Efficacy of rice flour fortification in Sri Lanka: A pilot study

Manjula Hettiarachchi[1], Chandrani Liyange[1], David C. Hilmers[2] and Steven A. Abrams[2]
[1]Nuclear Medicine Unit, Faculty of Medicine, University of Ruhuna, P. O. Box 70, Karapitiya, Galle, Sri Lanka;
[2]U.S. Department of Agriculture/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX, 77030.

Abstract

Rice flour has been proposed as a vehicle for iron and zinc fortification in Sri Lanka. Although widely consumed, rice flour has not been previously evaluated as a fortified food. We conducted a mini efficacy trial on fortified rice flour while determining the bioavailability of iron and zinc from a meal made out of this flour. We randomized 53 Sri Lankan schoolchildren aged 7-10 yr into 4 groups who consumed (daily) local dishes made out of 75 g of fortified flour in 4 types (1) $FeSO_4$ (2) $FeSO_4$+Na_2EDTA (3) $FeSO_4$+ZnO and (4) $FeSO_4$+Na_2EDTA+ZnO for a period of four weeks. The fortification levels of iron and zinc were 60 mg/kg; the rice flour also contained folate at 2 mg/kg in each group. Na_2EDTA was added at 1:1 molar ratio with iron. Forty-eight subjects completed the trial. The mean improvement in hemoglobin was 5.96 g/L ($p<0.001$) with the greatest gain showing in the group consuming rice flour with $FeSO_4$ + Na_2EDTA (group 2). The $FeSO_4$ plus zinc group (group 3) also showed a greater improvement, demonstrating that the added zinc had no deleterious effect on hemoglobin status. Overall, serum ferritin and serum zinc were improved in this short period of time by 1.51 ìg/L and 0.59 ìmol/L respectively, but these changes were not significant ($p>0.10$). All groups showed a statistically significant improvement (pd"0.03) in their serum folate status at the end of the study (overall 6.58 nmol/L). The results demonstrated a benefit in using Na_2EDTA to improve both iron and zinc absorption. We conclude that the fortification of rice flour is feasible, although additional strategies such as dephytinization or an increase in the level of iron and zinc fortification should be considered to obtain a higher proportion of the daily-absorbed iron and zinc contents.

Keywords: flour fortification, rice, micronutrient status

Introduction

Strategies used to combat micronutrient deficiencies worldwide include fortification of staples, supplementation, modification of traditional diets, and recently, fortification of beverages (1). Similar to many developing countries, Sri Lanka has a high prevalence of deficiencies of micronutrients such as iron, zinc, vitamin A, and iodine, particularly in children, pregnant and young women and laborers in tea plantations in the central region of the country (2-5). As approaches to resolving this problem, supplementation programs and wheat flour fortification, have been considered but such fortification has not been adopted in Sri Lanka. Although pill supplementation is considered to be useful for short-term interventions such as during pregnancy (6,7), it has not been found satisfactory in improving iron status. Moreover, it is not considered to be sufficiently sustainable for the population as a whole. Wheat flour has been used as a vehicle in many countries for food fortification, but the lack of local processing and low per capita consumption of this staple by target groups make it unsuitable for this purpose in other nations, including Sri Lanka (8).

Rice is a potential vehicle for fortification because of its widespread consumption by at-risk populations (9). Most Sri Lankans eat rice as their primary dietary staple with a low intake of animal products; it is thought that this diet may contribute to a high prevalence of multiple micronutrient deficiencies.

Therefore, we sought to evaluate the efficacy of fortified rice flour as a possible vehicle for a national food fortification program in Sri Lanka. Our primary objective was to evaluate the acceptability and effectiveness of rice flour-based meal fortified with iron and zinc. In order to test the efficacy of Na_2EDTA in overcoming the inhibitory effects of phytates and polyphenols, we included two groups in our study that received Na_2EDTA at a molar ratio of 1:1 with Fe, a ratio that has been shown to be both efficacious and safe in previous studies (10). Ferrous sulfate ($FeSO_4$) and zinc oxide (ZnO) were chosen as the sources of iron and zinc for fortification because of their established bioavailability and widespread availability (11). Periconceptional use of folate supplements for the primary prevention of neural tube defects by fortification is the preferred strategy in many countries because of its potential to reach the largest proportion of women of child-bearing age (12). Therefore we decided to add folate to the rice flour.

