BIOMEDICAL AND ENVIRONMENTAL SCIENCES **20**, 126-130 (2007)

NaFeEDTA Fortified Soy Sauce Showed Higher Iron Absorption Rate in Chinese Females[1]

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Objective NaFeEDTA was considered as a promising iron fortificant for controlling iron deficiency anemia. Soy sauce is a suitable food carrier for iron fortification and is a popular condiment in China. Iron absorption rates of NaFeEDTA and $FeSO_4$ were observed and compared in adult female subjects. **Methods** The stable isotope tracer method was used in Chinese females consuming a typical Chinese diet. Ten healthy young Chinese women were selected as subjects in the 15-day study. A plant-based diet was used based on the dietary pattern of adult women in the 1992 National Nutrition Survey. Six milligram of ^{54}Fe in $^{54}FeSO_4$ soy sauce and 3 mg ^{58}Fe in $Na^{58}FeEDTA$ soy sauce were given to the same subjects in two days. Food samples and fecal samples were collected and analyzed. **Results** Iron absorption rates of NaFeEDTA and $FeSO_4$ were 10.51%±2.83 and 4.73%±2.15 respectively. The ^{58}Fe (NaFeEDTA) absorption was significantly higher than that of ^{54}Fe ($FeSO_4$) ($P<0.01$). The iron absorption rate from NaFeEDTA was 1.2 times higher than that from $FeSO_4$ in Chinese adult women consuming a typical Chinese diet. **Conclusion** The higher absorption rate of NaFeEDTA suggested that NaFeEDTA would be a better iron fortificant used in soy sauce for the controlling of iron deficiency anemia in China.

Key word: NaFeEDTA; $FeSO_4$; Iron absorption; Stable isotopes; Dietary pattern

INTRODUCTION

NaFeEDTA, as an iron fortificant, was reported to have less adverse effects than that of commonly used iron salts on the organoleptic profiles of the food vehicle such as taste and color[1]. Animal and human studies suggested that iron absorption of NaFeEDTA was higher than that of other iron salts such as ferrous sulfate especially in diets high in cereal food, because inhibitors in cereal food affected iron absorption less on NaFeEDTA[2-3]. Pilot observations showed improvements of iron status of the population intervened with NaFeEDTA in Thailand[4], Central America[5] and South Africa[6-7]. Soy sauce is a popular condiment that has a 68% coverage and 12.6 mL average daily consumption in total Chinese population[8]. Soy sauce was selected as the food carrier for a nation-wide iron fortification program in China sponsored by ILSI (International Life Science Institute) project IDEA (Iron Deficiency Eliminating Action). To estimate the suitable iron concentration for iron fortified soy sauce products, information on Fe absorption in Chinese consuming typical Chinese diet is necessary. In this study, iron absorption rate of NaFeEDTA and $FeSO_4$ was compared in human subjects consuming a plant-based diet that was based on the dietary pattern of adult women in the 1992 National Nutrition Survey[8].

MATERIALS AND METHODS

Subjects

Ten healthy women aged 18-22 y were selected for the study [body weight ($\bar{x} \pm s$) 50.35 ±6.12 kg, height 157.30±4.69 cm]. Health status of the subjects were assessed at the pre-study screening, which included a medical history inquiry and physical examination. The subjects (blood Hb>110 g/L) were also required not to be on menses in the study period. The External Medical Ethics Committee of the Institute of Nutrition and Food Safety, Chinese Center for

[1]This research was supported by Institute of International Life Science.

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0895-3988/2007
CN 11-2816/Q

Disease Control and Prevention and all subjects signed consent forms.

Stable Isotopes

The stable isotopes were obtained from the Chinese Academy of Atomic Energy in the form of $^{54}Fe_2O_3$ and $^{58}Fe_2O_3$. The abundance of the different Fe isotopes in the two compounds were enriched ^{54}Fe: 96.9% ^{54}Fe, 3.1% ^{56}Fe, 0% ^{57}Fe, and 0% ^{58}Fe; enriched ^{58}Fe: 0.7% ^{54}Fe, 30.3% ^{56}Fe, 2.8% ^{57}Fe, and 66.3% ^{58}Fe.

