The Effect of Dietary Phytate Content on Iron Absorption and Status in Young Korean Women

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Abstract : This study investigated the effects of dietary phytate reduction on the apparent absorption and biochemical parameters of iron status in young Korean women. Fourteen healthy, young women consumed low and high phytate diets for ten days of each experimental period. Duplicate diet samples, a fasting blood sample on day 9, and complete fecal samples for five consecutive days starting from day 5 of each diet period were collected. The iron content of diet and fecal samples were analyzed to calculate apparent absorption. Serum samples were analyzed for iron, ferritin, transferrin receptor and TIBC; transferrin saturation was also calculated. The apparent absorption of iron tended to increase in the low phytate period (32.51%) compared to the high phytate period (17.91%), but the difference was not significant ($p=0.06$). Serum ferritin decreased and serum transferrin receptor increased significantly during the low phytate diet although the mean values were within the normal range. Serum iron and transferrin saturation did not change significantly. In conclusion, the results indicated that reducing dietary phytate for ten days negatively affected iron nutritional parameters, but it moderately and positively affected apparent iron absorption in young Korean women. Further research on the long-term effects of a low phytate diet with an adequate iron content for young Korean women is necessary.

Key Words : dietary phytate, iron absorption, iron status

1. Introduction

Phytate (myo-inositol 1, 2, 3, 4, 5, 6-hexa*kis*phosphate (InsP6)) is a potent inhibitor of mineral absorption (Congrove, 1966; Morris, Ellis, 1989). The phosphate groups in inositol hexaphosphate can form insoluble complexes with mineral cations such as zinc, iron, and copper; phytate-bound minerals will accordingly be excreted in the stool due to the lack of any significant phytase activity in the human gastrointestinal tract (Krebs, 2000). Previous studies have shown the inhibitory effects of phytate on iron absorption. Non-heme iron absorption in animals (Pallauf *et al.*, 1999) and in humans (Hallberg *et al.*, 1987; Hurrell *et al.*, 1992) was significantly decreased by phytate. However, iron absorption can be increased by the addition of

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ascorbic acid (Gillooly *et al.*, 1984), by the addition of EDTA (Hurrell *et al.*, 2000) and by the degradation or removal of phytic acid (Hurrell *et al.*, 1992; Hurrell *et al.*, 2003).

The iron nutritional status of young Koreans, for example, is relatively low compared to the previous reports from Western countries (West, 1996 : 789-790; Ministry of Health and Welfare, 1999 : 115-116). Previous studies have reported that 26-33% of Korean college women have low serum ferritin (<15ug/L) and 12-15% have low hemoglobin concentration (<12g/dL) (Hong *et al.*, 1999, Lee *et al.*, 1997), and between 30-46% of Korean girls in high school have had an iron deficiency based on hemoglobin concentration (<12g/dL) and serum ferritin (<15ug/L) (Kwon *et al.*, 2002). One of the reasons for the deficiencies might be the chronic low content of their diets and the low bioavailability of iron in foods composing their diets. The typical Korean diet is composed mostly of vegetables, grains, and cereals. These foods contribute about 80% of the iron intake, but they are rich in phytate which results in the low bioavailability of iron. This may be a cause of the poor nutritional status of iron in young Korean women(Kye, Paik, 1993). Not surprisingly, these iron deficiencies may exacerbate the problems caused by the nutritional need for iron in early pregnancy. Additionally, decreased iron nutritional status in prepregnancy may lead to poor pregnancy outcomes (Allen, 2001).

Therefore, dietary strategies to improve the iron nutritional status of young Korean women need to be developed. Dietary modification, such as reducing phytate intake to increase bioavailability of minerals, is a possible strategy. The present study was designed to examine the extent to which controlling dietary phytate intake affects the apparent absorption and nutritional status of young Korean women.

