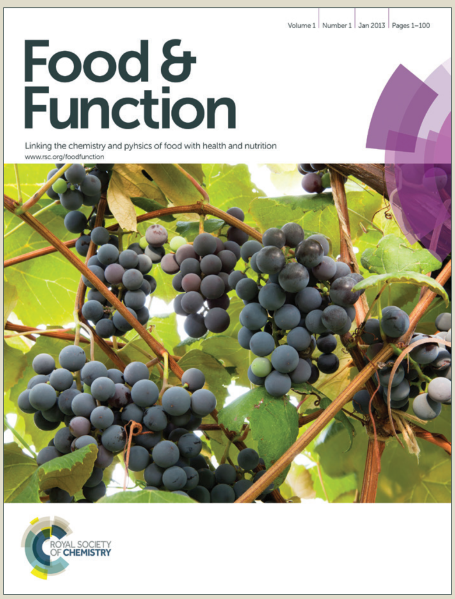
Food & Function

Accepted Manuscript



Effect of NaFeEDTA-fortified soy sauce on zinc absorption in children

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The work should be attributed to the Key Laboratory of Trace Element Nutrition MOH, Institute of Nutrition and Food Safety, Chinese Center for Disease Control.

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Supported by the research grant (81273073) from the National Natural Science Foundation of China.

Running title: zinc absorption

ABSTRACT

NaFeEDTA has been applied in many foods as an iron fortificant and is used to prevent iron deficiency in Fe-depleted populations. In China, soy sauce is fortified with NaFeEDTA to control iron deficiency. However, it is unclear whether Fe-fortified soy sauce affects zinc absorption. To investigate whether NaFeEDTA-fortified soy sauce affects zinc absorption in children. Sixty children were enrolled in this study and randomly assigned to three groups (10 male children and 10 female children in each group). All children received daily oral 3 mg of ^{67}Zn and 1.2 mg of dysprosium, while the children in the three groups were supplemented with NaFeEDTA-fortified soy sauce (6 mg Fe, NaFeEDTA group), $FeSO_4$-fortified soy sauce (6 mg Fe, $FeSO_4$ group), and no iron-fortified soy sauce (control group), respectively. Fecal samples were collected in during the experimental period and analyzed for Zn content, ^{67}Zn isotopic ration and dysprosium content. Fe intake from the NaFeEDTA-fortified and $FeSO_4$-fortified groups was significantly higher than that in the control group ($P<0.0001$). Daily total Zn intake was not significantly different among the three groups. There were no significant differences in fractional Zn absorption (FZA) (P=0.3895), dysprosium recovery (P=0.7498) and Zn absorption (P=0.5940) among the three groups. Therefore, NaFeEDTA-fortified soy sauce does not affect Zn bioavailability in children.

KEY WORDS: NaFeEDTA, soy sauce, zinc, iron, zinc absorption

INTRODUCTION

Iron (Fe) deficiency is a common nutritional deficiency and is the leading cause of anemia in China. Fe deficiency or anemia usually occurs in children and women at childbearing age, causing many healthy issues including delayed development of motorical abilities and mental function in children[1,2], preterm delivery[3] and upper respiratory infection in children[4]. Food fortification is the most common method to supplement nutrients in food and can effectively decrease the incidence of nutrient deficiencies[5]. NaFeEDTA, as an iron fortificant, has been applied in many foods and is used to reduce Fe deficiency in Fe-depleted populations[6-8]. In China, soy sauce is usually fortified with NaFeEDTA to prevent iron deficiency.

Zinc (Zn) deficiency is also a major global public health problem. Zn deficiency can lead to decreased immunity, growth retardation, and a decreased eugenics rate[9-12]. A few studies have demonstrated that NaFeEDTA may influence the absorption and utilization of Zn, copper and other trace elements[13,14]. Solomons et al. reported that the consumption of 15 mg NaFeEDTA (equivalent to 2 mg Fe) at one time does not affect the absorption and utilization of dietary Zn[13]. However, a study performed in China revealed that that plasma Zn levels of children aged 7-12 years old were significantly reduced after the 12-month intervention of NaFeEDTA-fortified soy sauce[15]. Thus, it is still controversial whether NaFeEDTA or NaFeEDTA-fortified soy sauce affects the absorption and utilization of trace elements such as Zn.

