Effect of Maternal Multiple Micronutrient vs Iron–Folic Acid Supplementation on Infant Mortality and Adverse Birth Outcomes in Rural Bangladesh
The JiVitA-3 Randomized Trial
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**IMPORTANCE** Maternal micronutrient deficiencies may adversely affect fetal and infant health, yet there is insufficient evidence of effects on these outcomes to guide antenatal micronutrient supplementation in South Asia.

**OBJECTIVE** To assess effects of antenatal multiple micronutrient vs iron–folic acid supplementation on 6-month infant mortality and adverse birth outcomes.

**DESIGN, SETTING, AND PARTICIPANTS** Cluster randomized, double-masked trial in Bangladesh, with pregnancy surveillance starting December 4, 2007, and recruitment on January 11, 2008. Six-month infant follow-up ended August 30, 2012. Surveillance included 127 282 women; 44 567 became pregnant and were included in the analysis and delivered 28 516 live-born infants. Median gestation at enrollment was 9 weeks (interquartile range, 7-12).

**INTERVENTIONS** Women were provided supplements containing 15 micronutrients or iron–folic acid alone, taken daily from early pregnancy to 12 weeks postpartum.

**MAIN OUTCOMES AND MEASURES** The primary outcome was all-cause infant mortality through 6 months (180 days). Prespecified secondary outcomes in this analysis included stillbirth, preterm birth (<37 weeks), and low birth weight (<2500 g). To maintain overall significance of α = .05, a Bonferroni-corrected α = .01 was calculated to evaluate statistical significance of primary and 4 secondary risk outcomes (.05/5).

**RESULTS** Among the 22 405 pregnancies in the multiple micronutrient group and the 22 162 pregnancies in the iron–folic acid group, there were 14 374 and 14 142 live-born infants, respectively, included in the analysis. At 6 months, multiple micronutrients did not significantly reduce infant mortality; there were 764 deaths (54.0 per 1000 live births) in the iron–folic acid group and 741 deaths (51.6 per 1000 live births) in the multiple micronutrient group (relative risk [RR], 0.95; 95% CI, 0.86-1.06). Multiple micronutrient supplementation resulted in a non–statistically significant reduction in stillbirths (43.1 vs 48.2 per 1000 births; RR, 0.89; 95% CI, 0.81-0.99; P = .02) and significant reductions in preterm births (18.6 vs 21.8 per 100 live births; RR, 0.85; 95% CI, 0.80-0.91; P < .001) and low birth weight (40.2 vs 45.7 per 100 live births; RR, 0.88; 95% CI, 0.85-0.91; P < .001).

**CONCLUSIONS AND RELEVANCE** In Bangladesh, antenatal multiple micronutrient compared with iron–folic acid supplementation did not reduce all-cause infant mortality to age 6 months but resulted in a non–statistically significant reduction in stillbirths and significant reductions in preterm births and low birth weight.

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Multiple micronutrient deficiencies are common among pregnant women in resource-poor regions of the world, especially in southern Asia.1-5 Coexisting with poor maternal nutrition across the region are excessive burdens of low birth weight (LBW) (8%-55%),6,7 preterm birth (7%-28%),8 small size for gestational age (22%-71%),7,9 stillbirth (-3%),10 infant mortality (0.6%-6.7%),7 and maternal mortality (0.2%-0.7%).11,12 Gestational micronutrient deficiencies may contribute to avertable adverse birth outcomes.13 For example, iron–folic acid supplementation is a standard of care during pregnancy in most low- and middle-income countries, as it reduces risks of anemia and LBW13,14 and thus represents a suitable intervention against which to evaluate multiple vitamin and mineral supplement use. A number of trials have compared effects of daily, antenatal, multiple micronutrients (MMs) with iron–folic acid supplementation, revealing modest increments in birth weight, with consequent reductions in LBW, but have generally lacked statistical power to discern effects on infant mortality.13 A cluster randomized trial in Indonesia reported a 14% reduction in LBW13-15 and 18% reduction in mortality through age 3 months among infants whose mothers received a daily antenatal MM vs iron–folic acid supplement.15 However, data for effects of antenatal MM supplementation on longer-term infant mortality are sparse for guiding policies in southern Asia.

We report here the findings of a cluster randomized trial to estimate effects of antenatal maternal MM vs iron–folic acid supplementation on infant mortality to age 6 months and adverse birth outcomes in rural Bangladesh.

Methods

We carried out from 2008 through 2012 a cluster randomized, double-masked trial to compare the efficacy of daily antenatal use of an oral 15-micronutrient supplement with an iron–folic acid supplement (standard of care) in reducing all-cause infant mortality to age 6 months (180 days). Second, the trial sought to assess effects of MM vs iron–folic acid supplementation on prespecified gestational outcomes of stillbirth, preterm birth, and LBW. The supplement’s effect on the relative risk (RR) of being born small in size for gestational age was explored as a post hoc analysis. Infant mortality was followed up through age 12 months (365 days). Additional effects of supplementation on maternal and infant morbidity, micronutrient status, and infant growth are among secondary outcomes that will be reported elsewhere.

