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Wheat Flour Fortified with Elemental Iron or $FeSO_4$ Provides Bioavailable Iron to Pakistani Children

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Abstract: Iron fortification of wheat flour is an accepted long term strategy to combat iron deficiency anemia worldwide. It is necessary to determine the relative bioavailability of various iron fortificants in local available flour prior to making any recommendation for wheat flour fortification. Relative bioavailability of various iron fortificants in 85% extraction wheat flour was evaluated. 54 school children, ages 5 to 10 years, were randomized in three groups, in order to receive a breakfast containing a wheat-based product, labeled with either 1) elemental ^{58}Fe, 2) $^{58}FeSO_4$, or 3) $^{58}FeSO_4$ + Na_2EDTA (1:1 molar ratio). Forty subjects successfully completed the trial. Using stable isotope methodology, the absorption of ^{58}Fe was determined. Overall, the two groups receiving $^{58}FeSO_4$ had significantly higher absorption (7.0%±3.8) than the group receiving elemental ^{58}Fe (4.1%±1.7). There was no significant difference found in the percent absorption of ^{58}Fe between the groups receiving $^{58}FeSO_4$ (7.4%±3.4) and those receiving $^{58}FeSO_4$ + Na_2EDTA (6.4%±4.4). Estimated absorbed iron from 300 g of wheat fortified at the tested concentrations would be approximately 730 µg, 670 µg and 380 µg from elemental Fe, $FeSO_4$ and $FeSO_4$ + Na_2EDTA respectively. Overall, iron absorption was in the range expected although the lack of efficacy of Na_2EDTA was unexpected.

Key words: Fortification, Pakistan, iron absorption

INTRODUCTION

Fortification of wheat flour has long been recognized as an effective tool in the global struggle against iron-deficiency anemia. Since wheat flour is widely consumed in Pakistan, it can be used to treat iron-deficiency in population. Guidelines for the optimization of flour fortification programs in individual countries include a multi staged process. First, thorough evaluation of the prevalence of iron-deficiency in the population should be established based on surveys of the iron intake of segments of the population most likely to be iron-deficient, such as women of child-bearing age. Next, the form, level and absorption of the fortificant should be optimized to provide the maximum acceptable amount of bioavailable iron without deleterious organoleptic changes. Finally, efficacy studies should demonstrate that the program is effective in decreasing the prevalence of anemia in the country (Hurrell and Lynch, 2004).

A variety of fortificants are available for wheat flour fortification. Globally, elemental iron powders are perhaps the most commonly used iron fortificants. They have desirable properties such as low cost, stable shelf life and reasonable taste qualities. The problem is the low bioavailability of iron powders with respect to other iron fortificants. A review by SUSTAIN Task Force concluded that all elemental iron powders are not equally bioavailable. Since prior studies to determine bioavailability had been based upon experimental and not commercial forms of the powders, the Task Force stated that new more data using commercial iron powders were needed (Hurrell *et al*., 2002). However, it is difficult to design human studies to determine the bioavailability of elemental powders because of lack of good markers. Commercially available iron powders are difficult to reproduce with either radioisotope or stable isotope labels. *In vitro* techniques and animal models have given differing results; therefore, the bioavailability of iron powders has not been easily elucidated. However, studies have shown that carbonyl and electrolytic iron have the best relative bioavailability value or RBV (approximately 0.5 - 0.6 of ferrous sulfate) (Swain *et al*., 2003).

Although ferrous sulfate is more bioavailable than elemental iron, it is more reactive and hence less stable. Moreover, it discolors wheat flour when exposed to humidity and long storage periods. The potential role of NaFeEDTA as a fortificant in developing countries has been widely discussed. It is much less affected by the inhibitors of iron absorption in the diets of populations in low socioeconomic settings. However, NaFeEDTA costs about six to eight times more than ferrous sulfate in

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terms of equivalent amounts of absorbed iron (Bothwell and MacPhail, 2004).

