Original Article

Therapeutic effects of NaFeEDTA-fortified soy sauce in anaemic children in China

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The therapeutic effects of NaFeEDTA-fortified soy sauce on anaemic students were investigated. Three hundred and four iron-deficient anaemic school children (11–17 years) were randomly assigned to three treatment groups: control group (consuming non-fortified soy sauce), low-NaFeEDTA group (consuming fortified soy sauce, providing 5 mg Fe/day) and high-NaFeEDTA group (consuming fortified soy sauce, providing 20 mg Fe/day). Blood haemoglobin (Hb) levels were determined before and after 1 month, 2 months and 3 months of intervention. In addition, serum iron (SI), serum ferritin (SF), free erythrocytic porphyrin (FEP), total iron binding capability (TIBC) and transferritin (TF) were measured before and after consumption of soy sauce for 3 months. The results obtained herein show that the parameters measured were not changed remarkably within the 3-month intervention in the control group ($P < 0.05$). However, increased Hb, SI, SF and TF levels and decreased TIBC and FEP levels were observed in both the high-NaFeEDTA group ($P < 0.01$) and the low-NaFeEDTA group ($P < 0.05$). The effectiveness of iron intervention in the low-NaFeEDTA group and high-NaFeEDTA group had no statistical significance after 3 months. It was concluded that nutritional intervention for anaemic students using NaFeEDTA-fortified soy sauce could play a positive role in the improvement of iron status and control of anaemia.

Key words: China, food fortification, Henan, iron deficiency anaemia, NaFeEDTA, soy sauce.

Introduction

Compared with the commonly used iron salt fortificants, NaFeEDTA is characterised by a higher absorption rate in the human body, less adverse effects on the organoleptic profile and intrinsic nature of its food vehicles and less influence on the bioavailability of other minerals.[1,2] As a result, increasing attention has been focused on the use of NaFeEDTA as a nutrient fortificant. Three major population-based intervention trials, in which NaFeEDTA was used as the iron fortificant, have been conducted:[1] (i) Garby and Areekul's 24-month population trial in Thailand in 1974, in which fish-sauce was selected as the carrier;[3,4] (ii) Viteri *et al.*'s 20-month population trial using sugar as the carrier in an urban community in Guatemala in 1983;[5,6] and (iii) Ballot's 24-month population trial in an urban community in South Africa in 1986, in which curry powder was selected as the carrier.[7,8] These studies showed that administering NaFeEDTA-fortified foods results in a promising improvement in iron deficiency anaemia (IDA) among populations. However, among the three reported trials, only the third trial conducted by Ballot in South Africa was double-blinded.

Iron deficiency anaemia is an important and urgent nutritional problem, and food fortification is considered an indispensable approach eliminating IDA from China. Soy sauce is a popular traditional condiment in China, making it a candidate for use as a carrier. The studies on soy sauce fortified with ferrous sulphate ($FeSO_4$) conducted by Dai in 1980s provided convincing results that the consumption of iron-fortified soy sauce improved iron status.[9–10] Unfortunately, the organoleptic profiles of soy sauce fortified with $FeSO_4$ were not acceptable because of significant precipitation of the $FeSO_4$. Our previous study reported that the absorption rate of iron in NaFeEDTA-fortified soy sauce was up to 10.51% in human subjects. It was higher than that of $FeSO_4$, with an absorption rate of 4.73%, suggesting that this fortified food may play a significant role in supplementing iron and thereby protecting against IDA. To investigate the effects of NaFeEDTA-fortified soy sauce on IDA in Chinese populations, a therapeutic trial in juvenile students suffering from IDA was conducted. The results obtained herein will serve as a basis for conducting further population-based effectiveness

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studies in areas with a high prevalence of IDA using NaFeEDTA-fortified soy sauce. These studies may offer a practical solution to the problem of IDA in some populations.

Materials and methods

The trial protocol was reviewed and approved by the External Medical Ethics Committee of the Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine.

Subjects

With the assistance of the Henan Provincial Center for Disease Control and Prevention and the Wancheng District Center for Disease Control and Prevention, Nanyang City, Henan Province, boarding students aged 11–17 years were screened to assess their appropriateness for inclusion as subjects in this study. The subjects had similar living standards and dietary patterns. They were chosen from three middle schools in the Wancheng District, Nanyang City, Henan Province.