The study protocol and amendments were approved by the Johns Hopkins Bloomberg School of Public Health institutional review board, Baltimore, Maryland, and the Bangladesh Medical Research Council, Dhaka, Bangladesh (trial protocol in Supplement 1 and amended trial protocol in Supplement 2). An international data and safety monitoring board annually reviewed the trial, procedures, and findings on primary and selected secondary outcomes. Oral informed consent was provided by study participants. No incentives were offered for study participation.

Participants and Population Site

The trial was conducted in a contiguous area of 18 of 82 unions (smallest rural governmental administrative unit) in Gaibandha plus 1 neighboring union (of 83) in Rangpur Districts of northern Bangladesh. The general area was selected in 2000 for an earlier trial,12 based on comparability with national rural topography, infrastructure, population density, ethnicity, health, nutrition, and services.16 Study unions were selected for their rural character, contingency, population size, and road access, leading us to define a study area of 450 km² with population of approximately 650 000. The 19 unions were subdivided into 596 sectors (Figure 1) with a median of 209 households each (inter-quartile range, 181-241), serving as units of randomization and assigned field work. All sectors were geographic information system (GIS)-mapped and households assigned an address. We used a preexisting organization of 596 resident, female staff (1 per sector), organized into 56 field teams, each with 1 to 2 trained interviewers/anthropometrists and a team leader, overseen by 18 coordinators and supervisors, and directed by a senior management team. We used a 5-weekly, home-based pregnancy surveillance system, capable of recruiting 5000 to 6000 newlywed couples and identifying 12 000 to 15 000 gravida annually.12,16 A substudy area of 44 sectors, average in study area characteristics and balanced by intervention group, was identified for biochemical and body composition studies.

Sample Size

We estimated a sample of 25 130 live-born infants (n = 12 565/group) would be required to discern a 15% or greater reduction in 6-month infant mortality based on an expectation of 60 deaths per 1000 live births in the iron–folic acid control group,12 accepting a type I (α) error of 5%, power (1-β) of 80%, a design effect of 1.15 for cluster randomization, and a 5% loss rate based on earlier studies. We estimated requiring 36 000 pregnancies, assuming a 30% loss from induced abortion, miscarriage, and stillbirth,12 and 2.5 years of recruitment, at 1 pregnancy per eligible woman under surveillance. However, after the 2008 financial crisis, we observed a decline in live births due to deferred pregnancies and increased abortions, requiring us to extend the trial by 1 year and amend the recruitment goal to 42 500 pregnancies, for which institutional review board approval was obtained in June 2010.

Randomization

We stratified the contiguous list of 596 sectors into 74 blocks of 8 each (for the first 592 sectors), plus a 75th block of 4 sectors. A sector-supplement code key with A or B was created by flip of a coin, reflecting assignment to iron–folic acid or MM supplementation, and duplicated, and each of 2 copies was sealed into an envelope by an uninvolved colleague at Johns Hopkins. We used an in-house program (VBScript, Microsoft) that recognized 70 possible permutations for n = 8 sectors and k = 2 supplement allocations and 6 for the last block of n = 4 sectors. Using this program, we randomized sectors within blocks to 1 of 2 codes such that each permutation had an equal probability of being chosen. The resulting 2 lists of sectors were securely transmitted to field headquarters. One envelope with the code key was securely transmitted to the supplement producer and the other sealed in an envelope and secured at Johns Hopkins Medical Research Council, Dhaka, Bangladesh.
Hopkins. At no time during the trial did study investigators or field or data management staff have access to the key.

**Study Supplements**

The MM supplement tested in this trial was equivalent to a formulation recommended by UNICEF for pregnancy, containing a recommended dietary allowance for vitamins A (770 μg retinol activity equivalents), D (5 μg, or 200 IU), E (15 mg), B₁ (thiamine, 1.4 mg), B₂ (riboflavin, 1.4 mg), B₃ (niacin, 18 mg), B₆ (pyridoxine, 1.9 mg), B₉ (folic acid, 600 μg), B₁₂ (cyanocobalamin, 2.6 μg), and C (85 mg); iron (27 mg); zinc (12 mg); copper (1000 μg); selenium (60 μg); and iodine (220 μg). The
control supplement contained 27 mg of iron and 600 μg of folic acid.

