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A Multiple-Micronutrient-Fortified Beverage Affects Hemoglobin, Iron, and Vitamin A Status and Growth in Adolescent Girls in Rural Bangladesh[1,2]

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Abstract

Adolescent girls have high nutrient needs and are susceptible to micronutrient deficiencies. The objective of this study was to test the effect of a multiple-micronutrient-fortified beverage on hemoglobin (Hb) concentrations, micronutrient status, and growth among adolescent girls in rural Bangladesh. A total of 1125 girls (Hb ≥70 g/L) enrolled in a randomized, double-blind, placebo-controlled trial and were allocated to either a fortified or nonfortified beverage of similar taste and appearance. The beverage was provided at schools 6 d/wk for 12 mo. Concentrations of Hb and serum ferritin (sFt), retinol, zinc, and C-reactive protein were measured in venous blood samples at baseline, 6 mo, and 12 mo. In addition, weight, height, and mid-upper arm circumference (MUAC) measurements were taken. The fortified beverage increased the Hb and sFt and retinol concentrations at 6 mo ($P < 0.01$). Adolescent girls in the nonfortified beverage group were more likely to suffer from anemia (Hb <120 g/L), iron deficiency (sFt <12 μg/L), and low serum retinol concentrations (serum retinol <0.70 μmol/L) (OR = 2.04, 5.38, and 5.47, respectively; $P < 0.01$). The fortified beverage group had greater increases in weight, MUAC, and BMI over 6 mo ($P < 0.01$). Consuming the beverage for an additional 6 mo did not further improve the Hb concentration, but the sFt level continued to increase ($P = 0.01$). The use of multiple-micronutrient-fortified beverage can contribute to the reduction of anemia and improvement of micronutrient status and growth in adolescent girls in rural Bangladesh. J. Nutr. 137: 2147–2153, 2007.

Introduction

Micronutrient malnutrition is recognized as an important public health problem affecting >2 billion people worldwide (1). The magnitude is much greater in low income countries where malnutrition, infection, and poverty are widespread and often interlinked (1,2). If left untreated, micronutrient deficiencies can have significant negative consequences on health and economic development (1). Among the most affected population groups are adolescents (10–19 y), who represent 20% of the world's population (2). Adolescence is the time when both males and females undergo the last intense phase of their growth to attain full adult height, concomitant with profound changes in body structure and physiology (3). As a result, nutrient demand is enhanced to support these developmental changes, particularly among females with the onset of menarche (4).

The major micronutrient deficiencies include iron, vitamin A, and iodine in adolescent girls living in developing countries (5). In addition, deficiencies of the B vitamins (folic acid, vitamin B-12, vitamin B-6, riboflavin, and niacin), vitamin C, and zinc often coexist with the 3 major problem nutrients (6). In Bangladesh, a large proportion (11–56%) of adolescent girls suffer from subclinical vitamin A deficiency (7,8). Moreover, recent surveys show that ~30–40% of adolescent girls in Bangladesh are anemic (9–11). Because 34% of Bangladeshi adolescent girls get married before the age of 18 y (12) and 33% have their first pregnancy by ages 15–19 y (13), they are at increased risk of developing anemia during pregnancy, compromising their health as well as that of their offspring. It is therefore essential to ensure that adolescent girls enter pregnancy with an adequate nutritional status.

To date, the main public health strategies to control micronutrient deficiencies include food diversification, supplementation, and food fortification. Recently, a new diet-based strategy, which delivers multiple vitamins and minerals at physiological levels in a single vehicle (fortified, fruit-flavored powdered beverage) has been proposed (14). Community-based trials in multiple

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countries showed that the consumption of multiple-micronutrient-fortified beverage is efficacious in improving micronutrient status in school children in Tanzania (15), Botswana (16), and the Philippines (17) and in pregnant women in Tanzania (18,19). This study was conducted to assess the effect of a similar multiple-micronutrient-fortified beverage on hematological status and levels of vitamin A, iron, zinc, and growth status among adolescent girls in Bangladesh.