If NaFeEDTA-fortified soy sauce affects dietary Zn absorption and utilization, then residents in China may acquire Zn deficiency due to the expansion of Fe-fortified soy sauce. Therefore, the present study was designed to evaluate the effect of NaFeEDTA-fortified soy sauce on the bioavailability of dietary Zn.

SUBJECTS AND METHODS

Subjects

Thirty male and thirty female children (13.0±1.1 years old) were recruited for this study. The inclusion criteria of subjects include not suffering from anemia, no history of chronic metabolic, no gastrointestinal diseases and have a routine medical examination.

All of the procedures involving human subjects were approved by the Ethical Committee of Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention. Informed written consent was obtained from the participants' parents prior to the beginning of the study.

Soy sauce sample

Soy sauce (Haitian company, Foshan, China) was purchased from the market. Soy sauces were fortified with NaFeEDTA (Sigma, St. Louis, MO) and $FeSO_4$ (Institute of Chemical, Zhuji City, China), which was completed by the Food fortification Office of China. The Fe level in the blank soy sauce, NaFeEDTA-fortified soy sauce and $FeSO_4$-fortified soy sauce was 0.041mg/ml, 0.293 mg/ml, and 0.286 mg/ml, respectively.

Experimental design and procedure

All of the subjects were randomly assigned to three groups: NaFeEDTA-fortified soy sauce group (NaFeEDTA), $FeSO_4$-fortified soy sauce group ($FeSO_4$) and blank control group (control). Each group consisted of 10 female and 10 male children. Female children should avoid the menstrual period and no significant differences in age, height and weight. The experimental procedure lasted ten days, which included three periods: adaptation period (Day 1 to Day 3), test period (Day 4 to Day 8) and post-test period (Day 9 to Day 10). At the adaptation period, the subjects were allowed to adapt to the Zn levels in the experimental diet. From the fourth day, the subjects in the NaFeEDTA and $FeSO_4$ groups were provided 2 mg of Fe and 1 mg of ^{67}Zn in the experimental diet (Chinese traditional diet) during each meal (breakfast, lunch and supper). The subjects in the control group were provided a similar volume of soy sauce and 1 mg of ^{67}Zn in the experimental diet during each meal. The actual intake of food was recorded. The concentration of the main macronutrients and energy in the food were examined. On the fourth day, a dysprosium fecal marker (0.4 mg Dy per meal) was administered along with the stable isotope to check the completeness of the fecal samples. On the fourth day and ninth day, subjects also received a 200 mg capsule of carmine red dye to determine the endpoint of the fecal collection. All samples were frozen at -20C until further analysis.

Stable-isotope labels

Zn isotopes were purchased from Trace Sciences International as oxide powder (^{67}Zn at 89.6% enrichment) (Richmond Hill, ON, Canada). The oral ^{67}ZnO powder was converted to $^{67}ZnSO_4$. For the preparation of $^{67}ZnSO_4$, oxide was dissolved in H_2SO_4 (0.5 mol/L) and diluted with ultrapure water to a concentration of 0.5 mg Zn/mL. The solution was filtered through a 0.22 μm filter and sent to the Institute of Drug Analysis to test if the preparation was safe and edible.

Detection of Zn in the fecal samples

The fecal samples were homogenized in a blender. Duplicate subsamples of the fecal powder were digested in a microwave oven (Excel, Shanghai, China). Zn was isolated from fecal samples by heating (120℃) 0.3 mL of the digested samples until all of the liquid had evaporated, followed by reconstitution in 1 mL of 3 mol/L HCl. The sample was subsequently heated (120℃) until dry. Digested fecal residue was re-dissolved in 1 mL of 3 mol/L HCl prior to anion exchange chromatography (AGMP1M). Columns were washed with 7 mL of 0.5 mol/L HNO_3 for three times and 2 mL of ultrapure water for three times, and then conditioned by 2 mL of 3 mol/L HCl for three times. Reconstituted fecal samples were loaded onto conditioned columns. Zn was eluted from the column using 2 mL of ultrapure water for five times. The final elute was dried on a hotplate and reconstituted in 2 mL of 2% HNO_3 before loading onto filaments for mass spectrometric analyses. All of the acids used in the digestions and chromatography were ultrapure grade. The Zn isotope ratio was measured using multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS, Isoprobe, GV, England).