Within the context of the above discussion, flour fortification studies were done in Pakistan. Like many other developing countries, Pakistan is a nation whose people have been widely affected by iron deficiency. In Pakistan anemia has been directly linked to the high incidence of maternal morbidity and mortality from childbirth, as well as premature births and miscarriage. There have been various studies and nutritional surveys done in Pakistan since 1965 which investigated the prevalence of iron deficiency in diverse regions and populations within the country (Siddiqui *et al*., 2004; Khan, 2001; Kazi and Qureshi, 2002; Planning and Development Division, 1978; Nutrition Division, 1988; Planning and Development Division, 2004; Paracha and Jamil, 1998). These have shown that the prevalence of anemia ranges from 42% to 66% in the rural population and from 41% to 56% of the urban population. Among preschool-aged children 33% to 65% were anemic while 45% of pregnant and lactating women were anemic. Iron deficiency was also found to be quite prevalent in Pakistan with low serum ferritin (<12 μg/L) in 66.5% of the children under the age of 5 and 45% of lactating mothers (Nutrition Division, 1988), indicating that iron deficiency is a primary cause of anemia in these groups. As part of a national policy to address these problems, several possible approaches have been considered. In the past the main strategy to control iron deficiency anemia has been iron supplementation of pregnant women through health outlets. Realizing the low acceptance of iron tablets by pregnant women and the very limited impact of this strategy, the Government of Pakistan decided to undertake food fortification to complement supplementation and food diversification. Wheat flour was chosen as a vehicle for fortification as it is the staple food of the Pakistani people with an average per capita monthly consumption of 9.72 kg (7.23 and 10.11 kg in urban and rural areas, respectively) (Nutrition Division, 1988).

In an attempt to evaluate the bioavailability of potential fortificants, three different iron compounds for wheat flour were tested in this study, an elemental iron powder, ferrous sulfate and ferrous sulfate with Na_2EDTA added, using stable isotope techniques at recommended fortification levels (Abrams *et al*., 2002). Evaluation of iron absorption was done in a group of school-aged children in Islamabad, who consumed a fortified wheat flour product as part of a typical breakfast meal.

MATERIALS AND METHODS

Subjects: The study received approval from the Pakistan Institute of Nuclear Science and Technology (PINSTECH) in Islamabad and from the Institutional Review Board for Baylor College of Medicine and Affiliated Hospitals in Houston, Texas, USA. Informed consent was obtained in Urdu from the guardians and consent was given by the individual subjects after explaining in detail the purpose, risks and benefits of the study. A total of 54 subjects between the ages of 6 to 10 years were enrolled in the study. Children were excluded if upon general examination by a doctor or taking medical history they found to have a chronic medical condition, were acutely ill, taking micronutrient supplements, or if they had a hemoglobin level (Drabkin's method) less than 85 g/L (Balasubramaniam and Malathi, 1992). Only one child had a hemoglobin level less than 70 g/L; who was referred for further evaluation and treatment. Childrens' height was measured to the nearest 0.1 cm with local manufactures scale and weight to the nearest 0.1 kg (HD-2006A1 Electronic Body Scale China). The male subjects came from a school for underprivileged children in Islamabad while girls were recruited from another school in Islamabad as both schools were not coeducational institutes. Albendazole (400 mg) was given to 54 subjects two weeks before study in a single dose. There were a total of 27 boys and 27 girls who met the eligibility criteria, received albendazole to remove intestinal parasites, were available for participation in the study and whose guardians provided informed consent. All boys and 25 girls completed the study. Two female participants were unavailable at the time of the follow-up blood draw. The blood samples were labeled appropriately sealed in zip lock bags, placed in container filled with enough dry ice and sent to resource lab. Although 52 subjects completed the study protocol, technical problems related to sample handling during transport from Pakistan to the United States limited the final results to 40 subjects.

