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Iron Fortification of Whole Wheat Flour Reduces Iron Deficiency and Iron Deficiency Anemia and Increases Body Iron Stores in Indian School-Aged Children[1–4]

Sumithra Muthayya,[5,9]* Prashanth Thankachan,[5] Siddhivinayak Hirve,[6] Vani Amalrajan,[5] Tinku Thomas,[5] Himangi Lubree,[7] Dhiraj Agarwal,[6] Krishnamachari Srinivasan,[5] Richard F. Hurrell,[8] Chittaranjan S. Yajnik,[7] and Anura V. Kurpad[5]

[5]Division of Nutrition, St. John's Research Institute, St. John's National Academy of Health Sciences, Bangalore, India; [6]Vadu Rural Health Program, and [7]Diabetes Unit, King Edward Memorial Hospital Research Centre, Pune, India; and [8]Human Nutrition Laboratory, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology, Zurich, Switzerland

Abstract

Wheat is the primary staple food for nearly one-third of the world's population. NaFeEDTA is the only iron (Fe) compound suitable for fortifying high extraction flours. We tested the hypothesis that NaFeEDTA-fortified, whole wheat flour reduces Fe deficiency (ID) and improves body Fe stores (BIS) and cognitive performance in Indian children. In a randomized, double-blind, controlled, school feeding trial, 6- to 15-y-old, Fe-depleted children (n = 401) were randomly assigned to either a daily wheat-based lunch meal fortified with 6 mg of Fe as NaFeEDTA or an otherwise identical unfortified control meal. Hemoglobin (Hb) and Fe status were measured at baseline, 3.5 mo, and 7 mo. Cognitive performance was evaluated at baseline and 7 mo in children (n = 170) at one of the study sites. After 7 mo, the prevalence of ID and ID anemia in the treatment group significantly decreased from 62 to 21% and 18 to 9%, respectively. There was a time x treatment interaction for Hb, serum ferritin, transferrin receptor, zinc protoporphyrin, and BIS (all $P < 0.0001$). Changes in BIS differed between the groups; it increased in the treatment group (0.04 ± 0.04 mmol/kg body weight) and decreased in the control group (−0.02 ± 0.04 mmol/kg body weight) ($P < 0.0001$). In sensory tests, NaFeEDTA-fortified flour could not be differentiated from unfortified flour. There were no significant differences in cognitive performance tests between the groups. NaFeEDTA-fortified wheat flour markedly improved BIS and reduced ID in Fe-depleted children. It may be recommended for wider use in national school feeding programs. J. Nutr. doi: 10.3945/jn.111.155135.

Introduction

Iron (Fe) deficiency (ID)[10] and ID anemia (IDA) are widespread globally. Forty percent of the world's children in their school-going years are reported to be anemic (1,2). Cereal flour fortification with Fe is the most cost-effective and sustainable way to improve its status in deficient populations (3). Wheat is currently the primary staple food for nearly one-third of the world's population (4), providing >50% of the total energy intake of people living in northern India.

Two major concerns when considering flour fortification with Fe have been the poor absorption of elemental Fe compounds when added to whole-grain flour and the abundant phytic acid present in the flour that hinders Fe absorption (5–7). NaFeEDTA protects Fe from the phytic acid present in foods by binding more strongly to ferric Fe at the pH of the gastric juice in the stomach and then exchanging the ferric Fe for other metals in the duodenum as the pH rises (8). It is 2- to 4-fold more bioavailable than ferrous sulfate, particularly in meals with a high-phytate content, thereby making it ideal for use in whole wheat flour (9–11). Its efficacy as a fortificant has been demonstrated in food vehicles such as curry powder, sugar, fish sauce, and maize flour (12–15). In a recent directive, the WHO and partner organizations, while providing guidance on national fortification of wheat and maize flours, have endorsed NaFeEDTA to be the

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[3] This trial was registered at www.clinicaltrials.gov as NCT00741143.
[4] Supplemental Figure 1 and Supplemental Table 1 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[9] Present address: Centre for Health Innovation and Partnership, NSW Health, Cumberland Hospital, North Parramatta, NSW 2151, Australia.
[10] Abbreviations used: BIS, body iron store; ID, iron deficiency; IDA, iron deficiency anemia; Hb, hemoglobin; SF, serum ferritin; TfR, serum transferrin receptor; ZnPP, whole blood zinc protoporphyrin.
* To whom correspondence should be addressed. E-mail: sumi.muthayya@gmail.com.