Zn absorption calculation

Fractional Zn absorption (FZA) was calculated with the following equation[16]:

$$FZA(\%)=\frac{^{67}Zn\text{I-}^{67}Zn\text{M}^{s}}{^{67}ZnI}\times 100$$

Where ^{67}ZnI is the oral ^{67}Zn intake (mg) and ^{67}ZnM^{s} is the unabsorbed ^{67}Zn in the feces (mg), which was determined as previously reported[17]. ^{67}Zn absorption (%) = FZA/Dysprosium recovery.

Statistical analysis

Results were expressed as means ± standard deviation (SD). ANOVA was used firstly to detect whether statistically significant difference in age, weight, height, BMI and ^{67}Zn absorption in different groups. If there is significant difference, take the Student-Newman-Keuls (SNK) test to perform pairwise comparison. All of the statistical analyses were performed with SAS 9.1 software. A value of $P<0.05$ was considered statistically significant.

RESULTS

Subject characteristics

Thirty male and 30 female children were recruited in this study, but two female children (menstruation during the study) and one male child (abnormal Zn absorption data) were excluded during the study. The cohort characteristics are described in Table 1. No significant differences were found in any of the measured physical characteristics among the groups.

Dietary composition

Intake of the main macronutrients, energy, Zn and Fe among the three groups is shown in Table 2. There were no significant differences in protein, fat, carbohydrate, energy and Zn intakes among the three groups. The children in the control group took in significantly less total Fe compared with the other two groups ($P<0.05$). Accordingly, Fe intake from soy sauce in the control group was significantly less than that in the other two groups ($P<0.0001$).

Zn absorption

FZA, dysprosium recovery and Zn absorption are shown in Table 3. There were no significant differences in FZA ($P=0.3895$), dysprosium recovery ($P=0.7498$) and Zn absorption ($P=0.5940$) among the three groups.

DISCUSSION

It is controversial whether NaFeEDTA or NaFeEDTA-fortified food affects Zn absorption[13-15]. In previous studies, plasma Zn was used as a marker for the status of Zn nutrition. However, plasma Zn is not sensitive and specific for evaluating the status of Zn nutrition. Nutrients can be divided into type 1 nutrients and type 2 nutrients[18]. Nutrients with specific functions usually belong to type 1 nutrients. Deficiencies of type 1 nutrients lead to microcytic anemia from a lack of iron, beriberi from thiamine, pellagra from niacin, scurvy from vitamin C, and macrocytic anemia from folic acid. Type 2 nutrients, including nitrogen, essential amino acids, magnesium, potassium and Zn, are required for multiple general metabolic functions. Zn deficiency is associated with diverse biochemical functions rather than a specific function, making it difficult to identify biomarkers for Zn deficiency[18,19].

Plasma Zn has been used as marker for NaFeEDTA and Zn, but it is not sensitive and specific. In other studies, the stable isotope method has been used for the evaluation of Zn absorption and bioavailability[20-22]. This method provides more reliable and credible results compared with plasma Zn. In the present study, we used the single-isotope tracer method for ^{67}Zn to assess the bioavailability of dietary Zn in children. We observed that the children in the NaFeEDTA and $FeSO_4$ groups had lower Zn absorption than those in the control group, but the difference was not significant. Absorption of iron and EDTA is an independent event in the gastrointestinal tract. EDTA has six coordinating atoms that combine with metal ions and form stable complex compounds. Therefore, once NaFeEDTA is dissociated, EDTA may combine with Zn and affect its absorption and utilization, which has been verified in an animal experiment[23]. In addition, the biological interaction between metal ions and ions with similar chemical structures is important. As a transition metal element, the outer electron configuration of Fe, Zn and copper is consistent, but the absorption and utilization of these metal elements may have antagonistic effects. Previous studies have demonstrated that excessive Fe can inhibit Zn bioavailability[24-26]. This may be due to competitive receptors or proteins in the intestinal cells for the absorption and transportation of Zn and Fe. Moreover, previous studies have shown that Fe supplements adversely influence Zn absorption in humans[27,28]. Therefore, we suspect that excessive Fe intake may affect Zn absorption.