Preparation of isotopes: ^{57}Fe and ^{58}Fe were obtained from Trace Sciences International, Toronto, Canada. ^{57}Fe (95% enrichment by mass) and ^{58}Fe (96% enrichment by mass) were provided in elemental metal form. Iron isotope solution was prepared, as the sulfate, at the mass spectrometry laboratory at PINSTECH using the methods described by Kastenmayer *et al*. (1994). The metal was dissolved in 0.03 mL of 7M nitric acid and 0.125 mL of 0.5M sulfuric acid for every mg of elemental iron. The solutions were dried at 120°C until it dried, at 230°C for 30 min and finally at 500°C for 30 min in a sand bath or furnace. After cooling, the final products were re-suspended in 0.2M sulfuric acid at 0.240 mL for every mg of iron. Deionized water was added to produce a solution yielding a unit dose of iron in the form of $^{58}FeSO_4$ for each 2.5 mL of liquid. Elemental iron was produced as a fine powder after dissolving ^{58}Fe nuggets in 7M nitric acid, drying at 120°, at 230°C and finally at 500°C for 30 min each in a sand bath. No further

Table 1: Calculated nutritional content of the test meal compared with daily requirements for children, age 7-10 years[1]

Nutrient	Amount calculated in meal	Daily requirement (7-10 years)	% Daily requirement provided by meal
Energy [kJ]	544.00		
Total fat [g]	14.10	65.0	22
Total carbohydrate [g]	87.24	300.0	29
Sugars [g]	24.62		
Protein [g]	22.27	50.0	45
Folic acid [µg]	73.22	400.0	18
Iron [mg]	4.13	18.0	23
Zinc [mg]	3.54	15.0	24
Calcium [mg]	176.67	1000.0	18
Phosphorus [mg]	491.60	1000.0	49
Potassium [mg]	609.76	3500.0	17
Magnesium [mg]	142.16	400.0	36
Sodium [mg]	126.17	2400.0	5
Selenium µg	81.00	70.0	116
Copper [mg]	0.36	2.0	18
Riboflavin [mg]	0.65	1.7	38
Vitamin A [µg][2]	134.20	1500.0	9
Vitamin B12 [µg]	0.98	6.0	16
Vitamin B-6 [mg]	0.40	2.0	20
Vitamin C [mg]	0.93	60.0	2
Dietary fiber [g]	10.61	25.0	42

[1]Analysis of the meal for nutritional content was performed using Nutrition Data Systems for Research (v4.03) program).
[2]Retinol

physicochemical characterization of the powder was attempted. Finally, Na_2EDTA was dissolved in deionized water and made in sufficient quantity to provide a 1:1 molar ratio solution with ferrous sulfate.

Test meal: The test meal was comprised of a "chapatti" (unleavened flat bread), a fried egg and a cup of tea given in breakfast after overnight fasting. This meal was chosen in order to test the absorption of iron from the fortified wheat flour in a typical meal eaten by Pakistani children. Although this meal contains large quantities of inhibitors of iron absorption, it was felt that by including these foods, we could best simulate "real-world" absorption. The chapatti was made with 140 g of dough consisting of 87 g of flour (85% extraction) and 53 g of water. The average tea serving was 216 g, including 98.5 g of water, 98.5 g of milk, 18.5 g of sugar and 0.5 g of tea. The average weight of the fried egg was 52 g. Macro and micronutrient-analysis of the meal was calculated using the Nutrition Data System for Research Program for dietary analysis (v 4.03, Nutrition Data System, Minneapolis, MN) (Table 1). The flour was labeled with 1.5 mg of ^{58}Fe in the form of either ferrous sulfate, ferrous sulfate with Na_2EDTA, or elemental iron. To insure the accurate measurement of percent absorption the following procedure was used:

1) Each child's chapatti was labeled with isotope and weighed individually rather than labeling the entire batch and then dividing it. This somewhat time-consuming process insured that the quantity of stable isotope that each child received was known precisely.
2) The total amount of flour used was the same for each child, insuring that the same amount of inhibitors was present. Unlabeled fortified flour with the same type of fortificant and at the appropriate concentration was added to the labeled flour in the required amount to provide 87 g of flour in a 140 g chapatti. The added isotope and ulabeled forms of iron was: Elemental Iron group [60 µg/g] 1.5 mg ^{58}Fe (25 g flour) and 3.7 mg ulabeled (62 g flour), $FeSO_4$ group [30 µg/g] 1.5 mg ^{58}Fe (50 g flour) and 1.1 mg ulabeled (37 g flour), $FeSO_4$ + Na_2EDTA [20 µg/g] 1.5 mg ^{58}Fe (575 g flour) and 0.2 mg ulabeled (12 g flour). Unlabeled fortificants were obtained from AIC (Kansas City, MO) as elemental iron, ferrous sulfate, or ferrous sulfate with Na_2EDTA. The elemental iron consisted of an electrolytically reduced powder that passed through a 100 mesh sieve (150 micron).
3) After carefully weighing each individual serving of dough, the dough was rolled flat and the carefully measured isotope was added to the serving. The iron isotope was added directly onto the Na_2EDTA in order to insure the formation of an iron-EDTA complex. The dough was mixed and then rolled once before cooking. Each chapatti was individually cooked at 250°C for 3 min.

One day prior to the test meal all children received a reference iron isotope dose orally, which consisted of 5 mg ^{57}Fe as iron sulfate dissolved in orange juice containing approximately 50mg of ascorbic acid. The reference dose was given to each subject early in the morning after an overnight fast. The following day, after

another overnight fast, each subject was given the single labeled chapatti with test meal. They fasted for an additional 2 hrs and then resumed their usual diet. There were no complaints of food discoloration or altered taste. Each child was monitored to insure that the entire chapatti was eaten; this was facilitated by the fact that no crumbs are produced when the chapatti is eaten because of its consistency. Iron isotope ratios were measured in the red blood cells collected 14 days after administration of the isotopes.

Isotope methods: The isotopes were prepared using the method described Abrams *et al*. (2002). Iron isotope ratios were measured in the red blood cells collected 14 days after meal administration with a thermal ionization magnetic sector mass spectrometer (MAT 261; Finnigan, Bremen, Germany). The results were expressed as the ratio of ^{58}Fe to ^{56}Fe. The ratio of the 2 non-administered isotopes (^{56}Fe and ^{54}Fe) was used to correct for temperature-specific differences in fractionation. Iron absorption was calculated from incorporation of ^{58}Fe into red blood cells, based on the assumption that 90% of the absorbed iron was incorporated into red blood cells.

The ratio of administered isotope (^{57}Fe or ^{58}Fe) is determined relative to ^{56}Fe in the sample of blood and the quantity of administered isotope incorporated in to erythrocyte (Fe_{inc}) is determined from enriched (enr) and baseline (base) isotope ratios as (for ^{57}Fe):

Statistical analysis: Based on the absorption from fortified wheat seen in a previous study in a similar population in Indonesia (Herman *et al*., 2002) the following assumptions were made in calculating the power required for the study:

1. Ferrous sulfate absorption will be 15.9% (SD 6.8%)
2. Elemental iron absorption will be 50% or less of ferrous sulfate absorption.
3. The addition of Na_2EDTA will increase $FeSO_4$ absorption by 50% to approximately 24% (SD 7.0%)

A sample size of 36 was predicted to give greater than 80% power to detect the assumed difference of 8% between the Fe sulfate Na_2EDTA group (total of 12) and the ferrous sulfate group (total of 12) and the 8% assumed difference between the $FeSO_4$ group and those taking elemental iron. The additional subjects in each group were added to accommodate drop-outs and lost data. Differences in iron absorption between groups were assessed using ANOVA and post-hoc pair wise comparison (Tukey's test with significance level set at 0.05). The groups were also compared while controlling for covariates such as gender and hemoglobin using analysis of covariance techniques. Statistical analysis was carried out using Stata 6.0 (Stata Labs, Inc., San Mateo, CA) and Minitab 13.0 (Minitab, Inc., State College, PA). Anthropometry was calculated using Anthro version 1.02, 1999 (Centers for Disease Control, Atlanta, GA). Descriptive statistics are expressed as the mean ± standard deviation. P-values less than 0.05 are considered to be significant.