II. Material and methods

1. Subjects

Sixteen healthy women aged 19-24 were recruited for this study by flyer which were distributed on the campus of Seoul National University in Seoul, Korea. The subjects were selected after an interview. Exclusion criteria included a body mass index of less than 17 or greater than 26 kg/m^2, smoking, chronic use of alcohol, prescription drugs, oral contraceptives, vitamin or mineral supplements, a hemoglobin level of less than 11 g/dl, and the presence of acute disease or chronic disease such as diabetes, hypertension, gastrointestinal disorder, and hyperlipidemia. Young women who had usual dietary zinc intake of less than 5 mg/d or greater than 15 mg/d were excluded. Each subject completed a 24-hour recall and 2 day diet records before the metabolic studies began.

All subjects gave their informed consent to participate in this study. Fourteen of the sixteen subjects completed the study. The study protocol was reviewed and approved by the Committee on Human Research of the College of Human Ecology at Seoul National University and the Office of Human Research Protection at the University of California at Davis. General characteristics of the subjects are shown in <Table 1>.

<Table 1> General characteristics of subjects at baseline (N=14)

	Mean	SD	Range
Age (years)	22.6	1.5	19~14
Anthropometric measurements			
Weight (kg)	53.9	5.8	47~67
Height (m)	162.3	4.4	156.0~171.0
BMI (kg/m^2)	20.5	1.9	18.0~24.8
Dietary intakes*			
Energy (Kcal)	1706.5	529.4	1034.9~3054.1
Fat (g)	45.6	16.9	24.6~86.1
Protein (g)	63.3	23.1	114.3~32.8
Iron (mg)	13.3	6.6	27.8~5.0
Zinc (mg)	7.6	2.8	3.6~3.3
Phytate (mg)	310.4	350.7	61~1350.5
Phytate: zinc molar ratio	4.0	4.1	0.6~17.4

*Values were estimated with database from Korean Nutrition Society (Korean Nutrition Society, 1998 : 266-463) and previous report(Lee *et al.*, 2003; Joung *et al.*, 2003)

2. Study design

The study design is shown in <Table 2>. The feeding study was divided into high and low phytate diet periods of ten days each with a ten-day washout period in between. Subjects lived in the metabolic unit during the metabolic period, and consumed diets without leftovers provided from the metabolic kitchen. Upon completion of the first high phytate diet period, subjects returned to their homes for a ten-day period before starting the second period. A fasting blood sample was collected from each subject on day 9 of each metabolic period. Serum was separated and kept at -70°C for analyses of iron status parameters. Complete fecal samples were collected for five consecutive days starting on day 5 of the diet in each period. Weight was frequently measured before breakfast to monitor any sudden changes.

<Table 2> Experimental design for metabolic periods of high and low phytate and Zn supplementation

High phytate diet		Low phytate diet	
Study days	1 - 10	11 - 20	21 - 30
Diet	High phytate controlled diet at metabolic unit	Free diet at home	Low phytate controlled diet at metabolic unit
Blood collection	Day 9		Day 29
Fecal collection	Days 5 - 9	-	Days 25 - 29

3. Experimental diets

Two menus composed of common Korean foods were designed during the first and second metabolic periods to contain different amounts of phytate (Table 3). All food and drinks were

<Table 3> Menus for controlled diets

	High phytate		Low phytate	
	Menu 1	Menu 2	Menu 1	Menu 2
Breakfast	Brown rice gruel Kimchi Grilled seaweed Grilled yellow croaker Seasoned lettuce Orange juice	Ham and cheese sandwich Orange juice	*Brown rice gruel* Kimchi Grilled seaweed Grilled yellow croaker Seasoned lettuce Orange juice	Ham and cheese sandwich Orange juice
Lunch	Cooked brown rice Steamed egg Grilled tofu with seasoning Seasoned bean sprouts Kimchi Milk Banana Tomato	Cooked brown rice with soybeans Kimchi Soybean paste soup with Chinese cabbage Braised lotus root Pan-fried fish Milk Apple	Cooked rice *Soybean curd residue stew (biji)* Seasoned cucumber Seasoned squash Yogurt Milk Tomato	Cooked rice with soybeans Kimchi Soybean paste soup with Chinese cabbage Braised lotus root Pan-fried fish Milk Apple, banana
Dinner	Cooked brown rice Kimchi Sea mustard soup Stir-fried chicken Potato salad Seasoned vegetables Water melon	Cooked brown rice Soybean curd residue stew (biji) Seasoned cucumber Seasoned squash Melon	Cooked rice Kimchi Sea mustard soup Stir-fried chicken Potato salad Seasoned vegetables Water melon	Cooked rice Steamed egg Grilled tofu with seasoning Seasoned bean sprouts Melon Kimchi

