Impact of a multiple-micronutrient food supplement on the nutritional status of schoolchildren

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Abstract

Background. Multiple-micronutrient deficiencies exist in many developing nations. A system to deliver multiple micronutrients effectively would be of value in these countries.

Objective. To evaluate the delivery of multiple micronutrients through the food route. The goal was to test the stability of the supplement during cooking and storage and then to test its bioefficacy and bioavailability in residential schoolchildren 5 to 15 years of age.

Methods. A pre- and post-test design was used to study children 5 to 15 years of age, with an experimental and a control group. The experimental group ($n = 211$) consisted of children from two residential schools, and the control group ($n = 202$) consisted of children from three residential schools. The experimental group received a micronutrient supplement containing vitamin A, vitamin B_2, vitamin B_6, vitamin B_{12}, folic acid, niacin, calcium pantothenate, vitamin C, vitamin E, iron, lysine, and calcium daily for 9 months. There was no nutritional intervention in the control group.

Children in the experimental and control groups were matched by socioeconomic status, age, and eating habits at baseline. All of the children in the experimental and control schools were dewormed at baseline, after 4 months, and at the endpoint. Biochemical measurements (hemoglobin, serum vitamin A, serum vitamin E, serum vitamin B_{12}, and serum folic acid) were measured at baseline, after 4 months, and at the endpoint (after 9 months). The heights and weights of the children were also measured at baseline and endpoint. Serum vitamins A and E were measured in a subsample of 50% and vita-min B_{12} and serum folic acid measured in a subsample of 25% of the children.

Results. In the experimental group, the mean gains in hemoglobin, serum vitamin A, serum vitamin E, serum vitamin B_{12}, and serum folic acid over 9 months were 0.393 g/dL, 6.0375 µg/dL, 1037.45 µg/dL, 687.604 pg/mL, and 1.864 ng/mL, respectively. In the control group, the mean losses in hemoglobin and serum vitamin A over 9 months were 0.9556 g/dL and 10.0641 µg/dL, respectively, and the mean gains in serum vitamin E, vitamin B_{12}, and folic acid were 903.52 µg/dL, 233.283 pg/mL, and 0.0279 ng/mL. The mean gain in all biochemical measurements was significantly higher ($p < .05$) in the experimental group than in the control group.

Conclusions. Vitamin A, vitamin E, vitamin B_{12}, folic acid, and iron are bioavailable from the multiple-micronutrient food supplement used in this study. This method of micronutrient delivery has been beneficial. We believe the study intervention was beneficial because of small doses of the micronutrients added but delivered many times through meals throughout the day, over a period of 9 months.

Key words: Food delivery, multiple-micronutrient supplementation

Introduction

Micronutrient deficiencies in developing countries are a consequence of the plant-based cereal diets typically consumed in these areas [1, 2]. Dietary phytate inhibits the absorption of many micronutrients, notably iron and zinc. Micronutrient deficiencies in infancy can cause impairments in physical development and cognition that may be irreversible [3–5]. Iron and iodine deficiencies affect more than 30% of the global population [6]. It has been suggested that supplementation with multiple micronutrients may be the best way to improve the nutritional status of malnourished

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populations [7]. Earlier studies targeted children within the family. Packaged in single-serving sachets, the fortificant (microencapsulated ferrous fumarate) is designed to be sprinkled onto complementary cooked foods, to be served to children, one sachet per meal. A randomized, controlled trial demonstrated that microencapsulated ferrous fumarate sprinkles were as efficacious as ferrous sulfate drops in the treatment of anemia in infants 6 to 18 months of age [8].

There are no data on the effects of adding multiple-micronutrient supplements to food during cooking in order to make the micronutrients available to the entire family. For this method to be successful, the micronutrients should not change the color, odor, or taste of the food, should be stable at cooking temperatures, and should be bioavailable. With these concepts kept in mind, a multiple-micronutrient food supplement was developed that contained vitamin A, vitamin B_2, vitamin B_6, vitamin B_{12}, folic acid, niacin, calcium pantothenate, vitamin C, vitamin E, iron, lysine, and calcium. The intention was that the supplement sprinkled on the food during cooking would supply these nutrients to the entire family. The advantage of this method of nutrient delivery is that all the family members would benefit from the supplement.