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only Fe fortificant suitable for use in high-extraction flours (16). Information on the efficacy of NaFeEDTA-fortified, whole wheat flour in improving Fe status is lacking and therefore much needed.

The impact of Fe fortification on the cognitive functioning of Fe-deficient, school-aged children has not been adequately studied. Reports of modest improvements in mental development scores in children older than 7 y following Fe supplementation have been mostly limited to initially anemic or IDA children (17). However, ID results in decreased brain Fe even before RBC production is affected (18), suggesting that cognitive effects may precede hematological changes. Data from the NHANES surveys (1988–1994) have revealed lower math scores among Fe-deficient, school-aged children, including those without anemia (19). We therefore hypothesized that fortification of whole wheat flour with Fe as NaFeEDTA would improve Fe status and body Fe stores (BIS) and would be beneficial for cognitive performance in Fe-depleted school children.

Methods

Study children and sites. This study was carried out between July 2007 and May 2008 among school children located in 2 sites in India: an urban primary school in Bangalore city, Karnataka state, and 2 primary schools in rural Vadu in Maharashtra state. These schools serve the educational needs of nearby poor communities at their respective locations. Neither region is endemic for malaria and the presence of intestinal parasitic infestation in this population is low (20,21). Children attending these schools were taught in Kannada and Marathi, the local languages spoken in Bangalore and Vadu, respectively. Informed, written consent was obtained from the parents of the children and oral assent was obtained from the children. The protocol of the study was approved by the ethical committees at St. John's National Academy of Health Sciences, Bangalore and the King Edward Memorial Hospital Research Centre, Pune, India.

Fortification of wheat flour with NaFeEDTA. Whole wheat flour manufactured by Christy Fried Gram Industry was used for this study. The whole wheat flour was produced by pulverizing dried and cleaned wheat kernels to a particle size of 500 μm. The resulting homogenous, whole wheat flour with a maximum moisture content of 6% was fortified with NaFeEDTA (AkzoNobel). The fortification level was set at 6 mg Fe/0.1 kg flour, well below the recommended maximum daily safe consumption level in humans of 0.37 mg Fe/kg body for NaFeEDTA and of 0.8 mg Fe/kg body for Fe from all sources present in the diet (22). The mixing of whole wheat flour with NaFeEDTA was carried out under the direct supervision of S. Muthayya and P. Thankachan. A total of 4000 kg of fortified flour was prepared. Because the ribbon blender available had only a 150-kg capacity, a premix of 20 kg was initially prepared and to each kilogram of premix, 100 kg of unfortified flour was added. Immediately after, the Fe-fortified and unfortified wheat flour were assigned color codes, and packed in color-coded, identical, 20-kg polyethylene bags under the supervision of an investigator not involved in the study; the bags were then dispatched to the study sites. The study investigators, assessors of cognitive tests, and study children were all unaware of the group assignments until the study was completed, all data were entered, and the analyses were performed.

Cooking of meals. The lunch meals using the NaFeEDTA-fortified and unfortified whole wheat flour were prepared daily at both study sites. Study research assistants, who were responsible for the meals each day, weighed out edible portions of all foods according to standard local recipes using electronic food scales (Soenle-Waagen; precision, 1 g) and supervised the cooking. The chapathi preparation was standardized such that each child received 3 standard-sized chapathis made from 100 g of wheat flour daily. The nutrient composition of the meals was calculated using Indian food conversion tables (23).

The meals were then transported to the school and individual portions of the lunch meal were served in color-coded plates, 6 d/wk (except on school holidays). At the school, the group assignment of the participating children was identified by using a color-coded personal badge. Three or 4 local recipes for vegetable or lentil dishes with different seasoning ingredients were presented along with the chapathis in a repeating sequence to maintain interest. The research staff ensured that the study children consumed their standard meals (3 chapathis and vegetable/lentil accompaniments) under their direct supervision. The staff at both the study sites were given adequate training on the measurement of leftovers on a visual scale to ensure standardization. At the end of the meal, the percentage leftover of chapathis was estimated in individual children using visual scales as percentages of 25, 50, 75, or 100 and recorded. A total 131 and 138 d of study intervention were completed at the Bangalore and Vadu sites, respectively.

