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Community-Level Micronutrient Fortification of School Lunch Meals Improved Vitamin A, Folate, and Iron Status of Schoolchildren in Himalayan Villages of India[1–3]

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Abstract

Anemia and micronutrient deficiencies are common among Indian schoolchildren. We assessed the effectiveness of micronutrient fortification of meals cooked and fortified at school on anemia and micronutrient status of schoolchildren in Himalayan villages of India. In this placebo-controlled, cluster-randomized study, 499 schoolchildren (6–10 y) received either multiple micronutrients (treatment group) or placebo (control group) as part of school meals (6 d/wk) for 8 mo. Both groups were dewormed at the beginning of the study. The micronutrient premix provided 10 mg iron, 375 μg vitamin A, 4.2 mg zinc, 225 μg folic acid, and 1.35 μg vitamin B-12 for each child per day (~75% recommended dietary allowance). Blood samples drawn before and after the intervention were analyzed for hemoglobin, ferritin, retinol, zinc, folate, and vitamin B-12. Baseline prevalence of anemia (37%), iron deficiency anemia (10%), low serum ferritin (24%), retinol (56%), zinc (74%), folate (68%), and vitamin B-12 (17%) did not differ between groups. Postintervention, fewer in the treatment group had lower serum retinol [odds ratio (OR) (95% CI): 0.57 (0.33–0.97)] and folate [OR (95% CI): 0.47 (0.26–0.84)] than the control group. The serum vitamin B-12 concentration decreased in both groups, but the magnitude of change was less in the treatment than in the control group ($P < 0.05$). Total body iron (TBI) increased in both groups; however, the change was greater in the treatment than in the control group ($P < 0.05$). Micronutrient fortification of school meals by trained school personnel was effective in improving vitamin A, folate, and TBI status while also reducing the magnitude of a decrease in vitamin B-12 status. J. Nutr. doi: 10.3945/jn.109.114751.

Introduction

Micronutrient deficiencies are a major public health problem in developing countries (1). The WHO estimates that ~2 billion people worldwide are affected by such deficiencies, of whom 85% reside in resource-poor countries (1). Although a modest amount of information exists on the nutritional status of schoolchildren, studies from several developing countries have demonstrated a high prevalence of micronutrient deficiencies in this age group (2–4).

Undernutrition in schoolchildren can lead to anemia (2) and negatively affect growth (5), motor and cognitive development (6), and immune function (7), all of which may adversely affect academic performance (8).

Low dietary intake and poor absorption (and/or bioavailability) are major causes of micronutrient deficiencies (1). However, the timely provision of micronutrients through nutritional interventions (sometimes accompanied by other health improvement measures such as deworming) often reverses such deficiencies and their associated developmental impairments (9,10). Conventional approaches to micronutrient interventions, which usually involve providing nutritional supplements or centrally processed fortified foods, have not been sustainable and have had less impact in rural areas due to high implementation costs, distribution constraints, low coverage, and noncompliance with supplement intake (11,12). Programs for addressing micronutrient malnutrition in India have focused mainly on preschool children and women of reproductive age.

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With the exceptions of deworming, iodization of salt, and fortification of commercially available foods, no clear policies exist on addressing micronutrient malnutrition among schoolchildren in most states of India. Even in places where these policies exist, implementation and coverage of these programs is usually poor in rural areas (13). All states have ongoing school feeding programs, which, however, were mainly designed to address protein-energy malnutrition and not micronutrient deficiencies. Therefore, there is a need for a simple and easy-to-transfer strategy that can be used to address micronutrient deficiencies in rural settings.

Efficacy trials of fortification interventions that involve the addition of a micronutrient premix to cooked food just before consumption have produced promising results in preschool and schoolchildren in India and other countries (14–16). However, effectiveness trials of such interventions have been successfully completed only in preschool children (17). There is still limited evidence among schoolchildren of the effectiveness of micronutrient fortification wherein the micronutrient premix is added to cooked meals at the school just before consumption.

Our purpose in this study was to determine the effectiveness of a school-level fortification of cooked lunch meals with a locally manufactured micronutrient premix (whereby the cooking and fortification are done at the school) on anemia and micronutrient status of schoolchildren in Himalayan villages of India.

Methods

Study area. The study took place in Tehri Garhwal district, a hilly agrarian community located in the mid Himalayan ranges of Uttarakhand State, ~250 km northeast of Delhi, India (**Supplemental Fig. 1**). The local population engages mainly in subsistence farming. This district is not malaria endemic and has 3 seasons: summer (April–June), rainy (July–September), and winter (October–February). However, there are 2 main agricultural seasons in the year: lean season (July–September), during which most households cultivate their lands, and postharvest season (April–June), the period after the main harvest. Food consumption patterns vary from extreme deprivation in the lean season (July–September) to high intakes of a greater variety of foods during the postharvest period (April–June). Thus, there is always the potential for seasonal changes in nutritional status of the population between these 2 periods each year. The main staple foods are wheat (prepared mainly as "chapati/roti") and rice, which are often eaten with "dhal" (sauce prepared using pulse/lentils) and/or vegetables. Being a predominantly Hindu community, consumption of animal products is limited, although consumption of buffalo milk, yogurt, and eggs is widespread.

At baseline, there were 1350 public primary schools with 71,423 children enrolled in the district. All schools had an ongoing lunch program in which children received meals cooked at school 6 d/wk. The program had a standardized menu that consisted mainly of rice and dhal or vegetables, aimed at providing each child with at least 1884 kJ/d and 12 g protein/d. No micronutrient intervention or deworming program was ongoing among schoolchildren in the district during the study period.