Supplements were produced in 2 batches 14 months apart. Premixes were produced by DSM, Singapore, and air-freighted to Dhaka, where Beximco Pharmaceuticals manufactured 8 million tablets of each formulation, identical in appearance. Tablets were packed into 200-count, opaque plastic bottles, affixed with codes A or B representing supplement content, and shipped to the field where logistics staff, unininvolved in the study, relabeled bottles with sector numbers (001-596) according to the random allocation list. Bottles remained under dark, temperature-controlled, and humidity-controlled conditions until dispensed to field.

Over the course of study, tablets were sampled from field depots 2 to 3 times annually and tested for nutrient content 10 times, blinded to code at either DSM without charge or by Medallion Laboratories for a fee. Supplement content ranged from 80% to 183% of declared values across all nutrients (eTable 1 in Supplement 3).

Procedures

Pregnancy surveillance began on December 4, 2007, and recruitment on January 11, 2008. Using a preexisting list of resident women, 596 female sector staff visited all households to confirm eligibility and enlist newly married women aged 12 to 45 years living with their husbands. Each year women were given a wall calendar on which to mark the first day of each menstrual period. Thereafter, every 5 weeks, staff revisited households to continue enlisting newlyweds and ask women about menstruation in the past 30 days. Women reporting amenorrhea were asked the date of the first day of their last menstrual period, checked against marked calendars, and offered a urinary human chorionic gonadotropin test (Orchid) to confirm pregnancy. Women with positive test results were visited within days by team leaders who provided information about the trial and requested participation. After women gave oral consent, recorded before a witness, they were enrolled and provided 14 tablets in address-labeled bottles per sector allocation. Staff counted and replenished tablets to 14 each week and recorded outcomes of fetal loss, live birth, and maternal or infant death. Women moving their residence within the study area were tracked and dosed by original sector allocation. Weekly resupply and monitoring visits continued through 12 weeks postpartum.

Fifty-six female interviewers experienced in maternal and newborn anthropometric assessment7,19 received refresher training in recording adult weight to the nearest 100 g on Seca 880 digital scales (Seca), standing height to the nearest 0.1 cm on portable stadiometers, and mid-upper arm circumference (MUAC) to the nearest 0.1 cm using insertion tapes. Refresher training was provided for taking newborn weight on Tanita BD585 scales to the nearest 10 g; length to the nearest 0.1 cm on local length boards; and head, chest, and arm circumferences to the nearest 0.1 cm using insertion tapes.

After providing consent, women were visited by interviewers who measured height, weight, and MUAC and collected baseline data on home assets; education; history of pregnancy and child mortality; and dietary intake, morbidity, and tobacco, betel nut, and alcohol use in the past week. In the sub-study, hemoglobin was assessed at home visits by HemoCue (Model Hb301).

Gestational age at pregnancy recruitment and outcome were calculated in the data center, based on the first day of last menstrual period immediately before positive urine test results and conveyed to field staff as needed. Miscarriage (spontaneous loss, <24 weeks’ gestation) and stillbirth (born ≥24 weeks without breathing, crying, or moving limbs) were investigated within a month. Live-born infants were assessed for size, delivery complications, and morbidity symptoms shortly after birth (median age, 8 hours). Newborns were given a 50 000-IU oral dose of vitamin A to reduce risk of infant mortality, demonstrated previously in this population.20 When the infants were aged 1, 3, and 6 months, interviewers assessed their vital status, anthropometry, breastfeeding status, and recent histories of morbidity, diet, and health care received. Infant death was investigated by a verbal autopsy interview with parents, data from which were reviewed by 2 physicians who reached consensus about primary and underlying causes. Six-month follow-up of infant status was completed by August 30, 2012.

Statistical Analysis

Baseline maternal and household characteristics, including a living standards index,21 were assessed for comparability across groups and differences tested with a t test and χ2 test for continuous and dichotomous variables, respectively. Adherence to supplementation was defined as the percentage of distributed supplements reported consumed from date of informed consent through 12 weeks after pregnancy outcome, calculated as the sum of initial supplements given plus those replenished weekly, based on tablet counts. Distributions (median and interquartile range) of adherence were compared by group.

