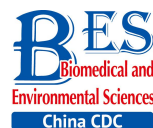


Original Article



Zinc Absorption from Representative Diet in a Chinese Elderly Population Using Stable Isotope Technique*

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Abstract

Objective To determine the dietary zinc absorption in a Chinese elderly population and provide the basic data for the setting of zinc (Zn) recommended nutrient intakes (RNI) for Chinese elderly people.

Methods A total of 24 elderly people were recruited for this study and were administered oral doses of 3 mg ⁶⁷Zn and 1.2 mg dysprosium on the fourth day. The primary macronutrients, energy, and phytic acid in the representative diet were examined based on the Chinese National Standard Methods. Fecal samples were collected during the experimental period and analyzed for zinc content, ⁶⁷Zn isotope ratio, and dysprosium content.

Results The mean (± SD) zinc intake from the representative Chinese diet was 10.6 ± 1.5 mg/d. The phytic acid-to-zinc molar ratio in the diet was 6.4. The absorption rate of ⁶⁷Zn was 27.9% ± 9.2%. The RNI of zinc, which were calculated by the absorption rate in elderly men and women, were 10.4 and 9.2 mg/d, respectively.

Conclusion This study got the dietary Zn absorption in a Chinese elderly population. We found that Zn absorption was higher in elderly men than in elderly women. The current RNI in elderly female is lower than our finding, which indicates that more attention is needed regarding elderly females' zinc status and health.

Key words: Zinc absorption; Chinese elderly people; Stable isotope technique

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INTRODUCTION

Zinc (Zn) is recognized as an essential micronutrient for humans, and it participates in all aspects of intermediary metabolism and transmission and regulates the structural and functional integrity of chromatin and biomembrane^[1-2]. Zn deficiency could lead to DNA

damage, acute infections and oral periodontal diseases, and decrease in immunity and cognitive functions^[3]. Studies have shown that approximately 33% of elderly people in the world are at risk of Zn deficiency^[4], which is caused by inadequate Zn intake^[1,4]. Even in developed countries, Zn deficiency, especially marginal Zn deficiency, is often observed in elderly subjects^[5-6].

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In China, the elderly population (aged 60 years and above) had totaled 194 million toward the end of 2012, contributing to about 14.3% of the total population. By 2030, China's elderly population is expected to reach 400 million, making the country to hold the largest elderly population^[7]. Thus, it becomes more important to maintain an optimal Zn status in the elderly population and to promote the health of Chinese elderly people. Data regarding Zn absorption are lacking for the Chinese elderly population, and the recommended nutrient intakes (RNI) of dietary Zn for elderly people is determined by western standards^[8]. However, controversy exists on whether Zn absorption is consistent among elderly people in different populations. Therefore, it is necessary to determine the dietary Zn absorption in the Chinese elderly people. Stable isotope technique is considered to be the most accurate and reliable for determining the absorption of minerals in human beings^[9-10]. The objective of the present study was to measure Zn apparent absorption in a Chinese elderly population using the stable isotope technique and provide the basic data for the setting of Zn RNI for Chinese elderly population.

METHODS

Subjects

Eleven male and thirteen female rural elderly

people (aged 60-70 years) were recruited for this study. All the subjects were in good health. In particular, accurate examinations were carried out to exclude subjects with anemia, gastrointestinal diseases, chronic metabolic diseases, or abnormal Zn nutrition status (plasma Zn < 10.7 μmol/L and C-reactive protein > 10 mg/L).
The trial was registered in the Chinese Clinical Trial Registry (No. ChiCTR-OOB-15007070). All the procedures involving human subjects were approved by the Ethical Committee of the Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention. All subjects gave their written informed consent to participate in this study.

Experimental Diet

According to the basic data of the typical rural dietary pattern obtained from the China National Nutrition and Health Survey 2010-2013 (CHNNS 2010-2013) and market supply, the varieties and quantities of the foods in the experimental diet were assorted to design a 3-day recipe and repeatedly served during the whole experimental period to each subject. The food met the principles of nutrient balance, and the energy values were distributed for each meal (breakfast, lunch, and supper) according to the energy requirement. Table 1 shows the 3-day recipe used in this study.