Study design. This was a single-blind, placebo-controlled, cluster-randomized study in which schoolchildren received either a micronutrient premix or placebo as part of their daily school meals for 8 mo (1 school year). Cluster randomization, wherein clusters of schools were randomly allocated to a type of treatment, was used rather than randomization at the individual level, because there was a substantial risk of confusing the micronutrient and the placebo premix if both were administered in the same school. The study took place from August 2007 to April 2008 and was interrupted by a 2-wk school vacation in December. In addition, there were 33 public holidays and 29 Sundays during which there was no school, so fortified meals could not be provided.

Sampling procedures. The sample comprised 6- to 10-y-old children in grades 1 to 4 in public primary schools. The sample size was calculated using a power of 0.8, significance level of 0.05 with a 2-sided CI, design effect of 1.58, and an assumed change in hemoglobin of 3.93 ± 11.2 g/L (mean ± SD) in the treatment compared with the control group after the intervention. The assumed change in hemoglobin was taken from results of a similar micronutrient intervention in Tamil Nadu, India (16). After an upward adjustment of 20% to account for losses to follow-up, our estimate of 486 was rounded up to a total of 500 children required for the study.

Twenty schools were selected for the study from across all the 9 blocks (subdistricts) in Tehri Garhwal district using a stratified random sampling procedure. A list of all public primary schools in the district was obtained from the Directorate of Primary Education in Uttarakhand. Schools were stratified by block and the names of the schools in each stratum were written on identical pieces of paper, which were folded and shuffled together. Two schools were picked randomly from each stratum to participate. The same procedure was used to assign 1 of the selected schools per strata to micronutrient premix (treatment) and the other to placebo premix (control). However, for 2 blocks (Bhilangana and Jounpur), the number of schools per block was about twice that of each of the other blocks. Therefore, 3 schools were selected from each of these blocks to allow for their proper representation in the sample. Two of the selected schools in Jounpur block and 1 school in Bhilangana block were assigned randomly to the treatment group. The others were assigned to the control group. Two schools were replaced using the same sampling procedure, because the paths to these schools were considered dangerous due to risk of wild animal attack. Within each selected school, names of all children in grades 1 to 4 between the ages of 6 and 10 y were obtained and a similar random sampling procedure was used to select 25 children for anthropometric, biochemical, and parasitological assessments.

All students in each school received lunch meals fortified with the assigned premix regardless of their participation in the study assessments. All schools in the district (regardless of their participation in the study) received the micronutrient premix after the baseline assessments with the exception of the control schools, which received the micronutrient premix after the trial.

Ethical considerations and exclusion criteria. Written informed consent was obtained from the district education authorities, school headmasters and cooks, community leaders, and the parents or legal guardian of all the children in each school. Separate consent was also obtained from parents of children selected for the anthropometric, biochemical, and parasitological assessments before their inclusion. Verbal consent of children aged ≥7 y was obtained before the assessments. The protocol was approved by the Ethics Committee of the office of the Chief Medical Officer in Uttarakhand and the Institutional Review Board of Tufts University (Institutional Review Board study no. 8208). Conditions for exclusion were severe anemia (hemoglobin <70 g/L) (2), sickle cell disease, HIV, and tuberculosis. However, none of the children suffered from any of these conditions.

Premix composition and school meal fortification procedures. The micronutrient premix consisted of vitamins and minerals carried in dextrose anhydrous powder and was composed such that every 0.25 g of the composite powder would provide 10 mg iron (NaFeEDTA), 375 μg vitamin A (retinyl acetate), 4.2 mg zinc (zinc gluconate), 225 μg folic acid, 90 μg iodine (potassium iodide), 26.25 mg vitamin C (ascorbic acid), 0.68 mg thiamine (thiamine mononitrate), 0.68 mg riboflavin (riboflavin 5-phosphate sodium), 9 mg niacin (nicotinamide), 1.35 μg vitamin B-12 (1% on manitol, as carrier), 0.75 mg vitamin B-6 (pyridoxine hydrocholoride), 3.75 μg vitamin D (ergocalciferol), 5.25 mg vitamin E (all-rac-α-tocopherol), and 0.45 mg copper [$CuSO_4.(H_2O)_5$] to each child. The premix provided ~75% of the recommended dietary allowance of the micronutrients for each child (Indian Council of Medical Research). The placebo was dextrose anhydrous powder with no added micronutrients.

The contents of the premix were decided by the World Food Program (Delhi, India) with the input of the lead author (A.K.O.) and were

produced by Nicholas Piramal India. The premix was provided as 500-g packs accompanied by 2 sets of standardized plastic spoons that measured 0.5 and 2.5 g of premix (the respective serving size for 2 and 10 schoolchildren). The manufacturer analyzed the micronutrient content of each premix before distribution. To facilitate blinding, both the micronutrient and placebo premix were carried in identical packets, which had no information easily identifying the contents. Instead, the micronutrient and placebo premix were labeled *Sampoorna* 1 and *Sampoorna* 2, respectively. Only the study coordinator and senior World Food Program officials in Delhi had access to the codes to the premix assignment. Schools were provided with a monthly supply of premix and dark brown plastic containers for storage after opening the packet to prevent breakdown of light-sensitive micronutrients.

Throughout the study period, school cooks prepared the school meals without interference by the study team. The amount of meals prepared was based on the total number of students present at school on each day. After meal preparation, the cook measured the appropriate number of spoons of premix (based on number of students present), which was mixed thoroughly with a small quantity of water and then added to the food at room temperature.

