riboflavin groups.

In the second phase, we used a mixed model of repeated-measures analysis of variance with correlated errors (30) to compare each supplementation group with the riboflavin group. We examined group differences in mental, motor, and behavioral development slopes over the 6-mo intervention period. The mixed-model technique has the advantage of modeling the error variance at 6 and 12 mo separately, along with their correlation, and allowing estimation of individual group changes. The analysis was conducted with unadjusted scores.

In the third phase, we conducted bivariate correlations to determine which demographic variables were associated with children’s development and behavior at 12 mo and should be used as covariates. We included the covariates and again ran the mixed-model repeated-measures analysis of variance.

Effect sizes were calculated by subtracting the mean at 12 mo from the mean at 6 mo and dividing by the SD of the difference between the 2 means by using estimates from the models adjusted for covariates (31). Comparisons between each group’s average change and the average change for the riboflavin group were adjusted by using Dunnett’s test.

The power for detecting differences between each supplementation group and the riboflavin group was determined by assuming that $\alpha = 0.05$, a sample size of 45 for the riboflavin group, a mean sample size of 44 for each supplementation group, and two-tailed tests. There is a power of $\geq 0.80$ to detect a difference of 0.60 SDs between the slope for each supplementation group and the riboflavin group. Because the measures of development and behavior are correlated over time, the power to detect effects $< 0.60$ may exceed 0.80, proportional to the amount of correlation.

**RESULTS**

**Attrition, supplementation success, and missing data**

There were 125 children (36%) who did not complete the 12-mo assessment, leaving a final sample of 221 children (Figure 1). No differential dropout was observed across supplementation groups, sex, maternal education, anemia, growth measures at 6 mo, or performance on the Bayley II Scales at 6 mo.

We calculated the supplementation success rate by dividing the received dose by the expected dose. The MM was not well tolerated, and many infants vomited after receiving it. In the MM group, the success rate was 78%. In the other 4 groups, the success rate varied between 88% and 91% ($P < 0.001$ compared with the MM group), indicating less success among infants in the MM group than in the other groups.

Thirty-six children (16.3%) did not undergo evaluation with the HOME scale, 11 (5.0%) did not have hemoglobin data at 12 mo, and 4 (1.8%) did not have anthropometric data at 12 mo but did have data available on all other measures. Staff availability, transportation, and inclement weather were the primary reasons for missing data, which suggested that the data were missing at random rather than systematically. Because the HOME scale plays an important role in children’s development and therefore should be included as a covariate, we used multiple imputation to supply values for the missing data (32). NORM 2.03 was used to...
develop the imputation model for variables with missing data, to construct imputed data sets, and to aggregate the parameter estimates from each data set (32).

When the trial began at 6 mo of age, there were no group differences in demographic variables (maternal age, maternal education, percentage married, monthly income, household size, and HOME score) or child variables (breastfeeding duration, hemoglobin concentration, and anthropometric measures; Table 1). The average maternal age was 28.1 y. Overall, 48.6% of the mothers had <5 y of schooling, and most were married (98.2%). The average household size was 6.4, and 72.5% of the children had older siblings. Stunting (length-for-age z score < -2) occurred in 18% of the infants; there was no evidence of wasting (weight-for-length z score < -2). More than two-thirds (68%) of the children were anemic (hemoglobin < 110 g/L). Performance on the Bayley II was within the normal range on all scales. There were no group differences in the children’s development and behavior at 6 mo (Table 2). At 12 mo, motor quality scores were lower in the children in the riboflavin group than in the children in the zinc, iron+ zinc, and MM groups.

At the end of the trial, when the infants were 12 mo of age, nutritional status had declined significantly, as indicated by changes in length-for-age (\( P < 0.001 \)), weight-for-length (\( P < 0.001 \)), and weight-for-age (\( P < 0.001 \)). There were no differences across groups in any of the growth measurements; at 12 mo, 37.6% of the infants were stunted and 8.3% were wasted. There were no significant differences in hemoglobin associated with supplementation (Table 1); at 12 mo 64% of the infants were anemic.

In the first phase of the analysis, the results of the repeated-measures multivariate analysis of variance assessing the effects of supplementation versus riboflavin on a composite of 5 developmental outcomes showed a significant effect of time (\( P < 0.001 \)) and a significant group-by-time interaction (\( P < 0.03 \)). There were significant group-by-time interactions for both PDI and orientation-engagement (\( P = 0.02 \) and \( P = 0.01 \), respectively).

