Community-level micronutrient fortification of a food supplement in India: a controlled trial in preschool children aged 36-66 mo[1–3]

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ABSTRACT

Background: Children participating in the Integrated Child Development Service (ICDS) in India have high rates of iron and vitamin A deficiency.

Objective: The objective was to assess the efficacy of a premix fortified with iron and vitamin A and added at the community level to prepared khichdi, a rice and dal mixture, in increasing iron and vitamin A stores and decreasing the prevalence of iron deficiency, anemia, and vitamin A deficiency.

Design: This cluster, randomized, double-blind, controlled trial was initiated in 30 Anganwadi centers (daycare centers) in West Bengal state, India. Children aged 36–66 mo ($n = 516$) attending village-based ICDS centers were randomly assigned to receive either a fortified or a nonfortified premix for 24 wk. Blood was drawn at 0 and 24 wk by venipuncture for the measurement of hemoglobin, serum ferritin, and serum retinol.

Results: The change in the hemoglobin concentration of anemic children was significantly different between fortified and nonfortified khichdi groups ($P < 0.001$). Prevalence rates of anemia, iron deficiency, and iron deficiency anemia were significantly lower after 24 wk in the fortified-khichdi group than in the nonfortified-khichdi group ($P < 0.001$). There were no significant differences in serum retinol concentrations or in the prevalence of vitamin A deficiency between the fortified- and nonfortified-khichdi groups.

Conclusion: A premix fortified with iron, vitamin A, and folic acid and added to supplementary food at the community level can be effective at increasing iron stores and reducing the prevalence of iron deficiency and anemia. *Am J Clin Nutr* 2007;85:1127–33.

KEY WORDS Iron deficiency, vitamin A deficiency, fortification, anemia, India, children

INTRODUCTION

Deficiencies in the essential micronutrients vitamin A and iron are major public health concerns in India. The prevalence of iron deficiency anemia in India has been estimated to be as high as 74% in preschool-age children (1). National prevalence data for subclinical vitamin A deficiency in this age group does not exist, but regional prevalence rates range from 26.3% in New Delhi to 80.1% in Hyderabad (2). Existing micronutrient programs in India target the distribution of iron tablets and high-dose vitamin A syrup to preschool-age children. These programs, in place since the 1970s, have had mixed results because of inadequate coverage and compliance (3).

The Integrated Child Development Service (ICDS) is the largest child nutrition program in the world, with 32 million children aged 3–6 y enrolled in 568 888 Anganwadi centers (daycare centers) distributed throughout India (NC Saxena and J Ravi, unpublished observations, 2005). A major focus of the ICDS is the distribution of a food supplement, often khichdi (a rice and lentil mixture), to these children. This supplement provision aims to improve the health and nutritional status of the children while relieving short-term hunger (4). Each 200-g serving of khichdi provides sufficient macronutrients (≈300 kcal and 8 g protein per serving); however, it lacks adequate iron and vitamin A.

The addition of a fortified premix to the ICDS supplementary nutrition program would be unique because the fortification would occur at the community level in Anganwadi centers. Most fortification programs combine either a fortified premix with the target food vehicle at a central processing factory or provide a fortified premix directly to the household (5–8). Fortification at the Anganwadi center is advantageous because instills ownership of the fortification program in the village community and demystifies the fortification process. The use of locally consumed food vehicles, such as khichdi, in community-level nutrition programs has been shown to increase the acceptability, sustainability, and adherence to the program while often generating employment for local women (9). To determine the efficacy of this local fortification of khichdi with encapsulated ferrous fumarate and vitamin A, we examined the effect of fortified khichdi on hemoglobin, serum ferritin, and serum retinol concentrations and on prevalence rates of anemia, iron deficiency, vitamin A deficiency, and low vitamin A status in children aged 3–6 y.

SUBJECTS AND METHODS

Study sites

This study was conducted in Anganwadi centers throughout Mahestala block in South 24 Parganas, West Bengal. Mahestala block is a semiurban area ≈60 km south of Kolkata. There is an

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average of 1 Anganwadi center per 1000 person in the general population and an average of 20–30 children per center. Prevalence rates of malaria, hookworm, and other intestinal parasites are low in this section of West Bengal (10).