Training of school cooks and teachers. The cook and 1 teacher from each school participated in a half-day training session on standard procedures for fortifying school meals (i.e. dosage and addition of premix to meals), proper handling and storage of the premix, and maintenance of monitoring registers before the intervention. Headmasters, district directors of basic education, and district directors of health received a half-day orientation on the study protocol.

Intervention monitoring. Each school's headmaster was given a notebook to document the school attendance of study children, number of days that meals were cooked and fortified, and amount of premix used. Study enumerators paid bi-weekly visits to study schools to assess these registers to ensure correct procedures were followed. On such visits, enumerators observed premix addition to meals, provided the schools with additional premix if needed, and collected the packaging material for the used premix.

A substudy was conducted in 4 of the treatment schools (selected randomly using the same sampling procedure) to assess micronutrient retention in the premix after 20 d of storage under school conditions and whether fortification of school meals improved its micronutrient content. Enumerators visited these schools 20 d after the first date of premix distribution to collect samples of the micronutrient premix, unfortified cooked food (plain "dhal"), and micronutrient-fortified cooked food (fortified "dhal") using airtight plastic containers. These samples were transported to Choksi Laboratories within 3 d of collection for analysis using the standards of the American Association of Cereal Chemists. The results for the micronutrients of greatest interest in this study (iron, zinc, vitamin A, folate, and vitamin B-12) are reported below.

Estimated compliance. At the end of the intervention, compliance with premix intake by the study participants was assessed as the ratio of total days of school attendance to the number of days a school meal was cooked during the study period. Our previous acceptability trial indicated that almost all schoolchildren in this district consumed school meals (98.9%), ~80% finished all the food served to them with no leftovers, and the micronutrient premix was highly liked (18).

Data collection techniques. At baseline, the birth date and sex of children were abstracted from school records; age (y) was determined from birth date. Before and after the intervention, enumerators conducted interviews with primary caretakers to obtain information on the study child's morbidity related to occurrence of diarrhea, fever, cough, runny nose, and vomiting in the 2 wk preceding the survey. The questionnaires were tested prior to the survey and all interviews were conducted in Garhwali (the local dialect).

Anthropometry. Weight and height of the children were measured according to WHO procedures (19). At each survey, duplicate measurements of weight and height were taken and the mean value was determined. Weight was measured in a school uniform to the nearest 0.1 kg using a portable electronic weighing scale (Seca) and standing height was measured to the nearest 0.1 cm with a locally made height rod.

Collection and processing of blood and stool samples. About 5 mL of venous blood (nonfasting) was collected from each child into trace metal-free yellow screw-top test tubes. Within 5 min after venipuncture, a small amount of blood was analyzed for hemoglobin using a portable HemoCue Hb 201$^+$ analyzer (Ängelholm). Blood spots were made on filter paper cards for laboratory determination of serum retinol. The cards and the remaining blood sample were wrapped in aluminum foil and transported to the field office where the blood was centrifuged at $1238 \times g$ for 15 min on the same day to obtain serum samples. Sera were aliquotted and stored in a freezer until transported in a cold box with the filter papers to the Molecular Diagnostic Laboratory in Lucknow for analysis. Serum ferritin and C-reactive protein (CRP)[11] were determined by ELISA (Biocheck). Serum soluble transferrin receptor (sTfR) was assessed by ELISA (Biovendor). Serum retinol was determined by HPLC (20). Serum zinc was measured by atomic absorption spectrophotometer (model 680 AA, Shimadzu). Serum folate and vitamin B-12 were determined by radioimmunoassay (Bio-Rad Laboratories).

For assessment of intestinal helminth infections, children were given plastic containers to collect their stool samples. Some children provided stool samples on the same day, whereas others took the containers home and returned them the next day. Fecal samples were prepared for microscopic examination using the guidelines of National Committee for Clinical Laboratory Standards (21). Fecal smears in saline and iodine solution were examined for ova and/or cysts of worms such as hookworm, *Ascaris lumbricoides*, *Trichuris trichiura*, and *Taenia saginata* under 40× microscopy. All children (both treatment and control groups) who were involved in the anthropometric, biochemical, and parasitological assessments (n = 25/school) were given sweets and/or fruit juice after these assessments and were administered an oral dose of 500 mg albendazole before beginning the fortification.

Altitude. The altitude of each village was measured at the school with a handheld GPS (model eTrex Vista HCx, Garmin). The altitudes of the schools involved in the study ranged from 889 ± 3 m to 1749 ± 3 m above sea level.

Anthropometric and biochemical reference values. Stunting, underweight, and wasting were defined as height-for-age Z score (HAZ) < -2, weight-for-age Z score (WAZ) < -2 and weight-for-height Z score (WHZ) < -2, respectively, using CDC/NCHS reference values (22). CRP was considered elevated at >10 mg/L (23). Anemia was defined as hemoglobin <115 g/L, after accounting for the effect of altitude on hemoglobin using appropriate WHO reference values (24). Low storage iron was defined as serum ferritin <15 μg/L (24) and children with both anemia and serum ferritin <15 μg/L were considered to have iron deficiency anemia (24). Elevated sTfR considered indicative of tissue iron deficiency was >8.5 mg/L (25). Definitions for low serum concentrations of retinol, zinc, folate, and vitamin B-12 were <1.05 μmol/L (1), <9.9 μmol/L (26), <13.6nmol/L (3), and <300 pmol/L, respectively (27).

Data management and statistical analysis. Data were entered with CSPro version 3.3 (US Census Bureau). WAZ, WHZ, and HAZ were computed using Epi-Info version 3.5 (CDC). Total body iron (TBI) was estimated from the formula: TBI in mg/kg body weight = − (log (sTfR: ferritin ratio) – 2.8229)/0.1207 (28). The values obtained were further converted to μmol/kg body weight.

