Efficacy of Different Iron Fortificants in Wheat Flour in Controlling Iron Deficiency[1]

JIAN HUANG, JING SUN, WEN-XIAN LI, LI-JUAN WANG, AN-XU WANG[*],
JUN-SHENG HUO[2], JUN-SHI CHEN, AND CHUN-MING CHEN

*Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing 100050, China; *Nanyang Wancheng District Center of Disease Control and Prevention, Nanyang 473009, Henan, China*

Objective   To observe the different impacts of electrolytic iron, $FeSO_4$, and NaFeEDTA on body iron store of anemic school students.   **Methods**   Four hundreds anemic students at the age of 11-18 years were divided into four groups. Of which, three consumed different iron fortificants from wheat flour as food vehicle for six months and one consumed non-fortified flour (control). The fortification level of electrolytic iron, $FeSO_4$, and NaFeEDTA was 60 mg Fe/kg, 30 mg Fe/kg, and 20 mg Fe/kg, respectively. Blood samples were collected at 0, 2, 4, and 6 months and hemoglobin (Hb), serum ferritin (SF), and transferrin receptor (TfR) were measured.   **Results**   The hemoglobin levels in three intervention groups increased, the increments of Hb in the NaFeEDTA group were significantly higher than that in the other groups. SF and TfR levels increased in the tested groups and body iron store in the NaFeEDTA group was higher than that in the other groups. These parameters did not show any significant changes in the control group.   **Conclusion**   NaFeEDTA and $FeSO_4$ fortified wheat flour has positive impacts on iron status in anemic students and NaFeEDTA is more effective than $FeSO_4$, while electrolytic iron is less effective in improving iron store in anemic students.

Key words: Body iron; Iron deficiency anemia; Wheat flour; Fortification; Electrolytic iron; $FeSO_4$; NaFeEDTA

INTRODUCTION

Iron deficiency (ID) and iron deficiency anemia (IDA) are major nutrition problems around the world. Iron fortification and supplementation are considered the major approaches to the control of ID and IDA. However, appropriate selection of iron fortificants remains an important technical issue[1]. Iron bioavailability and efficacy on ID and IDA, along with other factors such as organoleptic and price, are the necessary parameters for a proper selection of iron fortificant[2]. In recent years, body iron and serum ferritin (SF) and serum transferrin receptor (sTfR) are ordinarily used as parameters of iron status, but they are affected by inflammation and parasitic infection other than body iron status[3]. Elemental iron and $FeSO_4$ are the most commonly used iron sources in food fortification, while NaFeEDTA is used to fortify food containing high iron absorption inhibitors[4-5]. However, the cost-effectiveness of these fortificants is still controversial in iron intervention practices[6-7]. This study was to compare the effect of electrolytic iron, $FeSO_4$ and NaFeEDTA in wheat flour fortification on iron status of anemia students for the selection of proper iron fortificants in food fortification.

MATERIALS AND METHODS

Iron Fortificants

Electrolytic elementary iron, $FeSO_4$, and NaFeEDTA were selected as iron fortificants to be evaluated. Wheat flour with a 70% extraction rate was used as food vehicle. Three different kinds of fortified flour were produced from basal flour, including electrolytic iron fortified flour, $FeSO_4$ fortified flour, and NaFeEDTA flour. The three varieties of fortified flour and the basal flour were provided at free to four groups of subjects during the trial. The levels of iron fortified as recommended commonly were electrolytic iron in 60 mg Fe/kg,

$FeSO_4$ in 30 mgFe/kg, and NaFeEDTA in 20 mgFe/kg, respectively. Electrolytic iron and $FeSO_4$ were provided by SUSTAIN, and NaFeEDTA was provided by Beijing Vita Sci-Tech Co. Ltd.[8]. The contents of iron in electrolytic iron, $FeSO_4$, and NaFeEDTA were 98%, 32%, and 13%, respectively.

Subjects and Treatment

The World Health Organization diagnostic criteria for IDA were used for the diagnosis of anemia[9]. Four hundred and nine school students at the age 11-18 years, diagnosed as IDA from 4500 students in 4 schools in Nanyang city, Henan province, were divided into control group (n=109, 47 males, 62 females), electrolytic iron group (n=96, 42 males, 54 females), $FeSO_4$ group (n=107, 44 males, 63 females) and NaFeEDTA group (n=106, 40 males, 64 females) on school basis and supplied with different kinds of iron fortificants. The students in the four groups were from four nearby schools in the same area and had similar economic status, lifestyle and dietary pattern. The subjects students lived and took meals together in the schools, except for one-month vacation. The fortified and basal flour was also provided at free to the families of students so that the students and other family members had the same flour. The students were assured without other health problems through school health examination record. The intervention lasted 6 months.

The study protocol was reviewed and approved by the Ethical Committee of the Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention. Consent forms were obtained from each student and his/her guardian.