Dietary surveys were conducted using a three-day food weighing and recording method[11] in the cafeterias of the selected schools, followed by calculation of dietary iron intake. In addition, a health survey was carried out that included blood haemoglobin (Hb) concentration, age, nationality, height, weight, medical history, boarding or not and eating in the schools' cafeterias or not.

According to the Diagnosis Criteria for Anaemia recommended by the World Health Organization,[12] the students were diagnosed as anaemic in accordance with the following criteria: boys or girls younger than 15 years with Hb concentration < 120 g/L; girls aged 15 years or older with Hb concentration < 120 g/L; and boys aged 15 years or older with Hb concentration < 130 g/L. All students with anaemia were randomly assigned to one of the three treatment groups: (i) control group, consuming non-fortified soy sauce; (ii) low-NaFeEDTA group, consuming NaFeEDTA-fortified soy sauce containing 1 mg Fe/mL; and (iii) high-NaFeEDTA group, consuming NaFeEDTA-fortified soy sauce containing 4 mg Fe/mL. The soy sauce consumed was produced by Beijing Huwang Wadakan® Food Company using the low-salt solid fermented technique.

Study design

Soy sauce supplementation.

Based on the study protocol, 5 mL of soy sauce was administered daily to each subject at lunch in the form of soy sauce soup for a period of 3 months. In order to ensure accurate intake, the soup was prepared in the school cafeteria and consumed by the subjects under complete supervision from the teachers. At the same time, detailed information on the soy sauce consumption of each subject was recorded on a consumption sheet.

Sample collection.

A 10 mL sample of intravenous blood was collected from each subject before the study and after 3 months of soy sauce consumption. From this sample, 10 μL of blood was dropped onto a piece of filter paper for the free erythrocytic porphyrin (FEP) assay. Following centrifugation of blood samples at 500 g for 20 min, serum was separated and stored at – 20°C for subsequent determination of serum iron (SI), serum ferritin (SF) and transferritin (TF) concentrations, and for total iron binding capability (TIBC). Blood Hb was measured using fingertip blood taken before the study and after 1, 2 and 3 months of soy sauce consumption.

Laboratory analyses

Haemoglobin concentration was determined by the cyanomethemoglobin assay.[13] Van-Zij solution was purchased from the Institute of Hematology, Chinese Academy of Preventive Medicine. Quality-control serum samples with a known concentration (127 g/L) were provided by the Shanghai Institute of Clinical Examination. The determination of SI, SF and TF concentrations and TIBC were carried out using the respective assaying kits (Randox, Crumlin, UK), according to the instructions. FEP concentration was measured by fluorimetry, according to the method previously described.[14] A FEP standard was purchased from Sigma (St Louis, MO, USA). The STAGE Auto-biochemical Analyser (Nucleus and Electricity Company, Ohio, USA) and SN682 Counter (Rihuan Nucleus Instruments, Shanghai, China) were used for the analysis.

Results

Diet consumption and iron intake

According to the dietary surveys, the average daily iron intakes for the three schools were 17.62, 17.1 and 17.57 mg, respectively. Most of the daily iron intake was derived from cereals and vegetables, showing that the diet consumed is a plant-food-based diet (Table 1).

Prevalence of anaemia in the surveyed students

Among the 4022 eligible students in the three selected middle schools, 4008 students were available. The prevalence of anaemia diagnosed, based on Hb value, is shown in Table 2.

Three hundred and four students (155 boys and 149 girls) were diagnosed as anaemic and assigned to one of the three treatment groups. The incidence for each school was as follows: school 1, 55 boys and 47 girls in the control group (consuming non-fortified soy sauce); school 2, 39 boys and 63 girls in the low-NaFeEDTA group (consuming NaFeEDTA-fortified soy sauce containing 1 mg Fe/mL); school 3, 61 boys and 39 girls in the high-NaFeEDTA group (consuming NaFeEDTA-fortified soy sauce containing 4 mg Fe/mL).