RESULTS

Characteristics of study subjects: Baseline characteristics of the 40 individuals whose samples were analyzed are described in Table 2. There were more boys (n = 24) than girls (n = 16) and the girls were significantly younger ($p<0.01$). There was no significant difference in weight, height and age between groups, but the boys were significantly older ($p = 0.001$), heavier ($p<0.001$) and taller ($p<0.001$) than girls. There were no significant differences between the different iron groups in terms of height for age, weight for age, or weight for height. Girls had significantly lower weight for age ($p<0.01$) and weight for height ($p = 0.01$) than boys. The mean BMI was 14.9 with boys significantly higher than girls ($p<0.04$). The prevalence of under nutrition was assessed on the basis of weight-for-age and height-for-age using the CDC 2008 reference standards (CDC, 2008). Of those originally enrolled in the trial twenty-one percent had weight-for-age Z-scores <-2.0 and 17% had height-for-age Z-scores <-2.0. Baseline data from this study showed that 38% of the subjects had Hb<110 g/L and no subjects had Hb >120 g/L. There were no significant differences in hemoglobin between groups or sexes. These figures compare with the National Nutrition Survey (2001-2002) that revealed that 33% children under the age of 5 years were anemic and 2.6% were severely anemic (Planning and Development Division, 2004).

Iron absorption: Iron absorption from the reference dose of ^{57}Fe was similar among the 3 groups (Table 3), with a mean of 29.4% (individual values ranged from 2.1% to 69.1%). Absorption of ^{58}Fe was significantly different between groups ($p = 0.035$) by ANOVA. There was a significant difference in the absorption of ^{58}Fe between the $FeSO_4$ group (7.4%±3.4) and the elemental iron group (4.1%±1.7 $p<0.04$) using post-hoc pair wise comparisons, but the difference between the $FeSO_4Na_2EDTA$ group (6.4%±4.4) and the elemental iron group did not reach significance ($p = 0.12$). There was no statistical difference in absorption between the $FeSO_4$ and the $FeSO_4Na_2EDTA$ groups. Subgroup analysis was also done by combining the two groups taking $FeSO_4$ (with and without EDTA); there was a significant difference between absorption in the pooled group and the absorption from elemental iron ($p = 0.01$). In an analysis of covariance of fractional absorption of ^{58}Fe including the covariates of group, age, sex, ethnic group and initial hemoglobin, only group ($p<0.04$) and

Table 2: Anthropometric data and hemoglobin of children completing study[1]

Category	Age [mos]	Wgt [kg]	Hgt [cm]	Hb [g/L]	BMI
Boys [n = 24]	100.3±15.6	25.0±4.5	126.8±09.0	104±10	15.5±1.5
Girls [n = 16]	84.5±15.9	19.0±4.2	116.6±07.5	109±9	14.0±2.2
Elemental Iron group [n = 13]	93.6±15.4	22.4±4.2	122.5±05.2	108±7	14.8±2.0
$FeSO_4$ group [n = 15]	94.6±20.9	22.3±5.9	122.6±12.5	104±13	14.9±1.9
$Na_2FeEDTA$ group [n = 12]	93.6±16.1	23.2±5.9	123.6±10.6	107±9	15.0±1.9
Overall [n = 40]	94.0±17.4	22.6±5.3	122.9±09.8	106±10	14.9±1.9

[1]Mean±SD

Table 3: %Fe Absorption from reference dose (5 mg ^{57}Fe) and test meal (1.5 mg ^{58}Fe) and total predicted absorbed iron from 100g fortified flour at tested concentration[1]