The current study tested the acceptability of the supplement during cooking and storage and tested for the bioavailability of five important micronutrients: iron, vitamin A, vitamin E, vitamin B_{12}, and folic acid, as well as other B-complex vitamins, in the target group of children in residential schools. If the food supplement was found to benefit the children by improving their levels of serum vitamin A, serum vitamin E, hemoglobin, serum vitamin B_{12}, and serum folic acid, we could conclude that this method of micronutrient delivery was feasible, setting the stage for conducting larger-scale community studies.

This study aimed to evaluate the delivery of multiple micronutrients through the food route. The goal was to test the stability of the supplement during cooking and storage and then to test its bioefficacy and bioavailability in residential schoolchildren 5 to 15 years of age.

Methods

Subjects

The study had a pre- and post-test design with experimental and control groups. Two residential schools (A and B) were randomly selected as the experimental schools and three other residential schools (C, D, and E) as the controls from residential schools in the city of Chennai, Tamilnadu, South India. Schools A through E were chosen randomly from a computer-generated list and a random table. Study schools were chosen prior

to randomization, after a survey of residential schools was undertaken, because children at these schools had the lowest intake of outside (unfortified) cooked food and the schools had the fewest holidays when the children were allowed to go home, which would cause less disruption in the study. Children in the experimental schools were supplied with the multiple-micronutrient food supplement. Children in the control group, except those with severe anemia and serum vitamin A deficiency, who were treated for ethical reasons and excluded from the study, received no intervention other than deworming. The study began when the schools reopened after the summer vacation and continued for 9 months until the schools closed again for the next summer vacation. There were 211 children in the experimental group (169 in school A and 42 in school B) and 202 children in the control group (90 in school C, 70 in school D, and 42 in school E).

The experimental and control groups of children were selected after establishing their homogeneity in terms of age and socioeconomic status; the families of all the children had a monthly income of less than Rs1,500 ($US30). The supplement was provided to the experimental schools every month, and its use was monitored by counting the number of packets remaining in the school every week.

Manufacture and administration of supplement

The multiple-micronutrient food supplement was manufactured in a ribbon blender (Bhuvaneshwari Engineering, Chennai, India) at 50 rpm. The homogeneity of the supplement's micronutrient content was established at the manufacturing stage by assessing the micronutrient content in different parts of the blender. It was determined that all of the micronutrients were uniformly and homogeneously distributed within the product.

The dosage was 1 g of supplement per child per day. The required daily quantity for all the children in a specific school was premeasured, packed, sealed, and delivered to the schools, so that one packet could be cut open each day and added to the food during cooking, throughout the day. The supplement was dissolved in water and added to liquid food in the final stages of cooking, and it was sprinkled onto solid foods. The cooking staff of both the experimental schools certified that the supplement did not change the color or taste of any food. Each school has a central kitchen where the food is prepared and a central dining room where the resident children eat.

It was generally observed that there was no waste of the food prepared in the schools; all prepared food was consumed. The children were served the quantity of food they wished, and there was no food left over on the plate.

Blood collection and storage

Blood samples (5 mL) were drawn from each child at the schools and transferred to the laboratory within 2 hours of collection. During collection of these samples, 500 µL was transferred into vials with ethylenedi-aminetetraacetate (EDTA) as an anticoagulant. The hemoglobin measurements were performed on these samples within a few hours of blood collection. The remaining 4.5 mL of blood was transferred into vials covered with black paper to prevent exposure to light, and the blood was allowed to clot. Serum separation was performed and the samples were frozen at –20°C within a few hours after collection of blood. Analysis of serum for folic acid and vitamins B_{12}, A, and E was completed within a month after blood collection. The samples were processed in a dark room with yellow lighting to prevent retinol isomerization.