Effect of iron-fortified soy sauce on Hb levels in subjects

According to Table 3, the Hb levels before intervention were not statistically different between the three groups ($P > 0.05$), indicating the same anaemia profile among the three groups. Hb levels in the high-NaFeEDTA group were significantly higher than those in the control and low-NaFeEDTA groups after 1 month of intervention ($P < 0.05$). Hb levels in the low-NaFeEDTA group and high-NaFeEDTA group were significantly increased after 2 and 3 months of

Table 1. Daily food consumption and iron intake in surveyed students

School	Cereals		Non-staple foods		Condiments	
	Wheat	Rice	Vegetables	Meat and eggs	Salt†	Soy sauce
School 1						
Weight (g)	527.1	119.9	249.0	15.3	11.8	11.0
Iron (mg)	14.23	0.85	1.28	0.24	0.13	0.89
% of total food		69.3‡	26.7	1.6		2.4§
% of total iron		85.5‡	7.3	1.4		5.8§
School 2						
Weight (g)	502.0	101.4	260.1	17.4	13.2	12.5
Iron (mg)	13.55	0.72	1.34	0.28	0.13	1.08
% of total food		66.6‡	28.7	1.9		2.8§
% of total iron		83.5‡	7.8	1.6		7.1§
School 3						
Weight (g)	516.5	109.2	263.3	17.1	12.4	13.0
Iron (mg)	13.95	0.78	1.33	0.27	0.12	1.12
% of total food		67.2‡	28.3	1.8		2.7§
% of total iron		83.8‡	7.6	1.5		7.1§

†Salt listed here did not include that contributed from soy sauce. Values represent: ‡wheat + rice; §salt + soy sauce.

Table 2. Anaemia prevalence in surveyed students in selected schools

Schools	No. students (11–17 years)		Anaemia prevalence (%)
	Boys	Girls	
School 1 (Control)	849	614	13.5
School 2 (Low NaFeEDTA)	766	634	22.6
School 3 (High NaFeEDTA)	601	544	11.0

Table 3. Effect of iron-fortified soy sauce on haemoglobin levels in students with anaemia (g/L, Mean ± SD)

Groups	No.	Before intervention	1 month after intervention	2 months after intervention	3 months after intervention
Control	81	116.9 ± 5.5	117.9 ± 6.3	118.6 ± 5.3	118.5 ± 4.7
Low NaFeEDTA	82	115.4 ± 5.1	117.2 ± 8.5	$128.4 \pm 7.0^{a,bb}$	$135.7 \pm 8.5^{aa,bb}$
High NaFeEDTA	77	116.1 ± 5.1	$124.0 \pm 10.6^{a,bb}$	$131.6 \pm 11.6^{aa,bb}$	$140.0 \pm 9.5^{aa,bb}$

Compared with haemoglobin levels before intervention: $^{a}P < 0.05$; $^{aa}P < 0.01$. Compared with the control group: $^{b}P < 0.05$; $^{bb}P < 0.01$.

intervention, with significant differences compared with those in the control group ($P < 0.01$). Two subjects in the low-NaFeEDTA group and one subject in the high-NaFeEDTA group were still suffering from anaemia at the end of the study, and other subjects had all recovered from anaemia. The prevalence of anaemia in the control group, however, was up to 69.5%. The results showed that iron-fortified soy sauce significantly increased Hb levels in students with anaemia.

Effect of iron-fortified soy sauce on serum iron concentrations in subjects

Serum iron concentrations did change significantly after the consumption of soy sauce by the control group. However, in the low-NaFeEDTA and high-NaFeEDTA groups, SI concentrations were increased by 30 μg/dL and 24 μg/dL, respectively, after 3 months of intervention (Table 4).

Effect of iron-fortified soy sauce on serum ferritin concentrations in subjects

There was no significant change in SF concentration in the control group before or after the consumption of soy sauce (Table 4). However, the SF concentrations of both the low-NaFeEDTA group and the high-NaFeEDTA group increased significantly after consuming iron-fortified soy sauce ($P < 0.01$). Furthermore, the SI concentrations in these two iron intervention groups were significantly higher than in the control group ($P < 0.01$).

Effect of iron-fortified soy sauce on serum free erythrocytic porphyrin concentrations in subjects

According to the results shown in Table 5, the serum FEP concentration in the control group after the intervention was not significantly higher than that before the intervention ($P > 0.05$). However, serum FEP levels decreased by 12.13 μg/dL and 16.88 μg/dL, respectively, in both low-NaFeEDTA and high-NaFeEDTA groups after 3 months of intervention.