Group	Elemental iron [n = 13]	$FeSO_4$ [n = 15]	$FeSO_4Na_2EDTA$ [n = 12]	Overall [n = 40]
Reference Dose [^{57}Fe]	28.2%±14.6	30.0%±20.6	30.0%±20.9	29.4%±18.5
Test meal [^{58}Fe]	4.1%±01.7	7.4%±03.4	6.4%±04.4	6.0%±03.5
Absorbed iron from 100 g of fortified flour [µg]	243.0	223.0	126.0	-

[1]Mean±SD

hemoglobin ($p<0.01$) were significant. There was no significant difference between males (6.5%±4.3) and females (5.3%±1.8) in ^{58}Fe absorption over all groups. As expected, there was a strong negative correlation between hemoglobin level and iron absorption ($r = -0.52$, $p<0.01$).

Based on the results above, for each 100 g of fortified flour at the proposed fortification levels, the predicted amount of absorbed iron can be calculated in each group. For elemental iron (4.1% absorption) at 60 µg/g, 244 µg of elemental iron will be absorbed; for iron sulfate (7.4% absorption) at 30 µg/g, 222 µg will be absorbed and for ferrous sulfate plus Na_2EDTA (6.4% absorption) at 20 µg/g, 128 µg will be absorbed. For a child who eats 300 g of fortified flour per day, this represents iron absorption of 730 µg, 670 µg and 380 µg for the elemental iron, the $FeSO_4$ and the $FeSO_4Na_2EDTA$ groups, respectively from the iron fortificant. The daily absorbed requirement for children in this age group is estimated to be 700 to 800 µg per day for iron (Institute of Medicine, 2001). Thus, approximately 90% and 70% of the daily iron requirements of children in this age group could be met through the consumption of 300 g of wheat flour fortified with elemental iron or iron, respectively, at these fortification levels.

DISCUSSION

This trial represents an effort to establish a rational basis for the selection of a fortificant for a proposed wheat flour fortification program in Pakistan. Both elemental iron and ferrous sulfate were found to be potentially useful candidates for such a program and warrant further consideration. The form of elemental iron that was used as the unlabeled fortificant was obtained from the Micronutrient Initiative. It was in the form of a premix of an electrolytically reduced iron that was passed through a 100 mesh screen (150 micron). However, since the stable isotope form of elemental iron powder that was added as a label was prepared from larger pieces of ^{58}Fe in a laboratory in Pakistan and was not further characterized, we can not directly compare our results to those of other commercially available elemental iron powders. Nevertheless, the results obtained for the relative bioavailability values of the elemental powder were near the higher end of values seen in other studies. In a previous stable isotope study, Walter *et al.* (2004) examined the bioavailability of H_2-reduced elemental iron powder in white wheat bread made from 72% extraction flour in 5-7 year old children (Walter *et al.*, 2004). They found that the bioavailability of the iron from wheat bread rolls was 6.5% compared with 4.1% in our study. However, the particle size of their powder was 15 µm, smaller than the commercial specification of <45 µm, which probably enhanced its bioavailability. They calculated the relative bioavailability of elemental iron as 65% compared to $FeSO_4$. We reported a mean % absorption value for $FeSO_4$ of 7.4%; thus, the bioavailability value relative to $FeSO_4$ was 55%. Swain *et al.* (2003) measured the bioavailability value of six widely used elemental iron powders using a rat hemoglobin repletion bioassay. The results of this study showed relative bioavailability ranging from 21-64% with carbonyl iron having the highest value and reduced iron being the least bioavailable (Swain *et al.*, 2003). It would be expected that the 100 mesh electrolytically reduced powder supplied in the premix in our study would have a lower bioavailability than the 46% reported in Swain's study since its particle size is greater than the electrolytically reduced powders used in that investigation.

