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The Effect of Reducing Dietary Phytate Intake and Zinc Supplementation on the Iron Status of Elderly Korean Women

Hyojee Joung, Sook-Hyun Park[1], Sun-Kyung Choi[1], Shan-Ji Li[1], Jihye Kim[1] and Hee-Young Paik[1§]
The School of Public Health, Seoul National University, Seoul 151-742, Korea,
[1]Department of Food and Nutrition, Seoul National University, Seoul 151-742, Korea

To investigate the effects of dietary phytate reduction and zinc supplementation on biochemical iron parameters in elderly Korean women consuming inadequate iron, fifteen healthy women aged 64-75 years were recruited for a feeding study. A high-phytate diet (27.8 phytate:zinc molar ratio) was provided for 9 days, followed by a nine-day low-phytate diet(12.3) and a subsequent 28-day period of unregulated meals with zinc supplementation (22 mg/d as zinc gluconate). Serum iron increased significantly with the low-phytate diet (130.4 μg/L) but returned to the level observed during the high-phytate diet (113.0 μg/L) period when subjects were taking zinc supplements (105.8 μg/L). However, serum ferritin in the subjects decreased significantly with the low-phytate diet (73.8 μg/L) as well as with zinc supplementation (57.2 μg/L), compared to levels following consumption of the high-phytate diet (89.6 μg/L). Transferrin receptor and transferrin saturation were unchanged with the treatments. In summary, zinc supplementation might result in deteriorated iron status in elderly Korean women who consume inadequate iron, while there was no significant effect from reducing dietary phytate.

Key words: Dietary phytate, Zinc supplementation, Zinc, Iron, Elderly Korean women

INTRODUCTION

Inadequate dietary intake or low bioavailability of zinc and iron may lead to mild or undetected deficiencies. The ypical Korean diet contains lower zinc and iron content than is called for in the Korean Recommended Dietary Allowances. The diet is composed mostly of vegetables, grains, and cereals, which supply approximately 80% of total zinc and iron intakes and also contain high levels of phytate. Given the high-phytate content of the Korean diet and the ample experimental data showing that phytate inhibits mineral absorption, it is possible that the reduced bioavailability as well as low mineral contents of the diet contribute to the poor mineral status among Koreans.[1-3)]

Serum concentrations of zinc and iron reported in Korea are relatively low compared to reports from Western countries.[4-6)] In a previous study of rural Korean adults, 45% of the study subjects had low plasma zinc levels and 15-30% had low hemoglobin and serum ferritin levels.[3)] Serum and dietary intake levels of zinc and iron among the elderly (>70y) in Korea were reported to be significantly lower than those among younger adults.[1-3)] The Korean elderly have a higher prevalence of low zinc and iron statuses compared to younger adults and therefore are at elevated risk of deficiency.

Phytate is a potent inhibitor of mineral absorption.[7)] Studies have shown that non-heme iron absorption in humans is significantly decreased by phytate[8-9)] and high-phytate diets are associated with compromised zinc status in various population groups.[10-12)] Changing from a "mixed" to a lactoovovegetarian diet has also been shown to significantly reduce zinc absorption (35%) and serum zinc concentration(11-13%).[13-14)]

Strategies to improve the mineral status of the elderly in Korea could include mineral supplementation, which is becoming more common in Korea, and dietary modification involving phytate reduction to increase mineral bioavailability. Previous studies showed that fractional zinc absorption increased significantly with a low-phytate diet[13-16)] and that zinc supplementation for 28 days significantly increased serum zinc concentrations and urine excretion in the elderly.[17)] Regarding iron status, phytate-degraded food improved iron absorption from cereal porridges in younger adults.[18,19)] However, zinc supplementation may have caused decreased iron bioavailability due to competitive absorption. Considering that there are limited data on the effects of dietary phytate reduction and zinc supplementation on the nutritional parameters of iron in the elderly, the present study was designed to investigate these effects in elderly Korean women.

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§ To whom correspondence should be adressed.

MATERIALS AND METHODS

1. Subjects

Fifteen healthy women aged 64-75 years were recruited through flyers posted in areas neighboring Seoul National University, Seoul, Korea. Sixteen volunteers were selected for the study. Exclusion criteria included the presence of acute disease or chronic diseases (such as diabetes, hypertension, gastrointestinal disorder and hyperlipidemia), a body mass index of less than 17 or greater than 30 kg/m^2, hemoglobin levels of less than 10.5 g/dL, smoking and a history of chronic use of alcohol, prescription drugs, oral contraceptives and vitamin or mineral supplementation. Each subject completed a 24 hr dietary recall prior to the feeding study. Fifteen subjects completed the study. General characteristics of the subjects are shown in Table 1. There were no significant changes of body weight in the subjects.