Effect of iron-fortified soy sauce on total iron binding capability in subjects

In both the low-NaFeEDTA and high-NaFeEDTA groups, treatment for 3 months significantly decreased the TIBC levels ($P < 0.05$). In the control group, however, there were no significant changes in TIBC levels after soy sauce consumption compared with that before consumption (Table 5).

Effect of iron-fortified soy sauce on serum transferritin concentrations in subjects

Table 6 shows that TF concentrations in both the low-NaFeEDTA and high-NaFeEDTA groups were decreased significantly compared with that in the control group and that before intervention ($P < 0.01$).

Discussion

The present study shows that Hb, SI, SF, FEP, TIBC and TF concentrations in subjects in the control group were not changed remarkably after consuming non-fortified soy sauce for 3 months. However, all of the above parameters in both the low-NaFeEDTA group and high-NaFeEDTA group were significantly improved after consuming NaFeEDTA-fortified soy sauce. In the low-NaFeEDTA group, consumption of soy sauce for 1 month did not cause a remarkably increased Hb concentration. However, this was significantly increased after 2 and 3 months of intervention. The Hb levels in the two intervened groups did not differ significantly at the end of the study, showing that daily supplementation of 5 mg Fe as NaFeEDTA in soy sauce can effectively cure IDA within 3 months. The results also indicate that the daily supplementation of 20 mg iron in the form of NaFeEDTA in soy sauce could quickly cure IDA within 2 or 3 months

Soy sauce is a traditional condiment consumed commonly in all parts of China and used in all types of cooking and cuisines. The average consumption of soy sauce in China was around 12.6 g in the early 1990s and it is less varied in the different regions of China than other potential food carriers such as flour, rice, sugar and vinegar.[14] Because of its high salt content, the amount of soy sauce used in food preparation is self limited. Therefore, the chance of excess iron intake from iron-fortified soy sauce is negligible. Soy sauce fortified with NaFeEDTA retained the same features of regular soy sauce in terms of its flavour, colour and precipitant when compared with non-fortified soy sauce. This is a critical advantage of using NaFeEDTA over other commonly used iron compounds (such as $FeSO_4$) as a nutrient fortificant in soy sauce.

Table 4. Effect of iron-fortified soy sauce on SI and SF in students with anaemia (Mean ± SD)

Groups	No.	SI (μg/dL)		SF (ng/mL)	
		Before intervention	After intervention	Before intervention	After intervention
Control	81	97.69 ± 35.01	92.98 ± 30.76	48.33 ± 20.20	47.13 ± 15.65
Low NaFeEDTA	82	73.11 ± 19.45	103.04 ± 25.34^{aa,b}	43.69 ± 20.77	60.88 ± 19.93^{aa,bb}
High NaFeEDTA	77	80.45 ± 26.18	104.17 ± 32.36^{a,bb}	42.47 ± 16.44	58.12 ± 19.99^{aa,bb}

Compared with concentration before intervention: $^{a}P < 0.05$; $^{aa}P < 0.01$. Compared with the control group: $^{b}P < 0.05$; $^{bb}P < 0.01$. SF, serum ferritin; SI, serum iron.

Table 5. Effect of iron-fortified soy sauce on FEP and TIBC in students with anaemia (Mean ± SD)

Groups	No.	FEP (ug/dL)		TIBC (ug/dL)	
		Before intervention	After intervention	Before intervention	After intervention
Control	81	51.21 ± 24.80	57.05 ± 17.75	0.396 ± 0.059	0.390 ± 0.060
Low NaFeEDTA	82	41.71 ± 17.95	29.58 ± 12.97^{aa,bb}	0.416 ± 0.062	0.397 ± 0.068^{a,b}
High NaFeEDTA	77	45.64 ± 20.02	28.76 ± 8.31^{aa,bb}	0.421 ± 0.059	0.397 ± 0.068^{aa,b}

Compared with concentration before intervention: $^{a}P < 0.05$; $^{aa}P < 0.01$. Compared with the control group: $^{b}P < 0.05$; $^{bb}P < 0.01$.