Study design

This was a double-blind, cluster, randomized trial. Cluster randomization was used because it was determined that the separate preparation of the fortified and nonfortified premixes and separation of randomly assigned children in each Anganwadi center was impractical and would overburden the Anganwadi worker. Enrollment forms and Anganwadi monitoring forms were used to determine eligible centers. To be eligible, a center had to have >20 regularly attending children and a regular supply of rice and lentils from the ICDS, and Anganwadi personnel needed to be willing to participate. Participating centers ($n = 30$) were randomly assigned with the use of a random number table to receive either fortified khichdi or nonfortified khichdi. All attending children received a single 200-g portion of the khichdi treatment assigned to their Anganwadi center 6 times/wk for 24 wk.

The fortified premix has a dextrose anhydrous base prepared by Nicolas Piramals India Ltd (Mumbai, India). For each 200-g serving of khichdi, the premix provides 14 mg encapsulated ferrous fumarate, 500 IU vitamin A (retinyl acetate: particle size of 250; cold water soluble), and 0.05 mg folic acid. The placebo premix contained only dextrose anhydrous. Both premixes were packed in resealable polyethylene bags in 500-g increments. Each selected Anganwadi center received 500 g premix at baseline and after 3 mo of the intervention.

Study population

The subjects were children aged 36–66 mo from the 30 selected Anganwadi centers. Exclusion criteria included severe anemia (hemoglobin concentration <80 g/L) and a history of not attending the Anganwadi center ≥5 times/wk during the past 6 mo. Children with severe anemia were treated with a therapeutic course of oral iron. Children with severe vitamin A deficiency were not removed from the study. Consent for the study was obtained first from local Panchayet (village council) leaders and West Bengal public health officials. Informed consent for participation in the study was obtained from the mother or legal guardian of participating children in the study at enrollment. The study was approved by the Tufts University Health Science/Tufts–New England Medical Center Institutional Review Board.

Sample size

Assuming an estimated change in hemoglobin concentration of 0.6 g/L, an SD of 1.66, an α value of 0.05, and a β value of 0.20, 120 children per group would be required (11). A design effect of 2 was included to account for the cluster randomized design (12). This number was increased by 45%, to account for loss to follow up, for a total of 696 children enrolled.

Data collection

Height and weight were measured at 0 wk and 24 wk by using standardized techniques (13). At 0 and 24 wk, 5 mL whole blood was collected from enrolled children by venipuncture for the measurement of hemoglobin, serum ferritin, serum retinol, and C-reactive protein (CRP). Blood was collected by finger prick for the analysis of hemoglobin at 12 wk.

Training of Anganwadi workers and Panchayet monitors

Anganwadi workers from enrolled centers participated in a half-day training session before the initiation of the study. In training, Anganwadi workers were taught proper storage procedures for the fortified premix to ensure that the premix was not exposed to excessive light or high humidity. Anganwadi workers were also taught proper preparation techniques, ie, they were instructed to thoroughly mix the premix with the khichdi after the khichdi had cooled for 10 min to ensure a homogeneous mixture. The maintenance of monitoring forms was also reviewed during training. The Panchayet monitors attended a half-day training session at which they received instructions on the collection of program monitoring forms for the study.

Monitoring forms

Anganwadi workers maintained monitoring forms to record the daily attendance of enrolled children for receipt of the khichdi. Workers also recorded any vitamin A supplement use by the children during the intervention period. The Panchayet monitors collected weekly monitoring forms from 2 Anganwadi centers for the preparation of khichdi, the storage of the premix, and the amount of khichdi consumed by each enrolled child. All monitoring forms were collected by ICDS local supervisors and reviewed by ICDS Child Development Program Officers.

After 2 wk of the intervention, the monitoring forms indicated that 85% of Anganwadi workers were experiencing minor problems with the packaging of the premix, including breakage of the polyethylene bag and failure of the bag to properly seal. In an effort to reduce exposure of the premix, all Anganwadi workers were asked to place any torn or broken bags of premix into clean polyethylene containers provided by the ICDS.

