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ORIGINAL ARTICLE

Multiple micronutrient fortification of salt

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Background/Objective: To develop a salt fortified with multiple micronutrients, to test its stability during storage and cooking, and to assess its efficacy in improving the micronutrient status and the health of schoolchildren.
Subject/Methods: A salt fortified with multiple micronutrients was developed containing chelated ferrous sulfate and microencapsulated vitamins A, B1, B2, B6, B12, folic acid, niacin, calcium pantothenate and iodine. Its stability during 20 min of cooking and 6 months of storage was determined. Thereafter, the efficacy of the salt was assessed in 5- to 15-year-old schoolchildren in Chennai, India. For the experimental group ($N=119$), the food in the school kitchen was cooked with fortified salt for a period of 1 year. The control group ($N=126$) consisted of day scholars who did not eat at the school. Hemoglobin, red blood cell count, hematocrit, serum vitamin A and urinary iodine were measured at baseline and at the end of the study after 1 year.
Results: All micronutrients were stable during cooking and storage. Over the study period, there was a significant improvement ($P<0.05$) in hemoglobin, red cell count, urinary iodine and serum vitamin A in the experimental group, while there was a significant drop ($P<0.05$) in hemoglobin, hematocrit, red cell count and urinary iodine in the control group. In the experimental group, there was a mean increase of 0.55 g/dl in hemoglobin, 0.001 l/l in hematocrit, 0.470 million/mm^3 in red cell count, 212 μg/l in urinary iodine and 5.6 μg/dl in serum vitamin A.
Conclusion: The study shows that the salt fortified with multiple micronutrients is stable during cooking and storage and effective in combating multiple micronutrient deficiencies.
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Keywords: multiple micronutrients; salt fortification; iron; iodine; vitamin A; B complex vitamins

Introduction

In developing countries, there is a high prevalence of multiple micronutrient deficiencies, that occur particularly in the poorer segment of the population (United Nations Children's Fund, 2000; World Health Organization, 2000). However, the approach to combat micronutrient deficiencies is often applied to only one or two micronutrients such as, the supply of iron and folic acid tablets to pregnant women or the dispensation of vitamin A drops to children. Since multiple micronutrients are involved in erythropoesis, studies have shown that in populations where multiple micronutrient deficiencies exist, interventions with single micronutrients such as iron and zinc alone are not sufficient to mitigate the problem (Allen, 1994; Allen *et al*., 2000). There is a need to tackle multiple micronutrient deficiencies (UNICEF/UNU/WHO/MI, 1999). We therefore developed a multiple micronutrient-fortified salt containing vitamins A, B1, B2, B6, B12, folic acid, niacin, calcium pantothenate, iron and iodine.

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Subjects, materials and methods

Work on the development of the multiple micronutrient-fortified salt and its stability during cooking and storage was done from 2001 to 2002. All the ingredients used in the preparation of fortified salt were food grade or pharmaceutical grade and defined in the Codex. Our baseline dietary survey showed that the average salt consumption is about 10 g per child per day. The salt was fortified so as to provide an adequate quantity of the above-mentioned micronutrients per 10 g of salt. Ten grams of salt contained 3000 IU vitamin A, 2 mg vitamin B2, 2 mg calcium pantothenate, 30 mg niacin, 2 mg vitamin B1, 2 mg vitamin B6, 50 μg folic acid, 4 μg vitamin B12, 10 mg iron and 400 μg iodine. The formula was developed to provide approximately 1 RDA (recommended dietary allowance) of vitamin A after cooking

and 30% of the RDA for iron, because chelated iron with higher bioavailability was used. The dosage for B complex vitamins was approximately 2 RDA because of the very high prevalence (30.6%) of angular stomatitis in the children at baseline. The whiteness of the salt was measured using a reflectance meter (AIMIL Instrumentation, Chennai, India) and compared between fortified salt and raw salt.

Stability during cooking

Fortified salt was used under typical Indian cooking conditions. It was added to the Indian dish, sambar (lentil soup), which was cooked for 20 min. The required aliquots from the lentil soup were taken for the analysis of micronutrients. Six samples were taken before and after cooking. Concentrations of all added micronutrients were analyzed according to the Indian Pharmacopoeia (1996).

Stability during storage

Eight batches of the fortified salt were stored at 30 °C and 45% humidity for 6 months. Concentrations of all added micronutrients were determined before storage and 2, 4 and 6 months after storage. Analyses were done according to the Indian Pharmacopoeia (1996).

Bioefficacy study: This was done during the year 2002–2003.