Materials and Methods

Study area, subjects, and recruitment. The study was conducted in 54 nonformal primary education (NFPE)[9] schools operated by the Bangladesh Rural Advancement Committee (BRAC, one of the largest national nongovernmental organizations in the world) in Sherpur district, ~300 km northeast of Dhaka city. These schools were established for adolescent girls who have either withdrawn from the formal education system or never enrolled in school (20). A total of 54 NFPE schools were randomly selected from a list of 80 schools in Sherpur district. On average, each school had 30 pupils. The study area had similar geographical characteristics to the rest of the plain lands in Bangladesh, including high population density, fertile agricultural land, low risk of malaria, and minimal hookworm infection (21). No case of HIV was reported in the region among the study's age group (22).

All adolescent girls from each selected school were screened for micronutrient and health status. Students suffering from either severe micronutrient deficiencies [severe anemia, i.e. hemoglobin (Hb) <70 g/L, $n = 10$; clinical signs of vitamin A deficiency, i.e. night blindness, $n = 2$; and clinical signs of iodine deficiency, i.e. visible goiter, $n = 23$] or acute infection (clinical signs of fever or reportedly suffer from any infectious disease, $n = 9$) were excluded and referred to the nearby health center for appropriate treatment.

The study protocol was approved by the Bangladesh Medical Research Council and Government of Bangladesh. Before the start of the study, meetings were held with parents at the selected schools to inform them about the study. Informed written consents were obtained from the students as well as from each of their parents or guardian.

Study design and protocol. This study was a randomized, placebo-controlled, double-blind trial. Subjects from each school were randomly allocated to either a fortified or a nonfortified beverage group. Randomization was done by listing all selected children, assigning them with random numbers, and dividing the odd numbers from the even numbers to form the 2 groups. The test beverages were consumed 6 d/wk for 12 mo at the schools (no drink was provided during the school holidays, which totaled ~20 d) under the direct supervision of the schoolteachers with the assistance of the BRAC community health workers (*shastho shebikas*). One *shastho shebika* was assigned per school to prepare and distribute the drink. Schoolteachers maintained daily attendance sheets for tracking beverage consumption. Adolescent boys attending the schools (~30% of the students) were included in the randomization process and were provided the same beverages to avoid sharing. However, they were not included in any aspect of the data collection and analysis.

The fortified beverage was developed and produced by Procter & Gamble (14). It has been used earlier in clinical studies in school children (15,17) and pregnant women (18,19) with slight modification to meet the requirements of the study subjects. It is an orange-flavored powdered beverage, fortified with multiple-micronutrients and packaged in sachets (**Table 1**). Each sachet contained 45 g of powdered beverage. Procter & Gamble also provided an equal quantity of a nonfortified orange-flavored powdered beverage (identical to the fortified beverage in terms of weight, color, flavor, and appearance) as a control. The sachets containing the fortified and nonfortified beverage differed only in the sachet's color (blue or yellow). Researchers, schoolteachers, *shastho shebikas*, and students did not know whether the blue or yellow colored sachets contained the fortified beverage. The decoding was done only by the manufacturer after the study was completed and the data analyzed.

TABLE 1 Nutrient composition of the micronutrient-fortified powder in 1 serving (200 mL)[1]

		% RDA for girls per serving	
Nutrients	Amount per serving	9–13 y	14–18 y
Iron, *mg*	7.0	87	47
Vitamin A, *IU* (RE)	1296 (389)	43	56
Iodine, μg	75	62	50
Zinc, *mg*	7.5	94	83
Vitamin C, *mg*	120	267	185
Riboflavin, *mg*	0.91	100	91
Folic acid, μg	120	40	30
Vitamin B-12, μg	1.0	56	42
Vitamin B-6, *mg*	1.0	100	83
Vitamin E, *mg*	10	91	67
Niacin, *mg*	5.0	42	36

[1] IU, International unit; RDA, recommended dietary allowance (Dietary Reference Intake, Institute of Medicine) (23); RE, retinol equivalent.