Biochemical measurements

The concentrations of blood hemoglobin and serum vitamin A, vitamin E, vitamin B_{12}, and folic acid were measured. Hemoglobin was measured in all the children in both groups. Serum vitamin A was measured by high-performance liquid chromatography (HPLC) only in those children who were identified as having vitamin A deficiency by physicians who checked the eyes of the children for clinical signs of vitamin A deficiency, such as Bitot's spots or xerosis. Eighty-two children in the experimental group and 84 children in the control group had clinical signs of vitamin A deficiency. At baseline, only eight children in the experimental group had serum vitamin A levels under 20 µg/dL, the cutoff for vitamin A deficiency, as defined by the International Vitamin A Consultative Group (IVACG). Seven children in the control group had serum vitamin A levels under 20 µg/dL. For ethical reasons, these children were given therapeutic tablets of vitamin A to combat the deficiency and were excluded from further analysis. Analysis of data for serum vitamin A and E was performed only for the remaining 77 children in the control group.

Serum folic acid and vitamin B_{12} were measured in the 44 children in the experimental group and the 54 children in the control group who had the lowest hemoglobin levels. All biochemical measurements were carried out before the start of the study (baseline), 4 months after the start of the study (midpoint), and 9 months after the start of the study (endpoint).

Hemoglobin was estimated by the cyanmethemo-globin method with a spectrophotometer [9]. Serum vitamin A and E were measured by a rapid, reverse-phase HPLC method for simultaneous determination of retinol and α-tocopherol (vitamin E) [10]. Vitamin B_{12} and folic acid assays were performed with a para-magnetic particle, chemiluminescent immunoassay for the quantitative determination of levels in human serum using the Access immunoassay system (Beckman Instruments, Brea, CA, USA).

Validation of biochemical measurements

Hemoglobin levels were measured in duplicate in all of the samples; serum vitamin A, vitamin E, vitamin B_{12}, and folic acid were measured in duplicate in 10% of the samples. For validation of vitamin A measurements, the average of the two values was calculated and the percentage of deviation from the average determined. The permissible percentage deviation from average was 10% for vitamin A levels over 30 µg/dL and 15% for vitamin A levels between 15 and 30 µg/dL. The vitamin A levels of the subjects were within these averages. For validation of vitamin E measurements, the average of the two values was calculated and the percentage of deviation from the average determined. In all three rounds, the percentage of deviation from the average values did not exceed 10%.

Anthropometric measurements

The heights and weights of the children in the experimental and control groups were recorded at baseline and 9 months.

Deworming

Both the experimental and the control children were given a tablet of albendazole (400 mg) at baseline and at 4 and 9 months later. Deworming was done to ensure that there were no worms competing for the micronutrients and that the intestinal tract was clear for absorption of the micronutrients [11, 12].

Clinical assessment

Clinical assessment of angular stomatitis, a condition caused by deficiencies of B-complex vitamins, was conducted by physicians before the start of the study and after 9 months of intervention.

Administration of iron

In most of the studies reviewed in the literature, iron was administered in the form of ferrous sulfate tablets for periods ranging from 2 to 8 months [13–16]. In our study, the experimental group received 10 mg of elemental iron in the multiple-micronutrient supplement every day for 9 months. The iron was chelated ferrous sulfate along with malic acid as a biopromoter added. Chelated iron compounds have a much higher bioavailability than inorganic iron compounds. The studies

reviewed in the literature mentioned ferrous sulfate, ferrous fumarate, and other iron compounds but did not mention chelated iron compounds [17–20].

Measurement of stability of the supplement

To determine the stability of the supplement, its composition was analyzed initially, after adding it to an Indian dish—sambar (lentil soup) and cooking it for 30 minutes, and after storage for 10 months at 30°C and 45% humidity. The required aliquots from the lentil soup were taken for the analysis of micronutrients. Six samples were taken before and after cooking. Micronutrient composition was analyzed by methods described in the Indian Pharmacopoeia [21].

Statistical analysis

Statistical analysis was performed with SPSS 11.0 (SPSS Inc., Chicago IL, USA) and Microsoft Excel 2000 (Microsoft Corp., Seattle WA, USA). Analysis of variance (ANOVA) was used to compare the micronutrient status of the experimental and control groups and within the groups over time.