Methodology

Fifty three (53) subjects were recruited through a public advertisement. A meeting of potential participants and their families was held at the Faculty of Medicine in Galle, Sri Lanka. The study received approval from the Ethics Committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka and from the Institutional Review Board for Baylor College of Medicine and Affiliated Hospitals in Houston, Texas, USA. Informed written consent was obtained in Sinhalese from the parents after explaining in detail the purpose, risks and benefits of the study. Children between the ages of 7 to 10 were eligible for the study. Children were excluded if they had a chronic medical condition or were taking micronutrient supplements. Their weights and heights were recorded, a medical history obtained and, a physical examination was performed. Venous samples of blood (2ml) were obtained from each subject for the initial assessment of hemoglobin, serum zinc, folate and ferritin.

All study subjects were treated for parasites by administering oral mebendazole. The subjects were randomized into the following groups based on type of fortification of flour and stratified by gender; each group consumed a meal daily, of fortified rice flour (75g) containing:

group 1 - $FeSO_4$ and folate; group 2 - $FeSO_4$, Na_2EDTA and folate;
group 3 - $FeSO_4$, ZnO and folate; group 4 - $FeSO_4$, Na_2EDTA, ZnO and folate.

Na_2EDTA, disodium ethylenediaminetetraacetic acid; $FeSO_4$, ferrous sulfate; ZnO, zinc oxide.

Sixty (60) mg of elemental iron in the form of dried $FeSO_4$ and 60 mg of elemental zinc in the form of ZnO powder were added to each kg of rice flour to obtain the desired level of fortification. In addition, 385.08 mg of Na_2EDTA in dry powder form was added to each kg of rice flour in the appropriate groups in order to obtain a molar ratio of 1:1 of elemental Fe: Na_2EDTA and, 0.7:1 of elemental Zn: Na_2EDTA. Two (2) mg of folate was added per kg of rice flour in each group. Baseline and final biochemical parameters and anthropometry of the subjects were compared after providing the appropriately fortified rice flour over a period of four weeks. Forty-eight children completed the trial.

Rice flour preparation

Brown country rice was used for fortification. Grade 2 (well polished, 6-8% weight reduction on polishing) rice was powdered to the 300 - 500 mesh size using an electric grinder at the Industrial Technology Institute (ITI) Colombo, Sri Lanka. Mixing of food grade minerals with rice flour was accomplished at the ITI using a ribbon blender; iron and zinc levels were measured by f- AAS (flame Atomic Absorption Spectrometry). The fortificants ($FeSO_4$ & ZnO) were obtained from Dr. Paul

Lohmann, Germany, Na_2EDTA from AKZO NOBEL, Netherlands and folic acid from the Glaxo Welcome, India.

Analytical Methods

Hemoglobin concentration was measured by the cyanmethemoglobin method using a spectrophotometer at the Nutrition Research Laboratory of Faculty. Serum ferritin by immunoradiometric assay and folate by radioimmunoassay were analyzed with the provision of reagents by North East Thames Regional Immunoassay (NETRIA), London at the Nuclear Medicine Unit. Serum zinc was analyzed at the ITI, Colombo by f- AAS.

Statistical Analysis

Changes in biochemical parameters at the beginning and end of the study were analyzed using paired t-tests. Based on the absorption from fortified wheat seen in a previous study in a similar population in Indonesia (11) the following assumptions were made in calculating the power required for the study:

1. Ferrous sulfate absorption will be 15.9% with SD (±6.8%)
2. The addition of Na_2EDTA will increase $FeSO_4$ absorption by 50% to approximately 24% with SD (±7.0%)

A sample size of 18 was predicted to give greater than 90% power (alpha 0.05) to detect the assumed difference of 8% between the $FeSO_4$ + Na_2EDTA groups (total of 24) and the $FeSO_4$ groups (total of 24). The additional 6 subjects in each group were added to accommodate dropouts. Differences in serum biochemistry between groups were assessed using ANOVA and paired t-tests. Statistical analysis was carried out using Stata 6.0 (Stata Labs, Inc., San Mateo, CA). Anthropometric data were analyzed using Anthro version 1.02, 1999, Centers for Disease Control, Atlanta, GA. Descriptive statistics are expressed as the mean ± standard deviation (SD). P-values less than 0.05 were considered to be significant.

Results

Characteristics of Study subjects

There were 25 male and 28 female subjects enrolled with ages ranging between 7 and 10 years (Table 1). The prevalence of under nutrition was assessed on the basis of weight-for-age and height-for-age using the CDC 1978 reference standards (13). Twenty-one per cent 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 were anemic (Hb <120.0 g/L); 8% had low serum ferritin (<15.0 ìg/L); 36% were deficient in folate (<11.3 nmol/L); and 15% had zinc deficiency (<9.945 ìmol/L). There were no significant differences (table 1) between subgroups in terms of age, anthropometrics, and biochemical data (hemoglobin, ferritin, zinc, and folate).