Demographic, economic, and health survey

Mothers or legal guardians of enrolled children were surveyed at baseline and at the end of the study to assess their child's demographic, economic, and health status. The survey was based on the National Family Health Survey developed by the International Institute of Population Sciences in Mumbai, India (1). Enumerators carried out the entire survey at baseline and then repeated the economic and health sections at 24 wk. Health data collected in the survey included the history of fever, abdominal pain, blood in the stool, and cough occurring anytime in the 2 wk before the survey.

Laboratory analysis

Immediately after blood was collected, a few drops were placed into a separate vial for the measurement of hemoglobin with an AcT8 counter (Beckman Coulter, Krefeld, Germany) (14). The remaining collected blood was allowed to clot at room temperature and centrifuged at 3000 × g for 5 min. The serum was collected and transported to the Molecular Diagnostics Laboratory in Lucknow for further analysis. Serum ferritin was measured by enzyme-linked immunosorbent assays (RAMCO, Houston, TX) (15). CRP was measured by using nephelometry (TURBOX; Orion Diagnostics, Espoo, Finland) (16) and serum retinol by HPLC (17).

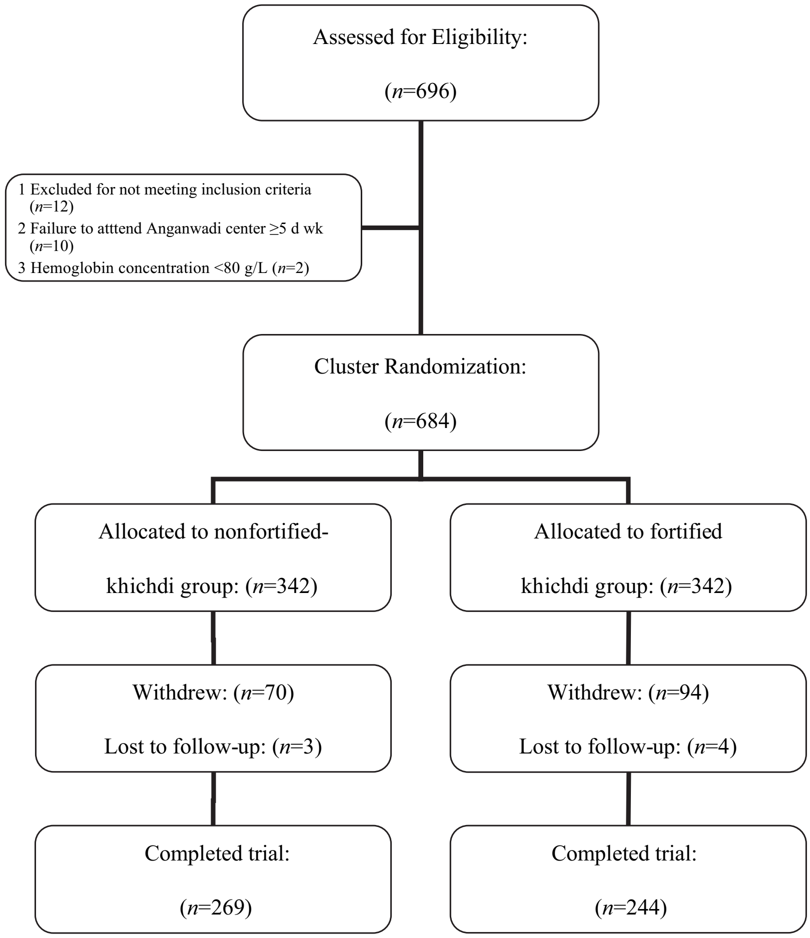

FIGURE 1. Enrollment procedure for the fortified-khichdi group.