Informed consent was obtained from all subjects and the study protocol was conducted in accordance with the standards set by Seoul National University's Committee on Human Subject Protection.

Table 1. General characteristics of subjects at baseline (N=15)

	Mean	SD	Range
Age(years)	69.9	3.1	64 ~ 75
Anthropometric Measurements			
Weight(kg)	58.7	6.3	46 ~ 69.5
Height(m)	1.58	0.06	1.48 ~ 1.72
BMI(kg/m^2)	23.6	2.9	18.0 ~ 29.2
Blood parameters			
Hemoglobin(g/dL)	12.4	1.2	10.5 ~ 15.3
Hematocrit(%)	37.6	3.7	33 ~ 47

2. Study Design

The study was composed of three metabolic periods (Table 2). In the initial phase, a high-phytate diet (27.8 phytate: zinc molar ratio, 5.9 mg of iron) was provided for 10 days to control the usual diet of subjects, followed by a 10-day low-phytate diet period (12.3 phytate:zinc molar ratio, 5.5 mg of iron) (Table 3, 4). Each controlled metabolic diet was composed of two daily menus and the subjects were provided menus alternatively. In the third, four-week period, the subjects consumed self-selected meals (6.2 phytate:zinc molar ratio) with zinc supplementation (22 mg/d zinc as zinc gluconate, SOLGAR, USA). A metabolic unit was set up in an apartment where the subjects lived together in order to facilitate and monitor meal consumption. Fasting blood samples were collected at three points in time, i.e. after nine days on the high- and low-phytate diets and 28 days after zinc supplementation. Complete 24-h urine samples were collected for five consecutive days, on days 5-9, during the high- and low-dietary phytate period, and on days 20-24 during the zinc supplementation period. Ten subjects completed the 24hr urine collection. Weight was measured frequently before breakfast to monitor for sudden weight changes. Precautions against environmental mineral contamination were taken for all meals and biospecimens during preparation, collection and analysis. Only new polypropylene containers were used and samples were prepared with triply deionized water and ultra high-purity reagents. All glassware was acid-washed in

Table 2. Experimental design for metabolic periods of high and low phytate and zinc supplementation

	High phytate diet period (MP1)	Wash-out period	Low phytate diet period (MP2)	Zn supplementation period
Study days	1-9	11-26	27-35	40-67
Diet	High phytate controlled diet	Self-selected diet but instructed to have low phytate diet	Low phytate controlled diet	Self-selected diet
Blood Samples	Days 9	-	Days 35	Days 67
24-hour urine collection	Days 5-9	-	Days 31-35	Days 59-63

Table 3. Menus for controlled diets

	MP1 (High phytate)		MP2(Low phytate)	
	Menu 1	Menu 2	Menu 1	Menu 2
Breakfast	***Brown rice gruel***	Rice gruel with beef	***Brown rice grue***[1]	Rice gruel with beef
	Kimchi	Orange juice	Kimchi	Orange juice
	Grilled seaweed	Grilled seaweed	Grilled seaweed	Grilled seaweed
	Grilled yellow croaker	Kimchi	Grilled yellow croaker	Kimchi
	Seasoned lettuce		Seasoned lettuce	
	Orange juice		Orange juice	
Lunch	***Cooked brown rice***	***Cooked brown rice with soybean***	Cooked rice	Cooked rice
	Soybean curd residue stew(biji)	Kimchi	***Soybean curd residue stew(biji)***[1]	Kimchi
	Seasoned cucumber	Soybean paste soup with Chinese cabbage	Seasoned cucumber	Soybean paste soup with Chinese cabbage
	Seasoned squash	Braised lotus root	Seasoned squash	Braised lotus root
	Soybean milk	Pan-fried fish	Soybean milk	Pan-fried fish
	Banana	Apple	Banana	Soybean milk
	Tomato	Soybean milk	Tomato	Apple
Dinner	***Cooked brown rice***	***Cooked brown rice***	Cooked rice	Cooked rice
	Kimchi	Steamed egg	Kimchi	Steamed egg
	Sea mustard soup	Grilled tofu with seasoning	Sea mustard soup	Grilled tofu with seasoning
	Stir-fried chicken	Seasoned bean sprout	Stir-fried chicken	Seasoned bean sprout
	Potato salad	Tangerine	Potato salad	Tangerine
	Seasoned spinach	Kimchi	Seasoned spinach	Kimchi
	Tangerine		Tangerine	