Table 1: Baseline anthropometric and biochemical statistics[1]

Parameter	Group 1	Group 2	Group 3	Group 4
n	M 6, F 8	M 7, F 6	M 6, F 7	Male 6, F 7
Age (months)	88.0 (± 18.7)	94.9 (± 19.5)	94.8 (± 19.2)	100.9 (± 15.9)
Weight (kg)	19.9 (± 3.8)	20.5 (± 3.6)	20.1 (± 3.9)	21.7 (± 4.5)
Height (cm)	119.2 (± 7.3)	122.0 (± 9.2)	121.0 (± 8.2)	124.5 (± 9.3)
BMI[2]	13.9 (± 1.7)	13.6 (± 0.9)	13.6 (± 1.3)	13.8 (± 1.4)
Hemoglobin (g/L)	119.9 (± 11.3)	121.9 (± 9.8)	123.1 (± 12.2)	121.9 (± 11.6)
Serum Zinc (ìmol/L)	12.2 (± 2.7)	11.8 (± 2.4)	11.8 (± 2.3)	12.1 (± 1.7)
Serum Folate (nmol/L)	12.8 (± 4.9)	12.4 (± 4.2)	12.1 (± 5.6)	13.0 (± 8.1)
Serum Ferritin (ìg/L)	47.3 (± 30.4)	52.9 (± 36.8)	51.9 (± 27.2)	42.0 (± 23.9)

[1]Results were expressed as mean (±SD)

[2]BMI was calculated using the formula [(weight (kg)/ height $(m)^2$]

Effects of fortified rice flour on anthropometry

The children who had completed the trial showed mean improvement of 680 g weight gain and 11 mm height gain (Table 2). A statistically significant improvement for all four age groups-was observed with a higher gain in two zinc groups. This provides a clear evidence of the positive impact of zinc on the growth of the child.

Table 2: The final anthropometry [mean (±SD)], and mean change following one month (comparing those who remained in the study) using paired t-test

Group		Weight (kg)	Height (cm)	BMI
1	Mean	19.9 (±2.7)	120.7 (±7.0)	13.6 (±0.9)
	Change	+0.52	+1.1	+0.11
	p-value	p=0.012	p<0.001	p=0.18
2	Mean	20.7 (±3.0)	122.4 (±8.2)	13.7 (±0.9)
	Change	+0.62	+1.1	+0.20
	p-value	p=0.033	p<0.001	p=0.25
3	Mean	20.9 (±4.3)	122.2 (±8.0)	(±1.4)
	Change	+0.78	+1.2	+0.24
	p-value	p<0.001	p<0.001	p=0.044
4	Mean	22.0 (±4.7)	125.1 (±9.8)	13.9 (±1.5)
	Change	+0.79	+1.0	+0.27
	p-value	p<0.001	p<0.001	p=0.003

Effects of fortified rice flour on iron and zinc status

The serum zinc, ferritin and folate levels were measured before and after the consumption of fortified rice flour for 30 days (figure 1). Among those subjects who completed the trial, the mean improvement in hemoglobin was 5.96 g/L ($p<0.001$) with the greatest gain showing in the group 2 ($FeSO_4$, Na_2EDTA). Group 3 ($FeSO_4$, ZnO) was the second best demonstrating that the added zinc had no deleterious effect on hemoglobin stores. Overall, levels-of serum ferritin and serum zinc were improved (by 1.51 ìg/L and 0.59 ìmol/L respectively) during this short period of time, but these changes were not significant ($p>0.10$). The fortified flour given to all groups was containing folate as well. All the groups showed significant improvement (pd"0.03) in their serum folate status at the end of the study (overall 6.58 nmol/L). The groups which received Na_2EDTA showed a greater increase in serum ferritin and hemoglobin in the Na_2EDTA groups was evident. However, the increased was statistically significant in hemoglobin only. The groups received zinc-fortified meals showed a gain in serum zinc, but did not reach a significant level.

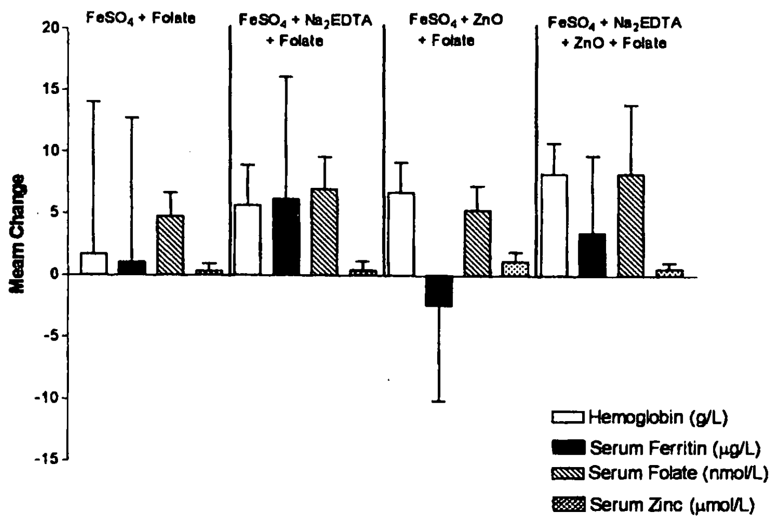

Fig. 1: Mean change (±SD) in biochemical status among study subjects following consumption of fortified rice flour for one month