Subjects

The research design was a pre-post test design with an experimental group and a control group. Although double-blind placebo-controlled trial would have been appropriate, it could not be carried out as the Institute Ethics Committee did not approve a placebo group in children. The survey was conducted at different schools in Chennai, India. A residential school in the suburb of Chennai, about 50 km from Chennai, in a predominantly agricultural area was chosen for the study. The residential school that was chosen had a minimum of outside cooked (unfortified) food served to the children and a minimum of holidays, which would cause a disruption in the study. The children in the school went home for only 10 days in the month of May. The experimental group consisted of children residing in the residential school ($N = 119$). The control group consisted of children who lived in communities nearby and attended the day school ($N = 126$).

We have considered an α of 0.05, power of 0.80 and a two-tailed test for all sample-size calculations. Our earlier studies had shown an increase of about 0.5 g/dl in hemoglobin with a s.d. of 1.25 and with these parameters the sample size required for this study, assuming a similar change in hemoglobin, is 100 children in each of the experimental and control groups. Therefore, a sample size of 100 children was chosen for all parameters of iron, namely hematocrit, red cell count and hemoglobin and the other biochemical parameter —urinary iodine. Other fortification studies had shown an increase of about 6 μg/dl in vitamin A with a s.d. of 12. Assuming similar changes, this study requires a sample size of 63 children to detect changes in vitamin A. Other studies measuring anthropometry when multiple micronutrients are given have shown a 1.5 cm gain in height in the experimental group over the control group with a s.d. of 2 and a 0.5 kg gain in weight of the experimental group over the control group with a s.d. of 1.25. Assuming similar changes, the study requires a sample size of 100 children in each group to detect changes in anthropometry. Eventually, since the gain in height of the experimental group over the control group was as high as 2.84 cm and the gain in hemoglobin was as high as 0.71 g/dl in some subgroups of children, we were also able to further break the sample agewise and genderwise and perform an analysis of iron parameters and anthropometry in the subsample of children, considering an α of 0.05 and power of 0.80.

The experimental and control groups were similar in terms of nutrient intake, socioeconomic status (monthly family income about Rs 1500 or US$30) and age. The mean age of the experimental and the control groups was 9.5 ± 2.3 and 9.1 ± 2.6 years, respectively.

Dietary assessment of the households of a subsample of the control group ($N = 25$), done by random sampling, showed that there were no significant differences in the micronutrient and macronutrient intake of both the experimental and control groups. The dietary consumption was calculated by assessing the details of food consumed in the past 3 days. The macronutrients and micronutrients were calculated by using the book 'Nutritive value of Indian foods' published by the National Institute of Nutrition, Indian Council of Medical Research.

The study was approved by the Institutional Ethics Committee of Sundar Serendipity Foundation and the doctoral committee of the MS Swaminathan Research Foundation. Informed written consent was obtained from the school director and informed oral consent was obtained from the parents/legal guardians of all the children. Children with severe anemia (Hb < 8 g/dl) were treated immediately and excluded from the study. All the children who were anemic at the end of the study were treated with ferrous sulfate tablets containing 60 mg elemental iron for 3 months. Children with biochemical vitamin A deficiency at baseline, that is, serum retinol < 20 μg/dl, were treated with vitamin A tablets and excluded from the study.

Study design

The multiple micronutrient-fortified salt was used to cook all meals for the children in the experimental group during a period of 1 year. There was no intervention in the control group except for deworming. The fortified salt was supplied to the school every month and its continuous use in daily cooking was monitored. Since this study was done in a residential school in Chennai, in this study only South Indian recipes were prepared.

Deworming was done in both groups by a single dose of albendazole 400 mg at baseline, after 6 months and after 1 year of intervention. Deworming was done to ensure that there are no worms to compete for the micronutrients and that the intestinal tract was clear for bioabsorption of the micronutrients as in other studies (Taylor *et al*., 2001; Olsen *et al*., 2003).

The biochemical parameters that were assessed were hemoglobin, hematocrit, red blood cell count (RBC), urinary iodine and serum vitamin A. Measurement of hemoglobin was carried out at baseline and at 6 months and 1 year after the start of the study. Hematocrit, RBC count, urinary iodine and serum vitamin A analysis were done at baseline and at the end of the study. Hemoglobin, hematocrit, RBC count and urinary iodine measurements were done in all the children, whereas serum vitamin A was measured only in those who had clinical manifestations of vitamin A deficiency at baseline (such as xerosis or Bitot's spot).

Height and weight of the children were measured in both groups at the start and the end of the study. The weights were measured using a portable weighing scale (Krups, Germany), which had been calibrated using standard weights. Height was measured using a measuring tape. Clinical assessment of vitamin A deficiency and angular stomatitis, a condition caused by vitamin B complex deficiencies, was done at baseline and at 6 months and 1 year after the start of the study by the same physician. We defined mild angular stomatitis as a condition with a thin mottled surface epithelium with superficial fissuring, and severe angular stomatitis as a condition with cracked surface epithelium with well-developed fissuring.