Before consumption, the contents of 2 sachets, which contained 90 g powder, were dissolved in 1000 mL of tube-well water. Each student received 200 mL of the reconstituted fortified or nonfortified beverage daily. Table 1 shows the nutrient composition and the percentage of the United States Recommended Dietary Allowances for adolescent girls contained in a 200-mL serving (23). Each participant had a plastic glass labeled with their name and color of their assigned beverage. To avoid arsenic contamination, each tube-well in the study was tested for arsenic content to ensure that the safety level (<0.5 mg/L arsenic) was not exceeded.

Baseline information, including socioeconomic status and menstrual history, was collected by trained BRAC program organizers through house-to-house interviews using structured questionnaires. Baseline information also contained individual food consumption data collected by expert field nutritionists using a 24-h recall method. Venous blood samples (3 mL) were collected by trained laboratory technicians from subjects at baseline, 6 mo, and 12 mo to assess Hb and serum levels of ferritin, retinol, zinc, and C-reactive protein (CRP). Anthropometric measurements including height, weight, and mid-upper arm circumference (MUAC) were determined by trained BRAC program organizers at baseline, 6 mo, and 12 mo at the schools. Wooden height scales were used for the measurement of height to the nearest 1 cm. Uniscales developed by the SECA Company (http://seca.accurate-scale.com) were used for measurements of weight to the nearest 0.1 kg. BMI was derived from weight and height measurements (kg/m^2) . Teaching-aids at Low Cost (http://www.talcuk.org) insertion tape was used to measure MUAC to the nearest 1 mm.

Laboratory analysis. Hb was measured by a trained technician using a portable photometer (HemoCue) immediately after the collection of blood (24). The HemoCue machine was standardized before each use for accuracy, using a standard microcuvette supplied by the manufacturer. Serum was separated at the field laboratory by centrifugation in a portable bench centrifuge (at 3600 × *g*; 15 min) before storage at −18 to −20°C. Frozen serum samples were then transported on dry ice and analyzed for ferritin, retinol, zinc, and CRP at the University of California Davis. Serum ferritin (sFt) was analyzed in duplicate by radioimmunoassay using a commercially validated kit (Diagnostic Products) and the interassay variation was <5%. Serum retinol was analyzed by HPLC and the interassay variation was <5%. Following acid digestion, zinc concentration in serum was determined by atomic absorption spectrophotometry relative to a standard curve (the National Institute of Standards and Technology) and the interassay variation was <10%. Serum CRP was analyzed by radial immunodiffusion (Nanorid; The Binding Site) and the concentration was determined relative to a calibration curve provided with the kit. For quality control, the laboratory

[9] Abbreviations used: BRAC, Bangladesh Rural Advancement Committee; CRP, C-reactive protein; Hb, hemoglobin; IDA, iron deficiency anemia; MUAC, mid-upper arm circumference; NFPE, nonformal primary education; sFt, serum ferritin.

used inter- and intra-assay variation (percent CV) of the standard curve and commercially available quality control samples.

Anemia was defined as Hb <120 g/L, depleted iron stores were defined as sFt <12 μg/L, and iron deficiency anemia (IDA) was defined by Hb <120 g/L and sFt <12 μg/L (25). Low serum retinol concentration was defined as serum retinol <0.70 μmol/L, low serum zinc was defined as serum zinc <10.7 μmol/L, and the presence of infection was defined as CRP >10 mg/L (26).

Sample size. A total of 1268 adolescent girls, 634 in the fortified group and 634 in the nonfortified beverage group, were planned to be recruited in the study. The sample size was calculated to detect within and between group differences in mean Hb, prevalence of anemia (Hb <120 g/L), mean serum retinol, and low serum retinol concentrations (serum retinol <0.70 μmol/L) at a power of 0.80, $\propto = 0.05$, and a lost-to-follow up of 25%.

Statistical methods. All forms were manually checked for completeness, consistency, and range. Data were coded, processed, and analyzed using SPSS for Windows (version 10.0) statistical software. When data were skewed, log transformation was used to perform statistical tests requiring normal distributions. Binary data were summarized with percentages. Paired *t* test was used for comparison of means within groups. Independent *t* test or general linear modeling was used for comparison of means and/or mean increment between groups. Wilcoxon's Signed Rank test was used for comparison of proportions between groups. Change in anemia prevalence within groups was assessed using McNemar's test. The OR for anemia, iron deficiency, IDA, and low serum retinol concentrations were computed using logistic regression. Differences were considered significant at $P < 0.05$.