Proportions, mean ± SD, or median [interquartile range (IQR)] were used for descriptive analysis. At baseline, differences in characteristics between the treatment and control groups were compared using a chi-square test for proportions, independent samples t test for means, and

[11] Abbreviations used: CRP, C-reactive protein; HAZ, height-for-age Z score; IQR, interquartile range; OR, odds ratio; sTfR, soluble transferrin receptor; TBI, total body iron; WAZ, weight-for-age Z score; WHZ, weight-for-height Z score.

Mann-Whitney U test for medians. Bivariate comparison of postintervention hemoglobin, TBI, and other serum micronutrient indicators to respective baseline values within each group was done using paired *t* test, Wilcoxon's test, and Pearson's correlations coefficients. Proportions were compared within groups using McNemar's test. Furthermore, multivariate analysis using repeated-measures ANCOVA (using proc mixed procedure in SAS) was used to compare postintervention-adjusted mean hemoglobin, TBI, and the other serum micronutrient indicators to respective baseline values. Between-group comparisons of these indicators were also done using ANCOVA. Variables exhibiting nonnormal distribution (serum ferritin, folate, and vitamin B-12) were first log-transformed to approximate normality before inclusion in the regression. Results from the ANCOVA are presented as either adjusted means ± SEM or adjusted geometric means (95% CI). In addition, multiple linear regressions were used to compare the postintervention hemoglobin, TBI, and other serum micronutrient indicators of the 2 groups while controlling for their respective baseline values and other covariates.

Logistic regression for binary repeated-measures (using generalized estimation equations, proc genmod in SAS) was used to study the effect of the intervention on prevalence of anemia and low serum ferritin, retinol, zinc, folate, and vitamin B-12 concentrations. Covariates in the ANCOVA, linear, and logistic regression models were child's age, gender, and CRP. No regression was conducted for prevalence of iron deficiency anemia because of the relatively small number of children with this condition. All regression models were conducted with and without dummy variables identifying each district and school. However, the results did not differ between the 2 models and therefore we present only the models without these 2 dummy variables. All analyses were performed using SPSS version 16.0 and SAS version 9.3 (SAS Institute). Statistical significance (α) was set at $P < 0.05$.

Results

Characteristics of study schools. Treatment and control schools had similar characteristics at baseline (**Table 1**). Schools were opened for 165 and 166 d (treatment and control, respectively) during the intervention period. Lunch meals were served on all days with the exception of 1 control school, where meals were not prepared for 4 d due to a shortage of rice.

TABLE 1 Baseline characteristics of study schools by treatment group and overall[1]

	Micronutrient	Placebo	Total
n	10	10	20
Classrooms, *n*	2.0	2.0	2.0
Grades, *n*	5.0	5.0	5.0
Children enrolled, *n*			
Boys	31.5 (21.3)	40.5 (14.3)	39.0 (15.5)
Girls	36.5 (18.0)	43.0 (16.0)	38.5 (14.3)
Attendance on baseline survey day, *n*			
Boys	28.0 (15.0)	31.0 (10.5)	30.5 (11.8)
Girls	32.0 (12.8)	30.0 (8.8)	31.0 (11.3)
School toilet, %			
Pit/latrine	60	60	60
No facility/bush	40	40	40
Place for cooking meal, %			
Separate room	90	90	90
Classroom	10	10	10
Place children eat school meals, %			
Corridor	90	100	95
Classroom	10	0	5
Storage place for meal supply, %			
Separate room	30	50	40
Headmaster office	60	30	45
Classroom	10	20	15

[1] Values are medians (IQR) or percent.

TABLE 2 Iron, vitamin A, zinc, folic acid, and vitamin B-12 content of micronutrient premix and of school meals[1]

	Micronutrient premix		Food sample[4]	
	Factory[2]	School[3]	Nonfortified	Micronutrient fortified
n	1	4	4	4
Iron, *mg*	10.1 (0.0)	10.1 (0.6)	4.1 (4.0)	12.1 (4.9)*
Vitamin A, μg	410.0 (0.0)	398.5 (16.0)	6.7 (24.1)	381.6 (21.3)*
Zinc, *mg*	4.24 (0.0)	4.2 (0.1)	0.6 (0.2)	4.5 (0.7)*
Folic acid, μg	223.8 (0.0)	235.5 (3.3)	0.0 (0.0)	224.5 (6.6)*
Vitamin B-12, μg	1.4 (0.0)	1.4 (0.0)	0.0 (0.0)	1.40 (0.0)*

[1] All values are median (IQR). *Different from nonfortified food ($P < 0.05$; Mann Whitney U test).
[2] Micronutrient content before premix left the factory.
[3] Micronutrient content after 20 d of storage of micronutrient sample in 4 treatment schools.
[4] Micronutrient content per 150 g of food sample taken from 4 treatment schools, before (non fortified) and after (micronutrient fortified) micronutrient fortification.

Retention of micronutrients in stored premix. The iron, zinc, vitamin A, folic acid, and vitamin B-12 contents of the premix did not change significantly after 20 d of storage at the school (**Table 2**). The micronutrient content of the nonfortified school meals was very low. The addition of the micronutrient premix significantly improved the iron, zinc, vitamin A, folic acid, and vitamin B-12 contents of the meals (Table 2).

Estimated compliance. Overall, estimated compliance was 91.2%, with no difference between the treatment (90.7%) and control (91.7%) schools. Absence from school was the only reason for noncompliance.