Sensory testing. At the Bangalore site, triangle tests (24) were performed to determine whether local women could distinguish the Fe-fortified flour from unfortified flour. The panel was composed of 18 middle-class Indian women. Three local dishes made of wheat flour (chapathi, poori, and dosa) and uncooked wheat flour were tested. The food samples were presented in a randomized block design. The cooked wheat dishes (from 30 g flour) and uncooked wheat flour (30 g flour) were presented on coded, polyethylene plates; 3 coded samples of each of the 4 dishes were given in random order in a semiprivate setting. The panelists determined which among the 3 samples differed from the other 2 samples and described how it differed in taste, smell, or appearance. The women were informed about the procedures of the test only after completion of the entire study.

Efficacy trial. A total of 756 children aged between 6 and 13 y at Bangalore and 561 children between 7 and 15 y at Vadu were invited to participate in the baseline screening of their anthropometric measurements, clinical health, and biochemical status for anemia, Fe status, and inflammation. Measurements of body weight and height and a brief medical history were obtained from each child. Five milliliters of whole blood was collected by veni-puncture for the determination of hemoglobin (Hb), serum ferritin (SF), serum transferrin receptor (TfR), whole blood zinc protoporphyrin (ZnPP), and serum C-reactive protein (CRP). Children were eligible for inclusion into the study if they were: *1*) apparently healthy, without any chronic illness and physical/mental handicaps; *2*) not severely anemic (Hb <80 g/L); *3*) Fe depleted (SF <20 μg/L or TfR >7.6 mg/L and ZnPP concentration >40 μmol/mol heme); *4*) not intending to use micronutrient supplements during the study; and *5*) planning to reside in the study area during the next 12 mo. In total, 1317 children from both sites were screened, of whom 916 children were not eligible, because they did not fulfill the inclusion criteria (**Fig. 1**). The remaining 401 children (194 children in Bangalore and 207 children in Vadu) were separately randomized at each site into 2 groups and enrolled into the trial. They were individually allocated into 1 of 2 groups to receive either an NaFeEDTA-fortified, wheat-based meal (treatment group) or an otherwise identical, wheat-based control meal with no fortificant Fe (control group). Randomization was performed by means of a computer-generated list in blocks of 8. The enrolled children, who were arranged in ascending order by grade at school and age in years, were assigned intervention codes in sequence. All measurements done at baseline were repeated at 3.5 mo (midpoint) and at 7 mo (endpoint) into the study. Severely anemic children received supervised treatment with oral Fe tablets [60 mg Fe (as ferrous sulfate) 4 d/wk for 12 wk]. Others who were diagnosed with illnesses were referred to a physician.

Cognitive measurements. Prior to start and at the end of the intervention, all randomized children at the Bangalore site were subjected to a battery of tests to assess cognitive performance. These tests were carried out in sound-protected cubicles at the St. John's Medical College premises, Bangalore. A few days prior to testing, the children were brought to these cubicles to orient them to the environment and the psychologists administering the tests. The cognitive measures consisted of a series of neuropsychological tests applicable for