In the second phase of the analysis, we used mixed-model repeated-measures analysis of variance to compare the PDI and orientation-engagement slopes from 6 to 12 mo. PDI scores for infants in the iron, zinc, and riboflavin groups decreased from 6 to 12 mo (Table 2). PDI scores decreased less from 6 to 12 mo in the infants in the iron+zinc and MM groups than in the children in the riboflavin group (\( P < 0.05 \), Dunnett’s test). Orientation-engagement scores decreased significantly from 6 to 12 mo for infants in the riboflavin group and significantly more than did scores for infants in the iron alone and iron+ zinc groups (\( P < 0.05 \), Dunnett’s test).

In preparation for the third phase of the analysis, we examined the correlation matrix between demographic variables and development and behavior scores at 12 mo, which showed that maternal education was associated with all indexes except emotional regulation, favoring infants with better-educated mothers (Table 3). Duration of breastfeeding was not associated with any of the measures, but others have reported associations between breastfeeding duration and children’s early developmental skills (33, 34). Neither hemoglobin at 6 mo nor the change in hemoglobin from 6 to 12 mo was related to any of the developmental or behavioral measures. The anthropometric measures were related to the Mental Developmental Index, the PDI, orientation-engagement, and motor quality. Changes in length-for-age and weight-for-length were correlated with the 6-mo measures of the same indexes (\( r = -0.45, P < 0.001 \) and \( r = -0.14, P < 0.05 \), respectively). We retained the 6-mo and the change from 6 to 12 mo measures of length-for-age and weight-for-length in the analyses to adjust for the children’s growth. We also controlled for duration of breastfeeding, maternal education, HOME score, and anemia because they have been associated with children’s development and behavior.

To complete the third phase, we used mixed-model repeated-measures analysis of variance, including covariates identified from the bivariate analyses. We repeated the analyses comparing the 6 to 12 mo slopes of the supplementation groups with the slopes of the riboflavin group. The results were similar to those
reported with the use of unadjusted scores. PDI scores for infants in the iron, zinc, and riboflavin groups decreased from 6 to 12 mo. Infants in the iron+zinc and MM groups experienced significantly smaller decreases in PDI scores than did the children in the riboflavin group (P < 0.05, Dunnett’s test) (Figure 2), with effect sizes in the moderate range (0.35 and 0.39, respectively). The effect sizes for differences in average change in the iron alone and the zinc alone groups in comparison with the average change in the riboflavin group were small (0.22 and 0.24, respectively) and were not significant.

In the reanalysis with covariates, only the riboflavin group experienced a significant decrease in orientation-engagement scores from 6 to 12 mo. Scores for infants in the riboflavin group differed significantly from the scores for the iron alone and iron+zinc groups (P < 0.05, Dunnett’s test). The effect sizes were moderate, ranging from 0.30 to 0.41.

**DISCUSSION**

Weekly administration of a micronutrient supplementation containing iron and zinc had a beneficial effect on infant motor development (PDI) and exploration (orientation-engagement). The protective effects of iron and zinc are important findings because infants who have well-developed motor skills and are explorative should be better prepared to take advantage of environmental resources and ultimately demonstrate better cognitive skills than do less motorically advanced children (35, 36).

Supplementation with iron and zinc together or with an MM containing iron and zinc protected Bangladeshi infants from the decline in motor development experienced by infants who received riboflavin from 6 to 12 mo. The effect sizes for children who received either iron alone or zinc alone were small and not significant. The effect size for the group that received iron and zinc together was similar to that for the MM group, which indicated that there was no additional benefit of the other vitamins and minerals on the children’s motor development.

Consistent with findings from other reports from Bangladesh, most of the infants’ developmental scores were within the normal range, but declined over time (37). There are several possible explanations for the reduction in motor development scores experienced by many of the infants across the second 6 mo of life. First, the attenuated growth that the infants experienced across the 6-mo study period may have compromised their developmental performance. Second, the infants were weaned during the second 6 mo of life, which meant that they had to compete with family members for limited access to nutritional food during a period of rapid growth and high nutritional demands (21). Thus, their vulnerability to micronutrient deficiencies may have been high during this period. Third, 7–12-mo-old infants frequently explore their environments by crawling and putting things into their mouths. In an environment that has high rates of fecal contamination, poor hygienic and food handling practices, and limited access to clean water, infants are at high risk of diarrhea and other infections, conditions that may create lethargy and interfere with exploration (38). Fourth, as maternally derived immunities wear off, children may become ill from exposure to infections and environmental contaminants. Finally, the developmental declines may represent the increasing differentiation in expectations for motor performance that occur with age. At 6 mo, infants are expected to demonstrate trunk control (eg, sitting), whereas at 12 mo, infants are expected to demonstrate more sophisticated trunk control (eg, sitting) and extremity control (eg, placing a peg in a hole).