Blood collection, storage and laboratory analysis

Five milliliters of venous blood was drawn from each child. The blood samples were transported to the laboratory within 2 h of collection at the schools. After drawing the blood, 500 μl of whole blood was transferred into vials containing EDTA as anticoagulant. Hemoglobin was analyzed in the above sample later in the laboratory. The other 4.5 ml of blood was transferred into vials wrapped in black paper to prevent sun exposure. When the blood was clotted, serum was separated from this sample in the laboratory and frozen within a few hours of blood collection at −20 °C. Analysis of serum vitamin A was completed within a month after blood collection. The samples were processed in a dark room with yellow lighting to prevent retinol isomerization.

Hemoglobin was assessed by the cyanmethemoglobin method as described by Dacie and Lewis (1995). The hematocrit was determined by centrifuging blood in wintrobe tubes (Dacie and Lewis, 1995). Red blood cell count was done by using the Neubauer counting chamber (Dacie and Lewis, 1995). Serum vitamin A was estimated by the Carrprice method (Jayaraman, 1996) using a spectrophotometer (UV double beam spectrophotometer, Shimadzu, Japan). Urinary iodine was measured by using the Sandell–Kolthoff reaction as modified by Pino *et al*. (1996) and readings were read using a spectrophotometer. Hemoglobin was analyzed in duplicate for all the samples and the analysis was repeated if the results differed by >5%. For serum vitamin A, urinary iodine, hematocrit and RBC, 10% of the samples were analyzed in duplicate for validation. In the vitamin A analysis, in the samples where the measurements were measured in duplicate, the average of the two values was calculated and the percentage of deviation from average was measured. The percentage deviation from average was less than 15%.

Statistics

Statistical analysis was done using SPSS 11.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2000 (Microsoft Corp., Seattle, WA, USA). '*t*'-tests and analysis of variance were done to compare the effects of the intervention between groups and over time. Analysis of covariance (ANCOVA) was done for hemoglobin, red cells and serum vitamin A. A multivariate regression analysis was done to compare which biochemical and anthropometric parameters contributed to the change in biochemical and anthropometric parameters in the experimental and control groups.

Results

Characteristics and stability of the fortified salt

When the fortified salt was prepared without vitamin B2, the color was almost as white as the color of raw salt. However, when vitamin B2 was added, buff-colored specks were seen in the salt. The stability of the micronutrients during cooking and storage is shown in Tables 1 and 2.

Efficacy study

Biochemical parameters. At baseline there were no significant differences between the experimental and the control

Table 1 Stability of the multiple fortified salt during cooking

Nutrient	*Concentration before cooking*	*Concentration after cooking for 20 min*	*% loss*
Vitamin A (IU/g)	327.54 ± 10.25	278.73 ± 11.25	14.9
Vitamin B2 (mg/kg)	212.19 ± 10.11	211.57 ± 9.56	0.29
Calcium pantothenate (mg/kg)	206.31 ± 5.13	204.75 ± 4.56	0.75
Niacin (g/kg)	3.05 ± 0.023	3.04 ± 0.051	0.33
Vitamin B1 (mg/kg)	210.8 ± 3.5	209.7 ± 3.41	0.52
Vitamin B6 (mg/kg)	215.2 ± 4.52	214.8 ± 3.85	0.18
Folic acid (mg/kg)	5.01 ± 0.062	5.00 ± 0.039	0.2
Vitamin B12 (μg/kg)	405.3 ± 5.21	404.6 ± 4.82	0.17
Iron (p.p.m)	1114 ± 39.6	1100 ± 43.5	1.25
Iodine (p.p.m)	42.2 ± 2.51	42.18 ± 2.46	0.05

Data given as mean ± s.d.

Table 2 Stability of the multiple fortified salt during storage at 30 °C and 45% relative humidity