In China, the NaFeEDTA content in fortified soy sauce is 175~210 mg/100ml, and daily Fe intake from fortified soy sauce is about 3 to 4 mg. In the present study, the daily Fe intake from soy sauce in the NaFeEDTA and FeSO4 groups was 5.18 mg and 5.06 mg, respectively, which is higher than that from soy sauce only. However, we did not observe significant differences in FZA and Zn absorption among the three groups. Our results indicated that the NaFeEDTA-fortified soy sauce did not affect the dietary Zn absorption in children. However, the effect of NaFeEDTA-fortified soy sauce on the absorption of other trace elements remains further investigation.

In conclusion, NaFeEDTA-fortified soy sauce does not affect the absorption and bioavailability of dietary Zn in children. Thus, NaFeEDTA-fortified soy sauce may be used as a safe and effective way to treat Fe deficiency in populations.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No.81273073).

We thank International Life Science Institute for providing convenient conditions in implementation of the project and Medjaden Bioscience Limited for assisting in the preparation of this manuscript.

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Table 1. Subject characteristics

	group	number	age（y）	Weight (kg)	Height (cm)	BMI
Female	NaFeEDTA	10	12.7±0.7	47.3±7.3	154±7	19.8±2.5
	$FeSO_4$	9	13.1±0.8	50.0±11.0	157±9	20.1±3.4
	control	9	13.2±0.7	47.1±4.9	156±5	19.4±2.1
Male	NaFeEDTA	9	13.6±1.2	47.3±15.5	156±9	19.0±3.8
	$FeSO_4$	10	12.9±1.3	46.4±8.0	158±10	18.7±3.4
	control	10	13.0±1.2	44.7±11.8	155±12	18.6±4.0

All data are expressed as mean ± standard deviation (SD).

Table 2. Nutrient intake

Group	Protein (g)	Fat (g)	Carbohydrate (g）	Energy (kcal)	Zinc (mg)	Total Iron (mg)	Iron from soy sauce (mg)
Male							
NaFeEDTA	96.3±18.6	54.6±12.8	464.6±125.8	2735±631	9.8±1.2	19.3±3.2^{a}	5.18±0.01^{a}
$FeSO_4$	91.7±15.5	49.6±8.6	413.4±114.4	2467±569	9.2±1.2	17.1±2.4^{a}	5.05±0.01^{a}
control	93.0±9.2	51.4±6.6	403.1±55.2	2447±277	9.4±0.7	14.0±1.7^{b}	0.73±0.01^{b}
Female							
NaFeEDTA	83.9±14.8	48.9±8.2	337.0±48.3	2124±261	8.9±1.0	16.9±1.8^{a}	5.18±0.00^{a}
$FeSO_4$	86.6±16.6	52.2±11.6	380.3±83.1	2337±487	9.0±1.1	18.4±3.4^{a}	5.06±0.01^{a}
control	78.9±8.7	48.6±6.1	316.4±52.4	2019±244	8.7±0.5	13.1±1.5^{b}	0.73±0.00^{b}

Different letters in superscript represent statistically significant among groups. All data are expressed as mean ± SD.

Table 3. FZA, dysprosium recovery and zinc absorption

group	FZA (%)	Dysprosium recovery (%)	Zn absorption (%)
NaFeEDTA	22.1±7.5	93.9±14.4	25.4±12.9
$FeSO_4$	24.2±6.5	91.2±11.2	27.1±7.5
control	25.7±10.3	91.3±11.8	29.0±11.9

All data are expressed as mean ± SD.