We estimated RRs and 95% confidence intervals of dichotomous outcomes, using generalized logistic binomial regression equations (GEE), a log link function, and exchangeable correlation,22 with the group receiving iron–folic acid supplements as the reference. All outcomes were assessed on an intention-to-treat basis and adjusted for the cluster-randomized design effect. We first examined effects of maternal MM vs iron–folic acid supplementation on infant mortality at age younger than 6 months (180 days), the trial’s primary outcome, with post hoc exploration of effects on neonatal (deaths at ≤28 days) and postneonatal (deaths at 29-180 days) mortality. Infants lost to follow-up were few (n = 5) and assumed alive at 180 days. Interactions for mortality at age younger than 6 months were explored by prespecified effect modifiers of infant sex, birth weight (<2500 or ≥2500 g), preterm status at birth (<37 or ≥37 weeks’ gestation), season of birth (dry or rainy), maternal adherence to supplementation (below or ≥median), maternal age (<20 or ≥20 years), gestational age at dosing (<12 or ≥12 weeks), parity (0 or ≥1), height (<150 or ≥150 cm), body mass index (<18.5 or ≥18.5, calculated as weight in kilograms divided by height in meters squared), maternal MUAC (<21.5 or ≥21.5 cm), betel nut use (none or any), and household living standards index (below or ≥median). We tested for interactions on mortality to 6 months using GEE logistic regression models, with a P < .10 (vs <.05) to enhance power to detect potentially important interactions for this outcome.
We estimated RR s and 95% confidence intervals for effects on prespecified secondary outcomes of stillbirth (fetal death at ≥24 weeks’ gestational age); preterm birth (<37 weeks), including substrata of extremely (<28 weeks), very (28 to <32 weeks), and moderately (32 to <37 weeks) preterm birth; LBW (<2500 g); and small size for gestational age (weight <10th percentile, as post hoc analysis) using GEE logistic binomial regression equations, a log link function, and exchangeable correlation, with

Table 1. Maternal Characteristics at Pregnancy Enrollment (Baseline) by Supplement Allocation

<table>
<thead>
<tr>
<th></th>
<th>Iron–Folic Acid Group (n = 22 162)</th>
<th>Multiple Micronutrient Group (n = 22 405)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yb</td>
<td>22 154 (22.8)</td>
<td>22 395 (22.3)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>6159 (27.8)</td>
<td>6118 (27.3)</td>
</tr>
<tr>
<td>20–29</td>
<td>11 362 (51.3)</td>
<td>11 465 (51.2)</td>
</tr>
<tr>
<td>≥30</td>
<td>4633 (20.9)</td>
<td>4812 (21.5)</td>
</tr>
<tr>
<td>Gestational age at urine test, wkb</td>
<td>21 919 (28.5)</td>
<td>22 161 (28.6)</td>
</tr>
<tr>
<td>≤7</td>
<td>6252 (28.5)</td>
<td>6346 (28.6)</td>
</tr>
<tr>
<td>8–12</td>
<td>10 961 (50.0)</td>
<td>11 123 (50.2)</td>
</tr>
<tr>
<td>≥13</td>
<td>4706 (21.5)</td>
<td>4692 (21.2)</td>
</tr>
<tr>
<td>Educationb</td>
<td>22 105 (14.6)</td>
<td>22 357 (14.7)</td>
</tr>
<tr>
<td>None</td>
<td>6795 (30.7)</td>
<td>6745 (30.2)</td>
</tr>
<tr>
<td>Class 1–4</td>
<td>3231 (14.6)</td>
<td>3281 (14.7)</td>
</tr>
<tr>
<td>Class 5–9</td>
<td>10 454 (47.3)</td>
<td>10 695 (47.8)</td>
</tr>
<tr>
<td>Class ≥10</td>
<td>1625 (7.4)</td>
<td>1636 (7.3)</td>
</tr>
<tr>
<td>Reproductive historyb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>22 112 (10.0)</td>
<td>22 361 (10.0)</td>
</tr>
<tr>
<td>0</td>
<td>6671 (30.2)</td>
<td>6647 (30.2)</td>
</tr>
<tr>
<td>1–3</td>
<td>13 255 (59.9)</td>
<td>13 520 (60.5)</td>
</tr>
<tr>
<td>≥4</td>
<td>2186 (9.9)</td>
<td>2194 (9.8)</td>
</tr>
<tr>
<td>≥1 Previous fetal loss</td>
<td>21 122 (10.0)</td>
<td>21 375 (10.0)</td>
</tr>
<tr>
<td>≥1 Prior infant death</td>
<td>15 439 (22.4)</td>
<td>15 715 (22.8)</td>
</tr>
<tr>
<td>≥1 Previous fetal loss</td>
<td>15 439 (22.4)</td>
<td>15 715 (22.8)</td>
</tr>
<tr>
<td>≥1 Prior infant death</td>
<td>15 439 (22.4)</td>
<td>15 715 (22.8)</td>
</tr>
<tr>
<td>≥1 Prior infant death</td>
<td>15 439 (22.4)</td>
<td>15 715 (22.8)</td>
</tr>
<tr>
<td>Living standards index ≥0.20859b,c</td>
<td>22 105 (10.0)</td>
<td>22 347 (10.0)</td>
</tr>
<tr>
<td>Meat or liver</td>
<td>18 763 (14.8)</td>
<td>19 160 (14.6)</td>
</tr>
<tr>
<td>Marine food</td>
<td>18 763 (63.9)</td>
<td>19 160 (64.4)</td>
</tr>
<tr>
<td>Egg</td>
<td>18 763 (17.7)</td>
<td>19 158 (17.4)</td>
</tr>
<tr>
<td>Milk products</td>
<td>18 763 (25.4)</td>
<td>19 159 (26.1)</td>
</tr>
<tr>
<td>Yellow vegetables and fruit</td>
<td>18 762 (19.0)</td>
<td>19 158 (19.2)</td>
</tr>
<tr>
<td>Dark green leafy vegetables</td>
<td>18 762 (22.4)</td>
<td>19 157 (22.1)</td>
</tr>
<tr>
<td>Productive cough</td>
<td>18 760 (14.2)</td>
<td>19 160 (14.0)</td>
</tr>
<tr>
<td>Dysentery</td>
<td>18 760 (4.6)</td>
<td>19 159 (4.8)</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>18 760 (1.2)</td>
<td>19 159 (1.3)</td>
</tr>
<tr>
<td>Night blindness</td>
<td>18 762 (1.1)</td>
<td>19 159 (1.3)</td>
</tr>
<tr>
<td>Any tobacco or betel nut use in past 7 d5f</td>
<td>18 759 (69.0)</td>
<td>19 157 (69.2)</td>
</tr>
<tr>
<td>Woman</td>
<td>18 759 (12.1)</td>
<td>19 154 (12.9)</td>
</tr>
<tr>
<td>Husband</td>
<td>18 759 (64.2)</td>
<td>19 157 (65.0)</td>
</tr>
<tr>
<td>Height ≤150 cm²</td>
<td>18 718 (52.9)</td>
<td>19 120 (51.8)</td>
</tr>
<tr>
<td>Body mass index &lt;18.5kgf</td>
<td>18 711 (39.9)</td>
<td>19 114 (40.4)</td>
</tr>
</tbody>
</table>