Results

Subject characteristics. We assessed 1171 adolescent girls for eligibility from 54 schools and 1125 enrolled (**Fig. 1**). Some were excluded ($n = 46$) because they did not meet the inclusion criteria ($n = 44$) or refused to participate ($n = 2$). Of those enrolled, 49 girls had missing data for Hb and 87 were lost to follow-up at 12 mo because of illness ($n = 34$) and refused to participate ($n = 53$).

Of the girls who completed the 12-mo trial and had complete data for Hb, the baseline characteristics did not differ between the 2 groups (**Table 2**). Thirty-four percent of the girls in the fortified group and 37% of the girls in the nonfortified beverage group were 13 y or older ($P = 0.35$). The mean age of onset of menstruation was 13.6 y and 23% of the girls had experienced menarche at the time of the study. The girls came from relatively low socioeconomic families as revealed by high levels of illiteracy among mothers, largely proportioned households without any cultivable land, and low per capita daily calorie intakes (Table 2).

Anthropometric status. At baseline, weight, height, MUAC, and BMI did not differ between the 2 groups (**Table 3**). After the 6-mo intervention, weight, height, MUAC, and BMI increased significantly in both groups as expected because of growth during adolescence. However, the increases in weight, MUAC, and BMI were higher in the group that received the fortified beverage compared with the nonfortified beverage group ($P < 0.01$). At the 12-mo follow-up assessment, weight, MUAC, and BMI units increased significantly from the 6-mo follow-up assessment in both groups, but mean increases did not differ between the groups (data not shown).

Hematological status. At baseline, there was no difference in Hb concentration between the 2 groups (**Table 4**). After 6 mo, mean Hb concentration increased in the fortified beverage group ($P < 0.01$) but did not change in the nonfortified beverage

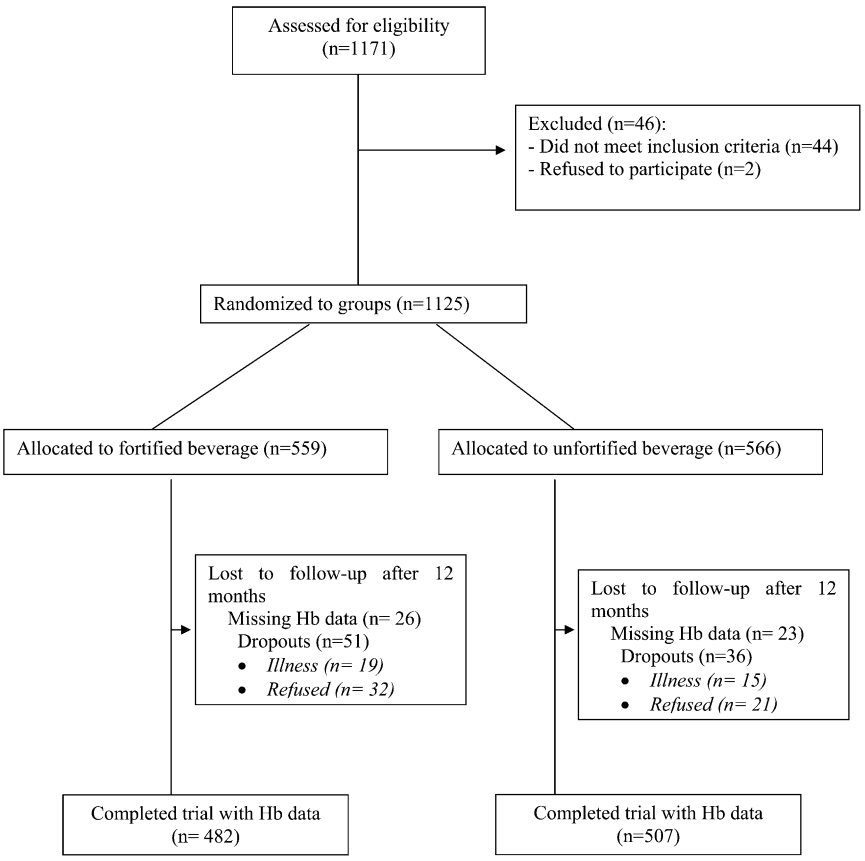

FIGURE 1 Trial profile. A total of 1171 adolescent girls attending 54 NFPE schools in Sherpur, Bangladesh were screened; 1125 girls participated in the study. Of them, 482 girls in the fortified powdered-beverage group and 507 girls in the nonfortified powdered-beverage group completed the 12-mo trial.