Children's characteristics. At baseline, anthropometric, biochemical, parasitological, and morbidity data were obtained from 499 children (treatment = 249 and control = 250). After the intervention, 44 children (9.0%) were lost to follow-up; thus, 455 children completed the trial (treatment = 220 and control = 235). The number of children who dropped out did not differ between the treatment (n = 29) and control (n = 15) groups (**Supplemental Fig. 2**) and there were no differences in baseline characteristics of the children who dropped out and those who completed the trial (data not shown). More than 87% (n = 437) and 99.6% (n = 453) of the children returned fecal samples at baseline and final surveys, respectively.

Children in the 2 groups did not differ in age, gender, anthropometric indices, intestinal parasite infection, recent morbidities, and circulating concentrations of hemoglobin, ferritin, retinol, zinc, folate, and vitamin B-12 at baseline (**Table 3**). The treatment group had lower sTfR than the control group ($P < 0.05$). However, TBI did not differ between the 2 groups (Table 3). The prevalence of children with anemia, iron deficiency anemia, elevated sTfR, and low serum concentrations of ferritin, retinol, zinc, folate, and vitamin B-12 did not differ between the 2 groups (Table 3). Few children had elevated CRP and neither this nor the serum concentration of CRP differed between the 2 groups (Table 3).

Intake of vitamin/mineral supplement and deworming tablets by children. Only 8.9% of the children were given

TABLE 3 Baseline characteristics of the children receiving micronutrient or placebo fortified food and the overall sample[1]

	Micronutrient	Placebo	Total
n	249	250	499
Demographics			
Age, *y*	7.0 (1.0)	7.0 (1.0)	7.0 (1.0)
Sex (girls), %	52.6	51.6	52.1
Anthropometry			
WAZ	−2.19 ± 0.77	−2.10 ± 0.77	−2.14 ± 0.77
HAZ	−2.20 ± 1.15	−2.13 ± 1.15	−2.17 ± 1.15
WHZ	−1.25 ± 0.79	−1.16 ± 0.72	−1.20 ± 0.76
Underweight (WAZ < −2), %	62.2	59.6	60.9
Stunting (HAZ < −2), %	58.2	54.0	56.1
Wasting (WHZ < −2), %	13.3	11.2	12.2
Parasite infection,[2] %			
Ascaris lumbricoides	8.5	10.3	9.4
Hookworm	7.6	7.5	7.6
Trichuris trichiura	1.3	1.9	1.6
Taenia saginata	2.2	0.9	1.6
Morbidity, %			
Diarrhea	12.9	14.8	13.8
Fever	54.6	39.6	47.1
Couth	23.3	16.8	20.0
Runny nose	15.7	18.0	16.8
Vomiting	8.0	9.2	8.6
Biochemical indicators			
Hemoglobin, *g/L*	121.4 ± 13.1	121.5 ± 12.3	121.0 ± 12.7
<115 g/L,[3] %	40.6	32.8	36.7
Serum ferritin, *μg/L*	38.7 (40.6)	42.2 (46.7)	39.7 (43.9)
<15 μg/L,[3] %	24.9	23.2	24.1
Iron deficiency anemia, %	10.8	9.6	10.2
Serum sTfR, *mg/L*	2.0 ± 0.8*	2.2 ± 1.1	2.1 ± 1.0
>8.5 mg/L, %	0.0	0.4	0.2
TBI, *μmol/kg*	148.6 ± 73.4	148.6 ± 77.0	148.6 ± 75.2
Serum retinol, *μmol/L*	1.03 ± 0.41	1.09 ± 0.37	1.06 ± 0.39
<1.05 μmol/L, %	59.0	53.2	56.1
Serum zinc, *μmol/L*	9.9 ± 2.7	9.7 ± 2.6	9.8 ± 2.7
<9.9 μmol/L, %	56.6	57.6	57.1
Serum folate, *nmol/L*	6.7 (12.1)	9.2 (15.1)	7.8 (13.8)
<13.6 nmol/L, %	72.7	63.2	67.9
Serum vitamin B-12, *pmol/L*	459.4 (332.1)	463.9 (361.8)	463.1 (350.2)
<300 pmol/L, %	18.1	16.8	17.4
Serum CRP, *mg/L*	1.2 (0.6)	1.2 (1.0)	1.2 (0.9)
>10 mg/L, %	2.8	2.8	2.8

[1] Values are mean ± SD, percent, or median (IQR). *Different from placebo, $P < 0.05$.
[2] $n = 437$.
[3] Anemia was defined as hemoglobin <115 g/L; and iron deficiency anemia as: hemoglobin <115 g/L and serum ferritin <15 μg/L.

deworming drugs and 15.5% were given vitamin or mineral supplements by their caretakers in the 6 mo prior to the baseline survey. At the postintervention survey, only 2.0% had taken deworming tablets and 5.9% of children had taken micronutrient supplements (besides what was provided by the intervention) in the 6 mo prior to the survey. The groups did not differ in intakes of deworming drugs or micronutrient supplements (data not shown).

Vitamin A, zinc, folate, and vitamin B-12 status. The serum retinol concentration increased in the treatment but not in the control group after the intervention ($P < 0.05$) (**Table 4**). The serum zinc concentration increased significantly in both groups. The geometric mean serum folate concentration increased in the treatment group but not in the control group ($P < 0.05$). The geometric mean serum vitamin B-12 concentration decreased in both groups, with a smaller decrease in the treatment group than the control group ($P < 0.05$) (Table 4). Even after adjusting for their respective baseline concentrations, age, gender, and CRP using multiple linear regression, the treatment group had higher postintervention serum retinol, folate, and vitamin B-12 concentrations ($P < 0.05$) than the control group (data not shown).