* Number of women out of total in the iron–folic-acid and multiple micronutrient groups assessed for each listed variable.

b Variables assessed at baseline household visit irrespective of pregnancy status at time of household visit. Missing data out of possible n = 22 162 and 22 405 women in the iron–folic-acid and multiple micronutrient groups, respectively, are as follows: age (n = 8 and 10), gestational age at urine test (n = 243 and 244), education (n = 57 and 48), parity (n = 50 and 44), prior fetal loss (n = 1 and 2) and prior infant death (n = 684 and 662) among women who reported a previous pregnancy (n = 16 123 and 16 377), and living standards index (n = 57 and 58). c Value of −0.20859 represents the median in the iron–folic-acid group based on derived index. d Variable assessment restricted to pregnancy, thus not assessed according to protocol if pregnancy terminated between dates of urine test and baseline interview. Missing data per variable out of possible n = 18 763 and 19 162 interviews in the iron–folic-acid and multiple micronutrient groups, respectively, are as follows: dietary intakes (n = 0–1 and 2–5), morbidity (n = 1–6 and 0–6), tobacco/betel nut use (n = 4 and 5–8), height (n = 45 and 42), and body mass index (n = 52 and 58). e Meat or liver: goat, lamb, beef, chicken, duck, goose, any animal liver; marine food: fish (fresh or dried), shrimp; dairy products: milk, yogurt; yellow vegetables and fruit: ripe pumpkin, mango, papaya, or jackfruit. f P < .05. g Calculated as weight in kilograms divided by height in meters squared.
the iron–folic acid group as the reference. Differences between groups on continuous secondary outcomes of gestational age and size measures at birth (weight; length; head, chest, and arm circumference; and ponderal index [calculated as weight in kilograms divided by height in meters cubed], measured ≤72 hours) were assessed by GEE linear regression analysis, with an identity link function and exchangeable correlation for normally distributed data. All secondary analyses were restricted to singletons, except stillbirth for which all births were included. P values for testing differences in outcomes were derived by 2-sided testing of each GEE regression coefficient (null hypothesis: β = 0), a procedure that accounts for design effect but not for multiple comparisons. To guide inference about statistical significance of 2-sided tests when comparing supplement effects across multiple study outcomes at an overall significance level at α = .05, we calculated 2 Bonferroni-corrected significance levels by dividing the conventional level of .05 by the number of study outcome comparisons: (1) α = .01 (ie, .05/5), to evaluate statistical significance of 1 primary plus 4 major secondary risk outcomes (<6-month infant mortality and stillbirth, preterm birth, LBW, and small size for gestational age, respectively) and (2) α < .007 (ie, .05/7) for evaluating significance of differences in continuous measures of gestational age; weight; length; head, chest, and arm circumference; and ponderal index at birth.

Effect sizes for continuous birth outcomes were calculated based on Cohen d. Birth weights were further examined by plotting normal probability distributions. Analyses were performed using SAS version 9.3 (SAS Institute).