TABLE 2 Baseline information of adolescent girls who participated in a fortified beverage-based intervention study[1]

	Fortified beverage, $n = 482$	Unfortified beverage, $n = 507$
Age, *y*	11.9 ± 1.9	12.0 ± 1.9
Family size, *n*	5.7 ± 1.8	5.6 ± 1.8
Mother able to read and write, %	14.2	15.1
Household with no land ownership, %	37.7	36.9
Energy intake,[2] *kcal/d*	1881 ± 588	1778 ± 393
Hb <120 *g/L*, %	32.4	30.4
sFt <12 $\mu g/L$, %	30.5	32.9
IDA, %	13.1	13.8
Serum retinol <0.70 $\mu mol/L$, %	11.2	10.3
Serum zinc <10.7 $\mu mol/L$, %	60.9	55.3
BMI <16 kg/m^2, %	79.3	80.3

[1] Values are means ± SD or percent.
[2] 1 kcal = 4.184 kJ.

group. Continued consumption of the fortified beverage during the next 6 mo did not show any further change in Hb; however, in the nonfortified beverage group, Hb slightly decreased from 123.8 g/L to 123.0 g/L ($P = 0.06$).

Similar to Hb, sFt concentration at baseline did not significantly differ between the 2 groups (Table 4). After 6 mo, sFt concentration increased significantly in both groups; however, the increase was higher in the fortified beverage group compared with the nonfortified beverage group ($P < 0.01$). Furthermore, sFt concentration increased between the 6- and 12-mo assessment in the fortified beverage group ($P < 0.01$), whereas no further increase was observed in the nonfortified beverage group.

TABLE 3 Effect of fortified powered beverage consumption on anthropometric indices among adolescent girls[1]

Indices	Fortified beverage		Unfortified beverage		P^2
Weight, *kg*	*n*		*n*		
Baseline	482	28.19 ± 5.92	507	28.24 ± 5.57	
Follow-up	410	30.20 ± 6.30	433	30.00 ± 5.75	
Change	410	+2.09 ± 1.21	433	+1.62 ± 1.17	0.001
P^3		0.001		0.001	
Height, *cm*					
Baseline	482	137.22 ± 8.65	507	137.40 ± 8.15	
Follow-up	408	139.41 ± 8.13	429	139.45 ± 7.83	
Change	408	+2.18 ± 1.09	429	+2.04 ± 1.11	0.104
P^3		0.001		0.001	
MUAC, *mm*					
Baseline	482	181.23 ± 18.97	507	181.22 ± 18.43	
Follow-up	413	187.81 ± 20.53	433	187.00 ± 19.31	
Change	413	+6.62 ± 5.15	433	+5.24 ± 4.88	0.001
P^3		0.001		0.001	
BMI, *kg/m^2*					
Baseline	482	14.80 ± 1.63	507	14.81 ± 1.57	
Follow-up	407	15.42 ± 1.81	429	15.27 ± 1.68	
Change	407	+0.61 ± 0.57	429	+0.41 ± 0.56	0.001
P^3		0.001		0.001	

[1] Values are means ± SD.
[2] Between group difference; General Linear Model controlling for baseline values and age.
[3] Within group paired *t* test.

Prevalence of anemia, iron deficiency, and IDA. After the 6-mo intervention phase, the prevalence of anemia in the fortified beverage group decreased from 32.4 to 19.1% ($P < 0.01$), with no significant change during the second 6 mo. In the nonfortified beverage group, prevalence of anemia after 6 mo (32.1%) did not differ from that of the baseline (30.4%). Furthermore, the prevalence of anemia in this group continued to increase to 36.5% at the 12-mo follow-up assessment, which was higher than the baseline prevalence ($P = 0.02$).