Ingredient	*Initial concentration*	*Concentration after 2 months of storage*	*Concentration after 4 months of storage*	*Concentration after 6 months of storage*	*% loss after 6 months*
Vitamin A (IU/g)	314.65 ± 14.8	283.72 ± 11.24	268.66 ± 15.1	211.02 ± 7.02	33
Vitamin B2 (mg/kg)	208.51 ± 7.41	208 ± 8.25	205.9 ± 8.62	205.48 ± 7.23	1.45
Calcium pantothenate (mg/kg)	202.91 ± 3.23	201.1 ± 3.51	202.8 ± 4.1	198.83 ± 5.21	2
Niacin (g/kg)	3.06 ± 0.063	3.03 ± 0.051	3.05 ± 0.023	3.01 ± 0.045	1.6
Vitamin B1 (mg/kg)	208.44 ± 3.81	206 ± 4.25	203.32 ± 3.51	200.19 ± 3.86	4
Vitamin B6 (mg/kg)	209.98 ± 6.27	207.52 ± 5.25	206.51 ± 6.5	205.38 ± 5.51	2
Folic acid (mg/kg)	5.04 ± 0.034	5.02 ± 0.028	5.03 ± 0.059	5.01 ± 0.045	0.6
Vitamin B12 (μg/kg)	408.465 ± 4.71	405.23 ± 5.21	403.5 ± 4.91	404.02 ± 4.53	1
Iron (p.p.m)	1043.37 ± 45.7	1032.5 ± 43.21	1030.5 ± 38.5	1029 ± 35.6	1.3
Iodine (p.p.m)	47.375 ± 3.53	47.2 ± 2.82	46.62 ± 3.41	46.6 ± 2.71	1.6

Data given as mean ± s.d.

Table 3 Biochemical and clinical parameters at baseline and at the end of the study

	Experimental group			*Control group*		
	N	*Baseline*	*Endpoint*	N	*Baseline*	*Endpoint*
Hemoglobin (g/dl)	119	9.631 ± 1.3[a,b]	10.147 ± 0.978[a]	126	10.294 ± 0.732[b,c]	10.085 ± 0.711[c]
Hematocrit (l/l)	119	0.3116 ± 0.0298	0.3127 ± 0.0275[d]	126	0.3092 ± 0.0243[c]	0.2916 ± 0.0239[c,d]
Red blood cells (million/mm^3)	119	3.474 ± 0.223[a,b]	3.94 ± 0.434[a,d]	126	3.814 ± 0.509[b,c]	3.521 ± 0.283[c,d]
Serum vitamin A (μg/dl)	85	35.55 ± 12.9[a,b]	41.11 ± 15.9[a]	71	43 ± 13.73[b]	45.8 ± 19.53
Urinary iodine (μg/l)	119	255 (100–620)[a]	506 (120–600)[a,d]	126	227.5 (5–600)[c]	55 (15–635)[c,d]
Angular stomatitis (%)	119	30.6	0	126	3.3	24.3

Data are given as mean ± s.d. for hemoglobin, hematocrit, red blood cells and serum vitamin A and as median (range) for urinary iodine and as percentage for angular stomatitis.
Significant group*time interaction, $P<0.001$ (ANOVA) for hemaglobin, hematocrit and red blood cells.
[a]Significant increase ($P<0.05$) from baseline to endpoint.
[b]Values differ significantly ($P<0.05$) at baseline between experimental and control groups.
[c]Significant decrease ($P<0.05$) from baseline to endpoint.
[d]Experimental group significantly higher ($P<0.05$) than control group at endpoint.

groups for hematocrit and urinary iodine. However, hemoglobin, red cell count and serum vitamin A were significantly lower in the experimental group than the control group at baseline (see Table 3). These differences at baseline were observed despite the fact that the nutrient intake in both groups was similar. There were eight children in the experimental group and one child in the control group whose baseline hemoglobin was less than 8 g/dl. For ethical reasons, they were treated with ferrous sulfate tablets and excluded from the study. There were no other children who had hemoglobin of less than 8 g/dl in the experimental or control group during midpoint and endline tests for hemoglobin.

Over the intervention period of 1 year, there was a significant improvement ($P<0.05$) in hemoglobin, red cell count, serum vitamin A and urinary iodine in the experimental group. In the control group, on the other hand, there was a significant decrease ($P<0.05$) in hemoglobin, hematocrit, red cell count and urinary iodine (see Table 3).

To assess the impact of the intervention in children of different age groups, the children were divided into two groups, one group comprising children in the age group of 5–10 years and the other group comprising children in the age group of 10.1–15 years. Each group of children was further divided into boys and girls and the impact of the intervention on hemoglobin, hematocrit and red cells in boys and girls of the above two age groups was studied and the data are given in Table 4. Broadly, the same trend in hemoglobin, red cells and hematocrit seen in the whole group of children is seen when the children are divided into different age groups genderwise.

If we consider the percentage prevalence of anemia over the intervention period, we find in the experimental group a distinct reduction in moderate anemia and increase in mild anemia and the nonanemic status, which shows the impact of the iron present in the fortified salt in reducing the prevalence of anemia. On the other hand, in the control group, we see an increase in the prevalence of moderate anemia and a reduction in the prevalence of mild anemia, a clear sign of increase in the prevalence of anemia. These data are given in Table 5.