Table 1. The Three-day Recipe in the Study

Meal Time	Day 1	Day 2	Day 3
Breakfast	Steam bread	Fried dough cake	Steam bread
	Stir-fried cauliflower	Stir-fried potato chips	Stir-fried lettuces
	Egg	Egg	Egg
	Millet congee	Millet congee	Rice porridge with bean
Lunch	Stir-fried pork slices with mushroom	Stir-fried pork slices with Chinese mushroom	Stir-fried pork with green pepper
	Stir-fried potato chips	Stir-fried spinach	Home-style bean curd
	Stir-fried egg with zucchini	Stir-fried egg with tomato	Stir-fried mushroom
	Steam bread	Steam bread	Steam bread
	Rice porridge	Cabbage soup	Tomato egg soup
	Apple	Banana	Orange
Supper	Stir-fried chicken	Stir-fried pork with green pepper	Stir-fried chicken
	Stir-fried pork with long bean	Stir-fried mushroom	Stir-fried zucchini
	Stir-fried cabbage	Stir-fried cabbage	Stir-fried potato chips
	Steam bread	Baked pancake	Baked pancake
Snack	Rice porridge with bean	Rice porridge with bean	Rice porridge with bean
	Bun	Bun	Bun

Stable Isotope Labels

Zn stable isotopes were obtained from Trace Sciences International as oxide powder (Richmond Hill, ON, Canada), and ⁶⁷Zn abundance was 88.55%. ⁶⁷ZnSO₄ for oral administration was converted by ⁶⁷ZnO powder that was dissolved in H₂SO₄ (0.5 mol/L) and diluted with ultrapure water to a concentration of 0.5 mg/L. This solution was sterilized to remove pyrogens and sent to the Institute of Drug Analysis to test if the preparation was safe and edible.

Administration of Meals and Sample Collection

The study flow chart is shown in Figure 1. After a 3-day adaptation period, 3 mg ⁶⁷Zn was administered with porridge and divided into three meals on the fourth day of the test period, which could meet the test requirements^[11]. Simultaneously, the rare earth element dysprosium (Dy 0.4 mg Dy per meal), which follows the same excretory pattern as unabsorbed Zn, was given along with the isotope to check the completeness of the fecal samples^[11-13]. On the fourth day and the eleventh day, capsules of carmine red dye were given to subjects before breakfast to determine the initiation and end of feces collection. The actual intake of food served for the elderly subjects every day was recorded. A duplicate dietary sample was collected for 3 days, and the concentrations of the primary macronutrients, energy, and phytic acid in the food were examined using the related routine protocols of the National Standard of the People’s Republic of China (GB 5009.5-2010, GB/T 5009.6-2003, GB/T 5009.153-2003).

Detection of Zn in the Fecal Samples

The fecal samples were homogenized in a blender, and the powder was digested in a microwave oven (Anton Paar, Austria). Total Zn contents of the

samples were measured by atomic absorption spectrophotometer, and Dy was analyzed by inductively coupled plasma mass spectrometry. Zn from 0.3 mL the digested samples was isolated by heating (120 °C) until all of the liquid had evaporated, and then the samples were redissolved in 1 mL of 3 mol/L HCl. The samples were again heated until dry. The digested fecal residue was then redissolved in 1 mL of 3 mol/L HCl before anion exchange chromatography. Columns were first washed three times with 7 mL of 0.5 mol/L HNO₃ and 2 mL ultrapure water and then conditioned three times using 2 mL of 3 mol/L HCl. The redissolved fecal samples were passed through the conditioned columns. Zn was eluted from the column five times using 2 mL ultrapure water. The final eluent was dried and reconstituted with 2 mL of 2% HNO₃ before mass spectrometric analyses. All the acids used in this experiment were of ultrapure grade. The Zn isotope ratio was detected by multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS; Isoprobe, GV, England).

Calculation of the Phytic Acid-to-zinc Molar Ratio

The phytic acid-to-zinc molar ratio (PZR) was calculated using the following Equation:

$$PZR = \frac{(A / 660.04)}{(B / 65.38)}$$

(1)

where A is the intake of phytic acid, and B is the intake of zinc.