* The foods in italics were treated with a phytase enzyme to reduce phytate content.

provided for the subjects during the metabolic periods. Dishes commonly consumed by Korean were selected for the experimental diets (Lee *et al.*, 2003). Menus for the high phytate diet were composed of common Korean foods and included dishes made with brown rice and soybean products. The average phytate:zinc molar ratio of the high phytate diet was 27.4. Low phytate diets were prepared by substituting brown rice with white rice, whole wheat bread with white bread, and by phytase treatment of the soybean dishes. These resulted in an average phytate:zinc molar ratio of 10.4. Detailed methodologies for the enzymatic reduction of phytate in some foods are described elsewhere (Lee *et al.*, 2003). For this investigation, phytase enzyme (5000 U/g, BASF,

Mount Olive NJ) from *Aspergillus niger* was added to the brown rice and soybean curd prior to cooking. The nutrient compositions of the controlled diets are shown in Table 4. Energy and macronutrients were calculated using the Korean Nutrient Composition Table provided by the Korean Nutrition Society (Korean Nutrition Society, 2000 : 266-463); phytate and mineral intake levels were measured as described in the following section. All menus were intended to satisfy the RDA nutrient requirements recommended for young Korean women and were designed to provide the same amounts between the two diets. Duplicate portions of the metabolic diets were collected for analysis of iron content.

4. Analyses of feces, meals and blood samples

Portions of each meal and every fecal sample were stored in polyethylene bags at -20°C. The protions were freeze-dried, homogenized by blender, and stored in the dessicator until analyses.

Freeze-dried meals and fecal samples (0.2-0.4g) were microwave-digested (MARS 5, CEM Corp., Matthews, NC), and then mineral content was determined by ICP-AES (Vista, Varian Inc., Walnut Creek, CA). Phytate content was determined by the anion exchange method described by Harland and Oberleas (1986). Phytate was extracted from the freeze-dried samples using 2.4% HCl, eluted with 0.7M NaCl solution on an ion exchange column with a small amount (0.5g) of anion exchange resin AGI-X4 (100-200 mesh, Cl- form). It was wet-digested with HNO_3/H_2SO_4 to release P, which was then measured colorimetrically at 640 nm. Phytate amounts were calculated as hexaphosphate equivalents. All measurements were done in triplicates. The apparent absorption of minerals was calculated from diet and fecal mineral contents : {(mineral intake-fecal mineral)/mineral intake} $\times 100$.

The serum levels of iron status parameters were measured using commercial kits: ferritin kit of cemiluminoscence immuno assay (Bayer, U.S.), TIBC kit (Eiken, Japan), and transferrin kit (Behring, Germany). Transferrin saturation (%) was calculated as the following equation: TIBC (umol/L) / serum iron (umol/L) x 100.

5. Statistical analysis

Results are expressed as mean (SD). Differences of means between high phytate and low phytate diet were tested by paired t-test.

III. Results

Menus for the experimental diets were designed to provide 14.50 mg/d of iron in the high phytate diet and 12.45mg/d of iron in the low phytate diet. The measurements were calculated by referencing the most commonly used nutrient database: The Korean Food Composition Table(Korean Nutrition Society, 2000 : 268-463). However, we found that the actual iron content of the experimental menus contained 5.57 mg/d in high phytate diet and 6.04 mg/d in the low phytate diet through the chemical analysis of composites in experimental diets (Table 4).