1) Phytase treated foods

Table 4. Nutrient compositions of phytate controlled diets

	High phytate diet	Low phytate diet	Korean RDA
Energy(Kcal)[1)]	1745.3	1758.7	1700
Protein(g)[1)]	77.3	75.2	55
Fat(g)[1)]	44.6	40.9	-
Iron(mg)	5.9	5.5	12
Zinc(mg)	6.3	6.3	10
Phytate(mg)	1722.6	782.1	-
Phytate: zinc molar ratio	27.8	12.3	-

1) Values were estimated with database from Korean Nutrition Society (1998)

10% nitric acid and rinsed three times with triply deionized water. The controlled diet preparation was explained in a previous report.[19)]

3. Analyses of Food, Urine and Blood Samples

At least twice during each of the three metabolic periods, composites of the prepared meals were freeze-dried (Bondyro, Ilsin Inc., Seoul, Korea), ground to homogeneity and kept in desiccators until the mineral and phytate analyses. All urine samples were collected in polypropylene containers. After collection, samples were combined, weighed and aliquoted into smaller containers for further analysis. Fasting blood samples were collected in plastic syringes (Sarstedt 8 mL Monovette syringes with ammonium heparin coated beads). Blood samples were kept on ice for a maximum of 2 hrs and then centrifuged at 3000 rpm for 10min at 4 ℃. After centrifugation, the serum was stored at -70 ℃ until analysis. Hemoglobin was measured at each visit using HemoCue (HemoCue AB, Angelholm, Sweden).

The total phytate content of the test meal was determined by Dionex Liquid Chromatograph System (Dionex Corp., Sunnyvale, CA, USA). Phytate was extracted from the freeze-dried diet composites (0.25 g) using 1.25% H_2SO_4 and eluted with 200 mM NaOH solution.

Food samples (0.2-0.4 g) were microwave digested (MARS 5, CEM Corp., Matthews, NC) and then zinc and iron contents were determined by Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES, Vista, Varian Inc., Walnut Creek, CA).

Urine samples were centrifuged at 1000 rpm (230 × g, 4 ℃, 10 min), using Allegra 6R (Beckman Coulter Inc., Palo Alto, CA) to remove solid material and then diluted with 1% nitric acid (Optima grade; Fisher Scientific; Pittsburgh, PA). Serum samples were centrifuged at 3000 rpm (2200 × g, 4 ℃, 15 min) using Allegra 6R centrifuge (Beckman Coulter Inc., Palo Alto, CA) and then diluted with 1 N nitric acid (Optima grade; Fisher Scientific; Pittsburgh, PA) before aspiration directly into an atomic emission spectrophotometer (Perkin-Elmer, Germany). Alkaline phosphatase activity was measured with a commercial diagnostic kit (Sigma Co, U.S.). Serum levels of iron status parameters were measured using commercial kits: Ferritin (Bayer, U.S.), TIBC (Eiken, Japan) and transferrin (Behring, Germany). Transferrin saturation (%) was calculated as serum iron (μmol/L) divided by TIBC (μmol/L)×100.

4. Statistical Analysis

Statistical analyses were conducted with SAS 8.0. Results are expressed as means ± SD. Differences of means among treatments were tested by repeated analysis of variance. Differences of $P<0.05$ were considered to be significant.

RESULTS

The effects of phytate reduction and zinc supplementation on the biochemical parameters of iron and zinc are shown in Table 5. In general, zinc supplementation caused more changes in the measured parameters compared to the phytate reduction. Serum zinc levels did not change but alkaline phosphatase activity increased significantly following both the low-phytate diet period (75.1 unit/L) and the zinc supplementation period (85.3 unit/L), as compared to the high-phytate diet period (66.3 unit/L). Urinary zinc excretion did not change following the low-phytate diet (368.1 μg/d) but did increase significantly after zinc supplementation (425.0 μg/d).

Serum iron concentration increased significantly following the low-phytate diet (130.4 μg/dL), but decreased again after

Table 5. Biochemical parameters for iron, zinc and copper in each experimental periods (N=15) (Mean ± SD)

	High phytate	Low phytate	Zinc supplementation	Normal range[27)]
Zinc status				
Serum zinc (μmol/dL)	112.6±31.3	121.3±15.2	115.9±18.8	10.7-18.4
Serum ALP (unit/L)	66.3±20.8[a1)]	75.1±21.1[b]	85.3±25.6[c]	39-117
Urinary zinc (μg/day)[2)]	348.1±68.6[a]	368.4±183.7[ab]	425.0±208.9[b]	300-600
Iron status				
Serum iron (μg/dL)	113.0±24.4[a]	130.4±24.7[b]	105.8±24.2[a]	115±50
Transferrin receptor (mg/L)	2.6±0.4	2.5±0.3	2.7±0.4	0.85-3.05
Serum ferritin (μg/L)	89.6±57.5[a]	73.8±44.3[b]	57.2±34.9[c]	100±60
Transferrin saturation(%)	27.1±5.0	26.6±3.8	24.9±5.6	35±15