Ethical issues

The study was approved by the institutional review board of the Sundar Serendipity Foundation. Informed written consent was obtained from the school directors, and informed oral consent was obtained from the parents or legal guardians of all of the children. The parents of the children in the experimental schools were informed about the use of the supplement in all meals served in the schools and about the blood tests to be performed. The parents of the children in the control schools were informed that blood tests would be performed on all of the children and that children with severe anemia (hemoglobin < 8 g/dL) or biochemical vitamin A deficiency (serum retinol < 20 μg/dL) would be treated immediately. Anemic children were treated with ferrous sulfate tablets (60 mg elemental iron) for a period of 3 months. Serum vitamin A-deficient children were treated with vitamin A tablets. These treated children were excluded from the study. All children who were anemic at the end of the study were treated with ferrous sulfate (60 mg elemental iron) daily for a period of 3 months. All children in the five schools were invited to participate in the study and all parents or legal guardians gave consent.

Results

Stability of the supplement

All micronutrients except vitamin A were very stable

after 30 minutes of cooking and 10 months of storage (**table 1**). A drop of up to 20% in the potency of vitamin A was observed during cooking, so the product was formulated to compensate for this loss of vitamin A.

Biochemical and clinical effects of the intervention

At baseline, the experimental and control groups had similar serum levels of vitamin E, vitamin B_{12}, and folic acid, but the hemoglobin level was significantly ($p < .05$) lower and the serum vitamin A level was significantly higher in the experimental group than in the control group. The differences between the groups could be due to differences between the schools in the diets consumed or in other, unknown, factors. Therefore all the biochemical measurements were analyzed schoolwise to study the impact of the supplementation.

At the end of 9 months of intervention there were significant ($p < .05$) improvements in the mean values of all of the biochemical measurements in the experimental group (**table 2**). This pattern was also seen when the results from each school were individually analyzed (**table 3**). In the control group, there were significant decreases from baseline to 9 months in mean hemoglobin and vitamin A levels, significant improvements in vitamins E and B_{12} levels, and no significant change in folic acid levels (**table 2**). This pattern was also seen

TABLE 1. Composition of the multiple-micronutrient food supplement and its stability during cooking and storage)

Micronutrient	Amount in the supplement		
	Initial amount	After 30 min cooking	After 10 mo storage
Vitamin A (IU/g)	3,000.00	2,425.00	2,326.00
Vitamin B_2 (mg/g)	1.00	1.00	1.00
Calcium pantothenate (mg/g)	1.00	1.00	1.00
Niacin (mg/g)	15.00	15.00	14.91
Vitamin B_6 (mg/g)	1.00	1.00	1.00
Folic acid (μg/g)	100.00	99.49	99.27
Vitamin B_{12} (μg/g)	1.00	1.00	1.00
Vitamin E (IU/g)	30.00	29.85	30.00
Vitamin C (mg/g)	30.00	28.01	23.96
Iron (mg/g)	10.00	10.00	10.00
Lysine (mg/g)	250.00	243.80	246.51
Calcium (% of weight)	13.75	13.75	13.75

when the results from each school were individually analyzed (**table 3**). At 9 months, the mean values of all the biochemical measurements except vitamin E were significantly higher in the experimental group than in the control group (**table 2**).

To determine whether the intervention had any effect on biochemical measurements, the changes in all of the biochemical measurements from baseline to 9 months were compared between the experimental and control groups (**table 4**). The increase was significantly greater ($p < .05$) in the experimental group than the control group for all measurements. This result demonstrated the bioabsorption of iron, vitamin A, vitamin E, vitamin B_{12}, and folic acid from the supplement.

The prevalence of angular stomatitis at the start of the study was 16.2% in the experimental group and 22.56% in the control group. At the end of the study, angular stomatitis had completely disappeared in the experimental group, whereas the prevalence remained at 25.40% in the control group (**table 2**).