Fecal frequency was 0.88 ± 0.25 times/day

<Table 4> The analyzed composition of the experimental diets

	High phytate diet	Low phytate diet
Energy (Kcal)*	1816.8	1829
Protein (g)*	78.5	78.1
Fat (g)*	51.6	48.8
Vitamin C (mg)*	159.5	163.5
Calcium (mg)	807.2	652.7
Phosphate (mg)	1472.5	1240.7
Magnesium (mg)	452.0	301.2
Phytate (mg)	1844.9	697.3
Zinc (mg)	6.5	6.4
Phytate: zinc molar ratio	29.9	10.8

* Nutrient contents were calculated by database from the Korean Nutririon Society

(range 0.46~1.41) in the high phytate period and 0.79±0.21 times/day (range 0.56~1.28) in the low phytate period. The fecal dry weight in the high phytate period showed significantly more than in low phytate period ($p<0.01$).

The analyzed content of iron in the diets was 5.57 mg/d in the high phytate diet and 6.04 mg/d in the low phytate diet. Fecal excretion of iron was 4.65 mg/d in the high phytate period and 4.12 mg/d in the low phytate period. Apparent absorption of iron was 17.91% in the high phytate period and 32.51% in the low phytate period, but the difference was not significant ($p=0.06$) (Table 5). During the total diet period, fecal iron was strongly correlated with fecal phytate ($r=0.65$, $p<0.01$) (Fig.1).

<Table 5> describes the changes in the serum biochemical markers of the iron nutritional status during the high and low phytate diet. Serum iron concentration increased following the low phytate diet, but the amount was not significant ($p=0.06$). Serum ferritin levels decreased significantly following the low phytate diet ($p<0.05$) and indicated deterioration of iron status. Transferrin receptor increased significantly, but the mean value was still within normal range. Transferrin saturation did not change significantly.

<Table 5> Apparent absorption of iron and nutritional status[1)]

	High phytate diet	Low phytate diet
Apparent absorption		
Diet iron (mg/d)	5.57±0.43	6.04±0.69
Fecal iron (mg/d)	4.65±1.79	4.12±1.91
Apparent absorption (%)[2)]	17.91±27.89	32.51±28.64
Iron status		
Serum iron (μg/dL)	117.84±45.72	121.69±43.05
Transferrin receptor (mg/L)*	1.94±0.87	2.15±0.96
Serum ferritin (μg/L)*	28.91±30.70	21.93±21.58
Transferrin saturation (%)	27.31±10.23	28.56±10.36

1) Subject 3 consumed 80% of the diet, thus 80% of the phytate content was used in the calculation. One subject was excluded as an outlier due to high excretion of iron, over 150% of the diet

2) Apparent absorption (%) = (diet iron-fecal iron) / diet iron × 100

* significant difference between groups at paired t-test ($p<0.05$)

Ⅳ. Discussion

Dietary phytate is a significant inhibitory factor accecting mineral absorption through the formation of insoluble complexes with mineral cations. Consequently, dietary phytate levels have been shown to influence mineral absorption in humans (Pallauf *et al.*, 1999; Hallberg *et al.*, 1987; Hurrel *et al.*, 1992; Manary *et al.*, 2002; Turnlaund *et al.*, 1998; Huddle *et al.*, 1998; Kotasaki-Kovatsi *et al.*, 2001). In order to investigate the effect of dietary phytate reduction on the iron status of young Korean women, we measured changes in their various biochemical parameters following the consumption of meals. These meals were prepared by substituting whole grains with refined grains and the enzymatic treatment of some phytate rich dishes.