Study definitions

Anemia was defined as a hemoglobin concentration <110 g/L, iron deficiency as a serum ferritin concentration <15 μg/L, and iron deficiency anemia as a hemoglobin concentration <110 g/L and a serum ferritin concentration <15 μg/L. A CRP concentration >10 mg/L was considered to be elevated. Vitamin A deficiency was defined as a serum retinol concentration <0.70 μmol/L, and low vitamin A status was defined as a serum retinol concentration >0.70 but <1.05 μmol/L.

Statistical analyses

Data processing and statistical analysis were performed with the use of SPSS 13.0 (SPSS Inc, Chicago, IL) and EXCEL (XP2002; Microsoft, Seattle, WA). When data were normally distributed, 2-factor repeated-measures analysis of variance was done to compare the effects of group × time for hemoglobin, serum retinol, CRP, and serum ferritin. If the interaction effect was significant ($P < 0.05$), Student's *t* tests between groups and paired Student's *t* tests within groups were conducted and adjusted for multiple comparisons by using a Bonferroni correction. Log transformation was used for serum ferritin, which was not normally distributed. Binary logistic regression was done to compare the effects of group × time for anemia, iron deficiency, iron deficiency anemia, vitamin A deficiency, and low vitamin A status. Proportions were compared by using Pearson's chi-square test. Significance was set at $P < 0.05$.

During the course of the study, 3 children received vitamin A supplementation and were removed from the analysis. No children received iron supplementation.

RESULTS

Baseline characteristics

In April 2005, 684 children were screened and enrolled in 30 Anganwadi centers (**Figure 1**). Of the children enrolled, 168 (24.5%) were lost to follow-up before the 24-wk assessment; thus, 516 completed the 24-wk trial. Reasons for loss to follow-up were refusal of further venipuncture ($n = 161$), change of location ($n = 5$), and low attendance at the Anganwadi center ($n = 2$). Most of the characteristics of the children who dropped out of the study did not differ significantly from those of the children who completed the trial, including age, sex, iron status, and mean hemoglobin concentration. However, the prevalence of anemia was significantly greater in the children lost to follow-up (35.1%) than in those who completed the trial (26.2%) ($P < 0.05$).

Age, sex, weight, and height did not differ significantly between the 2 treatment groups (**Table 1**). The hemoglobin, serum retinol, and CRP concentrations were significantly different at baseline between the fortified-khichdi ($n = 246$) and nonfortified-khichdi ($n = 270$) groups. Children in the

TABLE 1
Baseline characteristics of the nonfortified (NF) and fortified (F) groups

	NF (n = 130 F, 139 M)	F (n = 133 F, 113 M)	P^1
Age (y)	3.9 ± 0.6[2]	3.9 ± 0.7	0.322
Weight (kg)	12.1 ± 1.7	12.3 ± 1.7	0.146
Height (cm)	93.0 ± 5.5	94.3 ± 6.0	0.140
C-reactive protein (mg/L)	3.3 ± 4.0	2.2 ± 3.1	0.001
Hemoglobin (g/L)	120 ± 18	124 ± 15	0.003
Serum ferritin (μg/L)[3]	26.3 ± 2.0	25.1 ± 1.9	0.573
Anemia [% (n)][4]	32.6 (88)	19.1 (47)	<0.001
Iron deficiency [% (n)][5]	20.7 (56)	22.5 (55)	0.655
Iron deficiency anemia [% (n)][6]	9.6 (26)	4.9 (12)	0.039
Serum retinol (μg/L)	1.23 ± 0.51	1.10 ± 0.44	0.003
Vitamin A deficiency [% (n)][7]	13.0 (35)	17.5 (43)	0.174
Low vitamin A status [% (n)][8]	40.8 (110)	47.9 (117)	0.120

[1] Two-factor repeated-measures ANOVA and binary logistic regression.
[2] $\bar{x}$ ± SD (all such values, except where indicated otherwise).
[3] All values are geometric $\bar{x}$ ± 1 SD.
[4] Defined as hemoglobin < 110 g/L.
[5] Defined as serum ferritin < 12 μg/L.
[6] Defined as hemoglobin < 110 g/L and serum ferritin < 12μg/L.
[7] Defined as serum retinol < 0.70 μg/L.
[8] Defined as 0.70 > serum retinol <1.05 μg/L.