Calculation of Zn Absorption

Fractional Zn absorption (FZA) was calculated using the following Equation:

$$FZA (\%) = \frac{{}^{67}\text{ZnI} - {}^{67}\text{ZnM}^s}{{}^{67}\text{ZnI}}$$

(2)

where ⁶⁷ZnI is the oral ⁶⁷Zn intake (mg), and ⁶⁷ZnM^s is the unabsorbed ⁶⁷Zn in the feces (mg), which were determined as described previously^[11]. ⁶⁷Zn absorption (%) = FZA/dysprosium recovery.

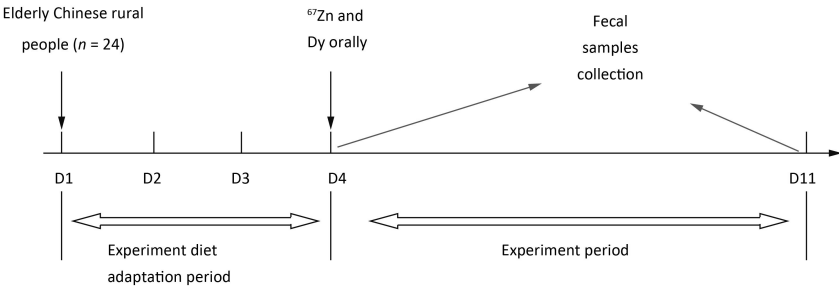


Figure 1. Experimental flow chart.

Calculation of Zn RNI

The most widely used method for estimating dietary Zn requirements is the factorial approach^[14]. The estimated average requirement (EAR) is derived by dividing the mean physiologic requirement of absorbed Zn by the estimated average absorption of Zn. The RNI for dietary Zn intakes is thus calculated as the EAR plus two times the CV and is equivalent to 120% of the EAR.

EAR= $\frac{A}{B}$ (3)

where A is the physiologic requirement of absorbed Zn, and B is the estimated average absorption of Zn

RNI = EAR × 1.2 (4)

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Data were analyzed using SPSS 17.0 software (SPSS Inc, Chicago, IL). Student's *t*-test was used to detect the presence of statistically significant differences between the male and female groups. *P* value < 0.05 was considered as statistically significant.

RESULTS

Subject Characteristics

Eleven male and thirteen female rural elderly people were recruited in this study, but one male individual was excluded during the study because of abnormal Zn absorption data. Anthropometric measurements of the subjects are described in Table 2. Except height, no statistically significant differences were found in the measured physical characteristics.

Daily Intake of Nutrients

Intake of the primary macronutrients, energy, Zn, phytic acid, and the PZR are shown in Table 3. The mean (± SD) Zn intake from the representative Chinese diet was 10.6 ± 1.5 mg/d. The zinc intake amounts in males and females were 10.7 ± 1.5 and 10.5 ± 1.5 mg/d, respectively. The PZR in the diet was 6.4, and it was significantly higher in males than in females (6.6 ± 0.4 vs. 6.2 ± 0.4, *P* = 0.03). There were no statistically significant differences in terms of protein, fat, carbohydrate, energy, Zn, and phytic acid intakes between the male and female groups.

Table 2. Subjects' Basic Characteristics

Characteristics	Mean	SD	Male		Female		<i>P</i>
			Mean	SD	Mean	SD	
Age (years)	63.9	3.6	65.2	2.8	62.9	3.9	0.13
Weight (kg)	66.1	10.3	67.5	9.1	65.0	12.0	0.59
Height (cm)	160.6	7.2	165.1	6.1	157.2	6.1	0.01*
BMI (kg/m ²)	25.5	3.1	24.7	2.5	26.2	3.3	0.27

Note. **P* < 0.05 represented statistically significant difference between males and females.