To find out whether the multiple micronutrient-fortified salt intervention has had any impact, the change (end-point values minus baseline values) of all the biochemical parameters

Table 4 Biochemical and clinical parameters at baseline and at the end of the study, agewise and genderwise analysis

Age and gender of children	*Experimental group*						*Control group*					
	N	*Hemoglobin (g/dl)*		*Red blood cells (million/mm^3)*		*Hematocrit (l/l)*	N	*Hemoglobin (g/dl)*		*Red blood cells (million/mm^3)*		*Hematocrit (l/l)*	
		Baseline	*Endpoint*	*Baseline*	*Endpoint*	*Baseline*	*Endpoint*		*Baseline*	*Endpoint*	*Baseline*	*Endpoint*	*Baseline*	*Endpoint*
Children, 5–10 years	62	9.51 ± 1.44[a,b]	9.98 ± 1.00[a]	3.46 ± 0.24[a,b]	3.97 ± 0.44[a,c]	0.3075 ± 0.0314	0.3087 ± 0.0230[c]	76	10.34 ± 0.63[b,d]	10.12 ± 0.74[d]	3.89 ± 0.52[b,d]	3.48 ± 0.29[c,d]	0.3053 ± 0.0240[d]	0.2845 ± 0.0230[c,d]
Children, 10.1–15 years	57	9.69 ± 0.97[a,b]	10.33 ± 0.92[a]	3.49 ± 0.21[a,b]	3.92 ± 0.43[a,c]	0.3161 ± 0.0276	0.3172 ± 0.0313[c]	50	10.29 ± 0.70[b,d]	10.05 ± 0.65[d]	3.70 ± 0.48[b]	3.58 ± 0.27[c]	0.3162 ± 0.0234[d]	0.3024 ± 0.0218[c,d]
Girls, 5–10 years	43	9.53 ± 1.55[a,b]	9.90 ± 0.94[a]	3.46 ± 0.20[a,b]	3.95 ± 0.47[a,c]	0.3065 ± 0.0260	0.3091 ± 0.025[c]	33	10.26 ± 0.72[b]	10.14 ± 0.78	3.72 ± 0.43[b,d]	3.49 ± 0.26[c,d]	0.3045 ± 0.0214[d]	0.2894 ± 0.0237[c,d]
Boys, 5–10 years	19	9.46 ± 1.22[a,b]	10.15 ± 1.16[a]	3.47 ± 0.32[a,b]	4.01 ± 0.39[a,c]	0.3097 ± 0.0420	0.3079 ± 0.019[c]	43	10.41 ± 0.55[b,d]	10.11 ± 0.73[d]	4.02 ± 0.54[b,d]	3.47 ± 0.31[c,d]	0.3058 ± 0.0263[d]	0.2807 ± 0.0213[c,d]
Girls, 10.1–15 years	24	9.57 ± 0.96[a,b]	10.28 ± 0.75[a,c]	3.42 ± 0.19[a]	4.06 ± 0.45[a,c]	0.3067 ± 0.025[a]	0.3217 ± 0.030[a,c]	25	10.40 ± 0.68[b,d]	9.87 ± 0.62[c,d]	3.64 ± 0.50	3.58 ± 0.24[c]	0.3176 ± 0.0196[d]	0.3000 ± 0.0189[c,d]
Boys, 10.1–15 years	33	9.78 ± 0.98[a]	10.38 ± 1.04[a]	3.53 ± 0.21[a,b]	3.82 ± 0.39[a,c]	0.3139 ± 0.033	0.3230 ± 0.028[c]	25	10.18 ± 0.70	10.24 ± 0.64	3.76 ± 0.47[b]	3.59 ± 0.31[c]	0.3148 ± 0.0269[d]	0.3048 ± 0.0245[c,d]

[a]Significant improvement ($P<0.05$) from baseline to endpoint.
[b]Significant difference ($P<0.05$) between experimental and control group at baseline.
[c]Experimental group significantly higher ($P<0.05$) than control group at endpoint.
[d]Significant decrease ($P<0.05$) from baseline to endpoint.

were compared between the experimental and the control groups. For all biochemical parameters, except vitamin A, the increment or change was statistically higher ($P<0.05$) in the experimental group than the control group (see Table 6).