nonfortified-khichdi group had a lower hemoglobin concentration (P = 0.003) and a higher prevalence of anemia (P < 0.001) than did children in the fortified-khichdi group. When the children were separated by anemia status, we observed that the mean hemoglobin concentration of the anemic children was not significantly different between the 2 groups (99.8 ± 1.3 compared with 98.9 ± 0.8 g/L). The serum retinol concentration was greater in the nonfortified-khichdi group (P = 0.003); however, the prevalence of vitamin A deficiency and low vitamin A status were not significantly different (Table 1).

There were significant differences in the prevalence of elevated CRP (P = 0.008) and the CRP concentration (P = 0.001) between the 2 groups (Table 1). Children with an elevated CRP concentration did not have a higher prevalence of recent fever, abdominal pain, bloody stool, or cough (data not shown), and no significant difference in these factors was found between the fortified- and nonfortified-khichdi groups.

Compliance

Compliance was high among enrolled children. Children in the fortified and nonfortified groups, on average, received the khichdi 90.2% and 89.3% of the days, respectively, during the study period. According to the monitoring reports, 98.9% of the enrolled children consumed 100% of the khichdi at each sitting. Weekly collected monitoring forms showed that the Anganwadi workers had no difficulties with the preparation or storage of the khichdi at the Anganwadi centers once the initial packaging problems were resolved. The workers strictly followed training instructions on the preparation and storage of the fortified premix.

Efficacy trial

As shown in **Table 2**, there was no significant difference in the mean hemoglobin concentrations of the participants in the 2

TABLE 2
Hemoglobin, hemoglobin in anemic children, and serum ferritin and serum retinol concentrations in fortified (F; n = 244) and nonfortified (NF; n = 269) groups over 24 wk

	Hemoglobin (g/L)		Hemoglobin in anemic children (g/L)[1]		Serum ferritin (μg/L)[1]		Serum retinol (μg/L)	
Time	NF	F	NF	F	NF	F	NF	F
0 wk	120 ± 18[2]	124 ± 15	98.9 ± 7.2	99.9 ± 9.1	25.7 ± 2.0[3]	25.1 ± 1.9	1.2 ± 0.5	1.1 ± 0.4
12 wk	124 ± 12	127 ± 12	116.2 ± 9.8	116.6 ± 11.2				
24 wk	124 ± 14	128 ± 11	109.9 ± 10.3[4]	116.9 ± 10.3[4,5]	22.9 ± 2.4[4]	35.5 ± 2.1[6,7]	1.4 ± 0.5	1.4 ± 0.4

[1] Significant treatment × time interaction, P < 0.01 (repeated-measures ANOVA).
[2] $\bar{x}$ ± SD (all such values, except where indicated otherwise).
[3] Geometric $\bar{x}$ ± 1 SD.
[4,6] Significantly different from baseline: [4] P < 0.05, [6] P <0.01.
[5,7] Significantly different from NF group: [5] P < 0.05, [7] P < 0.001.

TABLE 3
Prevalence of anemia, iron deficiency, iron deficiency anemia, vitamin A deficiency, and low vitamin A status in nonfortified (NF; $n = 269$) and fortified (F; $n = 244$) groups at 0 and 24 wk

	Anemia[1,2]		Iron deficiency[2,3]		Iron deficiency anemia[2,4]		Vitamin A deficiency[5]		Low vitamin A status[6]	
Time	NF	F	NF	F	NF	F	NF	F	NF	F
	n (%)		*n (%)*		*n (%)*		*n (%)*		*n (%)*	
0 wk	88 (32.6)	47 (19.1)[7]	56 (20.7)	55 (22.5)	26 (9.6)	12 (4.9)[7]	35 (13.0)	43 (17.5)	110 (40.8)	117 (47.9)
12 wk	36 (13.3)[8]	24 (9.8)[8]								
24 wk	56 (20.7)[8]	10 (4.1)[8,9]	82 (30.4)[8]	25 (10.2)[8,9]	25 (9.3)	1 (0.4)[8,9]	17 (6.3)	20 (8.1)	55 (20.4)	52 (21.5)