$^{54}FeSO_4$ and Na^{58}FeEDTA were prepared according to the method of Zhong *et al.*[9] and Jiaomai *et al.*[10]. In brief, $^{54}FeO_4$ powder was put in tube and heated for 4 h while hydrogen flowed through the tube. After the reaction $^{54}FeO_4$ was transformed to be ^{54}Fe powder. ^{54}Fe powder was mixed with 0.5 mol/L H_2SO_4 for 48 h. The light blue crystal formed was $^{54}FeSO_4$. Na^{58}FeEDTA was obtained by the method of Sawyer *et al.*[11] and Layrisse *et al.*[12]. $^{58}FeSO_4$ was prepared from $^{58}Fe_2O_3$ by the method mentioned above. Equal moles $^{58}FeSO_4$ and 2NaEDTA were dissolved in deionized water and the pH value of the solution was adjusted to 5. The precipitation of the reaction was refined by 90% ethanol water solution for three times. The Na^{58}FeEDTA product obtained was a light yellow powder.

Na^{58}FeEDTA fortified soy sauce was made by dissolving Na^{58}FeEDTA into soy sauce and the final ^{58}Fe concentration was 0.3 mg/mL. $FeSO_4$ soy sauce was made by the same method and the final ^{54}Fe concentration was also 0.3 mg/mL. The soy sauce with iron concentration 9.9 mg/100 mL was produced by Zhenji Fermentation Company, located in Shi Jiazhuang City, Hebei Province, China.

Dysprosium, a safety rare earth element, was used as nonabsorbable fecal marker[13-15]. $DyCl_3 \cdot 6H2O$ (Sigma Company) was dissolved in three-time distillated water with Dy concentration 4 g/L.

Diet

A three-day menu was designed and repeatedly used in 15 days (Table 1). The foods included in the menu were clustered from the dietary pattern of Chinese adult woman based on the national nutrition survey of China in 1992[8]. This diet simulated a typical dietary pattern of Chinese women who live in city and town. Averagely each subject took 17.25 mg iron from this dietary pattern and it meets the average iron intake of Chinese women. Subjects only consumed the served food during the trial and completely consumed the dishes, snacks and fruits. On the other hand, subjects freely consumed staple food (rice, steamed bread, *etc.*), but the consumption of staple was recorded during each meal. Boiled tap water was provided as drinking water in every morning and the water left over was weighted in the morning of next day.

TABLE 1

The Three-day Menu Used in the Study (g)

Day	1	2	3
Breakfast			
Deep-fried Wheat Stick	7	7	7
Steamed Bread	*	*	*
Millet Porridge		254	
Corn Porridge			246
Milk	100		
Tofu	20	20	20
Pickled Vegetables	6	6	6
Carrot Slices	17	17	17
Cucumber	12	12	12
Cowpea	28	28	28
Lunch			
Rice	*	*	*
Steam Bread	*	*	*
Egg Soup	350		
Fried Meat Slices	36	44	38
Fried Potato Slices	36	37	36
Fried Rape and Mushroom	64	68	62
Fried Egg	20	21	22
Fried Garlic Shoot	45		
Fried Green Pepper		76	
Fried Tomato			88

(to be continued)

(continued)

Day	1	2	3
Supper			
Rice	*	*	*
Steam Bread	*	*	*
Snack	30	30	30
Fruits	Water Melon 200	Banana 100	Honey Melon 100
Sea Food	Shell Fish 8	Shrimp 7	Sea Slug 7
Fried Eggplant	42		
Fried Bean	20	22	24
Fried Celery		23	28
Fried Cabbage With Vinegar	46	44	44
Fried Pork Meat	15	15	16
Fish			57
Roast Beef	37		
Chicken		41	
Egg Soup		350	350

Note. * Taken by the subjects according to their requirement.