Since at baseline the experimental group had significantly lower hemoglobin than the control group and at endline there were no statistical differences between the hemoglobin of the experimental and control groups, though there was a statistically significant ($P<0.05$) improvement of hemoglobin in the experimental group and a significant ($P<0.05$) decline in hemoglobin in the control group over 1 year, ANCOVA was done to find out what would be the probable trends in changes in hemoglobin if both the experimental and control groups started at the same hemoglobin level. The mean baseline hemoglobin of the experimental group was 9.63 g/dl and the mean baseline hemoglobin of the control group was 10.29 g/dl. The adjusted mean after the ANCOVA was 9.96 g/dl. The ANCOVA gave insights into what would have been the trend in hemoglobin changes if both the experimental and control groups started with the same mean hemoglobin of 9.96 g/dl. It was observed that in the experimental group hemoglobin would have increased from 9.96 to 10.31 g/dl, which is statistically significant ($P<0.05$). In the control group, hemoglobin would have decreased from 9.96 to 9.93 g/dl, although the change is statistically nonsignificant ($P>0.05$). At the end of the study, the endline hemoglobin of the experimental group (10.31 g/dl) is significantly more than the endline hemoglobin of the control group (9.93 g/dl) ($P<0.05$). Similarly, ANCOVA for RBCs and serum vitamin A was done because of significant differences in these parameters between the experimental and control groups at baseline, and it showed that if the means were adjusted at baseline there was a significant improvement in the experimental group over the control group ($P<0.05$) at endline.

Multivariate regression analysis showed that in the experimental group there was a relationship between the change in hematocrit, hemoglobin, RBCs, height and weight. In the control group, there was no contribution of the change in the biochemical parameters being influenced by anthropometric changes or vice versa. In the control group, there was no contribution of the change in hemoglobin, hematocrit or RBCs influencing each other. This shows that the changes in the experimental group are due to the intervention.

Angular stomatitis. In the experimental group, the prevalence of angular stomatitis due to vitamin B complex deficiencies was 30.6% at the start of the study, which was totally eliminated within a month of the start of the study and did not reappear throughout the whole study period. In the control group, the prevalence of angular stomatitis was 3.3% at the start of the study and increased to 24.3% until the end of the study (see Table 3).

Table 5 Prevalence of anemia % as per WHO criteria[a] in the experimental and control groups at baseline and endline

Prevalence of anemia (%)	*Experimental group*				*Control group*			
	Baseline	*Endline*	*Baseline*	*Endline*	*Baseline*	*Endline*	*Baseline*	*Endline*
	Children 5–11 years of age	*Children 5–11 years of age*	*Children 12–15 years of age*	*Children 12–15 years of age*	*Children 5–11 years of age*	*Children 5–11 years of age*	*Children 12–15 years of age*	*Children 12–15 years of age*
No anemia[b]	3.4 (4)	4.2 (5)	0 (0)	6.8 (8)	0 (0)	0.8 (1)	0 (0)	0 (0)
Mild anemia[b]	30.2 (36)	50.4 (60)	49.6 (59)	62.2 (74)	66.6 (84)	55.6 (70)	71.4 (90)	52.4 (66)
Moderate anemia[b]	66.4 (79)	45.4 (54)	50.4 (60)	31 (37)	33.4 (42)	43.6 (55)	28.6 (36)	47.6 (60)
Total	100 (119)	100 (119)	100 (119)	100 (119)	100 (126)	100 (126)	100 (126)	100 (126)

[a]World Health Organization. Iron deficiency anaemia: assessment prevention and control—a guide for program managers. NHD01.3 edn. WHO: Geneva, 2001.
[b]In children 5–11 years of age, nonanemic status is defined as hemoglobin greater or equal to 11.5 g/dl, mild anemia as hemoglobin 10–11.49 g/dl and moderate anemia as hemoglobin 7–9.99 g/dl. In children 12–15 years of age, nonanemic status is defined as hemoglobin greater or equal to 12 g/dl, mild anemia as hemoglobin 10–11.99 g/dl and moderate anemia as hemoglobin 7–9.99 g/dl. Number of children is given in parentheses.

Vitamin A deficiency. Serum vitamin A was measured only in those children who had clinical manifestations of vitamin A deficiency, that is, 95 children of the experimental group and 78 children of the control group. At baseline, 10 children of the experimental group had serum vitamin A $<20\,\mu g/dl$, the cutoff for vitamin A deficiency. In the control group, seven children had serum vitamin A $<20\,\mu g/dl$. For ethical reasons, children with serum vitamin A $<20\,\mu g/dl$ were treated with tablets of vitamin A and excluded from the study. No more children in the control group or experimental group had serum vitamin A less than $20\,\mu g/dl$ when tested at the end of the study period. The results of serum vitamin A are given in Tables 3 and 6.

Anthropometric measurements. For 111 children from the experimental group and 111 children from the control group, all the anthropometric measurements could be made. The rest of the children were absent for one or more of the measurements.

There were no significant differences between baseline heights and weights of the experimental and the control groups. However, at the end of the study, the children of the experimental group were significantly taller than the control children (see Table 7). Weight was not significantly different between the experimental and the control groups at the end of the study. However, if the increment in weight during the study was considered, we found that there was a significant improvement in the experimental group (see Table 8).