Dietary Survey and Blood Measurement

Dietary pattern of the students remained unchanged and was assured by food frequency survey[10] before and after the intervention. Five mL of intravenous blood samples was collected from each student at 0, 2, 4, and 6 months. Blood hemoglobin (Hb), SF, and sTfR were measured.

Hb was measured with Hemocue B-hemoglobin system (Hemocue Corporation, Sweden) using whole blood sample.

Each intravenous blood sample was centrifuged at 3000 rpm for 20 min. Serum was taken and stored at -80 ℃ for subsequent measurements. Test kits from the RANDOX Company (England) were used to measure SF (turbidity at 700 nm), and TfR was measured by ILISA at 450 nm and corrected at 540 nm with a Bio-Rad microplate manager spectrophotometer (R&D System, Inc. America).

Iron store was calculated following the equation[4]:
Body iron=-[log (sTfR/SF)-2.8229]/0.1207

Statistical Analysis

Data analysis was performed by Student t-test using the SPSS software.

RESULTS

SF and sTfR

SF level in the control and electrolytic iron groups did not change significantly during the trial period. However, SF level in the NaFeEDTA group increased significantly after 4 months, while SF level in the $FeSO_4$ group did not increase significantly at end of the trial. The SF levels in the NaFeEDTA and $FeSO_4$ groups were increased to 14.0 ng/mL and 9.5 ng/mL, respectively, after 6 months, compared with their baseline values (Table 1).

TfR levels in the control group did not change significantly in the trial. However, TfR levels in the NaFeEDTA, $FeSO_4$, and electrolytic iron groups decreased significantly after 4 and 6 months. The TfR levels in the 3 groups were decreased to 13.0 nmol/L, 8.0 nmol/L, and 3.7 nmol/L, respectively, after 6 months (Table 1).

Body Iron Store

Body iron stores in the NaFeEDTA and $FeSO_4$ groups continuously increased during the trial period, but not significantly changed in the electrolytic iron and control groups (Fig. 1). Body iron store in the electrolytic iron group did not change during the 4 months of trial, but notably increased after 6 months.
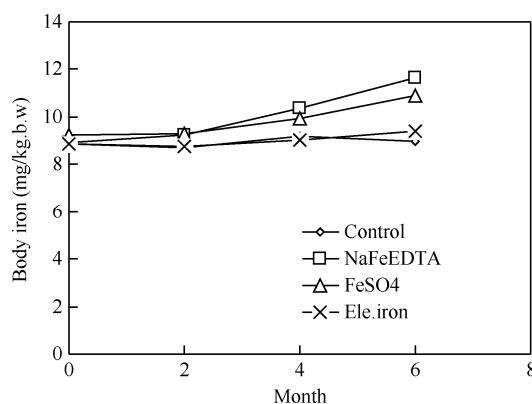

FIG. 1. Changes of body iron store during the trial.

Hemoglobin

The changes of Hb levels in the four groups during the trial are shown in Table 1. Before intervention, the Hb levels were not statistically different among the four groups, and the Hb level in

TABLE 1

Changes of Blood Parameters during the Intervention Trial ($\bar{x} \pm s$)

Groups	Months	Hemoglobin (g/L)	Serum Ferritin (ng/mL)	sTfR (nmol/L)
Control	0	114.5±5.3	48.9±19.4	37.3±8.5
	2	114.4±8.4	45.6±25.1	38.4±9.7
	4	114.8±7.2	47.8±21.4	35.2±8.0
	6	114.9±8.0	46.8±23.8	36.3±9.2
NaFeEDTA	0	114.9±5.0	46.0±20.5	36.4±7.2
	2	118.3±9.3[*]	47.5±19.6	34.2±8.3[※]
	4	122.9±9.7[‡,※]	55.3±21.3[‡,§]	30.0±7.1[‡,※]
	6	132.4±10.2[‡,※]	60.0±24.5[‡,※]	23.4±4.6[‡,※]
FeSO$_4$	0	114.5±6.5	49.0±19.8	35.5±7.7
	2	117.0±14.4	47.2±30.0	34.0±8.9[※]
	4	118.7±13.5[*,§]	50.8±19.4	30.8±7.4[‡,※]
	6	123.8±13.1[‡,※]	58.5±20.9[‡,※]	27.5±6.1[‡,※]
Electrolytic Iron	0	114.1±4.7	46.4±17.9	37.1±8.6
	2	117.3±8.4	43.3±18.9	35.4±8.5[§]
	4	117.6±12.4	44.4±20.1	34.1±8.1[*]
	6	118.0±10.9[*]	48.3±20.4	33.4±8.1[‡,§]

Note. [*]$P<0.05$ *vs* baseline (0 month), [‡]$P<0.01$ *vs* baseline (0 month), [§]$P<0.05$ *vs* control group, [※]$P<0.01$ *vs* control group.