After 6 mo, the prevalence of iron deficiency (defined as sFt <12 μg/L) decreased in both groups. In the fortified beverage group, iron deficiency decreased from 30.5 to 6.6% ($P < 0.01$). In the nonfortified beverage group, iron deficiency was reduced from 32.9 to 27.4% ($P < 0.01$). The reduction was greater in the fortified (22.9%) than in the nonfortified beverage group (6.6%) ($P < 0.01$). No further change was observed in the nonfortified beverage group between 6 and 12 mo. However, in the fortified beverage group, the prevalence of iron deficiency dropped further from 6.6 to 3.2% ($P < 0.01$).

After the 6-mo intervention phase, the prevalence of IDA (combination of anemia and iron deficiency) in the fortified beverage group decreased from 13.1 to 1.5% ($P < 0.01$), with no further change during the following 6 mo. In contrast, in the nonfortified beverage group, prevalence of IDA at 6 mo (14.0%) did not differ from the baseline (13.8%). No further change was observed during the following 6 mo. After 6 mo of the supplementation, the adolescent girls in the nonfortified beverage group were 2 times more likely to be anemic, 5 times more likely to be iron deficient, and 11 times more likely to suffer from IDA (**Table 5**).

Effect of the fortified beverage on anemia treatment and prevention. Among the adolescent girls who were anemic at baseline, the percentage who remained anemic after 6 mo of intervention was 12.2% in the fortified beverage group and 20.5% in the nonfortified beverage group. Therefore, the likelihood of remaining anemic was lower in the fortified beverage group than in the nonfortified beverage group (OR = 0.29, 95% CI = 0.18–0.49; $P < 0.01$).

Among the nonanemic girls at baseline, anemia developed in 6.8% of the girls in the fortified beverage group compared with 11.6% of the girls in the nonfortified beverage group after 6 mo of the intervention. Therefore, the odds of becoming anemic were lower in the fortified beverage group compared with the nonfortified beverage group (OR = 0.56, 95% CI = 0.36–0.88; $P < 0.01$).

Vitamin A and zinc status. Baseline mean serum retinol and zinc concentrations did not differ between the groups and a similar proportion of girls in the 2 groups had low serum retinol (serum retinol <0.70 μmol/L) and low serum zinc values (serum zinc <10.7 μmol/L) (Table 4). After 6 mo, serum retinol and zinc concentrations increased significantly in both groups. The increased serum retinol was significantly higher in the fortified beverage group compared with the nonfortified beverage group (Table 4). Between 6 and 12 mo, serum retinol and zinc concentrations decreased in both groups ($P < 0.01$).

From baseline to 6 mo, prevalence of low serum retinol dropped from 11.2 to 1.2% in the fortified beverage group ($P < 0.01$) and from 10.3 to 6.3% in the nonfortified beverage group ($P < 0.01$). The change was higher in the fortified beverage group ($P < 0.01$). After 6 mo of supplementation, the nonfortified beverage group was 5 times more likely to have a low serum retinol concentration compared with the girls of the

TABLE 4 Effect of fortified powdered-beverage consumption on Hb, sFt, serum retinol, and serum zinc status among adolescent girls[1]