Discussion

In this study, we examined the effects of multinutrient-fortified rice flour on the of growth and nutrition of young children in Sri Lanka for a period of four weeks, as a pilot study. Major outcomes were anthropometric, haematologic and biochemical indices of nutritional status.

The four groups were comparable at study entry. The improved growth in the study groups cannot be assumed, due to a higher energy intake from the rice flour itself, as a control group was not included. But there are other potential explanations. One of the cardinal features of zinc deficiency is growth failure; thus, it is possible that the increase in zinc intake facilitated an augmented lean tissue accretion or increased utilization of the energy provided. Such a possibility is described in the response of BMI in this sample. The concept of 'hidden hunger' associated with micronutrient deficiency has been described in recent years (14), and this may provide an alternative explanation for the improvement in growth. Lawless et al. (15) reported improvements in growth and appetite in Kenya as a result of iron supplementation on the basis of the results of an appetite test and questionnaires. Kanani and Poojara (16) reported an improvement in growth of adolescent Indian girls who were given folic acid and iron supplementation. It is therefore possible that the fortification may have had higher energy intake as a result of increased appetite from improvements in their folate and iron status. Alternatively, improvement in iron stores, serum folate concentrations and enhanced zinc status may also be important in limiting morbidity, which can lead to improved growth variables, especially in at-risk children in developing countries.

At the start of the study, low folate, ferritin and zinc values were relatively common. Even though some data are available on the micronutrient status of selected age groups (3-5), the status of zinc, ferritin and folate among children in this age group has not been investigated. The data presented in this study would represent the primary data available on primary school children in Sri Lanka. Differences observed at the end of the study strongly suggest a beneficial effect on zinc and folate status from consumption of the fortified rice flour. This may be clinically important, as has been shown, leading to enhanced growth and decreased morbidity and mortality from infectious diseases in developing countries (17).

Various studies have demonstrated the positive effects of Na_2EDTA on iron absorption from $FeSO_4$. Davidsson, et al, found that corn masa flour fortified with ferrous fumarate did not have enhanced fractional iron absorption with the addition of FeNaEDTA. However, absorption from $FeSO_4$ has been increased when Na_2EDTA was added at a molar ratio of 1:1(18). Hurrell, et al. measured iron absorption from weaning cereals fortified with $FeSO_4$ with or without Na_2EDTA (10).

The supplementation of diets with a specific food substance high in one or more micronutrients recognized as potentially deficient in the regular diet is not a new concept. Although it is well recognized that food fortification is among the preferred and cost-effective approaches in combating micronutrient malnutrition, its effectiveness in developing countries is yet to be demonstrated. One of the limiting factors is the lack of simple and affordable technology to fortify foods with stable and bioavailable nutrients without compromising commonly accepted taste and appearance (19).

There are some theoretical advantages of using rice flour to supply micronutrients to children. First, this strategy might increase the preferences of the country to improve paddy cultivation. It is unlikely that other kinds of flour especially wheat would be readily available to children of low-income families at a low cost in our country, if the government discontinue the subsidiary programme on wheat. Another concern is that the fiber content of rice might increase the gut transit time and the higher caloric content from the meal. Possible disadvantages might be initial high cost for the machinery needed for powdering rice and the higher cost over the unfortified flour. Weighing the apparent advantages from the higher micronutrient intake against the potential disadvantages of the product, the benefit: risk ratio is probably high, especially in populations with high rates of micronutrient deficiencies. Our study was limited by the inclusion of small study population. Although our results are likely to reflect the true benefit of the fortified rice flour, definitive randomized, placebo-controlled, double blind studies in multiple age groups over an extended period in a resource-poor setting should be conducted.

In conclusion, this study demonstrated that regular consumption of micronutrient-fortified rice flour for four week led to improvements in weight, height and several measures of micronutrient status in semi urban school children in Galle. The use of multiple fortification strategies has several advantages. These include ease of distribution, high levels of acceptance, the ability to lower substances such as phytates, which can inhibit nutrient absorption, and the potential for purchase by private consumers rather than reliance on government programs

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