Enhancement of stability and shelf life of the micronutrients

The supplement did not change the odor or color of any foods during cooking. The micronutrients in the supplement were also stable during storage and cooking. This was achieved by encapsulating the heat-labile vitamins, such as vitamin C, with food-grade cellulose acetate phthalate to provide a heat-resistant

TABLE 2. Biochemical and clinical measurements of children at baseline and 9-month endpoint (mean ± SD)

Measurement	N	Baseline	Endpoint	N	Baseline	Endpoint
		Experimental group			Control group	
Hemoglobin (g/dL)	211	10.99 ± 1.41^{ab}	11.38 ± 1.06^{be}	202	11.32 ± 1.13^{ac}	10.35 ± 0.99^{ce}
Serum vitamin A (μg/dL)	82	47.18 ± 19.66^{ab}	53.33 ± 25.82^{be}	77	43.62 ± 13.83^{ac}	32.08 ± 10.74^{ce}
Serum vitamin E (μg/dL)	82	910.90 ± 269.00^{b}	1960.00 ± 400.80^{b}	77	973.90 ± 314.00^{d}	1932.55 ± 575.80^{d}
Serum vitamin B_{12} (pg/mL)	44	193.66 ± 84.90^{b}	1030.60 ± 1232.00^{be}	54	217.29 ± 155.60^{d}	440.24 ± 269.00^{de}
Serum folic acid (ng/mL)	44	4.87 ± 1.31^{b}	6.75 ± 2.17^{be}	54	4.92 ± 1.09	5.08 ± 1.69^{e}
Angular stomatitis (% of subjects)	211	16.20	0	202	22.56	25.40

a. Values at baseline differed significantly ($p < .05$) between experimental and control groups.
b. Values increased significantly ($p < .05$) in experimental group from baseline to endpoint.
c. Values decreased significantly ($p < .05$) in control group from baseline to endpoint.
d. Values increased significantly ($p < .05$) in control group from baseline to endpoint.
e. Values in experimental group were significantly ($p < .05$) higher than in control group at endpoint.

TABLE 3. Biochemical measurements according to school at baseline and 9-month endpoint (mean ± SD)

Measurement	School	N	Baseline	Endpoint	School	N	Baseline	Endpoint
			Experimental group				Control group	
Hemoglobin (g/dL)	A	169	11.20 ± 1.40^{a}	11.60 ± 0.99^{a}	C	90	10.71 ± 0.76^{b}	10.38 ± 0.98^{b}
	B	42	10.14 ± 1.11^{a}	10.54 ± 0.90^{a}	D	70	12.14 ± 1.24^{b}	9.96 ± 0.93^{b}
					E	42	11.34 ± 0.59^{b}	10.99 ± 0.78^{b}
Serum vitamin A (μg/dL)	A	75	48.36 ± 19.64^{a}	54.56 ± 25.45^{a}	C	35	36.93 ± 12.34^{b}	29.80 ± 9.86^{b}
	B	7	34.57 ± 16.03	40.57 ± 28.20	D	28	46.83 ± 19.12^{b}	34.00 ± 10.37^{b}
					E	14	41.00 ± 12.56^{b}	32.43 ± 11.84^{b}
Serum vitamin E (μg/dL)	A	75	932.64 ± 262.00^{a}	$1,971.73 \pm 398.80^{a}$	C	35	976.65 ± 303.80^{a}	$2,071.25 \pm 739.00^{a}$
	B	7	715.77 ± 264.69^{a}	$1,862.22 \pm 428.80^{a}$	D	28	$1,098.30 \pm 283.60^{a}$	$1,916.20 \pm 362.37^{a}$
					E	14	699.50 ± 239.30^{a}	$1,618.57 \pm 305.58^{a}$
Serum vitamin B_{12} (pg/mL)	A	36	202.26 ± 84.40^{a}	$1,060.31 \pm 1,305.00^{a}$	C	26	253.15 ± 192.70^{a}	499.54 ± 341.00^{a}
	B	8	169.87 ± 83.00^{a}	815.25 ± 462.00^{a}	D	16	214.18 ± 122.00^{a}	340.25 ± 99.45^{a}
					E	12	141.27 ± 56.30^{a}	482.42 ± 236.90^{a}
Serum folic acid (ng/mL)	A	36	4.73 ± 1.28^{a}	6.83 ± 2.33^{a}	C	26	4.57 ± 1.07	4.79 ± 1.22
	B	8	5.53 ± 1.36	6.27 ± 1.27	D	16	5.144 ± 1.04	4.78 ± 1.92
					E	12	5.39 ± 1.06	5.97 ± 1.75

a. Significant improvement ($p < .05$) from baseline to endpoint.
b. Significant decrease ($p < .05$) from baseline to endpoint.