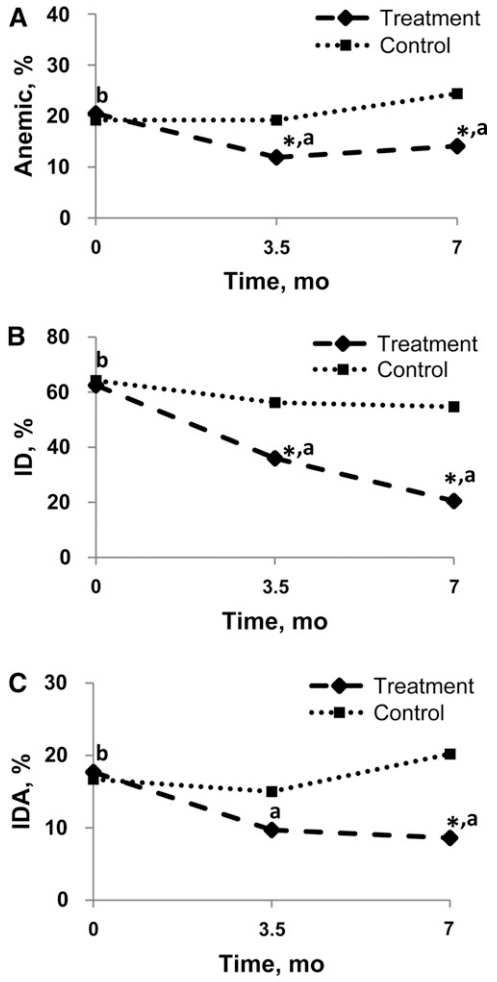

FIGURE 1 Prevalence of anemia (*A*), ID (*B*), and IDA (*C*) in children who received wheat-based meals that were or were not fortified with NaFeEDTA at baseline, 3.5 mo, and 7 mo. *Different from control, $P < 0.05$ (Pearson's χ^2 test). Percentages within each group with a superscript without a common letter differ, $P < 0.05$ (McNemar's test). ID, iron deficiency; IDA, iron deficiency anemia.

use in school-aged children related to specific cognitive domains (short-term memory and retrieval ability, cognitive speed, and fluid reasoning) consistent with the Carroll model (25). The cognitive battery included 3 core tests from the Kaufman Assessment Battery for Children (26) and additional tests (27–29) that underwent an extensive adaptation process to ensure their applicability in the local cultural context (30). The specific tests used were Atlantis (learning ability/long-term storage and retrieval scale), KOH'S Block Design (visuo-spatial ability), Word Order (sequential processing/short-term memory scale), Pattern Reasoning (planning/fluid reasoning scale), Verbal Fluency (broad retrieval ability), and Coding-WISC-III (cognitive speed). The tests were adapted for use in 7- to 15-y-old Kannada-speaking children of low socioeconomic status in Bangalore, India through an iterative process of translating, piloting, and modifying (30,31). These cognitive measures were previously shown to be sensitive to the effects of nutritional interventions (32) and were administered by trained masters-level psychologists in the local Kannada language. A team of 5 psychologists were extensively trained during 3 wk prior to the study to ensure standardization in the test administration and scoring procedures with retraining as needed throughout the study period. The training was repeated in the week prior to the cognitive assessments at 7 mo. To exclude individual variation, cognitive assessments for each child were conducted by the same psychologist at both baseline and the end of study. The tests were administered to each child during the morning hours between 0900 and 1100 h for a 25-min period. Care was taken to ensure that all children had breakfast before testing began in the morning, because omitting breakfast is known to impair cognitive performance (33).

Biochemical analysis. At baseline, midpoint, and endpoint, Hb was measured on hematology analyzers, on an AcT diff^2 (Beckman Coulter) at Bangalore, and an ABX Micros 60 (ABX Diagnostics) at Vadu. All other biochemical analyses for SF, TfR, ZnPP, and CRP were carried out at the Core Biochemistry Laboratory Facility at St John's Research Institute, where these assays have been established and validated against external control materials. SF was measured by electro-chemiluminescence on an Elecsys 2010 analyzer (Roche Diagnostics). External 3-level control material (WHO Standard 80/578; Ramco Laboratories) were used for this method. TfR and CRP were measured using immunoassays on a Hitachi 902 analyzer (Roche Diagnostics). The TfR values obtained from the Hitachi 902 analyzer were converted to results corresponding to the Ramco assay using the regression equation: Ramco TfR value = (Roche Tfr value $-0.299) \times 0.631^{-1}$ (34). ZnPP was measured on washed RBC with a hematofluorometer (Aviv Biomedical). Serum samples were aliquoted and frozen at −20°C until analysis. Anemia was defined as an Hb concentration <120 g/L in children aged ≥12 y and <115 g/L in children aged 5–11 y (1). SF values of children with elevated CRP (≥5 mg/L) were excluded from analysis (n = 5, 16, and 17 each at baseline, 3.5 mo, and 7 mo, respectively). ID was defined as having an SF <15 μg/L (35) or as TfR >7.6 mg/L plus a ZnPP concentration >40 μmol/mol heme (35–37). IDA was defined as anemia with ID using the above-mentioned criteria. BIS was calculated from the ratio of TfR:SF by using the method of Cook et al. (38). To convert the BIS values from mg/kg to mmol/kg, they were divided by 55.847. Only children with normal CRP concentrations were included in the calculation of BIS.