Table 3. Daily Intakes of Major Nutrients

Nutrient	Mean	SD	Male		Female		<i>P</i>
			Mean	SD	Mean	SD	
Energy (kcal)	3147.8	509.5	3240.5	518.6	3076.4	490.6	0.47
Protein (g)	99.9	16.7	102.5	17.0	97.9	16.3	0.53
Fat (g)	137.6	23.0	141.4	25.9	134.7	20.1	0.51
Carbohydrate (g)	375.4	67.2	387.3	62.3	366.2	69.4	0.48
Zinc (mg)	10.6	1.5	10.7	1.5	10.5	1.5	0.73
Phytic acid (mg)	686.3	115.0	715.8	89.0	663.7	126.9	0.30
Phytic acid/Zn	6.4	0.5	6.6	0.4	6.2	0.4	0.03*

Note. **P* < 0.05 represented statistically significant difference between the male and female groups.

Zn Absorption

FZA, dysprosium recovery, and Zn absorption are shown in Table 4. The absorption rate of ⁶⁷Zn was 27.9% ± 9.2%. The absorption rates of ⁶⁷Zn in males and females were 30.9% ± 8.3% and 25.5% ± 9.2%, respectively. There were no significant differences in FZA (*P* = 0.20), dysprosium recovery (*P* = 0.33), and absorption rate of ⁶⁷Zn (*P* = 0.18) between the male and female groups. The absorption of Zn was categorized according to the WHO standards as follows: high (> 30%-50%), moderate (15%-30%), and low (< 15%)^[15]. As shown in Table 4, the Zn absorption was categorized as high for males and moderate for females.

Zn RNI

Zn physiologic requirements as recommended by International Zinc Nutrition Consultative Group (IZiNCG) for adult males and females were 2.69 and 1.96 mg/d^[8,16]. Zn EAR and RNI are shown in Table 5. Calculating by the Zn absorption rate of the elderly people in this study, the EAR and RNI of Zn were 8.7 and 10.4 mg/d, respectively, in the elderly men and 7.7 and 9.2 mg/d, respectively, in the elderly women.

DISCUSSION

In our study, we first explored the Zn absorption in Chinese elderly people using stable isotope technique. Results showed that the mean dietary Zn absorption in Chinese elderly people was 27.9%. The

Zn absorption was categorized as high for elderly males and moderate for elderly females according to the WHO standards.

Studies from other countries have reported large variations in the mean dietary Zn absorption in the elderly people (range: 17%-35%), indicating the lack of agreement in relation to absorption of Zn both between and within countries. Couzy et al. showed that the apparent Zn absorption calculated from the isotope data was 35% in eight elderly men aged 70-83 years in the USA^[17], whereas Turnlund et al. reported that the mean Zn absorption was 17% when 15 mg Zn was taken by six elderly men aged 65-74 years in California^[18]. A study in Korea by Kim et al. reported that the absorption of Zn was 19% in elderly women given 6.2 mg Zn^[19]. Our study showed that the absorption of Zn from a representative diet in elderly people was lower than that reported by Couzy et al. and higher than those reported by Turnlund et al. and Kim et al.

Evidences obtained from human and animal model studies indicate that Zn absorption is regulated by intake of Zn to maintain homeostasis^[20-22]. With increasing amounts of dietary Zn in a meal, fractional Zn absorption will decrease^[20,23]. Tran et al. reported that absorbed Zn in healthy adults reached saturation with aqueous Zn doses > 20 mg^[24]. The present study showed that the level of zinc intake in our subjects was consistent with the level of zinc intake in rural elderly people surveyed by CHNNS 2010-2013^[25]. Thus, the measured fractional absorption of Zn could reflect the zinc absorption rate in the Chinese elderly population.

Table 4. FZA, Dysprosium Recovery, and Zinc Absorption

Items	Mean	SD	Male		Female		<i>P</i>
			Mean	SD	Mean	SD	
FZA (%)	24.3	7.4	26.7	6.8	22.5	7.3	0.20
Dysprosium recovery (%)	88.4	6.2	86.9	5.0	89.5	6.7	0.33
Zn absorption (%)	27.9	9.2	30.9	8.3	25.5	9.2	0.18

Table 5. Zn Estimated Average Requirement and Recommended Nutrient Intake

Items	Male		Female	
	Recommended ^a	Calculated ^b	Recommended ^a	Calculated ^b
Zn EAR	10.4	8.7	6.1	7.7
Zn RNI	12.5	10.4	7.5	9.2

Note. ^aZn EAR and RNI in Chinese Dietary Reference Intakes (2013). ^bThe values were calculated by the zinc absorption rate of the elderly in this study. EAR, estimated average requirement. RNI, recommended nutrient intakes.