Results
Among 127 282 women ever under pregnancy surveillance, 64 049 resided in the iron–folic acid and 63 233 in the MM supplement sectors, among whom 22 814 and 23 104, respectively, became pregnant (Figure 1). Among the 22 162 and 22 405 pregnancies recruited into each group, 22.7% (n = 5037 and 5089, respectively) and 10.6% (n = 2356 and 2378, respectively) ended in induced abortion and miscarriage, respectively. We observed 14 142 and 14 374 live-born infants in the iron–folic acid and MM groups, respectively, 99% of whom were singletons and all of whom contributed to the 6-month mortality analysis.

At baseline, about half of pregnant women in both groups were in their 20s, and 80% were in the first trimester (Table 1). Groups did not differ in age, gestational age (median, 9 weeks;
interquartile range, 7-12 weeks), education, parity, history of fetal and infant loss, living standard index, diet, recent morbidity, tobacco and betel nut use, height, or body mass index. Adherence was high, with half the women in both groups estimated to consume a median of approximately 95% (interquartile range, 84%-100%) of all distributed supplements; 80% in both groups consumed more than 80% of their intended tablets (eFigure 1 in Supplement 3). Hemoglobin distributions and anemia prevalence, measured in substudy participants, were the same by intervention group at baseline (22%), during the third trimester (42%), and at 3 months postpartum (12%) (eTable 2 in Supplement 3).

At 6 months (180 days), MM did not significantly reduce all-cause infant mortality; there were 764 deaths (54.0 per 1000 live births) in the iron–folic acid group and 741 deaths (51.6 per 1000 live births) in the MM group (RR, 0.95; 95% CI, 0.86-1.06; P = .36). Kaplan-Meier curves for both groups are shown in Figure 2. Stratification yielded RRs of 0.98 (95% CI, 0.88-1.20) and 0.81 (95% CI, 0.63-1.04) for neonatal and postneonatal mortality, respectively (Table 2). Mortality at less than 6 months did not differ by statistical effect modifiers except for maternal adherence, with RRs of 1.05 (95% CI, 0.91-1.20) and 0.83 (95% CI, 0.70-0.97) for infants in the MM vs iron–folic acid groups born to high (above the median) and lower maternal supplement consumers, respectively (interaction P = .03), and for sex with RRs of 1.02 (95% CI, 0.89-1.17) and 0.86 (95% CI, 0.74-1.00) for boys and for girls, respectively (interaction P = .10) (eTable 3 in Supplement 3).

Based on follow-up through 12 months (365 days), there was no difference in all-cause mortality, reflected by rates of 58.8 per 1000 live births (832 deaths) and 55.5 per 1000 live births (798 deaths) in the iron–folic acid and MM groups, respectively, and an RR of 0.94 (95% CI, 0.85-1.04).

Leading consensus causes of death at age younger than 6 months were sepsis (17 deaths per 1000 live births), prematurity/very low birth weight (14/1000), and birth asphyxia (14/1000). Relative risks of death attributed to sepsis, diarrhea/dysentery, and birth asphyxia among infants in the MM vs
iron–folic acid groups were 0.79 (95% CI, 0.66–0.96), 0.33 (95% CI, 0.12–0.90), and 1.29 (95% CI, 1.05–1.60), respectively (eTable 4 in Supplement 3).

As reported in Table 2, among offspring of MM vs iron–folic acid supplement mothers, there was a non–statistically significant reduction in stillbirth (RR, 0.89; 95% CI, 0.81–0.99; P = .02; Bonferroni-corrected α = .01). A significant reduction in preterm birth among newborns in the MM group was observed (RR, 0.85; 95% CI, 0.80–0.91; P < .001) with comparable RRs for extremely preterm, very preterm, and moderate-to-late preterm births (Table 3). A significant reduction was also observed in LBW among newborns in the MM group (RR, 0.88; 95% CI, 0.85–0.91; P < .001). A non–statistically significant reduction in rates of small size for gestational age was observed among newborns in the MM vs iron–folic acid group (RR, 0.98; 95% CI, 0.96–1.01; P = .13).

The mean duration of gestation at birth was 0.30 weeks (95% CI, 0.21–0.40) longer in the MM vs iron–folic acid group (P < .001), yielding an effect size of 0.10 (Table 4). Newborns of MM supplement mothers were larger by 54 g in weight; 0.20 cm in length; 0.10, 0.25, and 0.20 cm in arm, chest, and head circumferences; and 0.20 in ponderal index, respectively (all P < .001), with effect sizes ranging from 0.09 to 0.13. All continuous secondary birth outcome differences were significant by the Bonferroni correction (P < .007). Although boys were slightly larger, differences and effect sizes in gestational age and birth size associated with maternal MM vs iron–folic acid use were comparable in both sexes (eTable 5 in Supplement 3). Normalized probability distributions of newborn weight revealed a distributional shift to the right among infants born to MM vs iron–folic acid mothers (eFigure 2 in Supplement 3).