Various studies have demonstrated the positive effects of Na_2EDTA on iron absorption from $FeSO_4$. Davidsson *et al.* (2001) found that corn masa flour fortified with ferrous fumarate did not have enhanced fractional iron absorption with the addition of FeNaEDTA (Davidsson *et al.*, 2001). However, absorption from $FeSO_4$ increased from 5.5% to 9.0 (geometric mean) when Na_2EDTA was added at a molar ratio of 1:1 while in our study the

absorption with $FeSO_4$ was 7.4% and with Na_2EDTA added was 6.4%. However, the difference found in our study was not statistically significant. Hettiarachchi *et al.* (2004) studied the effect of Na_2EDTA on the absorption of $FeSO_4$ from rice flour fortified with iron and zinc using similar isotopic techniques and similar population (Hettiarachchi *et al.*, 2004). The fractional absorption of ^{58}Fe from a meal was significantly better ($p<0.01$) in the groups of schoolchildren receiving Na_2EDTA added to $FeSO_4$ (4.7%) versus those who received $FeSO_4$ alone (2.2%). In our study the Na_2EDTA was added in a 1:1 molar ratio with ferrous sulfate at a concentration of 20 µg/g iron and failed to show the expected increase in % absorbed values. This combination has been shown to be as effective as NaFeEDTA when the EDTA: Fe molar ratio is between 1:2 and 1:1 (Davidsson *et al.*, 2005). Because we used a molar ratio of Na_2EDTA: $FeSO_4$ of 1:1, the ratio of EDTA: Fe was slightly less than 1:1 (1:1.454). However, this should have been well within the range that has been investigated before and which has yielded improved absorption. We considered several methods of adding the Na_2EDTA and the $FeSO_4$, including first adding it to the flour and then mixing thoroughly, but we felt that adding it to the final individual dough portions would insure the most accurate results. We doubt that our technique resulted in lower absorption than expected due to inadequate formation of Fe and Na_2EDTA complexes, but we can not completely exclude that possibility. Some component of the meal used in the current study such as tea might have interfered with the efficacy of Na_2EDTA. The absorption of $FeSO_4$ was relatively high and it is possible that the addition of Na_2EDTA did not further improve the absorption. Finally, it is possible that some of the EDTA may have complexed with other minerals in the GI tract while still in solution, but this hypothesis awaits further investigation.

Additional valuable information could have been gained by better characterizing the final powder ^{58}Fe product although the inhomogeneity of the powder that we produced would have limited the usefulness of this information. Stable isotope labeling of iron powders that are physicochemically equivalent to commercial powders would be extremely useful in future studies, helping to verify the results of non-human studies. This could help guide flour fortification programs worldwide. What our investigation showed was that fortification of wheat flour with either ferrous sulfate at 30 µg/g or a laboratory-produced elemental iron powder at 60 µg/g can contribute substantially to the absorbed iron requirement of Pakistani children and could potentially be an effective strategy to combat the high prevalence of anemia in the country. There was general satisfaction with the organoleptic properties of the product and with the notable exception of the lack of efficacy of $FeSO_4Na_2EDTA$, the results were within the ranges found in other studies. These results are being used to design efficacy studies using the candidate fortificants. This investigation demonstrates the need for absorption studies in indigenous populations using locally consumed food products prior to embarking on an expensive food fortification program and underscores the continued need for the development of methods to determine the relative bioavailability value of elemental iron powders in human studies.

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REFERENCES

Abrams, S.A., I.J. Griffin and S. Herman, 2002. Using stable isotopes to assess the bioavailability of minerals in food fortification programs. Food Nutr. Bull., 23 (suppl 3): 158-165.

Balasubramaniam, P. and A. Malathi, 1992. Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. J. Postgrad. Med., 38: 8-9.

Bothwell, T.H. and A.P. MacPhail, 2004. The potential role of NaFeEDTA as an iron fortificant. Int. J. Vitam. Nutr. Res., 74: 421-434.