Statistical analysis. The baseline biochemical status of children (Hb and Fe indices), the main outcome variables for the study, were comparable between the sites (data not shown). Data from both sites were therefore combined for analysis. Data processing and statistical analysis were performed with SPSS (version 13.0, 2004) and with Microsoft Excel (XP 2006). The normality of the data was checked before analysis using the Shapiro-Wilk's test and by graphically evaluating Q-Q plots. Normally distributed data were expressed as mean ± SD and as median (quartile 1, quartile 3), if otherwise. Variables that were not normally distributed were log-transformed for further analysis. The biochemical and cognitive variables were compared between the treatment and control groups at baseline using the independent sample t tests. Repeated-measures ANOVA was performed to assess the effect of intervention on measures of Hb, SF, TfR, and BIS at the 3 time points by considering the group × time interaction effect. Bonferroni-adjusted post hoc comparisons were done for the significant main and interaction effects. The time effect for the binary variables of anemia, ID, and IDA was tested by using the McNemar's test and the group effect by using the Pearson χ^2 test. Results of the sensory study were evaluated using the binomial test, with an expected probability of correct detection in the triangle test to be 0.3. An ANCOVA was performed for the comparison of raw scores for all cognitive variables between the study groups at the end of study after adjusting for baseline values. The raw scores were also compared between baseline and endpoint within each group using paired t test with Bonferroni correction for multiple testing. Separate models with gender, SF group (group 1 with SF values less than median; group 2 with SF values greater or equal to median), and BIS group (group 1 with BIS values less than median; group 2 with BIS values greater or equal to median) as additional factors were performed to examine the interaction of the treatment effect with these variables. Log-transformed values were used when data did not follow normal distribution. Differences were considered significant at $P < 0.05$.

Results

Of the 401 children (n = 200 in the treatment group and 201 in the control group) who participated in the study, 379 children (n = 186 in the treatment group and n = 193 in the control group)

completed the study. The dropout rate was similar in the 2 groups (**Supplemental Fig. 1**). There were no significant between-group differences for any of the baseline characteristics (**Table 1**). The mean age of all the children in the study was 10.4 ± 2.6 y. Their overall prevalences of anemia, IDA, and ID were 20.4, 18, and 63.5%, respectively.

The study children consumed meals containing 100 g wheat flour/meal daily (**Table 2**). The daily lunch meals provided 11.2 ± 0.7 mg Fe in the treatment and 5.1 ± 0.6 mg Fe in the control groups. In the treatment group, the chapathis contained 6 mg of added fortificant Fe in the form of NaFeEDTA. The Fe content in the unfortified chapathi meal served daily with the added vegetable and/or lentil gravy is summarized in the table. On any given day, ~80% of the children at both sites had no leftovers on their plate after the meal. Compliance was estimated based on the mean consumption of the cooked meal per day per child throughout the study period. The mean compliance with the intervention in the Bangalore and Vadu sites was estimated to be 85 and 78%, respectively. While compliance in the treatment and control groups at the Bangalore site was 84.3 and 85.7% respectively, the figures for Vadu were 78.7 and 76.5%, respectively. The level of compliance between the intervention groups was comparable throughout the study period.

The sensory tests indicated that at 2 levels of fortification, i.e., 6.0 and 10 mg Fe/100 g flour, NaFeEDTA-fortified and unfortified wheat flour in the raw form were comparable in taste, flavor, color, and odor (data not shown). Similarly, in all of the cooked recipes, namely chapathi, poori, and dosa, the meals containing wheat fortified flour at both 6 and 10 mg Fe/100 g were indistinguishable from the respective meals containing unfortified wheat flour.

Both the treatment and control groups showed similar, marked gains in weight and height after 7 mo of intervention when compared with their baseline values (**Table 3**). However, gains in body weight of 3.2 ± 2.1 kg (n = 371) and height of 4.5 ± 2.0 cm (n = 364) did not differ between the groups over the duration of the study.