The concentration of Zn in blood plasma or serum is currently the most common indicator of Zn status in a population, and Zn status plays an important role in Zn bioavailability^[3]. In this study, the plasma Zn of all the subjects was $> 10.7 \mu\text{mol/L}$, and the individual Zn status was good. Except Zn intake and Zn status, Zn absorption is largely determined by the presence of specific inhibitors and enhancers of Zn absorption^[3,26]. It is well known that phytic acid is the major dietary factor that inhibits Zn absorption^[3,27]. The phosphate groups in phytate can form strong and insoluble complexes with Zn, and because the gastrointestinal tract of humans lack any significant phytase activity, phytic acid-bound Zn will be excreted in the feces^[3,26]. Therefore, the PZR was used to estimate the negative effect on Zn absorption. As suggested by the WHO, the PZR is between 5 and 15, which does not influence Zn absorption^[14,16]. In our study, the PZR was 6.4, indicating that phytate intake in our study was appropriate.

In general, protein is a major source of dietary Zn. The amount of protein in a meal positively correlated to Zn absorption, especially animal protein, which could facilitate Zn absorption from single meals^[26,28]. Our research used typical Chinese diets for the subjects, which were based on Chinese National Nutrition Survey^[29]. The intake of protein met the recommended dietary intakes for Chinese rural population^[8]. However, the protein intake pattern that 54.6% of the protein among Chinese rural people comes from cereal grains and 29.9% from legumes and animal food^[25] may decrease the Zn absorption.

The establishment of recommended nutrient intakes is a common foundation for all countries to develop food-based dietary guidelines for their populations. The RNIs are used as yardsticks for the assessment of dietary surveys and food statistics, providing guidance on appropriate dietary composition and meal provision, and formulating food labeling^[30]. In our study, the RNI of Zn calculated by the absorption rate in elderly men and women were 10.4 and 9.2 mg/d, respectively. The RNI of elderly men in this study was lower than the recommended values^[8], whereas that of elderly women was higher. Thus, elderly women may be at risk of Zn deficiency when using the current RNI. Further studies should verify these results and pay more attention to the zinc status and health of elderly females.

There are some limitations in our study. First,

our study used a single-labeled stable isotope technique, and the result is apparent Zn absorption. Although double-labeled stable isotope technique is more precise than the single-labeled technique, the venous stable isotope natural abundance is lower, and that method is traumatic with a lower acceptance, which is not conducive to carry out for large-scale samples. In our previous study, we found that true Zn absorption in women of childbearing age is only 2% higher than apparent Zn absorption^[31]. The endogenous fecal Zn excretion did not differ in the young and elderly people^[32-33], and the difference between apparent Zn absorption and true Zn absorption in the elderly will be smaller. Our study can also reflect the Zn absorption in the Chinese elderly and provide a reference for further studies. Further research needs to confirm the difference between the two methods for Zn absorption in the Chinese elderly population. Second, because of the demand of analytical sensitivity, the dose of ^{67}Zn was approximately one-third of the daily dietary zinc; ^{70}Zn could be used to solve the issue of high dose in further study. In addition, we recruited elderly people from a rural area, wherein the Zn absorption may be different to that in an urban area. More studies are needed to explore the Zn absorption in the elderly people.

In summary, our study determined the dietary Zn absorption in the Chinese elderly population, and the typical meal among the elderly people in the rural region provided an adequate intake of zinc. We found that Zn absorption was higher in elderly men than in elderly women. The current RNI in elderly females is lower than our calculation result. Elderly women may be at risk of Zn deficiency when using the current RNI. Further studies should pay more attention to the zinc status and health of elderly females.

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AUTHORS' CONTRIBUTIONS

YXG and YLC designed the research; MYX recruited the subjects; LM and PJH supervised the

research; LXB, LWD, WM, and YLL conducted the research; RTX and WJ analyzed the samples; YC analyzed the data; and LYJ wrote the paper. YXG had the primary responsibility for the final content. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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