If the children are divided into two groups of 5- to 10-year-olds and 10.1-to 15-year-olds and analyzed for change in heights and weights in 1 year, it can be seen that only in children 10.1–15 years of age was there a significant improvement ($P<0.05$) in the gain in height and weight in the experimental group when compared to the control group. This may be due to the adolescent spurt in growth taking place in this age. There is no significant difference in the gain in height and weight in children in the 5–10 years age group between the experimental and control groups. These data are given in Table 8. The stunted and underweight children of the experimental group significantly ($P<0.05$) gained more height and weight than the control group during the study (see Table 7).

Discussion

Many other studies have used only inorganic iron compounds without biopromotors (Mannar and Diosady, 1998; Sivakumar *et al*., 2001; Zimmermann *et al*., 2004). Zimmermann *et al*. (2003) have used ferrous sulfate encapsulated with hydrogenated soybean oil. This double-fortified salt developed yellow coloration when the moisture of the salt was 3–4%, although the intervention with this salt was able to increase mean hemoglobin by 14 g/l. Another study tested the stability of 16 forms of encapsulated iron in salts of

Table 6 Change in the biochemical parameters during the study over 12 months

Biochemical parameters	*Experimental group*		*Control group*	
	N	*Change (end point value minus baseline value)*	N	*change (end point value minus baseline value)*
Hemoglobin (g/dl)	119	0.550 ± 1.04[a]	126	−0.226 ± 0.728[a]
Hematocrit (l/l)	119	0.001134 ± 0.0308[a]	126	−0.01787 ± 0.0244[a]
Red blood cells (million/mm^3)	119	0.470 ± 0.475[a]	126	−0.290 ± 0.567[a]
Serum vitamin A (μg/dl)	85	5.56 ± 19.6	71	2.82 ± 19.34
Urinary iodine (μg/l)	119	212.2 ± 178.7[a]	126	−166.94 ± 167.5[a]

Data given as mean ± s.d.
[a]Group mean significantly higher in the experimental group than the control group ($P<0.05$).

Table 7 Anthropometric measurements during the study (over 1 year)

Parameter	*Experimental group*				*Control group*			
	N	*Baseline*	N	*Endpoint*	N	*Baseline*	N	*Endpoint*
Height (cm)	111	124.87 ± 11.35	111	131.131 ± 12.55[a]	111	125.42 ± 13.88	111	127.158 ± 14.18[a]
Weight (kg)	111	23.44 ± 5.6	111	26.6 ± 6.95	111	22.57 ± 7.89	111	25.29 ± 8.86
Children's height less than −2 s.d. (stunted), height (cm)	37	124.02 ± 11.31	37	131.2 ± 12.6[a]	18	118.16 ± 11.04	18	122.4 ± 11.44[a]
Children's weight less than −2 s.d. (underweight), weight (kg)	25	21.82 ± 5.51	25	25.1 ± 7.43[a]	42	19.23 ± 4.51	42	21.6 ± 5.3[a]

Data given as mean ± s.d.
[a]Values of the experimental group significantly higher ($P<0.05$) than the control group at endpoint.

Table 8 Mean change in anthropometric parameters in 1 year, agewise and genderwise analysis

Age and gender of children	*Experimental group*			*Control group*		
	N	*Change in height (cm)*	*Change in weight (kg)*	N	*Change in height (cm)*	*Change in weight (kg)*
Whole group	111	6.26 ± 2.91[a]	3.17 ± 2.11[a]	111	4.68 ± 1.91[a]	2.78 ± 1.49[a]
Children, 5–10 years	57	5.33 ± 2.82[b]	2.29 ± 1.07[b]	71	4.85 ± 2.03[b]	2.06 ± 1.04[b]
Children, 10.1–15 years	54	7.24 ± 2.69[a]	4.33 ± 2.32[a]	40	4.40 ± 1.66[a]	3.64 ± 1.75[a]
Girls, 5–10 years	38	5.32 ± 3.37[b]	2.34 ± 1.27[b]	32	4.75 ± 2.78[b]	2.07 ± 1.10[b]
Boys, 5–10 years	19	5.35 ± 1.20[b]	2.24 ± 0.88[b]	39	4.92 ± 1.13[b]	2.05 ± 0.94[b]
Girls, 10.1–15 years	22	7.49 ± 2.14[a]	5.20 ± 1.86[a]	21	4.41 ± 1.76[a]	3.95 ± 1.94[a]
Boys, 10.1–15 years	32	7.07 ± 3.04[a]	3.73 ± 2.44[b]	19	4.38 ± 1.59[a]	3.29 ± 1.48[b]

[a]Significant improvement in the experimental group when compared to the control group ($P<0.05$).
[b]No significant difference between the experimental and control groups.