There was a time × treatment interaction for the prevalence of anemia, ID, and IDA ($P < 0.01$) (Fig. 1). The prevalence of anemia, ID, and IDA significantly decreased from baseline at 3.5 and 7 mo in the treatment group. During the 7 mo, the anemia prevalence in the treatment group decreased (20.5 to 14.1%; $P < 0.05$), whereas it increased in the control group (19.2 to 24.4%; $P = 0.05$). The prevalence of ID decreased in the treatment group (62.5 to 20.5%; $P < 0.001$) but did not change in the control group. Fe fortification resulted in a decrease in IDA prevalence from 17.7 to 8.6% ($P < 0.001$), whereas in the control group there was no change.

There was a progressive change in Hb, SF, TfR, ZnPP, and BIS concentrations after 7 mo (Table 3). A time × treatment interaction was observed for Hb, SF, TfR, ZnPP, and BIS (all $P < 0.0001$). Changes in BIS differed between the groups; it increased in the treatment group (0.04 ± 0.04 mmol/kg body weight) and decreased in the control group (−0.02 ± 0.04 mmol/kg body weight) ($P < 0.0001$). Blood Hb, SF, ZnPP, and TfR (all $P < 0.0001$) significantly differed between groups after 7 mo. There was no significant change in Hb, SF, TfR, and BIS in the control group at the end of study at 7 mo.

There was no overall effect of treatment on cognitive performance at the end of the intervention period after adjusting for baseline scores for each of the tests (**Supplemental Table 1**). The interaction effects of treatment with either gender or grouping by SF or BIS status were also not significant for any of the cognition variables.

TABLE 1 Age, gender, and anthropometric and hematological characteristics of the children who received wheat-based meals that were or were not fortified with NaFeEDTA at baseline[1]

	Treatment group	Control group
n	200	201
Age, *y*	10.8 ± 2.6	10.7 ± 2.7
Child gender, % (male:female)	50:47	56:41
Weight, *kg*	26.5 ± 8.0	26.2 ± 7.8
Height, *m*	1.34 ± 0.15	1.33 ± 0.15
Hb, g/L	125 ± 13	125 ± 12
SF, *μg/L*	12.8 (6.9, 22.2)	12.2 (6.7, 22.2)
TfR, *mg/L*	5.8 ± 3.4	5.8 ± 2.8
BIS, *mmol/kg body weight*	0.03 ± 0.05	0.03 ± 0.05
Anemia, %	21.5	19.4
IDA, %	19.0	17.0
ID, %	62.5	64.5

[1] Data are mean ± SD, geometric mean (± SD), or percentage. There were no differences between groups. BIS, estimated body iron store; ID, iron deficiency IDA, iron deficiency anemia; Hb, hemoglobin; SF, serum ferritin; sTfR, serum transferrin receptor.

Discussion

Whole-grain wheat flour is one of the most inhibitory food vehicles for Fe absorption. Through fortification with NaFeEDTA, in this controlled study, whole wheat flour has been demonstrated to be suitable as a highly effective vehicle for Fe fortification in markedly reducing anemia (35%), IDA (51%), and ID (67%) in Fe-depleted, school-going Indian children. These figures remained unchanged, except for a slight increase in anemia prevalence in the control group. Consumption of whole wheat flour fortified with NaFeEDTA providing 6 mg Fe/ 100 g flour resulted in a marked and sustained improvement in Fe status, as indicated by their increases in SF and BIS. A similar study with school children in Kenya (15) consuming fortified whole-grain corn flour showed that whereas the addition of 56 mg/kg of elemental Fe did not result in a reduction in the prevalence of IDA or ID and an improvement in Fe status any better than in the control group, adding 28 or 56 mg/kg of Fe as NaFeEDTA did significantly reduce ID among these school children by 70 and 91%, respectively. Ferrous sulfate due to both its affordability and high bioavailability is often the preferred choice for fortification. However, Fe absorption from $FeSO_4$ used to fortify cereal foods has been shown to be unacceptably low due to the natural presence of phytic acid (39).