[1] Defined as hemoglobin < 110 g/L. Significant difference between time-and-group model and time-only model, $P < 0.01$ (binary logistic regression). Significant difference between time-and-group model, $P < 0.001$ (binary logistic regression).
[2] Significant treatment × time interaction, $P < 0.01$.
[3] Defined as serum ferritin < 15 μg/L.
[4] Defined as hemoglobin < 110 g/L and serum ferritin < 12 μg/L.
[5] Defined as serum retinol < 0.70 μg/L.
[6] Defined as 0.70 > serum retinol < 1.05 μg/L.
[7,9] Significantly different from NF group: [7] $P < 0.05$, [9] $P < 0.01$.
[8] Significantly different from baseline, $P < 0.01$.

groups between baseline and the end of the study. Because of a significant interaction between anemia, treatment group, and time ($P < 0.01$), a subgroup analysis was performed in the children with anemia. Hemoglobin concentrations in the anemic children increased significantly in the fortified group when compared with the nonfortified group from weeks 0 to 24 ($P = 0.04$). After 24 wk, serum ferritin significantly increased in all participants in the fortified group, and the change in serum ferritin was significantly different from that of the nonfortified group ($P < 0.001$). Although serum retinol increased significantly in both groups during the study period, there was no significant difference in serum retinol between the 2 groups at the end of the intervention.

As shown in **Table 3**, the prevalence of anemia, iron deficiency, and iron deficiency anemia were significantly lower after 24 wk in the fortified group ($P < 0.001$). A continued reduction trend in anemia was found in the fortified group while prevalence of anemia in the non-fortified group increased between weeks 12 and 24. The prevalence of iron deficiency decreased significantly in the fortified group, and the change in iron deficiency was significantly different from the nonfortified group after the 24-wk intervention ($P < 0.001$). The prevalence of iron deficiency anemia was nearly eliminated in the fortified group, whereas it remained almost unchanged in the nonfortified group ($P < 0.001$). There was no significant difference in the prevalence of vitamin A deficiency or low vitamin A status between the nonfortified and fortified groups after 24 wk of intervention.

DISCUSSION

This study clearly showed that khichdi locally fortified with an encapsulated ferrous fumarate and vitamin A premix was efficacious in improving iron status and reducing the prevalence of anemia, iron deficiency, and iron deficiency anemia in West Bengal preschool children. Although previous studies have reported the efficacy of iron-fortified salt, fish sauce, sugar, beverages, and complementary food, this study was unique in that it used an existing targeted supplementary nutrition program to provide community-level fortification. The addition of a fortified premix to prepared khichdi in the ICDS Anganwadi centers provides an excellent opportunity to prevent and reduce micronutrient deficiencies in at-risk children without the additional costs associated with the development and implementation of a new program infrastructure.

The fortified khichdi significantly increased iron stores, despite the low absorption of dietary iron from the khichdi and the utilization of no additional absorption promoter in the fortified premix. The estimated iron absorption from the fortified khichdi was ≈4%, which was due to the high content of phytic acid, low consumption of ascorbic acid, and absence of heme iron in the khichdi (18). Although this level of iron absorption is not different from that estimated in other studies, we chose not to integrate EDTA or ascorbic acid into the fortified food vehicle to enhance the absorption of available iron. Although beneficial, EDTA and ascorbic acid add to the expense of the fortified premix, and losses of ascorbic acid would be unacceptably high under the normal storage conditions of the fortified premix used in this study (19). The increase in iron stores of the fortified-khichdi group may have been facilitated by the estimated low prevalence of malaria and severe helminth infection in the study population, which would keep iron losses low (10, 20).