Table 6. Effect of iron-fortified soy sauce on TF in students with anaemia (mg/dL, Mean ± SD)

Groups	No.	TF before intervention	TF after intervention
Control	81	266.79 ± 55.16	259.94 ± 54.25
Low NaFeEDTA	82	264.19 ± 44.69	240.94 ± 47.10^{aa,bb}
High NaFeEDTA	77	269.02 ± 50.50	237.76 ± 42.40^{aa,bb}

Compared with TF before intervention: $^{aa}P < 0.01$; Compared with the control group: $^{bb}P < 0.01$. TF, transferritin.

The present study shows that NaFeEDTA-fortified soy sauce is highly effective in treating IDA. It presented evidence that this fortified soy sauce could be recommended for potential wider use in China. Based on the well-established processes in our Institute, NaFeEDTA made with the same specifications as products from other foreign companies is currently available. NaFeEDTA-fortified soy sauce has been approved for manufacture systemically in two big food industries by the Chinese Ministry of Health. In addition, NaFeEDTA-fortified soy sauce will be an acceptable condiment for the public in that its cost is only 20 cents higher than traditional soy sauce, per serving (500 mL). There is a promising future for IDA elimination in China through food fortification. To obtain further data supporting its effect on IDA and to put it into application sooner, however, it is necessary to carry out further studies on NaFeEDTA-fortified soy sauce, including large scale intervention trials in populations with high anaemia prevalence.

References

1. Hurrell RF. Preventing iron deficiency through food fortification. Nutr Rev 1997; 55: 210–222.
2. Hurrell RF, Ribas R, Davidsson L. Sodium iron EDTA as a food fortificant: Influence on zinc, calcium and copper metabolism in the rat. Br J Nutr 1994; 71: 85–93.
3. Garby L, Areekul S. Iron supplementation in Thai fish-sauce. Ann Trop Med Parasitol 1974; 64: 467–476.
4. Garby L. Condiments. In: Clydesdale FM, Wiemer KL, eds. Iron Fortification of Foods. Orlando, FL: Academic Press, 1985; 165–170.
5. Viteri FE, Alvares E, Torun B. Prevention of iron deficiency by means of iron fortification of sugar. In: Vnderwood BA, ed. Nutrition Intervention Strategies in National Development. New York: Academic Press, 1983; 287–314.
6. Viteri FE, Garcia-Ibanez R, Torun B. Sodium iron NaFeEDTA as an iron fortification compound in Central America. Am J Clin Nutr 1978; 32: 961–971.
7. Ballot DE, Macphail AP, Bothwell TH, Gillooly M, Mayet FG. Fortification of curry powder with NaFe (III) EDTA in an iron-deficient population: Report of a controlled iron-fortification trial. Am J Clin Nutr 1989; 49: 162–169.
8. Ballot DE, Macphail AP, Bothwell TH, Gillooly M, Mayet FG. Fortification of curry powder with NaFe (III) EDTA in an iron-deficient population: Initial survey of iron status. Am J Clin Nutr 1989; 49: 156–161.
9. Dai RT. Study on iron fortified soy sauce I. Primary study on $FeSO_4$ fortified soy sauce. Acta Nutrimenta Sinica 1984; 6: 149–153 (in Chinese).
10. Dai RT. Study on iron fortified soy sauce II. Absorption of iron fortified soy sauce in human. Acta Nutrimenta Sinica 1984; 6: 231–238 (in Chinese).
11. Wang XS, Yin T-A, Liu JP, Wang WG, Yan HC, Gao XL. Methods for Evaluation of Nutritional Status in Humans. Tianjin: Tianjin Science and Technology Press, 1989; 4–31 (in Chinese).
12. Wang XS. Methods for biochemical determinations. In: Wang XS, Yin TA, Liu JP, Wang WG, Yan HC, Gao XL, eds. Methods for Evaluation of Nutritional Status in Humans. Tianjin: Tianjin Science and Technology Press, 1989; 139–141.
13. Chen XC. Applied Nutrition. Beijing: Public Health House, 1984; 292–296 (in Chinese).
14. Ge KY. The Dietary and Nutritional Status of Chinese Population in 1990s. National Nutrition Survey, 1992, vol. 1. Beijing: People's Public Health House, 1996 (in Chinese).