The proportion of children with low serum concentrations of retinol and zinc decreased in both groups ($P < 0.05$). At the end of the intervention, the difference in the proportion of children with low serum retinol between the treatment and control groups was marginally significant ($P = 0.06$) (Table 4). The prevalence of children with low serum folate concentrations decreased in the treatment group ($P < 0.05$) but not in the control group (Table 4). In both groups, the proportion of children with low serum vitamin B-12 concentrations was higher at the end of the intervention compared with baseline ($P < 0.05$). However, the increase in prevalence was less in the treatment group than in controls (Table 4).

Effect of study intervention on anemia and iron status. Hemoglobin concentrations did not change in either group (**Table 5**). The geometric mean serum ferritin concentration increased significantly in the treatment but not in the control group (Table 5). The sTfR concentration decreased in both groups ($P < 0.05$) (Table 5). No child had elevated sTfR at the end of the intervention. TBI increased in both groups, with a greater change in the treatment compared with control group ($P < 0.05$) (Table 5). After controlling for baseline values and potential covariates using multiple linear regression, the postintervention TBI was higher in the treatment compared with the control group ($P < 0.05$) (data not shown).

The prevalence of anemia decreased in the treatment group but not in the control group ($P < 0.05$) (Table 5). The proportion of children with iron deficiency anemia and low serum ferritin decreased in both groups ($P < 0.05$) (Table 5).

Multivariate logistic regression. In the repeated-measures logistic regression analysis, only the models involving low serum retinol, folate, and vitamin B-12 concentrations as dependent variables had a significant treatment × time interaction, indicating that the change in log odds of children having low serum retinol, folate, and vitamin B-12 was different for the treatment and control groups (**Table 6**). At the end of the intervention, children in the treatment group were 43% less likely to have low serum retinol [odds ratio (OR) (95% CI): 0.57 (0.33–0.97)], 53% less likely to have low serum folate [OR (95% CI): 0.47 (0.26–0.84)], and 59% less likely to have low serum vitamin B-12 [OR (95% CI): 0.41 (0.22–0.86)] compared with children in the control group.

Changes in inflammation and infections. The median (IQR) CRP concentration decreased between baseline [1.2 (0.9)] and 8 mo of intervention [0.3 (1.3)] ($P < 0.001$). However, the prevalence of children with elevated CRP did not change (2.6% at baseline vs. 3.3% at 8 mo). The median CRP concentration and prevalence of children with elevated CRP did not differ between the 2 groups.

Among children who submitted stool samples at both baseline and postintervention, the prevalence of intestinal parasites (defined as absence or presence of any kind of worm)

TABLE 4 Serum concentrations and prevalence of low serum retinol, zinc, folate and vitamin B-12 of the children receiving micronutrient or placebo fortified food before and after 8 mo of treatment[1]

	Micronutrient premix, *n* = 220		Placebo, *n* = 235	
	Baseline	8 mo	Baseline	8 mo
Retinol, *μmol/L*	1.03 ± 0.03	1.29 ± 0.03*	1.10 ± 0.02	1.19 ± 0.03
<1.05 μmol/L, %	58.2	29.5*	54.0	38.3*
Zinc, *μmol/L*	9.9 ± 0.2	10.7 ± 0.2*	9.7 ± 0.2	10.8 ± 0.2*
<9.9 μmol/L, %	55.0	41.4*	57.9	39.6*
Folate, *nmol/L*	6.4 (5.3–7.7)	9.5 (8.3–10.8)*#	7.9 (6.8– 9.2)	6.3 (5.2–7.6)
<13.6 nmol/L, %	73.2	57.7*	64.3	66.0
Vitamin B-12, *pmol/L*	454.6 (395.8–522.2)	277.8 (261.2–295.4)*#	477.7 (454.8–511.9)	222.9 (207.5–239.5)*
<300 pmol/L, %	19.5	53.6*#	17.0	70.2*

[1] Values are adjusted mean ± SEM, adjusted geometric mean (95% CI) or percent. Means and geometric means are adjusted for age, sex and CRP. *Different from baseline, $P < 0.05$; #Different from corresponding placebo, $P < 0.05$.

increased between baseline and postintervention in the treatment (19.9% vs. 32.7%) and control (21.4% vs. 36.3%) groups ($P < 0.05$). Most of the children infected with worms at the end of the intervention were uninfected at baseline and a low percentage of children in each of the treatment and control groups were infected with intestinal parasites at baseline but tested negative for parasites at the end of the intervention (**Table 7**). The type of intestinal worm infections also differed between the baseline and postintervention surveys. Overall, the types of worm infestation at baseline were: *A. lumbricoides* (10.1%), hookworms (7.8%), *Trichuris trichiuira* (1.3%), and *Taenia* (1.5%), with none of the children having more than 1 type of intestinal worm. At postintervention, children with mono-infections had *A. lumbricoides* (3.5%), hookworms (2.0%), *E. histolytica* (17.4%), *T. trichiura* (1.0%), *Taenia* (0.8%), *Giardia* (0.5%), and *H. nana* (0.3%). In addition, 9.1% of the children had more than 1 type of worm infestation.

The prevalence of diarrhea, fever, cough, runny nose, and vomiting decreased similarly in the 2 groups between baseline and postintervention surveys ($P < 0.05$) (Table 7).

Anthropometry. Height, weight, HAZ, WAZ, and WHZ increased in both groups over the study period, with no significant benefit of the intervention (data not shown). Wasting decreased significantly from baseline to postintervention in the treatment (12.7 vs. 8.4%) and control (11.9 vs. 7.4%) groups. The prevalence of underweight also decreased significantly from 60.5 to 51.8% in the treatment group and from 59.6 to 51.5% in the control group. The changes in wasting and prevalence of underweight did not differ between the groups. The prevalence of stunting did not change significantly from baseline to postintervention in either the treated (57.7 vs. 54.5%) or control (53.6 vs. 51.5%) groups.