North and West Africa, and the authors found that the encapsulated ferrous iron caused unacceptable color changes in the salt (Wegmuller *et al*., 2003). The authors assumed that this might be because current encapsulating technology uses hydrogenated plant oils, which do not sufficiently prevent moisture penetration and because iron solubility and capsule integrity are further compromised by mechanical abrasion during salt mixing. The capsules also melt at 45–50 °C and may cause unwanted sensory changes during food preparation (Wegmuller *et al*., 2003). In our study, we have also used ferrous sulfate as the iron source but have chelated it with malic acid and sodium hexa meta phosphate and have added sodium dihydrogen phosphate as a biopromotor. Our studies showed that when the iron is well chelated, it does not react with the salt to produce discolorations and does not produce sensory changes. We feel that chelated ferrous sulfate with biopromotors has a higher bioavailability but without the problems caused by ferrous sulfate, such as coloration of the salt or the food during cooking. If a high-quality salt is used with low calcium and magnesium content, then the chelated iron compound can be directly added to the salt and no microencapsulation is required. However, if it has to be added to salt with high magnesium, calcium or moisture content, then microencapsulation of the iron compound with compounds like glyceryl stearate or edible waxes would be preferable. In an earlier bioefficacy study on double-fortified salt, we have found an improvement in hemoglobin in tea pickers along with an improvement in productivity (Rajagopalan and Vinodkumar, 2000).

The bioavailability of all the fortificants used in this study has been established extensively in the past when the fortificants have been delivered as supplements—as tablets or liquid formulations. What has not been done in the past and what has been established in this study is the bioavailability of these fortificants when they are used to fortify common salt, which is used in cooking. The fortificants have to be stable in salt and during the cooking process. Most micronutrients were stable in the salt during cooking and storage. Only vitamin A concentration dropped during cooking and storage but with microencapsulation and adding of overages, its shelf life can be extended considerably. Still, the fortified salt has increased serum retinol levels in the experimental group, and is therefore effective. The B complex vitamins and iodine were very stable during cooking and storage.

One limitation of this study is that the experimental group of children is in the residential school and the control group of children resides in communities near the school. In this study, we have observed a drop in all biochemical parameters in the control group. The drop in iron in the control group might have been due to the reduced availability of iron from food during the growing age when iron requirement is needed most. Similar drops in iron levels in control groups of schoolchildren have been seen in other studies (Madhavan Nair *et al*., 1998; Zimmermann *et al*., 2004). The drop in urinary iodine values may be due to the interruption in the use of iodized salt by families who were previously consuming it. This issue was brought to the notice of the communities and the people were advised to consume iodized salt only.

Several factors contributed to the improvement of the micronutrient status of the children in this study. We provided adequate quantities of micronutrients through salt. The average consumption of salt was 10 g per day, and the children of the experimental group consumed three meals per day and an afternoon snack, and all the food was prepared with fortified salt. The micronutrient delivery took place in repeated small doses throughout the day, which enhances micronutrient absorption, as fractional absorption of non-heme iron increases with decreasing doses (Skikne and Baynes, 1993). In this region, anemia is due to low dietary iron bioavailability and not due to increased iron losses. Malaria is not a problem and neither is hookworm infestation. However, infection due to other helminthes such as ascaris is quite common and we had controlled it in our study by periodic deworming with albendazole.

The results of anthropometry are similar to other studies, which have shown an improvement in height and weight of children due to multiple micronutrient supplementation (Rivera *et al*., 2001; Yang *et al*., 2002).

The experimental residential group of children received a fixed menu throughout the year, irrespective of the seasonal variations, but the diet of the control group of children depended a lot on what was growing in the fields around their homes. The prevalence of angular stomatitis, therefore, fluctuates between 3.3 and 24.3% as seen in the control group. This may be due to the seasonal variations in the types of food consumed over 1 year in the control group. If a dietary survey had been done in different seasons, a better picture of the nutrient status due to seasonal variations could have been found out, but the dietary survey was done only at baseline. This may be another limitation of the study. Angular stomatitis may be due to infections when it responds to topical applications of antibiotics or gentian violet, as in earlier studies (Bamji *et al*., 1979) or due to micronutrient deficiencies (Blanck *et al*., 2002). Since in our study angular stomatitis disappeared when fortified salt was used, it may be assumed that the cause of angular stomatitis in our study might have been micronutrient deficiencies and not infections.

This study was carried out in a controlled atmosphere of a residential school. It needs to be repeated in the form of larger field trials in various communities.

In conclusion, we have shown a significant improvement in serum retinol status, iron status and iodine status in the experimental group. The effect of B complex vitamins was seen by the elimination of angular stomatitis. Thus, we feel that salt can be an effective carrier for multiple micronutrients and be used as an effective strategy to combat micronutrient malnutrition in developing countries.

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