After consuming the low phytate diet for 9 days, there was a significant decrease in plasma ferritin and a significant increase in transferring receptor which indicated a deteriorating iron status. Plasma iron and transferrin saturation, as well as the apparent absorption of iron, did not change significantly with the reduction of dietary phytate. The results imply that the short period of dietary modification in this study may not have had a positive effect but, rather, a slightly negative effect on the iron nutritional status of young Korean women with marginal iron status.

The reasons for the negative effects of dietary phytate reduction on iron nutritional status may be explained by the short period of intervention as well as the low iron content of experimental diet. First, the consumption of a low phytate diet for 9 days may not be sufficient enough to induce significant positive changes in the biochemical parameters measured in our study, even though modification did result in a moderate increase of apparent absorption ($p=0.06$). Secondly, the dietary intake of iron during the experiment was very low. The actual iron content of the experimental diets was 5.57 mg in the high phytate diet and 6.04 mg in the low phytate diet. These amounts represent only 34.8% and 37.8% of the Korean RDA, respectively. As shown in Table 5, the analyzed dietary iron content of the diets was very low compared to the amount we had originally intended to provide in the experimental diets when we calculated the values using the Korean Food Composition Table (Korean Nutrition Society, 2000 : 266-463). Our calculations using the iron database of the Korean Food Composition Table resulted in an overestimation of almost 3 times that of the actual dietary iron contents. Thus, the iron content of some foods must be over represented in the Korean Food Composition Table. Considering the high prevalence of marginal iron deficiency in Korean women (Kye, Paik. 1993), the iron content of Food Composition Table must be investigated and corrected so that the iron intake of the population can be calculated more accurately. Another possibility that might account for the low effect of dietary phytate intake on the iron nutritional status is its nutrient interaction with other substance such as protein and vitamin C. However, the intake of protein and vitamin C between the high and low metabolic periods was not different in our study; all of our subjects consumed only the experimental diets provided by the metabolic unit.

The biomarkers of iron status do have

limitations. Plasma iron or transferrin saturation levels are useful for screening purposes, but they are not useful for determining iron status because many diseases can induce secondary changes in plasma iron transport (Cook, 1994). Serum transferrin receptor assays provide more reliable information on iron status since non-nutritional factors and chronic diseases have little effect on transferrin receptor status (Sauberlich, 1999 : 343-346). In our study, transferrin receptor levels increased and serum ferritin levels decreased following phytate reduction which indicated a depletion of stored iron.

Further research is needed to confirm the effects of long-term dietary phytate modification on subjects who consume an adequate level of dietary iron intake. Research is also needed to develop more practical methods of reducing dietary phytate as the phytase treatment used in the present study cannot be easily reproduced in the average household. There have been, however, several trials to improve the iron bioavailability of meals with high phytate content. Bach *et al.* (2003a) have reported that increasing cooking temperature does not affect non-heme iron absorption in women from phytate rich meals, but in another study, they found that non-heme iron absorption from a phytate rich meal increased with the addition of a small amount of pork meat (Bach *et al*, 2003b). This strategy could improve iron nutritional status in Korea, especially in its young women.

It should also be noted that Graf and Eaton (1993) have explained that dietary phytate chelates with various minerals and prevents iron-mediated generation of the hydroxy radicals. This keeps intestinal epithelial cells from oxidative damage and protects against colon cancer. In their study, the differences between the dry weight of feces during the high and low phytate diets support the possibility that the effects of phytate prevent colon cancer. However, it is not clear whether these effects were caused solely by phytate since dietary fiber may also a factor. Due to the lack of a complete DB of dietary fiber in the Korean Food Composition Table, dietary fiber of the two diets could not be calculated. The interrelationships of phytate and fiber on mineral bioavailability and the prevention of cancer should be addressed in the future research.

V. Conclusion

In conclusion, reducing dietary phytate for a short period affected some iron nutritional parameters negatively but tended to increase apparent iron absorption in young Korean women. Considering the high prevalence of marginal iron deficiency in Korean women, more long-term studies to confirm the beneficial effects of phytate reduction on iron status and various strategies to improve iron absorption in meals with a high phyate content are needed.

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