When children’s behaviors were considered, children in the riboflavin group scored lower on orientation-engagement at 12 mo than at 6 mo. In contrast, children who received iron alone, zinc alone, or iron and zinc in combination (but not the MM), achieved orientation-engagement scores that did not differ significantly from their 6-mo scores and were protected from the decline that they would have experienced in the absence of the supplement.

The protective effect of iron and zinc supplementation on the infants’ exploratory behavior lends support to the functional isolation hypothesis (22). Exploratory behavior, measured by scores in orientation-engagement, is an important component of infant development (35, 36). These findings are consistent with data from an iron supplementation trial in Chile in which the supplemented children were more socially interactive and displayed more positive affect than did the nonsupplemented children (10). Iron and zinc supplementation may have protected the infants from the decline in motor development experienced by infants in the riboflavin group by protecting them from the reductions in exploration (indexed by orientation-engagement) experienced by the infants in the riboflavin group. This finding illustrates the importance of examining changes in children’s behavior, related to their micronutrient status.

It is not clear why the children in the MM group were not protected from the decline in orientation-engagement experienced by the children in the riboflavin group. In addition to iron
and zinc, the MM contained 16 other vitamins and minerals. One possibility is that there were interactions among micronutrients in the MM group that limited the effectiveness of the intervention. Not only were infants in the MM group more likely to reject the supplement, but in the larger trial, infants in the MM group had a higher incidence of diarrhea than did infants in the other groups (23). Infants with frequent diarrhea may be uncomfortable and have less energy to explore than do infants with less frequent diarrhea. Further investigation is necessary to examine the effects of other ingredients in the MM on infants’ behavior and development. In the future, it would be useful to include information from caregivers regarding the acceptability of the supplementation and the infant’s behavior during supplementation.

The weekly iron supplement had no effect on the children’s hemoglobin concentration, and hemoglobin concentration was not related to children’s development and behavior at 6 or 12 mo. Although we eliminated children with severe anemia, approximately two-thirds of the children were mildly anemic at recruitment and remained anemic throughout the trial. These findings are consistent with those of other trials that have found no effects of iron supplementation on hemoglobin concentration among mildly anemic children (9), possibly suggesting that mild anemia may have causes other than iron deficiency.

The results from this trial may clarify findings from previous studies that have reported either no differences or very small effects on development and behavior related to single micronutrient supplementation. In this trial, when iron and zinc were administered alone, the effects on children’s psychomotor development were in a positive direction, although they were small and not significant. However, when iron and zinc were given in combination, the effect sizes were moderate and significant. Other investigators, many of whose studies had small sample sizes, may have lacked the power to detect small effects. In addition, many did not control for differences in the infants’ growth or caregiving environment.

There are several methodologic limitations that should be considered before interpreting the data. Small sample sizes and limited power may have interfered with our ability to detect differences. In addition, without biochemical indicators of the infants’ iron and zinc status, we have to rely on their response to supplementation and cannot be sure of the mechanisms linking supplementation with developmental and behavioral changes.

Most previous micronutrient trials administered supplements daily. The finding that weekly administration of the supplement helped to protect the infants from declines in development and behavior has substantial public health importance. Because weekly administration is much lower in cost and easier to implement than is daily administration, it should result in better compliance and enable more children to receive the supplements. This trial has advanced our knowledge of the protective effects of iron and zinc supplementation, when administered together, on the development and behavior of infants in low-income countries. It also points to the importance of incorporating behavioral indexes into investigations of early development and lends support to the importance of the functional isolation hypothesis in considering how micronutrient supplementation may influence infant development. There is a need for more trials that evaluate the administration of combinations of micronutrients and that examine the mechanisms linking micronutrient supplementation and development.

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AHB and REB designed the overall study and obtained the funding. KZ, LAP, and SEA participated in the design, supervision, and monitoring of the study. MMB, AHB, JDH, and REB designed the developmental subsyudy. MP and JDH provided training and supervised the data collection. KL and SWM assisted with the data analysis. MMB prepared the initial draft. All authors made critical comments during the preparation of the manuscript and accept responsibility for the work. None of the authors had a personal or professional conflict of interest.

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