TABLE 4. Changes in biochemical measurements from baseline to 9-month endpoint (mean ± SD)[a]

| | Experimental group | | Control group | |
Measurement	N	Change	N	Change
Hemoglobin (g/dL)	211	0.393 ± 1.12	202	−0.956 ± 1.33
Serum vitamin A (μg/dL)	82	6.038 ± 25.43	77	−10.064 ± 17.82
Serum vitamin E (μg/dL)	82	1,037.450 ± 393.00	77	903.520 ± 618.50
Serum vitamin B_{12} (pg/mL)	44	687.604 ± 1,110.00	54	233.283 ± 195.20
Serum folic acid (ng/mL)	44	1.864 ± 1.99	54	0.028 ± 1.60

a. The change was significantly ($p < .05$) better in the experimental group than the control group for all measurements.

TABLE 5. Anthropometric measurements of children 5 to 10 years of age at baseline and 9-month endpoint (mean ± SD)

| | Experimental group | | | Control group | | |
Measurement	N	Baseline	Endpoint	N	Baseline	Endpoint
Height (cm)	59	118.63 ± 7.07	122.15 ± 7.19	100	117.06 ± 10.59	119.99 ± 10.46
Weight (kg)	59	20.71 ± 3.28	22.53 ± 3.42[a]	100	19.57 ± 4.38	21.22 ± 4.97[a]

a. Values were significantly ($p < .05$) higher in the experimental group than in the control group at endpoint.

coat. Similarly, the B-complex vitamins were coated with glyceryl stearate or other edible waxes or gums to protect them and to mask the unpleasant taste of some B-complex vitamins. Vitamin A is already encapsulated in gum acacia and sugar by the manufacturers. Lysine, vitamin E, and calcium pantothenate were left uncoated. The iron source was ferrous sulfate, with chelating agents and biopromoter added to enhance its bioavailability even in the presence of dietary phytates.

Anthropometric effects

The heights and weights of 197 children in the experimental group and 196 children in the control group were measured at baseline and at 9 months. There were no significant differences between the groups in changes in height or weight from baseline to 9 months. Therefore, we analyzed the data from children 5 to 10 years of age and the data from children 11 to 15 years of age separately. Among children 5 to 10 years of age, the mean weight at the endpoint was significantly greater ($p < .05$) in the experimental group than in the control group, although at baseline there was no significant

TABLE 6. Changes in anthropometric measurements of children 5 to 10 years of age from baseline to 9-month endpoint (mean ± SD)

Measurement	N	Experimental group	N	Control group
Height (cm)	59	3.53 ± 1.14[a]	100	2.93 ± 1.38[a]
Weight (kg)	59	1.82 ± 0.93	100	1.65 ± 1.11

a. Values were significantly ($p < .05$) higher in the experimental group than in the control group at endpoint.

difference between the mean weights of the two groups (**table 5**). The mean increase in height was 3.525 cm in the experimental group and 2.93 cm in the control group ($p < .05$), but the mean increase in weight did not differ between the groups (**table 6**).

Discussion

The aim of this study was to test the efficacy of the delivery of multiple micronutrients in cooked food in residential schools. If the multiple-micronutrient food supplement remained stable during cooking and storage and was efficacious in the improvement of serum biochemical measurements, this study would provide basic evidence that this method of delivery works and should be considered for larger field community trials.

Since multiple micronutrients are responsible for erythropoiesis, earlier studies have shown that in populations where multiple micronutrient deficiencies exist, interventions with single micronutrients alone, such as iron and zinc, are not sufficient to mitigate the deficiencies [22, 23]. Earlier studies have shown that when multiple-micronutrient supplements are administered, it is the iron and zinc that cause linear growth in children [24]. In the present study, there were no significant differences in anthropometric measurements between the experimental and control groups when each group was considered as a whole. However, if the children in the 5- to 10-year age group are studied separately, the increase in height is significantly greater in the experimental group. The difference in the result when the younger age group was considered separately may have been due to the occurrence of growth spurts

at different ages.