Reductions in ID and IDA in the present study were of a similar magnitude, as observed earlier in a 7-mo, randomized, controlled study of Fe-fortified rice, where Indian school children received nearly 3 times the quantity of Fe as micronized ferric pyrophosphate than the Fe in the NaFeEDTA-fortified flour meal (40). Clearly, Fe present in NaFeEDTA is better absorbed by at least 3 times that of other Fe fortificants (10–12). In communities that typically have a low consumption of meat and meat products that are rich in the highly bioavailable heme Fe and use wheat flour as a major staple in their diet such as in North India, NaFeEDTA added as a Fe fortificant may be the best alternative for improving Fe status. The drop in anemia prevalence was more pronounced in the first half of the

TABLE 2 Nutrient composition of the wheat-based chapathi meals per daily serving

	Bangalore site			Vadu site			
Nutrients	Meal 1 + tomato curry	Meal 2 + green pea curry	Meal 3 + chick pea curry	Meal 1 + potato curry	Meal 2 + moth beancurry	Meal 3 + kidney beancurry	Meal 4 + cauliflowercurry
Energy, *kJ*	1810	1880	2130	2320	2100	2390	2180
Protein, *g*	13.4	14.5	17.3	16.3	16.7	19.1	16.7
Fat, *g*	8.0	8.0	9.4	6.9	5.1	6.8	6.3
Carbohydrate, *g*	77	80	89	107	97	108	99
Fe, *mg*	4.74	4.84	5.84	5.29	4.56	5.03	4.92

intervention, after which it remained unchanged in the treatment group. The improving Fe status and/or a possible simultaneous worsening of micronutrient deficiencies (riboflavin, folate, and/or vitamin B-12) that may have impaired Fe utilization over the 7-mo period could have resulted in the less-pronounced drop in anemia prevalence observed in the latter part of the study.

NaFeEDTA also has excellent organoleptic properties as has been shown in the results of the triangle tests. Women in the present study rated wheat flour (in cooked and raw form) fortified with 6 and 10 mg Fe/100 g as comparable with unfortified wheat flour. In addition, children participating in the study did not react unfavorably to the color or taste of the wheat-based meals during the course of the study. Addition of NaFeEDTA increases intrinsic Fe absorption and improves the apparent absorption of zinc in the diet (41,42). It has no effect, however, on copper, calcium, or magnesium absorption (43) or of heavy metals (41). It has also been reported not to cause fat oxidation during long periods of storage at ambient temperature, unlike ferrous sulfate, when added to wheat flour (42). A limitation of our study is that we did not evaluate the population acceptability and the long-term stability of the fortified wheat flour and cooked, wheat-based meals. The long-term potential of NaFeEDTA fortification to induce Fe overload has also been a concern, but the available evidence suggests that homeostatic controls would prevent excess Fe accumulation in the normal population (41). Like most EDTA-metal complexes, NaFeEDTA also dissociates in the gut to a bioavailable form of Fe and an EDTA salt, with almost all the EDTA excreted in the stool (44). In rat studies, NaFeEDTA has been demonstrated to have an oral toxicity similar to that of ferrous sulfate (45).

At 7 mo, both groups showed impressive, significant increases in body weight (3.2 kg) and height (4.5 cm) from their baseline values. The observed changes exceeding that of Indian growth norms (46) may possibly be attributed to the additional energy and protein in the lunch meal. Although the provision of Fe to Fe-deficient children has been shown to improve growth (47–50), our study did not demonstrate any effects of Fe on growth. A possibility, however, for the significant changes in both weight and linear growth in all the study children could be attributed to the extra protein consumed in the meal that contained lentils (which are high in lysine content). Because most protein in the diet of the study children comes from rice at Bangalore and pearl millet at Vadu, the protein quality of their habitual diet could have been suboptimal. Lysine is the limiting amino acid in a cereal-based diet and the intake of 35% of the children in our study at Bangalore did not meet the estimated average requirements of lysine (S. Muthayya, unpublished observations). Where undernutrition, namely underweight, stunting, and ID often coexist, adding Fe to a food vehicle that is rich in lysine could be considered a public health strategy to address these problems among marginally deficient school children.