Discussion

The addition of a micronutrient premix to school lunch meals by trained school personnel was effective in improving the micronutrient status of schoolchildren in Himalayan villages of India. The intervention was associated with improved mean serum retinol and geometric mean serum folate concentrations and reduced the magnitude of a decrease in geometric mean serum vitamin B-12 concentrations. There was no significant effect on mean serum zinc and mean hemoglobin concentrations. However, iron status was increased by the intervention as shown by the significant improvement in geometric mean serum ferritin concentrations as well as increased mean TBI associated with the intervention.

Consumption of the micronutrient-fortified food was also associated with a significant decrease in the prevalence of children with low serum retinol and folate as well as a reduction in magnitude of an increase in proportion of children with low serum vitamin B-12 concentrations. There was a significant reduction in the prevalence of anemia associated with the intervention in the bivariate analysis but not in the multivariate analysis.

TABLE 5 Indicators of iron status and prevalence of anemia, among children before and after 8 mo of treatment[1]

	Micronutrient premix, *n* = 220		Placebo, *n* = 235	
	Baseline	8 mo	Baseline	8 mo
Hemoglobin, *g/L*	122.0 ± 0.1	123.2 ± 0.1	121.7 ± 0.1	122.5 ± 0.1
<115 g/L,[2] %	39.5	26.8*	32.5	28.9
Ferritin, *μg/L*	28.6 (25.1–32.7)	34.7 (32.8–36.7)*	30.3 (26.3–34.8)	32.8 (30.9–34.8)
<15 μg/L,[2] %	23.6	2.7*	23.8	6.0*
Iron deficiency anemia, %	10.5	2.3*	9.8	4.3*
sTfR, *mg/L*	2.0 ± 0.1	1.2 ± 0.1*	2.2 ± 0.1	1.3 ± 0.1*
TBI, *μmol/kg*	148.6 ± 0.1	220.5 ± 0.1*#	148.1 ± 0.1	197.6 ± 0.1*

[1] Values are adjusted mean ± SEM, adjusted geometric mean (95% CI) or percent. Means and geometric means are adjusted for age, sex, and CRP. *Different from baseline value, $P < 0.05$; #Different from corresponding placebo value, $P < 0.05$.
[2] Anemia was defined as hemoglobin <115 g/L and iron deficiency anemia as: hemoglobin <115 g/L and serum ferritin < 15 μg/L.

TABLE 6 Repeated-measures logistic regressions for assessing the effect of the micronutrient fortification on changes in prevalence of anemia and low serum concentrations of ferritin, retinol, zinc, folate, and vitamin B-12 after 8 mo of treatment[1]

	Binary outcome variable[2,3]					
	Anemia	Low serum ferritin	Low serum retinol	Low serum zinc	Low serum folate	Low serum vitamin B-12
Treatment	0.31 (−0.08–0.70)	−0.02 (−-0.42–0.46)	0.16 (−0.21–0.53)	−0.12 (−0.50–0.25)	0.43 (0.03–0.83)*	0.19 (-0.29–0.66)
Time (8 mo)	−0.18 (−0.52–0.16)	−1.63 (−2.23 to −1.03)*	−0.64 (−1.01 to −0.28)*	−0.74 (−1.09 to −0.40)*	0.06 (−0.35–0.46)	2.46 (2.03–2.90)*
Treatment × time	−0.40 (−0.90–0.09)	−0.82 (−1.85 – 0.22)	−0.57 (−1.10 to −0.03)*	0.19 (−0.30–0.68)	−0.75 (−1.34 to −0.17)*	−0.89 (−1.49 to −0.28)*
Constant	0.31 (−0.84–1.45)	0.87 (−0.65–2.39)	0.32 (−0.69–1.32)	−0.19 (−1.22–0.83)	1.21 (0.26–2.16)*	−1.20 (−2.32 to −0.07)*

[1] Values are regression coefficients (95% CI), n = 455 for each model. * $P < 0.05$.
[2] Cut-offs for definition of each dependent variable is given in the Methods section.
[3] All the models were adjusted for the child's age, sex, and CRP.

The lack of a significant effect on anemia in multivariate models despite an improvement in iron status in the treatment group can be explained by several factors. At baseline, iron deficiency anemia (10.2%) accounted for only one-third of all anemia cases (36.7%) (Table 1). The low prevalence of iron deficiency anemia suggests that factors other than iron status may influence anemia in this population and these factors might have muffled the impact of the intervention on anemia. Baseline hemoglobin was a predictor of postintervention hemoglobin (r = 0.36; $P < 0.05$). Therefore, if the intervention had any impact on hemoglobin, we would expect a greater change among anemic children at baseline compared with the entire study population. Subgroup analysis involving only anemic children at baseline revealed an increase in hemoglobin during the study period ($P < 0.05$), with a marginally significant improvement in the treatment over the control group (P = 0.05). The resurgence of helminth infection together with changes in types of intestinal worms among children during the study may have contributed to the lack of an effect on anemia. Analysis of baseline data revealed an association between prevalence of helminth infection and anemia (29).

Micronutrient deficiencies, including vitamins A, B-2, B-6, B-12, and folate, may contribute to anemia (30,31). There was a high prevalence of low serum retinol, folate, and vitamin B-12 in our study; vitamin B-2 and B-6 were not assessed. Various hemoglobinopathies could have also limited the impact of the intervention on hemoglobin, but these were not assessed.