Centers for Disease Control and Prevention (CDC), 2008. National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention.

Davidsson, L., E. Ziegler, C. Zeder, T. Walczyk and R.F. Hurrell, 2005. Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: Erythrocyte incorporation of iron and apparent absorption of zinc, copper, calcium and magnesium from a complementary food based on wheat and soy in healthy infants. Am. J. Clin. Nutr., 81: 104-109.

Davidsson, L., T. Walczyk, N. Zavaleta and R.F. Hurrell, 2001. Improving iron absorption from a Peruvian school breakfast meal by adding ascorbic acid or Na_2EDTA. Am. J. Clin. Nutr., 73: 283-287.

Herman, S., I.J. Griffin, S. Suwarti, F. Ernawati, D. Permaesih, D. Pambudi and S.A. Abrams, 2002. Cofortification of iron-fortified flour with zinc sulfate, but not zinc oxide, decreases iron absorption in Indonesian children. Am. J. Clin. Nutr., 76: 813-817.

Hettiarachchi, M., D.C. Hilmers, C. Liyanage and S.A. Abrams, 2004. Na_2EDTA enhances the absorption of iron and zinc from fortified rice flour in Sri Lankan children. J. Nutr., 134: 3031-3036.

Hurrell, R.F., T. Bothwell, J.D. Cook, O. Dary, L. Davidsson and S. Fairweather-Tait, 2002. The usefulness of elemental iron for cereal flour fortification: A SUSTAIN task force report. Nutr. Rev., 60: 391-406.

Hurrell, R.F. and S. Lynch, 2004. Iron Fortification: The Science Base. Flour Fortification Workshop: Current Applications and Practical Applications-Discussion Paper. Cuernevaca, Mexico. 1-3 December 2004. [cited 2005 Nov 15] Available from: http://www.sph.emory.edu/wheatflour/CKPAFF/CKPAFFpdfs/R.HurrellandS.Lynch.pdf.

Institute of Medicine, 2001. Iron. In: Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy Press, Washington DC., pp: 328.

Kastenmayer, P., L. Davidsson, P. Galan, F. Cherouvrier, S. Hercberg and R.F. Hurrell, 1994. A double stable isotope technique for measuring iron absorption in infants. Br. J. Nutr., 71: 411-424.

Kazi, A.Q. and M.M. Qureshi, 2002. A critical analysis of Nutritional studies in Pakistan (1965 to 1987). Pakistan Council for Science and Technology, Islamabad.

Khan, M.M., 2001. Effect of maternal anaemia on fetal parameters. J. Ayub Med. Coll. Abbottabad, 13: 38-41.

Nutrition Division, 1988. National Institute of Health, Government of Pakistan National Nutrition Survey 1985-87, 1988; pp: 163.

Paracha, P.I. and A. Jamil, 1998. Assessment of micronutrient (Iron, Vitamin A, Zinc) status in preschool children of N.W.F. Province, Pakistan. Survey report published by UNICEF.

Planning and Development Division, 1978. Government of Pakistan Micronutrient Survey of Pakistan 1976-77, 1978; pp: 61.

Planning and Development Division, 2004. National Nutrition Survey, 2001-02. Planning and Development Division, Government of Pakistan National Nutrition Survey 2001-02, 2004; pp: 41-45.

Siddiqui, I.A., M.A. Rahman and A. Jaleel, 2004. Efficacy of daily vs. weekly supplementation of iron in school children with low iron status. J. Trop. Pediatr., 50: 276-278.

Swain, J.H., S.M. Newman and J.R. Hunt, 2003. Bioavailability of elemental iron powders to rats is less than bakery-grade ferrous sulfate and predicted by iron solubility and particle surface area. J. Nutr., 133: 3546-3552.

Walter, T., F. Pizarrro, S.A. Abrams and E. Boy, 2004. Bioavailability of elemental iron powder in white wheat bread. Eur. J. Clin. Nutr., 58: 555-558.