Discussion

In this typical rural Bangladesh setting, we found no overall effect on mortality to age 6 months, the primary outcome, including the neonatal period, among infants born to mothers who received daily supplements of MM vs iron–folic acid from early pregnancy through 12 weeks postpartum. Our study’s null finding is in agreement with a small number of trials that have provided an antenatal MM vs iron supplement, with or without folic acid, and found no effect on neonatal mortality.13 However, trials have often not been designed or powered to detect differences in later infant mortality or multiple maternal-fetal and infant outcomes.25,26 Among those that have, our finding is not consistent with an 18% reduction in 3-month infant mortality after antenatal MM vs iron–folic acid supplementation in Indonesia15 or a significant reduction in infant mortality to a year elsewhere in Bangladesh when MM supplementation was combined with food provision early (although not later) in pregnancy.27

We observed, however, differences in risk of adverse birth outcomes. Infants born to MM vs iron–folic acid supplement mothers had a non–statistically significant reduction in risk of stillbirth, supporting findings from smaller trials in which mothers were given MMIs from the first trimester onward.13 Infants of MM supplement mothers had a reduction in the risk of preterm birth, including very and moderate-to-mild preterm birth. These findings were consistent with slightly longer gestation, a 55-g increase in birth weight that is equivalent to an average 2-day ponderal increment late in gestation,28 small proportionate increases in other body dimensions, and reduction in LBW. This size difference is consistent with a 53-g increment and RR of 0.86 for LBW obtained from systematic reviews of MM supplementation trials across low-income regions, including South Asia.13,15,19 However, unlike findings from overviews,13,25,26 we found little difference in the risk of infants born small for gestational age and thus attribute observed gains in size to longer gestation.

Antenatal MM supplementation may have affected gestational and fetal nutrition through metabolic pathways other than those regulated by iron and folate. These include potential influences on the immune system that could reduce ma-

Table 4. Gestational Age and Newborn Size in Singleton Births by Maternal Supplement Allocation

<table>
<thead>
<tr>
<th>Birth sizea</th>
<th>No. of Live Births</th>
<th>Mean (SD)</th>
<th>No. of Live Births</th>
<th>Mean (SD)</th>
<th>Difference (95% CI)b</th>
<th>Effect Sizeb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>10 530</td>
<td>2531 (415)</td>
<td>10 642</td>
<td>2585 (407)</td>
<td>54 (41-66)</td>
<td>0.13</td>
</tr>
<tr>
<td>Length, cm</td>
<td>10 332</td>
<td>46.46 (2.26)</td>
<td>10 442</td>
<td>46.67 (2.17)</td>
<td>0.20 (0.13–0.27)</td>
<td>0.10</td>
</tr>
<tr>
<td>Arm circumference, cmc</td>
<td>10 510</td>
<td>9.46 (0.85)</td>
<td>10 617</td>
<td>9.56 (0.82)</td>
<td>0.10 (0.07–0.13)</td>
<td>0.12</td>
</tr>
<tr>
<td>Chest circumference, cm</td>
<td>10 468</td>
<td>30.70 (2.08)</td>
<td>10 575</td>
<td>30.95 (2.01)</td>
<td>0.25 (0.18–0.31)</td>
<td>0.12</td>
</tr>
<tr>
<td>Head circumference, cm</td>
<td>10 445</td>
<td>32.49 (1.57)</td>
<td>10 563</td>
<td>32.69 (1.51)</td>
<td>0.20 (0.15–0.25)</td>
<td>0.13</td>
</tr>
<tr>
<td>Ponderal indexd</td>
<td>10 331</td>
<td>25.11 (2.41)</td>
<td>10 442</td>
<td>25.32 (2.41)</td>
<td>0.20 (0.12–0.29)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Notes: P < .001 for all between-group comparisons. Applicable (7-comparison Bonferroni-corrected α = .007. 95% confidence interval adjusted for design effect by generalized estimating equations.

a Effect size estimated by Cohen d expressed as a standard normal deviate.24
b Missing data for gestational age at birth: n = 569 in the iron–folic acid group and n = 649 in the multiple micronutrient group.

c Singleton infants for whom any anthropometry was measured ≤72 hours after birth: n = 10 532 in the iron–folic acid group and n = 10 642 in the multiple micronutrient group.

d Ponderal index is an indicator of wasting or adequacy of weight adjusted for length, calculated as weight in kilograms divided by height in meters cubed.

* Left mid-upper arm circumference.
ternal, placental, and fetal inflammation from infection\textsuperscript{32-34}; oxidative metabolism that could reduce pathological stress, improve maternal-placental function, and reduce risk of hypertensive disease\textsuperscript{32-34}; and hormonal balance and epigenetic programming that could improve nutrient utilization and embryofetal growth and development.\textsuperscript{32,35} One pathway that appeared to be ruled out by our findings and those from Nepal\textsuperscript{36} could be through effects of added nutrients on hemoglobin concentration or risk of anemia, which remained unchanged between groups. Mechanistic studies embedded within human trials have been few and have yet to show compelling evidence of improved oxidoreductive,\textsuperscript{33,34,37} host defense,\textsuperscript{36,39} or endocrine\textsuperscript{40} effects to explain influence on the population, revealing future research priorities.