1) Mean values with different superscript in the same row are significantly different by repeated analysis at α=0.05.
2) Ten subjects completed 24-hr urine collection for 5 consecutive days in all three periods.

zinc supplementation (105.8 μg/dL). After the initial high-phytate diet, serum iron concentrations were 113.0 μg/dL. Serum ferritin levels decreased significantly following the low-phytate diet (73.8 μg/L) and zinc supplementation (57.2 μg/L) compared to the high-phytate diet period (89.6 μg/L), indicating deterioration of iron status. Transferrin receptor and transferrin saturation did not change significantly.

DISCUSSIONS

Dietary phytate is a significant inhibitory factor for mineral absorption through formation of insoluble complexes with mineral cations. Dietary phytate levels have been shown to influence mineral absorption in humans.[8-11] In order to investigate the effect of phytate reduction on the iron status of elderly Korean women, we measured changes in various biochemical parameters of iron following consumption of meals prepared by the substitution of brown rice with refined white rice and enzymatic treatment of some phytate-rich dishes.

After consumption of the low-phytate diet for nine days, there was a significant increase in serum alkaline phosphatase activity, a zinc dependent enzyme, and a non-significant increase in serum and urinary zinc levels in the subjects. The results imply that zinc status tended to be temporarily or partially enhanced by a phytate-reduced diet. However, more long-term studies are needed to confirm the beneficial effects of phytate reduction in various populations with marginal trace element status and a more practical method to reduce dietary phytate should be developed, considering that the phytase treatment used in this study cannot be easily reproduced in the average household.

The findings also showed that elderly Korean women who received zinc supplementation for 28 days had increased urinary zinc excretion and serum alkaline phosphatase activity, implying that zinc supplementation might enhance the zinc status of elderly subjects. Zinc supplementation has been used to increase zinc status and health functions in various subpopulations, including lactating women and their infants[20-23] and cirrhosis patients.[24] On the basis of the findings in our study, supplementation is a possible method to improve the zinc status of elderly Korean women. Regarding supplementation strategies to improve zinc status in populations, such as elderly Korean women, who have marginal zinc and iron statuses, interactions with other trace elements and their overall nutritional status must be considered.

With regard to the measured indicators of iron, the reduction of dietary phytate possibly contributed to a significant increase in serum iron. But, in contrast, a significant decrease in serum ferritin was observed. Larsson et al.[25] reported that reduced phytate intake resulted in increased iron absorption, so increased serum iron concentration after the low-phytate period might be due to increased iron absorption. It must be considered that the low-phytate diet was consumed for only nine days and the observed increase in serum iron may have been temporary. The nine-day period may not have been long enough to increase iron stores, expressed in serum ferritin level. The low content of iron in the controlled diets also should be considered in interpreting this data. With analyzed data our controlled high and low phytate diets contained only 5.9 mg/d and 5.5 mg/d, respectively, which is about 49% and 46% of the Korean RDA, even though the contents of iron were intended to have 12mg/d with the calculation based on the Korean Food Composition database.[26] Thus, the intake of the controlled diet with low iron content during the experimental period might have deteriorated the iron status of the subjects, especially their serum ferritin levels, indicating the depletion of stored iron. Further research is needed to confirm the effects of long-term diet modification with different levels of usual iron intakes.

After the four-week zinc supplementation (22 mg/d), the serum iron and ferritin levels of the subjects decreased and transferrin receptor levels and transferrin saturation did not change. Donangelo et al. reported similar effects of zinc supplementation in young women with low iron reserves, as zinc supplementation showed improved zinc indices and appeared to induce cellular iron deficiency.[27] The possible reasons for the decrease in serum iron and ferritin after zinc supplementation in our study might be due to the competition of zinc with iron during absorption.

In summary, strategies to reduce dietary phytate may contribute to improving zinc status in Koreans, especially in the elderly subpopulation. Enhancing the bioavailability of minerals by reducing phytate intake may be a possible strategy for overall improvement of mineral status. Although zinc supplementation for a short period might have a positive effect on zinc status, it could have a negative effect on iron status. Thus, supplementing with only one trace element should be done with caution since it may result in a compromised status of another trace mineral.

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