Indices	Fortified beverage		Nonfortified beverage		P^2
Hb[2], *g/L*	*n*		*n*		
Baseline	482	123.2 ± 11.7	507	124.1 ± 11.3	0.258
Follow-up after 6 *mo*	482	126.8 ± 10.0	507	123.8 ± 10.2	0.001
Change after 6 *mo*	482	+3.6 ± 10.8	507	−0.23 ± 9.6	0.001
P^3		0.0001		0.588	
Follow-up after 12 *mo*	482	126.6 ± 9.6	507	123.0 ± 11.0	0.001
Change between 6–12 *mo*	482	−0.17± 8.8	507	−0.84 ± 10.1	0.268
P^3		0.664		0.061	
sFt, *μg/L*					
Baseline	458	18.00 (10.00, 34.00)	482	17.00 (9.00, 31.00)	0.105
Follow-up after 6 *mo*	477	34.00 (22.00, 53.00)	498	21.00 (10.00, 36.00)	0.001
Change after 6 *mo*	454	+12.5 (2.00, 28.00)	473	+3.00 (−6.00, 15.00)	0.001
P^3		0.001		0.001	
Follow-up after 12 *mo*	444	35.00 (25.00, 53.00)	464	23.00 (11.00, 37.00)	0.001
Change between 6–12 *mo*	442	+2.50 (−11.00, 15.00)	460	0.00 (−8.00, 9.00)	0.234
P^3		0.001		0.145	
Serum retinol, *μmol/L*					
Baseline	460	1.00 (0.81, 1.20)	486	1.02 (0.83, 1.19)	0.947
Follow-up after 6 *mo*	479	1.29 (1.10, 1.52)	499	1.08 (0.89, 1.28)	0.001
Change after 6 *mo*	458	+0.29 (0.72, 0.51)	478	+0.070 (−0.11, 0.24)	0.001
P^3		0.001		0.001	
Follow-up after 12 mo	452	1.25 (1.05, 1.45)	479	0.97 (0.80, 1.18)	0.001
Change between 6–12 mo	450	−0.055 (−0.28, 0.17)	471	−0.090 (−0.27, 0.086)	0.180
P^3		0.001		0.001	
Serum zinc, *μmol/L*					
Baseline	446	9.74 (8.06, 11.65)	470	10.05 (7.99, 11.90)	0.249
Follow-up after 6 mo	452	13.31 (10.40, 16.98)	475	12.88 (10.10, 16.22)	0.303
Change after 6 mo	433	+3.87 (0.65, 7.54)	457	+2.84 (0.026, 6.70)	0.390
P^3		0.001		0.001	
Follow-up after 12 mo	448	9.94 (8.04, 11.92)	475	9.22 (7.75, 11.45)	0.086
Change between 6–12 mo	436	−3.26 (−7.64, −0.34)	460	−3.42 (−7.18, −0.56)	0.714
P^3		0.001		0.001	

[1] Values are means ± SD or medians (25th–75th percentiles).
[2] Between-group independent *t* test.
[3] Within group paired *t* test.

fortified beverage group (Table 5). After 6 mo, the prevalence of low serum zinc dropped from 60.9 to 25.6% in the fortified beverage group ($P < 0.01$) and from 55.3 to 25.9% in the nonfortified beverage group ($P < 0.01$). Between 6 and 12 mo, the serum zinc decreased in both groups (Table 4).

The prevalence of elevated CRP was low (<3%) in both groups at baseline, after 6 mo, and after 12 mo of intervention, and the groups did not differ (data not shown).

TABLE 5 Effect of fortified powdered beverage consumption on anemia, iron deficiency, and IDA among adolescent girls[1]

	OR	95% CI
Anemia (Hb <120 *g/L*)	2.04	1.52–2.74
Iron deficiency (ferritin <12 *μg/L*)	5.38	3.57–8.09
IDA	11.19	5.09–24.61
Vitamin A deficiency (<0.70 *μg/L*)	5.47	2.27–13.22

[1] Values from binary logistic regression adjusted for age. IDA defined as Hb <120 g/L and sFt <12 μg/L.

Discussion

The results from this study showed that regular (6 d/wk) consumption of the multiple-micronutrient-fortified beverage for 6 mo significantly increased Hb concentration and improved iron (in terms of sFt concentration) and vitamin A (in terms of serum retinol concentration) status in adolescent girls. We also observed that the fortified beverage effectively decreased the prevalence of anemia and iron deficiency. Furthermore, consumption of the fortified beverage was effective in preventing further development of anemia and depletion of iron stores. Consumption of the fortified beverage for an additional period of 6 mo did not affect Hb levels. It is possible this lack of further increase in Hb could be related to factors other than the micronutrients present in the test samples (15). However, continued consumption of the fortified beverage significantly increased sFt during the next 6 mo. Thus, consumption of the micronutrient-fortified beverage during adolescence could play a critical role in building iron store, which is important in preventing anemia, particularly during pregnancy (27).