the control group remained unchanged throughout the 6-month trial. The Hb level in the NaFeEDTA group increased significantly from month 2 to month 6. The Hb level in the FeSO$_4$ group increased significantly at month 4 and month 6 compared with the baseline and the control group. The Hb level in the electrolytic iron group was significantly higher than baseline and in the control group at month 6. The Hb levels were positively correlated with body iron (Fig. 2). The Hb levels in the control and electrolytic groups did not change much during the 6-month intervention. The body iron store in the control and electrolytic groups also changed very little. On the other hand, body iron increased in the NaFeEDTA and FeSO$_4$ groups along with increased Hb levels.
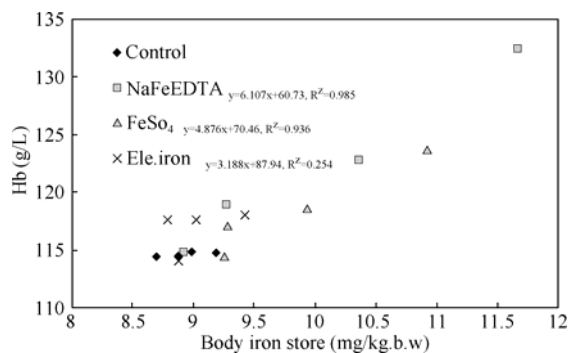

FIG. 2. Relation between body iron store and Hb levels. (Hb data from Table 1, iron store data from Fig. 1)

DISCUSSION

It has been widely recognized that iron deficiency has adverse effects on child growth (physical and mental), immune function, and productivity even before anemia occurs. Therefore, it is important to identify iron deficient subjects as early as possible, and several blood indicators such as serum iron (SI), total iron binding capability (TIBC), free erythrocyte protoporphyrin (FEP), serum ferritin (SF), and serum transferrin receptor (sTfR) have been used to assess iron status[11]. However, these indicators are considered not ideal because of their poor sensitivity to and correlation with each other. Cook *et al.*[4] have developed a new assessment method which combines SF and sTfR to estimate body iron store. Body iron store measurement on the basis of body weight could avoid possible confounding because of the differences in body weight. Since measurement of body iron is independent of hemoglobin determination, it can be used to distinguish iron deficiency anemia from other anemia[12]. However, more researches are needed on body iron store in different populations since body iron store data are lack in the Chinese population[4]. In this study, body store iron was positively correlated with Hb level, which supports the hypothesis that iron in consumed food is stored iron after absorption, and then available as part of hemoglobin through a biochemical mechanism (Fig. 2).

Iron fortification in food can be dated back to more than 60 yearsago[13]. Elemental irons including reduced iron and electrolytic iron and $FeSO_4$ are the most widely used iron fortificants in wheat flour[14-15], because they are inexpensive and readily available. A number of studies on absorption or bioavailability of iron fortificants in wheat flour revealed that iron fortificants, such as elemental iron, ferrous sulfate, NaFeEDTA, and ferrous fumarate, have different absorption rates or bioavailability[16-23].

Most iron intervention studies have focused on the effects of elemental iron, $FeSO_4$, and ferrous fumarate. Elwood et al.[24] found that H-reduced iron-fortified bread can increase Hb after 9 months of intervention. Elwood[25] reported that H-reduced iron-fortified bread has no beneficial effect on Hb levels within 3-6 months. Most scientists believe that the absorption of elemental iron in humans is poorer than that of other iron fortificants. However, electrolytic iron is considered a better iron source than H-reduced iron[26], showing that electrolytic iron has a lower efficacy on ID and IDA than $FeSO_4$ and NaFeEDTA.

In the middle of last century in South America, application of $FeSO_4$ (30 mg/kg) fortified flour successfully reduced the rate of IDA to less than 1% in Chile[27]. $FeSO_4$ has been used commonly in many iron fortified foods, because it is instable in food and easy to be affected by iron absorption inhibitors.

NaFeEDTA, a new iron fortificant, has a number of advantages, e.g. high absorption in humans consuming plant-based diet, less affected by iron absorption inhibitors such as phytic acid and polyphenol, and stable in food vehicles. However, the bioavailability of NaFeEDTA in fortified flour has not been reported.

In conclusion, NaFeEDTA in flour can improve body iron store. Hb level is remarkably higher in NaFeEDTA than in electrolytic iron and $FeSO_4$. $FeSO_4$ with a double concentration in flour as NaFeEDTA shows an appreciable impact on body iron and Hb. Thus, NaFeEDTA should be recommended as an iron fortificant of flour.