Stable Isotope Administration

Subjects had the given diet for 15 days. After 3-day preliminary diet for adaptation, subjects were given ^{54}Fe, ^{58}Fe, and Dy in breakfast, lunch and supper in egg drop soup from the fourth day to fifth day. The given amounts in breakfast, lunch and supper accounted for 20%, 40%, and 40% of the amounts of ^{54}Fe, ^{58}Fe, and Dy. The equal amounts of ^{54}Fe, ^{58}Fe, and Dy were given in each day and the total amount of ^{54}Fe, ^{58}Fe, and Dy were 6 mg, 3 mg, and 6 mg respectively. ^{54}Fe ($FeSO_4$) soy sauce, ^{58}Fe (NaFeEDTA) soy sauce, and Dy solution (as a marker of fecal excretion) were weighted and added to a bowl of egg drop soup (300 mL). This should be done right before the meal to prevent Fe exchange between the different iron compounds. Subjects were asked to completely eat the bowl of soup. From the 6th to 15th day, the feces samples of subjects were collected.

Absorption Studies

The diet samples were collected every day from one day before iron isotopes administration for 10 days. The samples included three composites, i.e. staple food (wheat flour and rice include snacks like candies), food from plant source (such as vegetables and fruits) and food from animal source. The samples were homogenized and kept in freezer (-80℃). Three drinking water samples were randomly collected from the water supplied during the trial.

Complete 24-hr feces of each subject were collected every morning for 10 days. The fecal samples were homogenized and kept under -80℃.

Iron contents of food and fecal samples were analyzed by atomic absorption spectrometry (Varian 20, USA) after the stool samples were digested with H_2NO_3[16]. Dy content and isotope ratios of ^{54}Fe, ^{56}Fe, ^{57}Fe, and ^{58}Fe in fecal samples were determined by ICP-MS (Finigan ELEMENT, Germany) according to the method of Ellen *et al.*[17].

Data Calculation

The iron contents of the food and fecal samples and the ratios of ^{54}Fe and ^{58}Fe in the isotopes calculated the amounts of ^{54}Fe and ^{58}Fe in samples. Amounts of ^{54}Fe and ^{58}Fe in feces samples were corrected by the recovery of Dy in feces. Total ^{54}Fe and ^{58}Fe intakes were calculated from the amounts of ^{54}Fe and ^{58}Fe in food samples and the amount of foods consumed in 10 testing days.

Dy recovery = (Total Dy intake –Total Dy in feces)/Total Dy intake

^{58}Fe absorption rate (%)= (Total ^{58}Fe intake – corrected total ^{58}Fe content in feces)/total ^{58}Fe intake

^{54}Fe absorption rate (%)= (Total ^{54}Fe intake – corrected total ^{54}Fe content in feces)/total ^{54}Fe intake

Statistical Analysis

All statistical analyses were performed by using SPSS (SPSS Inc.) computer statistical packages. Absorption rates of ^{54}Fe (from Fe_2SO_4) and ^{58}Fe (from NaFeEDTA) were compared by paired Student's t tests (with 95% levels of significance).

TABLE 2

Amounts of Dy, ^{54}Fe and ^{58}Fe in Diet and Feces ($\bar{x} \pm s$)

Subject	Diet (mg)	Feces (mg)
Dy	1.34±0.06	1.34±0.04
^{54}Fe	8.00±0.30	7.62±0.33
Corrected ^{54}Fe		7.63±0.37
^{58}Fe	2.25±0.06	2.01±0.08
Corrected ^{58}Fe		2.01±0.07

Note. Corrected data were calculated by the recovery of Dy.

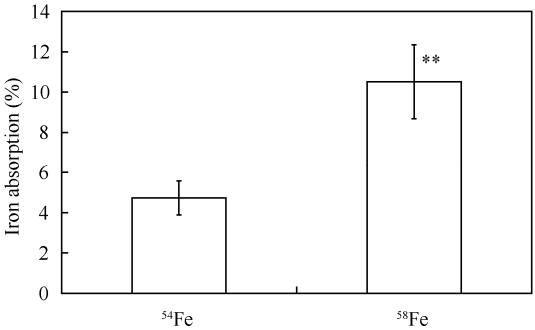

FIG. 1. iron absorptions of ^{54}Fe and ^{58}Fe.$^{**}P$<0.01 compared with ^{54}Fe.