Another reason for the development of the multiple-micronutrient food supplement is the cost factor. The cost of the supplement is about 45 paise (1 US cent) per person per day. Without lysine, the cost would be only 0.5 US cent per person per day. This is extremely low compared with the cost of multiple-micronutrient tablets. Moreover, focus group discussions with people in Tamilnadu have shown that the people perceive tablets as medicine and would not consume them unless they were sick. When they were educated about the supplement and the need to add it to food during cooking, they readily accepted the concept.

The use of the supplement for 9 months resulted in a significant ($p < .05$) improvement in all biochemical measurements. Clinical signs of angular stomatitis, a condition caused by a deficiency of B-complex vitamins, especially vitamin B_2, completely disappeared in the experimental group, whereas the prevalence of the condition remained the same in the control group. It could be inferred that the B-complex vitamins in general, and vitamin B_2 in particular, were absorbed from the supplement and that the higher level of ingestion of these micronutrients was a factor in the disappearance of angular stomatitis in the experimental group.

The hemoglobin level was significantly ($p < .05$) lower in the experimental group than in the control group at baseline, but at the endpoint it was significantly higher in the experimental group than in the control group. Over the 9-month period, there was a significant increase in hemoglobin level in the experimental group and a significant decline in the control group. This same pattern was seen when each of the schools was individually analyzed. The mean increase in hemoglobin level in the experimental group was 0.393 g/dL, and the mean decrease in hemoglobin level in the control group was 0.9556 g/dL. A statistically significant decline in hemoglobin in children aged 5 to 15 years has been observed elsewhere [25]. It may be due to the insufficient bioavailability of iron from the predominantly vegetarian cereal-based diets of these children or the diversion of iron to myoglobin in the muscles as the children grow. This, however, needs to be verified by conducting further prevalence studies on anemia over a period of 10 months to one year in a similar population.

The serum vitamin A level at baseline was significantly ($p < .05$) higher in the experimental group than in the control group. However, over the 9-month period, vitamin A values significantly increased in the experimental group and significantly decreased in the control group. At endpoint, the mean serum vitamin A level in the experimental group was significantly higher than in the control group. This trend was also seen when each school was individually analyzed. No illness or infection was observed in any of the control schools. The reason for the decline in serum vitamin A in the control schools is unknown, and similar prevalence studies in similar children are warranted.

The normal ranges of serum vitamin B_{12} and E levels are 200 to 950 pg/mL and 500 to 1,800 μg/dL, respectively. The same method (HPLC) was used to measure both vitamins A and E; the times of elution of both of these compounds are a few minutes apart. There was a significant increase in serum vitamins E and B_{12} in both groups. The same trend was seen when the schools were individually analyzed. The increases in the levels of these two vitamins were significantly ($p < .05$) higher in the experimental group than in the control group.

The normal range of serum folic acid levels is 3 to 17 ng/mL. In the experimental group, there were three children with serum folic acid levels < 3 ng/mL at baseline, and there were none in the experimental group at endpoint. In the control group, there were three children with serum folic acid < 3 ng/mL at baseline, and at endpoint there were two children with serum folic acid < 3 ng/mL. There was no significant difference between the experimental and control groups in serum folic acid at baseline. There was no change in folic acid levels in the control group, whereas there was a significant increase ($p < .05$) in the experimental group at endpoint. The same pattern was seen when the data from each school were individually analyzed.

These trends in biochemical measurements demonstrate the bioavailability of the micronutrients in the supplement. Similar improvements in vitamin A and hemoglobin levels have been seen in other trials in which multiple-micronutrient tablets have been administered to schoolchildren [26–28]. Thus, it can be concluded that the delivery of multiple micronutrients by the method of supplementation described in this study, i.e., through the food route, is as efficient as the conventional method of supplementation through tablets.

Conclusions

Vitamin A, vitamin E, vitamin B_{12}, folic acid, the other B vitamins, and iron are bioavailable from a multiple-micronutrient food supplement added to food during cooking. Long-term community trials should be undertaken to establish the efficacy of this pathway of supplementation to combat micronutrient malnourishment.

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