Cluster randomization was largely effective in this study, although there were baseline differences in hemoglobin concentration, serum retinol concentration, and the prevalence of anemia between the 2 groups. The mean hemoglobin concentration was significantly higher in the fortified-khichdi group, whereas the mean serum retinol concentration and the prevalence of anemia were higher in the nonfortified group. The demographic, economic, and health survey results indicated that the study population was remarkably homogeneous, and no significant differences between the fortified and nonfortified groups were observed in any of the measures (data not shown). The baseline differences in hemoglobin concentration, serum retinol concentration, and prevalence of anemia may have resulted because of the high dropout in the study (24.6%). The dropout was unevenly distributed between the fortified and nonfortified groups—a higher proportion of children who dropped out of the study were from the fortified group (28% compared with 21%). The dropout children from the fortified group had a lower mean hemoglobin concentration at baseline than did the children in the fortified group enrolled in the study. Although significantly different, the mean hemoglobin concentrations of both treatment groups were well above 110 g/L, and both groups had a lower prevalence of anemia than expected for this population. Although the prevalence of anemia was significantly greater in the nonfortified group, the hemoglobin concentrations in the anemic children were not different between groups. This finding indicates that the severity of anemia was not different between groups, but that the nonfortified group included more children who were anemic. This may have been attributed to the large dropout in the study and the unequal distribution of dropouts in the fortified group.

The hemoglobin concentration was relatively high in both the fortified and nonfortified groups at baseline, and the prevalences of anemia and iron deficiency anemia were lower than in previous studies (1, 21, 22). This may be explained by the exclusion of children under the age of 2 y in this study. Almost all previous reports on the prevalence of anemia and iron deficiency anemia in India included children aged 0–60 mo inclusive. The inclusion of children aged <24 mo skewed the prevalence data downward because this age group is at a much greater risk of anemia and iron deficiency anemia than are slightly older children. This greater risk was attributed to a rapidly expanding blood volume, which requires high levels of iron for erythropoiesis (23). Children older than 24 mo experience a dramatic decrease in their growth rate and, therefore, have lower requirements for iron. India-wide research by the Indian Council Medical Research corroborates our findings in their 1997 report, which found the prevalence of anemia to be 63% in children aged 1–3 y and 44% in children aged 3–5 y (24).

The failure of the fortified khichdi to increase serum retinol concentrations or to reduce the prevalence of vitamin A deficiency and low vitamin A status might have resulted because of the deterioration of vitamin A in the fortified premix. Despite the provision of resealable polyethylene bags and plastic containers, neither prevented light exposure of the premix. Because of the length of the duration of light exposure, it may be assumed that losses of vitamin A were very high during the intervention. The abundance of inexpensive fresh fruit and vegetables during the study period might also have accounted for the increase in serum retinol in both study groups. The provision of proper packaging for the fortified premix was expected to increase the fortified premix's ability to increase serum retinol concentrations and decrease vitamin A deficiency and low vitamin A status. Further studies to determine the efficacy of the fortified premix after the provision of proper packaging are needed.

The addition of an encapsulated ferrous fumarate– and vitamin A–fortified premix to the ICDS Anganwadi khichdi program was efficacious in reducing iron deficiency and anemia in preschool-age children. The use of locally fortified khichdi can therefore be considered an effective means of addressing both macro- and micronutrient malnutrition in preschool-age children in India. Its high adaptability suggests that it also would be an effective means of meeting the micronutrient needs of pregnant and lactating women and of younger children who are consuming solid food. Given its integration with the existing ICDS food-supplementation program, expansion of such local fortification would be relatively simple to implement and inexpensive. The fortification of khichdi presents a locally acceptable and self-sustaining micronutrient program, compared with the distribution of iron and vitamin A supplements, and an effective alternative to home fortification (25). The addition of a fortified premix to khichdi in ICDS Anganwadi centers provides an excellent opportunity to provide the needed micronutrients to children with or at risk of micronutrient deficiencies throughout India.

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JLV, RS, MGVM, FJL, and DHH designed the study. JLV and SD conducted the fieldwork, collected the data, and performed the final laboratory and data analysis. JLV conducted the statistical analysis and wrote the first draft of the manuscript. FJL and DHH edited the manuscript. None of the authors had any financial or personal conflicts of interest in regard to this study.

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