REFERENCES

1. Ricardo U, Hertrampf E, Reddy M (2002). Iron fortification of foods: overcoming technical and practical barriers. *J Nutr* **132**, 849-852.
2. Boccio J R, Iyengar V (2003). Iron deficiency: causes, consequences, and strategies to overcome this nutritional problem. *Biol Trace Elem Res* **94**(1), 1-32.
3. James D Cook, Carol H Flowers, Barry S Skikne (2003). The quantitative assessment of body iron. *Blood* **101**(9), 3359-3364.
4. Hurrell R F (2002). Fortification: overcoming technical and practical barriers. *J Nutr* **132**(suppl), 806S-812S.
5. Hertrampf E (2002). Iron fortification in the Americas. *Nutrition Reviews* **60**(Suppl. 1), 22-25.
6. Dary O (2002). Lessons learned with iron fortification in central America. *Nutr Rev* **60**(Suppl. 1), 30-33.
7. Osler M, Milman N, Heitmann B L (1999). Consequences of removing iron fortification of flour on iron status among Danish adults: some longitudinal observations between 1987 and 1994. *Prev Med* **29**(1), 32-36.
8. Joint FAO/WHO Expert Committee on Food Additives (1999). Report of 53rd Meeting of the Joint FAO/Who Expert Committee on Food Additives 1999 FAO/WHO Geneva, Switzerland.
9. Wang G Y (1992). The Chinese food composition table. Beijing: People's Medical Publishing House (In Chinese).
10. Chen X C (1984). Apply Nutrition. China: People's Medical Publishing House, 292-293. (In Chinese)
11. Cohen J H, Haas J D (1999). The comparison of mixed distribution analysis with a hree-criteria model as a method for estimating the prevalence of iron deficiency anaemia in Costa Rican children aged 12-23 months. *Int J Epidemiol* **28**, 82-89.
12. Punnonen K, Irjala K, Rajamaki A (1997). Serum transferring receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood* **89**, 1052-1057.
13. Venkatesh M (2000), Food fortification as a major strategy to address micronutrient malnutrition in Asia. In Manila Forum 2000: Strategies to fortify essential foods in Asia and the Pacific. ADB Press, 23-31.
14. Martinez-Torres C, Racca E, Rivero F, *et al.* (1991). Iron fortification of pre-cooked maize flour. *Interciencia* **16**, 254-260.
15. Hurrell R F, Furniss D E, Whittaker P, *et al.* (1989). Iron fortification of infant cereals: a proposal for the use of ferrous fumarate or ferrous succinate. *Am J Clin Nutr* **49**, 1274-1282.
16. L Hallberg, M Brune and L Rossander (1986). Low bioavailability of carbonyl iron in man: studies on iron fortification of wheat flour. *Am J Clin Nutr* **43**, 59-67.
17. Hurrell R F, Reddy M B, Burri J, *et al.* (2000). An evaluation of EDTA compounds for iron fortification of cereal-based foods. *Br J Nutr* **84**, 903-910.
18. Bilal R, Roohi S, Ahmad T, *et al.* (2002). Iron fortification of wheat flour: bioavailability studies. *Food Nutr Bull* **23**(Suppl. 3), 199-202.
19. Davidsson L, Dimitriou T, Boy E, *et al.* (2002). Iron bioavailability from iron-fortified Guatemalan meals based on corn tortillas and black bean paste. *Am J Clin Nutr* **75**(3), 535-539.
20. Lysionek A, Zubillaga M, Salgueiro J, *et al.* (2001). Bioavailability studies of a new iron source by means of the prophylactic-preventive method in rats. *Biol Trace Elem Res* **84**(1-3), 123-128.
21. Walter T, Dallman P R, Pizarro F, *et al.* (1993). Effectiveness of iron-fortified cereal in prevention of iron deficiency anaemia. *Pediatrics* **91**, 976-982.
22. Walter T, Pizarro F, Boy E, *et al.* (2004). The poor bioavailability of elemental iron in corn masa flour is not affected by disodium EDTA. *J Nutr* **134**, 380-383.
23. Walter T, Pizarro F, Olivares M (2003). Iron bioavailability in corn-Masa tortillas is improved by the addition of disodium EDTA. *J Nutr* **133**, 3158-3161.
24. Elwood P C, Waters W E, Sweetran P (1971). The haematinic effect of iron in flour. *Clin Sci* **40**, 31-37.
25. Elwood P C (1963). A clinical trial of iron fortified bread. *BMJ* **1**, 224-227.
26. Hurrell R, Bothwell T, Cook J D, *et al.* (2002). SUSTAIN Task Force, The usefulness of elemental iron for cereal flour fortification: a SUSTAIN Task Force report. Sharing United States Technology to Aid in the Improvement of Nutrition. *Nutr Rev* **60** (12), 391-406.
27. García-Casal M.N, Layrisse M (2002). Iron Fortification of Flours in Venezuela. *Nutr Rev* **60**(Suppl. 1), 26-29.