Although Fe fortification resulted in significant increases in Fe status indicators among Fe-depleted children in the present study, it did not reflect concurrent, significant improvements in their cognitive performance. There may be several reasons for the lack of effect of Fe fortification on cognitive measures. First, as suggested by 2 recent reviews, which include data from India, beneficial effects of Fe treatment on cognition were particularly apparent in children that were anemic or had IDA (17,51). Second, in many studies, the intervention occurred during infancy (52–54). There are suggestions that a Fe-deficient state during early life is associated with delayed neuronal

TABLE 3 Anthropometry and Fe status indices in children who received wheat-based meals that were or were not fortified with NaFeEDTA at baseline, 3.5 mo, and 7 mo[1]

			Time	
	n	Baseline	3.5 mo	7 mo
Body weight, *kg*				
Treatment group	182	27.0 ± 8.1	29.2 ± 9.0	30.2 ± 9.3
Control group	180	26.0 ± 7.1	28.1 ± 8.8	29.3 ± 9.2
Height, *m*				
Treatment group	172	1.34 ± 0.15	1.36 ± 0.14	1.38 ± 0.14
Control group	180	1.32 ± 0.15	1.35 ± 0.15	1.37 ± 0.15
Hb, *g/L*				
Treatment group	185	125 ± 11^{a}	128 ± 10^{b*}	129 ± 11^{b*}
Control group	193	124 ± 11	125 ± 12	123 ± 13
SF, *mg/L*				
Treatment group	166	14.6 (9.3, 17.9)^{a}	17.9 (11.8, 26.9)^{b*}	26.6 (17.8, 35.5)^{c*}
Control group	180	13.9 (9.2, 17.6)	13.4 (8.7, 19.8)	14.5 (9.3, 21.1)
sTfR, *mg/L*				
Treatment group	186	5.57 ± 2.36^{b}	4.82 ± 2.08^{a}	5.59 ± 2.03^{c*}
Control group	193	5.81 ± 2.80	5.46 ± 2.61	6.70 ± 3.05
ZnPP, *μmol/mol heme*				
Treatment group	186	61.0 (48.7, 80.0)^{b}	61.7 (48.0, 81.0)^{b}	54.3 (42.3, 69.7)^{a*}
Control group	193	62.7 (49.2, 80.7)	65.3 (50.0, 91.3)	62.0 (47.0, 84.2)
CRP, *mg/L*				
Treatment group	185	0.0 (0.0, 0.2)	0.0 (0.0, 0.4)	0.1 (0.0, 0.7)
Control group	192	0.0 (0.0, 0.2)	0.0 (0.0, 0.4)	0.1 (0.0, 0.5)
BIS, *mmol/kg*				
Treatment group	164	0.03 ± 0.05^{a}	0.06 ± 0.05^{b*}	0.07 ± 0.05^{b*}
Control group	179	0.03 ± 0.05	0.04 ± 0.06	0.03 ± 0.06

[1] Data are mean ± SD, median (quartile 1, quartile 3). *Data different from control at that time, $P < 0.001$. Means in a row with superscripts without a common letter differ, $P < 0.05$. BIS, estimated body iron store; Hb, hemoglobin; SF, serum ferritin; sTfR, serum transferrin receptor; ZnPP, erythrocyte zinc protoporphyrin.

development that might persist despite correction of the Fe-deficient state (55). Therefore, it is possible that the children participating in this study may have been Fe deficient during early life, with consequent irreversible changes in neural mechanisms. Third, there is a possibility that the effect of Fe fortification on cognitive measures was not strong enough to overcome the adverse impact of poverty and poor socioeconomic status on a child's psychomotor development. Finally, although the sample size in the present study was sufficient to detect a significant change, it is possible that the study duration of 7 mo was insufficient to significantly affect cognitive abilities of the study children.

In summary, the findings of this study support the recent fortification guidelines that suggest NaFeEDTA to be the ideal Fe compound for whole wheat flour fortification. Whole wheat flour fortified with NaFeEDTA was efficacious in markedly reducing ID prevalence and improving BIS and Fe status in Fe-depleted Indian school children. This is particularly relevant to the improvement of BIS in girls of school age in preparation for their higher daily Fe requirements during puberty and later during pregnancy. NaFeEDTA-fortified wheat flour may be recommended for wider use in national school feeding programs for children where there is a high prevalence of ID and in public distribution systems among vulnerable populations.

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