In the group receiving the nonfortified beverage, mean Hb remained low and overall anemia prevalence continued to

increase. This may be due to the increased need of iron for growth not being met. A higher proportion of girls in the nonfortified beverage group developed anemia during the study. The prevalence of iron deficiency and suboptimal vitamin A status was also significantly higher in the girls receiving the nonfortified beverage after 6 mo. Our findings thus are in agreement with previous studies using a similar multiple-micronutrient-fortified beverage that demonstrated significant improvements in iron and vitamin A status in school children and pregnant women (15–19).

Despite the high prevalence of low serum zinc at the start of the study, we did not observe a positive effect of the fortified beverage. The prevalence of low serum zinc decreased significantly and to a similar extent in both study groups within 6 mo. The lack of effect of the fortified beverage on zinc status compared with the nonfortified beverage could be due to the dose of zinc being too low or to a possible interaction between iron and zinc for absorption (28). Indeed, recent studies showed that iron inhibits zinc absorption (29) (and vice versa). However, iron has little effect on zinc absorption when zinc-iron ratios are 1:1, which is similar to the ratio in our study (30). Therefore, the interaction of iron and zinc from the fortified beverage should be further investigated and the dosage of zinc reevaluated.

In this study, girls who consumed the fortified beverage had a higher mean increase in weight, MUAC, and BMI over 6 mo compared with girls in the nonfortified beverage group. These results are consistent with those from the other studies that have used a similar multiple-micronutrient-fortified beverage in school children (15,16,31). In one of the previous reports (16), significantly increased weight and MUAC were obtained after 8 wk of fortified beverage consumption. In the other studies, significantly increased weight was shown after 6 mo (15) and 14 mo of supplementation (31). Similarly, a recent trial in adolescent girls in India reported increased weight gain with the use of iron supplements for a period of 3 mo (32). As speculated by others (15,33), the significantly greater weight and MUAC gains in the girls receiving the fortified beverage could be due to increased appetite. With our study design, however, it was not possible to determine whether these improvements in anthropometric measurements were due to a single vitamin or mineral (like iron) or to a combination of vitamins and minerals. Unlike that of the school children study in Tanzania (15), consumption of the fortified beverage did not significantly affect height gain during the duration of the trial. This inconsistency could be due to differences in age and gender. The mean age for the Tanzanian school children was 10 y (the subjects included boys and girls). In contrast, the mean age for the adolescent girls in this study was 12 y and boys were not included.

The results of the current study should be applied in the context of the following study limitation. Although strongly related to anemia, parasitic infection was not determined at baseline. Because BRAC does not provide deworming treatment for NFPE school students as a regular intervention, we opted not to test for it to create a situation that would be similar to that found in the current programmatic setting.

The advantages of using a fortified beverage in a school-based setting as reported here include: *1*) the ability to address more than 1 micronutrient deficiency simultaneously; *2*) the ability to provide the beverage isolated from meals containing inhibitors of mineral absorption such as phytate; and *3*) the likelihood of higher adherence due to the supervision during beverage preparation and consumption and due to the pleasant fruit flavor of the beverage. We demonstrated that it is efficacious to provide a multiple-micronutrient-fortified beverage in a school-based setting to improve anemia and other vitamin and mineral status of vulnerable adolescent girls as well as to lead to improvements in anthropometric measurements. The fortified beverage studied not only treated but also prevented anemia from developing. The findings in this study may have further implication in terms of pregnancy outcomes, because there is a positive interaction between maternal BMI at conception and birth weight (27). Indeed, women with low BMI who fail to gain adequate weight during pregnancy are at a higher risk of having a low birth weight babies.

The results of this study could be generalized to the rest of the rural areas in the country, because the study population was typical for rural Bangladesh. Future studies should investigate a number of operational issues, including the optimum duration of the use of the fortified beverage and feasibility of distributing the product through different channels such as schools (as used in this study), adolescent clubs, and also through the private sector, using a social marketing approach. As a public health strategy, this intervention has the potential to improve adolescents' overall nutritional status, which would in turn improve pregnancy outcomes.

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