RESULTS

All the ten subjects completed the study according to the protocol. One subject felt dizzy for about 15 min and the symptom disappeared after one hour's rest. Watching a picture of a seriously burned woman, would be the possible reason of the symptom. There were no evidence that this short time dizzy would affect iron absorption.

The mean amount of Dy intake and excretion were 1.34±0.06 mg and 1.34±0.04 mg respectively (Table 2). The recovery of Dy was 100.1%±2.4%. This result suggested that there were no losses in the diet and fecal sample collection.

The amounts of ^{54}Fe ($FeSO_4$) and ^{58}Fe (NaFeEDTA) administrated with diet were 8.02±0.30 mg and 2.25±0.06 mg respectively (Table 2). The mean amount of ^{54}Fe and ^{58}Fe in feces after corrected by Dy recovery was 7.63±0.37 and 2.01±0.07 respectively. The small variations of ^{54}Fe and ^{58}Fe intakes and excretion among the ten subjects suggested that the trial was successful.

The corrected absorption rate of ^{54}Fe and ^{58}Fe was 4.73%±2.15% and 10.51%±2.83% respectively (Fig. 1). The ^{58}Fe (NaFeEDTA) absorption was significantly higher than that of ^{54}Fe ($FeSO_4$) (P<0.01). This result suggested that iron absorption rate from NaFeEDTA was 1.2 times higher than that from $FeSO_4$ in Chinese adult women consuming a typical Chinese diet.

DISCUSSIONS

Soy sauce is one of the most popular condiments in China. In addition to its flavor and taste enhancing effect for a wide variety of dishes, soy sauce reportedly had promoting effect on iron absorption in human subjects which makes it a suitable food vehicle for iron fortification. The reporter[18] thought the reason of the promotory effect of soy sauce on iron absorption is that the soy sauce is fermentation products and the protein was degraded to amino acid which is beneficial to the iron absorption. The coverage of soy sauce will be increasing along with the continuous progress in the nation's economy[8]. At the same time, the centralization of soy sauce industry in China will be improved.

Studies on the iron absorption of NaFeEDTA had been carried out in various food diets. The reported data showed that the iron absorption rate of NaFeEDTA (4.1%-18.0%) was 2-3 times higher than that of $FeSO_4$ (1.6%-12.4%)[3, 5]. Hurrell[1] reported that the iron absorption inhibitors from the food of plant source was less affected when NaFeEDTA was used and the data showed that iron absorption rate from NaFeEDTA was higher than that of $FeSO_4$ when consuming a plant food diet. The iron absorption rate of NaFeEDTA (10.51%) and $FeSO_4$ (4.73%) showed a ratio of 2.2:1 when consuming typical Chinese diet, which was consistent with the results of previous studies[3]. Phytic acid and polyphenols are potent inhibitors of native and fortification iron absorption and low absorption of iron from cereal- and/or legume-based complementary foods is a major factor in the etiology of iron deficiency[19-20]. NaFeEDTA may increase the bioavailability of the intrinsic non-heme iron in plant foods rich in inhibitory factors such as phytate and polyphenols[21].

In this study the subjects administrated both sources of iron ($FeSO_4$ and NaFeEDTA) at the same time that enables a better comparison of iron absorption of the two sources of iron. MaPhail *et al*.[19] prepared the two sources of iron in different food vehicles right before meal and the iron absorption data also showed a better iron absorption of NaFeEDTA than that of $FeSO_4$. Some studies suggested that iron exchange may happen among different iron sources, therefore a long time mixture of different iron sources, which labeled by different iron isotopes may result in a same iron absorption rate among different iron compounds. In this study $^{54}FeSO_4$ soy sauce and Na^{58}FeEDTA soy sauce were prepared less than 10 minute before the meal, therefore there should be little iron exchange occurred according to the results of MacPail *et al.*[22].

In conclusion, the result of this study showed that consuming a typical Chinese women diet, the iron absorption rate of NaFeEDTA in soy sauce was significantly higher than that of $FeSO_4$. The results support the use of NaFeEDTA fortified soy sauce as one of the major strategies for iron deficiency anemia in China and other developing countries in Asia.

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(*Received June* 30, 2005 *Accepted September* 06, 2006)