Reasons for a null effect on postnatal survival after improvement in some birth outcomes with antenatal MM supplement use remain unknown but may reflect a complex interplay between maternal and newborn sizes and differential responses to supplementation by causes of death. For example, we\textsuperscript{38,41-43} and others\textsuperscript{27} have raised concern about cephalopelvic complications leading to neonatal asphyxia when birth size is increased among rural South Asian women of short stature, which could potentially mask longer-term survival benefit. In the present study, in which more than half of women were less than 150 cm (4 feet 9 inches) in height, birth size increments attributed to MM supplementation were comparable by sex although boys remained larger in size (eTable 5 in Supplement 3) and twice as likely to die from birth asphyxia (eTable 4) than girls in the MM group, who in contrast were smaller in size at birth, exhibited no increased birth asphyxia-related mortality, and were more likely than girls in the iron-folic acid group to survive through 6 months (eTable 3). A lower risk of diarrhea and sepsis (eg, infection)-related mortality among infants exposed to MM vs iron-folic acid (eTable 4) would be consistent with known roles of several provided micronutrients (eg, vitamins A and D and zinc) in fetal ontology of the immune system, which could enhance postnatal defenses.\textsuperscript{30,31}

Findings from this study should be generalized within its context and design. The study area is typical of rural Bangladesh\textsuperscript{46} but one where, although affected by anemia, little maternal iron deficiency was found, attributable to high iron content of potable ground water.\textsuperscript{44,45} Second, we adopted an iron-folic acid supplement, a standard of care, rather than a placebo as a control. These factors suggest caution when drawing inferences about effects among iron-deficient populations who do not receive supplements. All newborns received a 50 000-IU oral dose of vitamin A to reduce risk of mortality,\textsuperscript{20} possibly explaining the overall 10% to 15% lower 6-month mortality we observed compared with that estimated from previous data.\textsuperscript{12} It is possible that this intervention, not yet implemented in South Asia, could have altered proportionate mortality ratios and thus all-cause infant mortality response.

Conclusions

In Bangladesh, antenatal MM vs iron-folic acid supplementation did not reduce all-cause infant mortality to age 6 months but did result in a non-statistically significant reduction in stillbirth and statistically significant reductions in preterm birth and LBW.

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**ARTICLE INFORMATION**

**Author Contributions:** Drs West and Christian had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** West, Shamim, Labrique, Klemm, Christian.

**Acquisition, analysis, or interpretation of data:** West, Shamim, Mehra, Labrique, Ali, Shaikh, Klemm, Wu, Mitra, Haque, Hanif, Massie, Merrill, Schulze, Christian.

**Statistical analysis:** Wu, Christian.

**Obtained funding:** West.

**Administrative, technical, or material support:** Mehra, Shamim, Ali, Shaikh, Mitra, Haque, Hanif, Merrill.

**Study supervision:** West, Shamim, Mehra, Labrique, Ali, Shaikh, Klemm, Merrill, Christian.

**Conflict of Interest Disclosures:** All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr West reported a grant from DSM awarded to the Program in Human Nutrition at Johns Hopkins Bloomberg School of Public Health and having given 2 scientific presentations in 2 consecutive years at DSM in Basel, Switzerland, with accommodations provided.

Dr Christian reported giving a presentation at DSM in Basel with accommodations provided. No other disclosures were reported.

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**Role of the Funder/Sponsor:** The Bill and Melinda Gates Foundation, DSM, Sight and Life, and Beximco Pharmaceuticals had no role in the design of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

**Additional Contributions:** Oversight with respect to data and safety monitoring was provided by an international board. Nancy Sloan, DrPH, Columbia University, United States (chair); James Tonascia, PhD, Johns Hopkins University, United States; Anthony de Costello, FRCP, University College London, United Kingdom; Shamsun Nahar, PhD, King Khalid University, Saudi Arabia; Md. Khaleguzzaman, Bangladesh; and Tahmeed Ahmed, PhD, International Centre for Diarrheal Disease Research, Bangladesh. Joanne Katz, ScD, Johns Hopkins University, assisted in analyses for data and safety monitoring board reports. Alfred Sommer, MD, Johns Hopkins University, and Mahbub Rashid, PhD, Partners in Population and Development, provided technical guidance. Field management was overseen by Azaduzzaman, Rhonda Skinner, Johns Hopkins University, assisted with the formatting of the manuscript. No one received compensation besides salary.

**REFERENCES**