The lack of impact on zinc status could be due to the potential interference of iron with zinc absorption or the high phytate diet common in the study area. The inhibitory effect of iron on zinc absorption has been shown to occur when the molar ratio of iron:zinc is high (usually >1) (32). In our study, the molar ratio of iron:zinc in daily servings of the micronutrient premix per child was 2.8:1, thus raising the possibility of iron interfering with zinc absorption. Several studies have shown a negative impact of phytates on zinc and iron absorption (26,33). Although not measured in this study, the school meals are likely to have high phytate levels, because they consist mainly of rice and lentils. The lack of the intervention's impact on zinc status can partly be explained by serum zinc not being an adequate indicator of individual zinc status (26). The duration of exposure of children to the fortified food may also have been too short to elicit a significant intervention effect on some micronutrient indicators or to eliminate their deficiencies, because schools opened and prepared meals for only 165 d during the intervention.

The control group also had significant improvements in vitamin A, zinc, folate, and iron status. Schools involved in the study were very far apart (~25 km). Therefore, exchange of the premix between the treatment and control schools was unlikely. Improvement in micronutrient status of the control group can be attributed partly to seasonal changes in dietary intake. The baseline study was conducted in the lean season when household food consumption was likely to be lower. However, the final survey was conducted during the postharvest season when food consumption was likely to be at its peak. It was not possible to conduct the 2 surveys at the same time of the year without interrupting the intervention because of the timing of the academic calendar, which ends in May of each year, followed by a 3-mo school vacation. Deworming of all children prior to the intervention may have contributed to improvement in the micronutrient status of the control group, as shown by other studies (34,35). However, this is less likely, because more children had intestinal worm infections postintervention compared with baseline. There were also many types of helminth infections and more children had multiple infections at postintervention than baseline.

The significant decrease in vitamin B-12 status of children in the treatment group was unanticipated and inexplicable. We doubt there was an assay problem, because all analyses were cross-checked against quality control samples using similar

TABLE 7 Status of infections among children before and after 8 mo of treatment

	Micronutrient	Placebo
n	196	201
Intestinal parasite infections	%	
Chronically infected: baseline (+) and 8 mo (+)	6.1	9.0
Infected during intervention: baseline (−) and 8 mo (+)	26.5	27.4
Baseline infection cleared: baseline (+) and 8 mo (−)	13.8	12.4
Never infected: baseline (−) and 8 mo (−)	53.6	51.2
Morbidity[1]		
Diarrhea		
Baseline	12.9	15.3
After 8 mo	3.4	5.1
Fever		
Baseline	52.8	44.3
After 8 mo	20.8	20.5
Cough		
Baseline	25.3	19.9
After 8 mo	12.9	10.8
Runny nose		
Baseline	18.0	21.0
After 8 mo	2.2	1.7
Vomiting		
Baseline	7.9	10.2
After 8 mo	0.6	2.3

[1] n = 178 for micronutrient group and n = 176 for placebo group.

procedures and reagents from the same manufacturer. However, because the baseline and 8-mo serum samples were analyzed at different seasons, there was a potential for variability between samplings. Vitamin B-12 deficiency in our sample may be associated with the consumption of a primarily vegetarian diet, because 98.2% of our study participants were Hindus. It is also possible that the increased worm infection during the study period contributed to poor vitamin B-12 status of the children. Despite the decrease, the postfortification vitamin B-12 status of the treatment group was not as low as that of the control group, suggesting that the intervention had a beneficial effect on vitamin B-12 status.

The strengths of the study are that it used a representative sample of schoolchildren chosen randomly from all subdistricts of Tehri Garhwal district. The high compliance, relatively low attrition rate (9.0%), and lack of any significant differences between the dropouts and participants in baseline characteristics strengthen the study's internal validity. The trial reflects what can happen with micronutrient fortification programs under real community circumstances, because the study team did not interfere with school lunch preparation or food intake by the children. However, limitations of the study include the short duration of the intervention due to resource constraints and unanticipated public holidays that caused interruptions in delivery of the micronutrient intervention. The study did not assess hemoglobinopathies, intensity of helminth infection, or *Helicobacter pylori* infection, which could have helped explain the lack of effect on anemia. Despite these minor limitations, our findings add important information to the growing body of evidence that community level micronutrient fortification of staple foods is a feasible approach to reduce micronutrient deficiencies in rural communities.

In summary, micronutrient fortification of cooked school meals by trained school authorities was effective in improving vitamin A, folate, and iron status and reducing the magnitude of a decrease in vitamin B-12 status of schoolchildren in Himalayan villages of India. School feeding programs can therefore serve as a suitable vehicle for addressing micronutrient malnutrition among rural schoolchildren in our study district. The prevalence of helminth infection in our sample at both the baseline and postintervention surveys was above the threshold (20–50%) recommended by WHO for annual mass treatment of all schoolchildren in any community with albendazole or mebendazole (36). This prevalence together with the resurgence of intestinal helminth infection among children during the study period suggests that policy makers should include at least once a year deworming as part of micronutrient control strategies in this district.

Our results can be potentially generalized to children living under similar socioeconomic conditions in India and other countries. The addition of micronutrient premix to meals at school has potential advantages of being locally acceptable, sustainable, and lower implementation cost, because it uses the infrastructure of an already existing program. However, adoption of this fortification strategy in other areas will require adjustment to the micronutrient levels of the fortificant based on the micronutrient status and/or consumption profile of the population to avoid excessive intake. Further research is needed to assess the impact of this type of intervention on anemia as well as the cost effectiveness of delivering micronutrients through this strategy to schoolchildren.

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